Author’s response to reviews

Title: Adherence to the World Cancer Research Fund/ American Institute for Cancer Research cancer prevention recommendations and risk of in situ breast cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort

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Author’s response to reviews:

Our manuscript on the Adherence to the World Cancer Research Fund/ American Institute for Cancer Research cancer prevention recommendations and risk of in situ breast cancer

Editorial Comments:
As you will see from the reports, all three reviewers are enthusiastic about the manuscript. However, Reviewer 2 has made the point that this might be somewhat of a missed opportunity to comprehensively examine the relationship between established and putative carcinogenic exposures for invasive breast cancer and risk of BCIS in a high-quality cohort. We would encourage you explore whether or not that could be remedied.
Thank you for this comment. We do agree with Reviewer 2 that only a few BCIS-specific analyses have been published and that there is great potential for further BCIS-specific projects. However, as explained in the point-by-point reply to the reviewer, the pre-specified, approved aim of the present project was to assess whether adherence to the WCRF/AICR cancer prevention recommendations was associated with BCIS risk, rather than identifying new potential risk factors for BCIS.

Reviewer reports:
We thank the three reviewers for their knowledgeable and constructive comments, which have very much helped us to improve our manuscript. Please find below our point-by-point reply.

Reviewer #1 Tari King:
This is a very well written paper examining the relationship between lifestyle and the risk of in-situ breast cancer. The methodology and strengths and limitations of the findings are well described.
We very much appreciate the positive feedback from Dr. King.

1. I have only one comment as to whether the authors would consider showing the differences in the in-situ breast cancer populations (DCIS vs LCIS). I realize that the population is skewed and is predominantly DCIS yet these data could be interesting and hypothesis generating as we know there are differences between the two lesions at the molecular level.
We agree that the distinction between DCIS and LCIS is important. Results stratified by morphological subtype were included in the manuscript (Lines 303-306; Supplementary Table 3). However, given the limited power for these analyses especially in the screening-based EPIC cohorts, we decided not to make these results the focal point of our manuscript.

We state now in the discussion that better powered analyses on BCIS subtypes are needed (Lines 385-389).

Reviewer #2 Claire Vajdic:
The authors have examined the association between a lifestyle score and the incidence of in-situ breast cancer (BCIS) in the EPIC cohort. The EPIC cohort is high-quality and large, the baseline questionnaires were validated, the BCIS diagnoses were histologically confirmed, and sensitivity analyses were performed. The manuscript is well-written.
We thank Professor Vajdic for her positive feedback.

The authors observed no association between the lifestyle score and BCIS risk for the full cohort, and a modest protective association for the sub-cohort recruited mainly via mammographic screening.
1. However, I think that this is a missed opportunity to comprehensively examine the relationship between established and putative carcinogenic exposures for invasive breast cancer and risk of BCIS in a high-quality cohort. The composite lifestyle score categorises 8 factors (7 in previous studies) into one of three categories (4 categories for consumption of wholegrains, vegetables, fruit and beans), thus ruling out an opportunity to examine plausible exposures to the extent usually performed in large, well-characterised cohorts.

We thank the reviewer for this comment. We do agree that only a few BCIS-specific analyses have been published from the EPIC cohort and other cohorts and that there is great potential for further BCIS-specific projects. However, the pre-specified aim of the present project (that was approved by the EPIC steering committee in its present form) was to assess whether adherence to the WCRF/AICR cancer prevention recommendations was associated with BCIS risk, rather than identifying new potential risk factors for BCIS.

We decided to use categorized lifestyle factors and their composite score for the sake of comparability with previous publications from the EPIC cohort, particularly the one including invasive breast cancer by Romaguera et al. (Am J Clin Nutr. 2012). Admittedly, such classifications may artificially reduce information, but tests for trend modeling exposures on the continuous scale were in line with the results we present for the categorized exposures.

Other established risk factors for invasive breast cancer (e.g. reproductive factors) and smoking, were included in our analysis as covariates, and more in depth-analyses of EPIC data on etiological questions (which we agree are of great importance) rather than adherence to lifestyle recommendations will be carried out in the future.

2. Also, for some lifestyle factors the categorisations are potentially misleading. For example, the "be a healthy weight" component gives 1 point to normal weight, half a point to overweight, and no points to those either underweight or obese, thereby misclassifying a likely carcinogenic exposure given the established evidence that body fatness is a risk factor for postmenopausal invasive breast cancer (Lauby-Secretan NEJM 2016).

We generally agree that the individual classifications of risk factors as part of lifestyle scores are simplistic, as they may not represent actual shapes of associations between lifestyle factors and disease risks. Concerning breast cancer risk, we acknowledge that associations with BMI strongly depend on age and menopausal hormone therapy use. Previous studies and ours observed positive associations among older non-users of menopausal hormone therapy, but inverse associations among premenopausal women. However, recommendations for primary prevention in the general population can hardly account for such differential associations, and normal weight is considered ideal for primary prevention, rather than overweight, obesity or underweight. Therefore, we think that assigning zero points to obese study participants is actually in line with the IARC’s (Lauby-Secretan et al. NEJM 2016) and the WCRF/AICR's assessment of obesity as a causal risk factor for postmenopausal breast cancer and the majority of other cancers.

Assigning zero score points to underweight individuals as well appears justified against the background of multiple studies suggesting that underweight is associated with higher risks of chronic diseases including cancer due to various reasons such as unintentional weight loss, low muscle mass, severe comorbidities, or heavy smoking (outlined by D.K. Tobias in 2017 in Diabetes Care: https://www.ncbi.nlm.nih.gov/pubmed/28733375). Notwithstanding these considerations, the proportion of EPIC participants with BMI values <18.5 was very low (2 %).

In addition, as stated above, trend tests for individual score components on the continuous scale revealed results consistent with those from models, in which categorical variables were used. With regard to BMI, the only significant association we found when modeling BMI on the continuous scale was a linear inverse association between BMI and BCIS risk among premenopausal women, in line with our finding from analyses across categories (Supplementary Table 4) and previous studies. For
these reasons, we consider the simplified categorical analyses as appropriate for the present purpose, also in view of comparability with previous similar studies (see point 1 above).

3. Additionally, given the consistent evidence that 1 glass of alcohol per day increases the risk of postmenopausal invasive breast cancer (and emerging evidence of the same for premenopausal breast cancer), the categorisation of alcohol consumption in terms of BCIS risk is not on strong ground epidemiologically, as 1 point is given to $\geq 10$g/d ($\geq 1$ glasses per day).

This is a valid point. Again, our categorization was motivated by previous analyses from the EPIC cohort, and we adopted previous items for comparability. The cut-point at 10 g of alcohol per day was initially motivated by the fact that participants were recruited in the EPIC cohort during the 1990s, when the harmful effects of moderate alcohol consumption were less known, and the notion of potential cardiovascular benefits of moderate alcohol consumption was widespread (popularity of the “French Paradox”). Accordingly, the prevalence of abstainers was low among women in the EPIC cohort (16 %). Another motivation not to restrict the category reflecting the greatest adherence to the WCRF/AICR recommendations to abstainers was that many previous studies showed “sick quitter effects”, i.e. a higher proportion of persons with impaired health in the non-drinker category (Fekjaer, Addiction 2013), introducing reverse causality. Similar findings were also supported in EPIC publications (Rohrmann et al., Am J Epidemiol. 2006).

4. Furthermore, the utility of a lifestyle score to strengthen health promotion efforts in cancer control is not justified by the authors.

Despite our finding of an inverse association between the WCRF/AICR score and BCIS risk among women regularly participating in breast cancer screening, we believe that our evaluation of the WCRF/AICR lifestyle score in relation to BCIS risk cannot provide direct proof for the utility of the specific recommendations by the WCRF/AICR for health promotion, as the recruitment of the study took place in the 1990s before the WCRF/AICR were established. Ideally, the appropriateness and reach of the WCRF/AICR recommendations should be evaluated in more recent studies, possibly by other methods than standard dietary assessment and anthropometry, but such analyses were beyond the possibilities and scope of our project.

Other issues
1. I recommend the abstract list the component parts of the WCRF/AICR lifestyle score, especially because it differs from previous studies

This is a good suggestion, and we have added the individual WCRF/AICR cancer prevention recommendations used to construct the score to our abstract (Lines 106-108).

2. The abstract conclusion, that lifestyle is associated with BCIS risk among women with regular screening participation appears too strong given the lack of data on screening participation in this cohort, the stated paucity of prior evidence, and the upper bound of the 95% confidence interval.

We have modified the abstract conclusion (Lines 119-122).

3. Can the authors clarify whether the decision to stratify by recruitment centre type (screening, other) was made a priori?

Since BCIS is diagnosed mainly via mammographic screening and attendance of mammographic screening was not routinely collected in all centers of the EPIC cohort, we had a-priori decided to stratify our analyses by recruitment center type (screening, other), which we state in the Methods section (Lines 267-270). Of note, we also observed a statistically significant interaction between WCRF/AICR score and center type in relation to BCIS risk ($p=0.02$), even though the necessity to take screening into account was agreed on in the earliest phase of our project.
4. It is now usual practice to perform stratified analyses (by menopausal status) for invasive breast cancer. Can the authors clarify why that was not the primary analysis in this study, particularly given the justification for the study given in the introductory text?
We agree with the reviewer. Given the different results by menopausal status and menopausal hormone therapy use reported for invasive breast cancer, we have performed stratified analysis of the main results by menopausal status (Lines 298-300; Table 2). Additionally, we investigated the association between each WCRF/AICR cancer prevention recommendation and BCIS by menopausal status and menopausal hormone therapy use (Lines 311-313; Supplementary Table 4).

5. The missing data is a limitation that is not acknowledged in the discussion. From Table 1, the missing data do not appear to be missing at random - they are almost always greater for the women recruited via the non-screening compared to the screening centres. Given this, what is the potential impact of the imputation on the study findings?
We acknowledge that missing covariate data are a limitation of our study. However, when performing complete cases analyses, the results did not differ substantially (HRtotal=0.96, 95% CI: 0.91-1.02; HRScreening=0.86, 95% CI: 0.74-1.00; HROther=0.98, 95% CI: 0.92-1.04, per one unit of increase in the WCRF/AICR lifestyle score, in the multivariable models).

6. On what basis were the cut-points for the categories WCRF/AICR lifestyle score chosen?
We adopted a WCRF/AICR score similar to that used in previous analyses in the EPIC Cohort for the sake of standardization and comparability (Romaguera et al., AJCN 2012), which we state in the Methods section (Lines 186-200).

7. Discussion page 14, line 328. ".. that we observed in the present study is overall consistent with findings from studies on invasive breast cancer risk [18, 24-26], even though for some individual components (e.g. alcohol consumption) associations have been detected [4, 23, 26]". This statement appears to downplay an association that IARC has classified as having sufficient evidence of carcinogenicity (alcohol and invasive breast cancer). I recommend this paragraph be re-written with reference to the many high-quality cohort studies that have examined lifestyle exposures and invasive breast cancer risk, unconfined by the crude categories of a 'lifestyle score'.
As stated by the reviewer, there is no doubt about the carcinogenicity of alcohol consumption, and we have slightly modified our statement to avoid confusion (Line 342). In more general, the assessment of etiological associations was not the objective of the present study. In our statement in the discussion, we intended to compare our data with those of other groups, who had published on adherence to the WCRF/AICR cancer prevention recommendations using similar scores. We have clarified now that we only refer to studies investigating similar scores (Line 342). In addition, we state in the limitations section that more in-depth analyses on BCIS risk factors are needed, but that such analyses were beyond the scope of our a priori-defined project (Lines 385-389).

8. Discussion page 15, line 349. I don't believe there is sufficient evidence from this study to support the claim "Thus, associations between pre-diagnostic lifestyle and risk of BCIS as well as invasive cancer could suggest that lifestyle acts at a relatively early stage of breast carcinogenesis".
We agree that our study alone would not justify this claim. However, our statement was made also taking into account previous studies on the WCRF/AICR lifestyle score in relation to mammographic density and invasive breast cancer risk, as well as studies on possible mechanisms cited in the paragraph. We think that in context and with the cautious tone ("could suggest") our statement is reasonable.

Minor issues
1. As per the STROBE guideline, the numbers of women excluded, and the reasons for exclusion, should be reported.
   We have added the numbers of women excluded and the reasons for exclusion (Lines 175-184).

2. A sensitivity analysis using the WCRF/AICR lifestyle score used by previous studies would allow better comparison with those studies.
   We have carried out sensitivity analyses using the slightly different score previously used in the EPIC cohort, and the results were highly similar (HRtotal=0.98, 95% CI: 0.92-1.03; HRscreening=0.83, 95% CI: 0.71-0.98; HRowther=0.99, 95% CI: 0.94-1.06, per one unit of increase in the WCRF/AICR lifestyle score, in the multivariable models). We report on these sensitivity analyses now in the results section (Lines 314-316).

3. All references to associations with invasive breast cancer risk should clarify whether it applied to post-menopausal women, pre-menopausal women, or all women.
   We have added this information to the manuscript (Lines 328-330).

Reviewer #4 Arlene Naranjo:
This is a well-written and informative paper describing the relationship between adherence to cancer prevention lifestyle recommendations and in situ breast cancer (BCIS) risk.
We very much appreciate the positive feedback from Professor Naranjo.

1. Lines 134-135, clarify whether increasing/decreasing BMI was associated with decreased risk of BCIS. Also, define BMI.
   We clarified that higher BMI was associated with decreased BCIS risk and we gave the definition of BMI (Line 139).

   Lines 240-242, clarify whether participants that were lost to follow-up, experienced end of follow-up, or died, were censored in the Cox proportional hazards analysis.
   We have added two sentences to make clear that participants who were not diagnosed with BCIS were censored at the date of loss to follow-up, end of follow-up or death, whichever came first (Lines 250-253).

   Line 253, clarify what is meant by composite outcome in the sentence "BCIS was evaluated as a composite outcome..."
   We evaluated BCIS separately for each of the two main morphological subtypes (DCIS, LCIS), as well as a composite outcome (i.e. DCIS, LCIS and all other in situ lesions), which we have clarified now (Lines 265-266).

   Line 270, is the median follow-up time reported for all participants or only those that were not a BCIS case? Please clarify.
   The median follow-up time is reported for the whole cohort, not only for the non-cases. We have added the phrase “an overall” (Line 281) to make it clear we refer to the entire cohort.

   Supplementary Table 2, commas should be replaced by periods in the % column.
   We have replaced the commas by periods.

   Lines 272-273, missing [in] in the sentence "... the age-standardized incidence rate of BCIS was higher [in] the screening-recruited cohorts"
   We have added the missing “in” (Line 284).
Another limitation, since this was not a randomized study, is that there may be other latent factors beyond those studied that may affect occurrence of BCIS. There should be a bit more discussion about limitations in general with observational studies.
We have added a sentence on this aspect to the study limitations section (Lines 388-389).

Sincerely,

Nena Karavasiloglou
Tilman Kühn
(Corresponding authors on behalf of all co-authors)