Author's response to reviews

Title: Inorganic phosphate and the risk of cancer in the Swedish AMORIS study

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Author's response to reviews: see over
RESPONSE TO THE REVIEWERS

Reviewer: Janneke Hogervorst

Major compulsory revisions
1) The introduction is too brief and vague. What’s really missing in the Introduction is in what raw foods Pi occurs, in what foods it occurs as a processing agent and in what kind of beverages it occurs. If people would want to reduce their Pi intake because of the message in your paper, they would not now know of which foods they would to reduce intake. Also, not knowing the foods makes it very hard to judge the potential for confounding in your analyses.

RE: Thank you. It is indeed through that examples of dietary Pi sources would be useful to the readers and we have now added this information in the manuscript as follows: “Besides being naturally present in raw food including meats, fish, eggs, dairy products and vegetables, Pi is also found as an additive in processed food such as hamburgers and pizza, and as phosphoric acid in soda beverages”. Additionally, we have also rephrased the introduction to provide a clearer background for the current study.

What does “hidden” mean?
RE: ‘Hidden’ phosphate refers to the phosphate content in food and drink, including those used as part of food additives, which is not listed as an ingredient. We have now also clarified this in the manuscript as follows: “Mostly, these Pi content is not listed as an ingredient per se, and it was reported that this ‘hidden’ Pi content of food with Pi-containing additives is nearly 70% higher than in food without additives”.

The sentence starting with “Despite” is hard to follow. It may help to elaborate a bit more on the subjects in this sentence; there is room in the Introduction, I think. The transitions between parts of the introduction are not always logical to me, eg., “However” in the last sentence does not seem to follow logically from the previous sentence. Also “Despite” seems odd to me in the sentence starting with “despite”.

RE: We have now restructured this part as follows. “In the human body Pi is known to be mainly regulated by a set of hormonal and metabolic factors which tightly control calcium homeostasis, i.e. vitamin D and parathyroid hormone (PTH), and a recently identified Pi-regulating hormone, fibroblast growth factor 23 (FGF-23). However, intestinal absorption of Pi is efficient and minimally regulated, so that high Pi supplementation results in markedly elevated levels of serum Pi”.

2) The history of lung disease (which ones?) sounds like a very poor proxy for smoking to me, especially for the amount of smoking. Is asthma included as well, for instance? I think that people with asthma smoke less than people who don’t have asthma. Since the risk of many cancers (e.g. for pancreatic and lung cancer smoking is the strongest known risk factor) is increased by smoking, this worries me. Of course, the severity of confounding here depends on how strong the correlation between serum Pi and smoking is, but right now that is impossible to judge from your paper, because there is no information on the food sources of Pi. Another question: does smoking have an effect on Pi levels?
RE: Thank you. The history of lung disease used in this study refers to those under the ICD-7 codes 470-527, which mostly include upper and lower respiratory tract infections and did not include asthma. We agree that the lack of data on smoking status is a limitation of the current study, and although we used the history of lung disease, it may not be able to completely convey the effects of smoking on cancer risk. However, smoking has been strongly linked to an increased risk of respiratory tract infection (1) and therefore we use lung disease/respiratory tract infection to reduce the effects of smoking on cancer risk.

As for the link between Pi and smoking, previous studies have shown a positive relation between smoking and FGF23 and a negative association with PTH (2, 3), thus smoking may indirectly affect Pi levels. However, it is unclear to what extent smoking modifies Pi levels or whether it could directly affect serum Pi.

Minor essential revisons:

-Abstract first sentence: what is meant by extracellular?
  RE: Apologies for the confusion. We have now changed it into “…serum levels of inorganic phosphate” to make emphasis on the Pi quotient detectable in blood.

-Abstract, results: the “e.g. is a bit odd. It should be oesophagus, not stomach, in Women
  RE: Thank you for clarifying this. We have changed oesophagus into stomach cancer also removed the “e.g” in the abstract.

-Introduction: better: “denoted the potential link between Pi and the development of cancer in humans”
  RE: We have now modified the sentence as suggested.

-Last sentence of introduction: better: “cancer risk in humans”
  RE: We have now added this in the Introduction.

-Abstract: the conclusion does not seem to convey the main findings.
  RE: We have now added the main finding as follows, “Abnormal Pi levels are related to development of cancer”.

-Page 2: please write out AMORIS in full first
  RE: Thank you. This has now been added in the text.

-Page 2: Should it be Registry instead of Register?
  RE: It is National Cancer Register as previously described (4, 5).

-Page 2: How can one unite “healthy” with “outpatients” in the description of the CALAB database?
  RE: We have now removed the ‘healthy’ in the sentence as the pooled database did not contain information whether the participants were healthy or outpatients.
Page 3: write SES in full first
   RE: Thank you. This has been added in the Methods.

Page 3: “the censuses”?
   RE: This refers to the consecutive Swedish Censuses during 1970-1990. We have now added this information in the Methods.

Page 3: a reference to the method to measure Pi would be useful, same for other compounds that were measured.
   RE: Thank you. We have now added the references for methods used to measure Pi and other variables in the methods.

Page 4: you adjust for glucose, but since diabetes modifies Pi metabolism, it would be good to also check if diabetes is an effect modifier of the association between Pi and cancer.
   RE: We have included history of diabetes in the Cox models, and found that it is an effect modifier of the association between Pi and cancer.

Page 4: I am not sure if you should adjust for glucose (and history of diabetes: double adjustment!), alkaline phosphatase, season and creatinine. They all potentially influence serum Pi levels and correcting for them may lead to overcorrection. Are the risk estimates different if you do not adjust for those variables?
   RE: The risk estimates did not markedly differ with the models adjusted for glucose, history of diabetes, alkaline phosphatase, season and creatinine (e.g. HR: 0.97 (0.96-0.98) and 0.97 (0.96-0.99) for every SD increase of Pi in the unadjusted and adjusted models, respectively), thus an event of overcorrection is unlikely. However the above factors may potentially affect the relation between Pi and cancer and thus remained adjusted in the models.

Page 4: The results in Table 1 are not reported in the results section, except for example. Are there any meaningful differences between the two groups?
   RE: We have now added the comparison between the two groups in the Results as follows “The age of the participants, serum glucose, alkaline phosphatase and creatinine were higher in the population with cancer than those without cancer. Pi levels were slightly higher in the group without cancer, while no marked difference in Pi levels and calcium was noted between the two groups.”

Page 5: it is Multivariable-adjusted, not multivariate
   RE: We have now changed it as suggested.

Page 5: The first sentence relates to men and women together, please state so.
   RE: Thank you. We have now clarified that the result belongs to men and women together.

Page 5: the results after exclusion of first 3 years of FU seem the same to me.
RE: We agree that the exclusion of first 3 years of FU resulted in no marked difference in cancer risk, but we observed that the trend for Pi quartiles in men was no longer statistically significant and thus we stated that the association in men was slightly weakened.

-Page 5: why only mention the HR per SD of Pi for men and women together? For increases or decreases in risk, you should mention whether they are statistically significant or not (throughout whole paper).
  
  RE: Thank you. We have now added the P-value to the manuscript.

-Page 5: part about different cancers in men: please indicate that these results can be found in Table 3, same for part about women (table 4)
  
  RE: Thank you. We have now added this information in the Results.

-Page 5: for men: also other cancer was statistically significantly increased.
  
  RE: This has now been added in the Results.

-Page 5: There was an association between NHL and standardized (please add “standardized” all the time) Pi levels in men, but the dose-response relationship over the quartiles was not linear. That should be added.
  
  RE: We have now added this in the Results as follows, “Using standardized Pi, a positive association was also observed between increasing standardized Pi and the risk of non-Hodgkin’s lymphoma in men, but no linear association was observed using quartiles of Pi”.

-Page 5: endocrine apart (other than is better than apart from) from the thyroid. Also apart from prostate and testis, right? Please add this. What then is included in this category? Also, the trend over the quartiles is not linear.
  
  RE: Thank you. We have now added this in the Results. We used the ICD 7 code 195 to select other endocrine cancer, and among those included in this category are cancer of the adrenal, parathyroid and pituitary glands.

-Page 5: men: there is also a borderline statistically significant inverse relationship with colorectal cancer.
  
  RE: We have now added this in the Results as follows, “There was also a borderline inverse association between standardized Pi and risk of colorectal cancer, but this was not confirmed by Pi quartiles.”

-Page 5: women: there is also a (borderline) statistically significant positive trend (as indicated by p for trend) over the quartiles for laryngeal cancer, although case numbers are very small.
  
  RE: Thank you. We have now added this information in the Results as follows, “The test for trend also showed a borderline positive association with risk of laryngeal cancer, but the limited number of cases resulted in low statistical power.”

-Page 5, part about women: what cancers are in the other endocrine category?
RE: Please see the above answer for other endocrine cancer.

-Page 6: first sentence: increase in colorectal cancer risk not reflected by quartile analysis. please add.
  
  RE: We have now added this in the Results.

-Page 6: it is cancer of the endometrium, not endometrial
  
  RE: Thank you. We have now corrected this.

-Page 6: First part of the discussion: also briefly describe here the results for the individual cancer types.
  
  RE: We have now added the brief results of the individual cancer as follows, “Higher Pi quartiles in men was related to pancreatic, lung, thyroid, bone and other cancers. In women, a positive trend was observed between Pi quartiles and risk of oesophageal, lung, and nonmelanoma skin cancer, while a negative association was seen in breast, endometrial, and other endocrine cancer.”

-Page 6: second section: “Recent experimental studies in rodents”
  
  RE: We have now modified the sentence as suggested.

  
  REL Thank you. We have now rephrased the sentence as following, “Elevated levels of extracellular Pi were found to enhance gene expression as well as protein translation regulating cell proliferation in vitro”

-Page 6: “promote colonic cell hyperplasia and hyperproliferation”, but in men you rather see the opposite.
  
  RE: We have now added this sentence into the Discussion, “For colorectal cancer, results in women corroborated the positive link with Pi as shown in experimental findings in mice, but opposing results were found in men”

-Page 6: so there appears to be a U-shaped dose-response curve for Pi and cancer risk in mice?
  
  RE: Based on the results from two experimental studies in mice (6, 7), both low and high Pi are related to increased carcinogenesis and thus a U-shape association is likely to occur.

-Page 7, first sentence: “Consistent with previous experimental studies in rodents”. However, you did not describe that the sex difference you observed was also shown in the rodent studies, so what is consistent? Strictly speaking, you showed that higher Pi was related to an decreased risk of overall cancer in women, not that lower Pi was related to an increased risk.
  
  RE: We have now removed this first sentence from the text.

-Page 7: sentence about weakening after exclusion of first 3 years can be deleted as this weakening was almost negligible. Reverse causation is unlikely to explain both a less decreased risk in women and a less increased risk in men.
RE: Thank you. We have now changed this sentence into “These associations remained clear after excluding first three years of follow-up, thus no reverse causation was indicated.”

Page 7: second section: it should be non-melanoma skin cancer.
RE: Thank you. We have now corrected this in the text.

Page 7: “consistent positive association between standardized Pi levels”: standardized can be deleted? I do not understand how the positive association you saw here in humans can corroborate the prior biological findings linking BOTH HIGH AND LOW dietary Pi to a significantly increased tumor formation in mice.
RE: We have now modified the sentence as follows, “The consistent positive association between Pi levels and lung cancer corroborated prior biological findings linking high dietary Pi to a significantly increased tumor formation in mouse models of lung cancer”

Page 8, first sentence: non-melanoma
RE: This has now been corrected in the text.

Page 8, sentence starting with “On the other hand”: suggest to rephrase: For cancer of the brain/central nervous system, we observed no clear association with Pi levels, despite…”
RE: Thank you. We have now modified the sentence as suggested.

Page 8: please provide references for “not confirmed in observational studies”
RE: Apologies for the confusion. We have now changed the sentence into “However there is lack of observational studies linking Pi and brain cancer.”

Page 8: how does estrogen directly regulate circulating Pi?
RE: It is suggested by Uemura et al in their study that estrogen may act directly to suppress the capacity for Na/Pi cotransport at the renal brush border membrane (8).

Page 8: insert gynecological before cancer in the sentence with “inverse association between Pi levels and cancer risk in women”
RE: This has now been added in the Discussion.

Page 8, last sentence: it should be encoding
RE: Thank you. We have now corrected this.

Page 9: “mainly selected by analyzing”, rephrase e.g. in “mainly selected based on the availability of blood samples from health (not “healthy”) check-ups…”
RE: We have now modified the sentence as suggested.

Page 9: the last two sentences of the discussion seem to be put there as “oh yeah, and also this is kind of a drawback”, but actually this missing information on smoking (!) and alcohol may severely compromise the validity of your whole study.
RE: Thank you. We are aware that smoking status and alcohol are strongly related to cancer risk and thus may potentially affect the relation between Pi and cancer, thus we
have stated the lack of these information in our study population as a limitation of this study.

Page 9, conclusion: biological studies? Our findings provide, not provides. Also, again you say increased risk in women with low Pi, but better: decreased risk in women with high Pi. You should also mention that some cancers show a positive association in women, and that the inverse association for total cancer seems driven by the endocrine cancers.

RE: We have now rephrased the conclusion as follows, “Our findings provide novel epidemiological evidence revealing a decreased cancer risk in women with high Pi and increased risk in men with high Pi. However, women with high Pi displayed a higher risk for developing some specific cancers including oesophageal, lung, and nonmelanoma skin cancer. The persistent negative link between Pi levels and the risk of breast, endometrial and other endocrine cancers which drove the inverse relation between Pi and overall cancer risk in women may imply that Pi rather serves as a proxy for underlying hormonal or metabolic factors instigating carcinogenesis. Further study in this field should take into account these hormonal and metabolic factors involved in Pi metabolism and also the role of dietary Pi.”

In discussion and conclusion, I miss suggestions on how to go further from here. What other research is needed, can any dietary recommendations be given, etc.

RE: We have now modified the conclusion as follows, “Our findings provide novel epidemiological evidence revealing a decreased cancer risk in women with high Pi and increased risk in men with high Pi. However, women with high Pi displayed a higher risk for developing some specific cancers including oesophageal, lung, and nonmelanoma skin cancer. The persistent negative link between Pi levels and the risk of breast, endometrial and other endocrine cancers which drove the inverse relation between Pi and overall cancer risk in women may imply that Pi rather serves as a proxy for underlying hormonal or metabolic factors instigating carcinogenesis. Further study in this field should take into account these hormonal and metabolic factors involved in Pi metabolism and also the role of dietary Pi.”

References: some journal titles are written in full, some are abbreviated. Sometimes epub is put after the page numbers. Please remove.

RE: We have now used the BMC Cancer’s reference style for the manuscript.

Table 3 and 4 titles: part about adjustment should be in the legend of the tables.

RE: Thank you. We have now put this part in the legend of the tables.

Table 4: the HRs and CIs of the 3rd and 4th quartile for endometrial cancer are exactly the same. Please check if this is correct.

RE: We have checked this and got the same results.
Reviewer: Martin Almquist

This is a large epidemiological study into serum phosphate as a risk factor for cancer. The study is well performed and clearly written. Overall conclusions are well founded.

RE: Thank you.

A major limitation is the lack of data on levels of other phosphate regulating hormones such as vitamin D, parathyroid hormone and calcium, which the authors also point out, and the lack of information on a large number of general and specific risk factors for cancer, such as smoking, reproductive factors, and so on. This restricts the possibility to draw firm conclusions regarding any causal relationship between phosphate and cancer, ie confounding is a possible explanation for all the observed associations between phosphate and cancer.

RE: Please see our response to similar comments by Reviewer 1.

Major compulsory revisions
I would like a more detailed description of the large part of the original cohort that was excluded from analysis (almost half). Did this cause any selection bias?

RE: It has been shown that the AMORIS cohort is similar to the general working population of Stockholm County in terms of SES and ethnicity (9), and so is the subcohort used in this study, thus a selection bias is unlikely to occur.

Are levels of phosphate stable over time, i.e. do the values measured several years pre-diagnosis reflect an individual's true phosphate levels?

RE: For the current study we did not have repeated measurements of phosphate available. However, as alteration in phosphate levels is likely to occur in specific conditions, i.e. kidney disease, rickets and diabetes, we adjusted the models for these diseases using serum creatinine, alkaline phosphatase, glucose and history of diabetes in order to more accurately reflect phosphate levels. Furthermore, a single measurement of phosphate has been used in many published studies to measure the relation between phosphate metabolism and other diseases (10, 11).

Minor essential revisions
The diary number for the ethical approval should perhaps be mentioned.

RE: The diary number from the Ethics committee of Karolinska Institutet is 2010/1047-31/1 and this has now been mentioned in the Methods.

It seems from the paper that the same lab and the same method was used for allphosphate analyses in study cohort. If this truly is so maybe this could be more explicitly stated.

RE: Thank you. We have now clarified this as follows, “All laboratory examinations were performed using the described methods above with automated and calibrated instruments in the same laboratory”

The abbreviation SES needs to be explained.
RE: We have now explained this in the Methods.

References