Author’s response to reviews

Title: Low-carbohydrate, high-protein diet score and risk of incident cancer; a prospective cohort study

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Version: 3 Date: 17 April 2013

Author’s response to reviews: see over
Reply to reviewers NJ:

Reviewer 1, RK:

RK1. The authors state that „LCHP scores have increased in VIP participants with repeated samples 10 years apart”. On the other hand, excluding all participants with more than one sampling occasion, the median follow-up time was 9.7 years. That means that many of the subjects with low LCHP score probably changed their eating habits towards an higher LCHP score. Although the study has many strengths that have been discussed, the authors should discuss in more detail these weaknesses of the study design. What was the assumption that underlies the extrapolation of a one-point-in time assessment of eating habits towards the development of detectable tumors?

REPLY: Repeated sampling certainly adds strength to this type of study. However, after all other exclusions, only 22% of our study subjects had repeated sampling occasions. This was the reason for using a one-point-in-time assessment of dietary habits for the main analyses. As noted in the methods, line 96, subjects with repeated sampling occasions were not completely excluded. Rather, for these subjects, only data from the first sampling occasion were included.

We do appreciate this reviewer comment, and we agree that the effect of temporal changes deserves more attention. In the VIP cohort, the change in trend towards increased intake of fat occurred approximately year 2002 (reference number 2). We have, therefore, added a sub-analysis of the cohort to the manuscript, with follow-up ending on December 31, 2002 (Table 4 in the revised manuscript). During the resulting study period, 1990-2002, LCHP score was relatively stable on a population level. Interestingly, in this analysis, LCHP score was positively associated with the risk of total cancer in women, with a similar tendency in men, though no other significant associations were found. It should be noted that reducing the follow-up time also resulted in a major reduction in available cancer cases, from 3,059 to 531. This sub-analysis is now described in methods lines 155-6, results lines 196-201, discussion lines 259-262, conclusion lines 293-94).

RK2. Concerning the arguments above, it seems incorrect to state in the conclusions that “these largely null results provide important information concerning the long-term safety of moderate carbohydrate reduction...”. Again, this would assume that the participants have not changed their diet over a long time scale, which is certainly not true as the example of the rise in LCHP score over the years shows. This issue should be discussed in more detail.

REPLY: We agree, and we have modified the discussion to include this concern (discussion lines 259-262).

Minor Essential revisions:

RK3. In the references section, there are some inconsistent abbreviations. For example, Ref. 4 has the journal name written in full, while the same journal name is abbreviated in Ref. 5. Please check all references for consistency!
REPLY: Done!

RK4. The contradictory finding of increased risk for colorectal cancers with increasing LCHP score based on vegetable protein in men, but decreasing LCHP score in women with high saturated fat intakes is interesting and does not support the hypothesis that high animal protein intake increases the risk for these cancer types. Furthermore, there was no association between higher protein intake and increased risk in any other subgroup. How is this compatible with the “convincing evidence that a high consumption of protein sources such as red and processed meat is associated with increased colorectal cancer risk [33]? The latter reference is also not cited correctly, see bottom of first page in this reference.

REPLY: 1. We agree. This is emphasized in the revised manuscript (lines 229-31)
2. The reference is edited according to the first page of the reference.

Minor discretionary revisions:

RK5. In the discussion, the role of carbohydrate restriction in tumorigenesis is briefly discussed. The authors state that carbohydrate restriction might have different roles in the initiation and progression of some tumors. Which roles do they refer to? Are there some known mechanisms for the specific cancer sites considered by the authors?

REPLY: This is an interesting topic. For the sake of brevity we included two unspecific examples for effects in tumor progression, and we have modified the text appropriately (line 245-47). However, there are undoubtedly site-specific effects as well.

RK6. A reference for the ICD-7 codes could be provided.

REPLY: Done! (line 103)

Reviewer 2, KB:

KB1. Table 3, in the table text: Add information that the HR is for one point increment in the LCHP score.

REPLY: Done! (footnote 1)

KB 2. A) Please clarify in the text what energy percentage of carbohydrate and protein that is commonly considered a low-carbohydrate and high-protein diet, respectively?

REPLY: There is no uniform definition, but examples of extremely and modestly low carbohydrate diets have been added to line 45.

KB2B) Also, how do theses (more or less established) E% of carbohydrate and protein in popular press and scientific papers relate to the E% of carbohydrate and protein for the categories low, median and high LCHP score in your analyses? Is there a big difference?
REPLY: The macronutrient intakes in the study population were not nearly as extreme as many diets in the popular press and in some scientific papers. We emphasize this throughout the paper by describing the high LCHP scores in our study as moderately low in carbohydrates and moderately high in protein, for example in lines 204-5, and in the discussion lines 240-242.

Reviewer 3, AK:

Major Compulsory Revisions:

I have remarks regarding the description of the cohort; choices and description of exclusions; and the handling of BMI in the analysis.

AK 1. It is not clear form the ‘Study design and cohort’ section during which time period recruitment took place.

REPLY: This is now clarified in lines 81-2.

AK 2. Line 77: What does the year range 1990-1996 stand for? Is it the time period when 30 year old individuals were recruited?

REPLY: Yes. This is now clarified in line 78.

AK 3. Lines 87-95: Were exclusions individuals or participation occasions? Or all exclusions reported as excluded subjects had only one sampling occasion?

REPLY: In all stages of the selection, participation occasions were excluded. This is now clarified in lines 90-93.

AK 4. Lines 93-94: Is it necessary to exclude more than 15% of participant occasions because of repeated sampling? With changes in dietary habits over time, repeated measures provide additional exposure information, which can be analyzed in a time-varying analysis. Furthermore, the exclusion might introduce bias.

REPLY: This is an important point, and although we have not performed time-varying analyses, we have added a sub-analysis to the revised manuscript focusing on a time period in which LCHP score was relatively stable in the VIP population. Please see our response to Reviewer 1 (RK1-2).

AK 5. Lines 130-133: BMI is a potentially important confounder in this analysis. Dichotomizing BMI leads to a loss of information, and in my view, is unnecessary. The violation of the proportional hazard assumption does not justify dichotomizations, as there are various ways to deal with the violation of this assumption.

REPLY: Including BMI instead of the dichotomous obesity variable had essentially no effect on results. For this reason, we have not changed the analyses presented.

Minor Essential Revisions
AK 6. Line 108: Please indicate the response alternatives, or the most extreme alternatives.

REPLY: Done! (line 110)

AK7. Please indicate in the ‘Statistical analysis’ section that sex-specific analyses were performed.

REPLY: Done! (lines 128-29)

AK8. Lines 136-140, and tables 2 and 3: Were multivariable models adjusted for energy intake as presented in the tables, or were not adjusted as indicated in the text?

REPLY: Energy intake was included as a covariate. This was a text-error, which has now been corrected (line 141).

AK9. Please consider presenting person-time at risk in the cohort for exposure categories in tables 2 and 3, to allow calculation of incidence densities.

REPLY: We prefer not to. Person-years at risk may instead be approximated from data in Table 1.