Author’s response to reviews

Title: Metabolic risk factors for esophageal squamous cell carcinoma and adenocarcinoma. A prospective study of 580 000 subjects within the Me-Can project.

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Author’s response to reviews: see over
Dear Editor,

We are grateful for the possibility to submit a revised version of this manuscript. The reviewers have given several useful comments and that we have addressed below. Text that has been added in the new version is marked in blue and text that has been removed is marked in strikethrough red.

Referee 1
This large cohort study explores the associations between obesity, other metabolic risk factors and the risk of esophageal adenocarcinoma and squamous cell carcinoma. The findings confirmed previous observation that high body mass index increases the risk for esophageal adenocarcinoma, but decreases the risk for esophageal squamous cell carcinoma. In general, this is a well-conducted study and the results are clearly presented.

Referee 1, Discretionary Revisions

The finding between hypertension and esophageal squamous cell carcinoma is intriguing. The authors did not explain this association enough in the Discussion. Confounding might be one explanation. Smoking and alcohol drinking are main risk factors for squamous cell carcinoma in Western population, but in the regression model, only smoking was adjusted. Another explanation might be due to central obesity. In a recent report from EPIC cohort, when controlled for BMI, waist circumference and waist-hip-ratio were positively with esophageal squamous cell carcinoma (Steffen, A et al, CEBP, 2009). If the authors have data (or in the subset of cohort) on waist and/or hip circumference, it would be worthwhile to additionally adjust these variables.

Response, referee 1, discretionary revisions

In the study by Steffen et al a clear and negative association between BMI and ESCC risk but no association between waist/hip ratio was observed in a model adjusted for sex, education, smoking status, smoking duration, baseline alcohol consumption, lifelong alcohol consumption, physical activity and intake of fruits, vegetables, and meat. However, when BMI was entered into this model, waist/hip ratio was positively associated with the risk for ESCC and the inverse association between BMI and ESCC was strengthened. The authors concluded “general obesity may be associated with lower risk of ESCC on the one hand, but (...) abdominal fat distribution seems to be a risk factor for ESCC on the other hand.” We had no possibility to adjust for central obesity since data on waist and/or hip circumference was not available in our dataset.

As pointed out by the reviewer, another weakness in our study is the fact that we were not able to adjust for alcohol consumption. Given the previously well-established association between alcohol consumption and hypertension and alcohol consumption and ESCC, confounding by alcohol has to be considered. Both alcohol consumption and
waist/hip ratio are associated with hypertension but there is more evidence for an
association between alcohol and ESCC than for an association between waist/hip ratio
and ESCC. Therefore, we believe that alcohol is the most probable and first factor that
needs to be considered as a confounder. This was not given appropriate attention in the
first version of the manuscript.

The following amendments have been made in the manuscript in relation to this
comment:

Abstract

In accordance with previous studies, high BMI was associated with an increased risk of
EAC and a decreased risk of ESCC. An association between high blood pressure and risk
of ESCC was observed but alcohol consumption is a potential confounding factor that we
were not able to adjust for in the analysis. An association between high blood pressure
and the risk of ESCC was observed for the first time. The MetS was associated with EAC
but...

Discussion

The inverse association between BMI and risk of ESCC in the present study was similar
to the above-mentioned studies with a RR of 0.62 (95% CI, 0.50-0.79) per increment of 5
in BMI. Despite an inverse association between BMI and ESCC, Steffen et al recently
observed a positive association between waist-hip-ratio and risk of ESCC in a model
adjusted for BMI [33]. We had no possibility to investigate the association between
waist-hip ratio and ESCC risk since this information was not available in the Me-Can
cohort. The association between BMI and ESCC was...

and

We found a strong and dose dependent association between mid BP and risk of ESCC.
However, alcohol consumption is a known risk factor for hypertension [45] and has also
consistently been associated with the risk for ESCC [5]. It is therefore likely that alcohol
consumption is a confounder in the observed association between hypertension and
ESCC. An increased risk of esophageal cancer in general related to hypertension
diagnosed below the age of 60 years was recently reported in a study from the
Saskatchewan Health database[41], but we know of no studies to date, exploring the
possible association between hypertension and ESCC or EAC.[41]. The association
between hypertension and cancer in general has been explored in previous studies
finding either no[42] or a modest positive association[43, 44]. Common genetic
predisposition to both cancer and hypertension has been proposed as a possible
explanation but the potential mechanisms linking hypertension to cancer are largely
unknown[43].

Conclusion

...we found a positive association between blood pressure and ESCC. To the best of our
knowledge, this latter association has never been described before. However, this
finding has to be confirmed in studies where proper adjustment for alcohol
consumption is possible.
Minor Essential Revisions

Referee 1, comment 1
Page 6, ‘End-point assessment’. It is difficult to understand the first 2 sentences. It would be better to rephrase the sentences.

Answer to Referee 1, comment 1
These two sentences have been rephrased as follows:
The seven cohorts were linked to the respective National registers for a) cancer diagnosis, b) migration status (if available) and c) vital status. End of follow-up was 2006 in the Swedish cohorts, 2005 in the Norwegian cohorts and 2003 in the Austrian cohorts. Migration status was available in all cohorts except for the Australian cohort. End of follow-up for each cohort were as follows: The Austrian cohort a) 2003, b) no information available, c) 2003; the Norwegian cohorts a-c) 2005; and the Swedish cohorts a-c) 2006.

Referee 1, comment 2
Statistical analysis. The authors should describe how those covariates were selected in the final model. Further, did the authors check the proportional hazards assumption?

Answer to referee 1, comment 2
Selection of covariates in the final model
As stated in the methods section, the final model in the analysis of metabolic risk factors categorized in quintiles was adjusted for age, smoking status, quintiles of BMI. In the footnote of table 2, “sex” was lost among the listed variables, this has been corrected. These variable were selected for the final model for the following reasons:
Age and sex: not controversial.
Smoking: Known riskfactor for ESCC.
Quintiles of BMI: BMI was the only metabolic risk factor that was selected for the final model. The reason why BMI was included was that there is a strong correlation between BMI and all metabolic risk factors and BMI has also been associated with both types of esophageal cancer in previous studies. Adjustment for BMI was considered necessary to rule out that any association between other metabolic risk factors and esophageal cancers were not merely a BMI-effect. Bringing all metabolic factors into the final model would introduce a risk for over-adjustment.

The following amendment has been done in the manuscript in relation to this comment:

Methods
Relative risks were adjusted for age at baseline as a continuous variable and for sex, smoking status and quintile levels of BMI as categorical variables. We decided to include BMI in the final model due to the association between BMI and EAC and ESCC and the well-established association between BMI and other metabolic factors. The p-value for trend over...

The proportional hazards assumption
All adjusted models were stratified for study cohort and birth-year cohort and adjusted for age at baseline, sex and smoking habits. The proportional hazards assumption was
checked by log-log plots. This has been included in the methods section:

Cox proportional hazards analysis was used to calculate relative risks (RR) with 95% confidence interval (CI) for EAC and ESCC related to quintile levels of all five components of the MetS. The proportional hazards assumption was met in all analyses as verified by log-log plots. Attained age...

Referee 1, comment 3
Discussion, page 13. The authors argued that misclassification between esophageal squamous cell carcinoma and adenocarcinoma is unlikely. That is true. But the potential misclassification between esophageal adenocarcinoma and cardia cancer cannot be ignored.

Answer to referee 1, comment 3
This is correct. Tumors in the region of the esophagogastric junction can be impossible to classify as either esophagus or gastric cancer. However, misclassification in this regard will probably not have any major importance on estimates of RR in relation to BMI since most studies have reported that the association between BMI and esophageal adenocarcinoma is highly similar to the association between BMI and gastric cardia carcinoma (see for instance Lindblad et al, Cancer Causes and Control (2005) 16:285-294).

In order to acknowledge the potential misclassification between cardia cancer and EAC, we have made the following amendment to the manuscript:

Discussion
...the value of previous studies analyzing all esophageal cancer together can be questioned. Differentiation between distal EAC and adenocarcinoma of the gastric cardia may in some cases be difficult and some misclassification of gastric cardia cancers as EAC has most probably occurred in this study. However, adenocarcinoma of the gastric cardia and EAC are associated with BMI and smoking in a similar manner [28] and limited misclassification between these cancers will not have any major impact on investigated risk factors. Differences in measurement methods between the different cohorts...

Referee 2

General comments
The manuscript by Lindkvist B and colleagues presents findings from a large prospective study to support previously reported associations of obesity with increased risk of esophageal adenocarcinoma and decreased risk of esophageal squamous cell carcinoma. This manuscript also demonstrated an association between features of the metabolic syndrome and EAC. While this paper presents important, interesting findings regarding associations between metabolic risk factors and esophageal cancer, there are multiple issues that should be addressed before this paper will be in a form appropriate for publication.

Major compulsory revisions
Referee 2 comment 1
Abstract: Define mid blood pressure for clarity in the results section of the abstract.

Response to referee 2, comment 1
We have made the following amendment to the abstract in relation to this comment:

The mean value of systolic and diastolic blood pressure (mid blood pressure) was associated with the risk of ESCC (RR 1.77 (1.37-2.29)).

Referee 2 comment 2
Abstract: It would be important for readers to understand the implications for this study's findings. Thus, a sentence or two should be added to the conclusion section of the abstract to make clear what the findings add to the literature and if there are important implications that should be noted.

Response to referee 2, comment 2
The conclusion section of the abstract has now been modified as follows:

In accordance with previous studies, high BMI was associated with an increased risk of EAC and a decreased risk of ESCC. An association between high blood pressure and risk fo ESCC was observed but alcohol consumption is a potential confounding factor that we were not able to adjust for in the analysis. An association between high blood pressure and the risk of ESCC was observed for the first time. The MetS was associated with EAC but not ESCC. However, this association was largely driven by the strong association between BMI and EAC. We hypothesize that this association is more likely to be explained by factors directly related to obesity than the metabolic state of the MetS, considering that no other metabolic factor than BMI was associated with EAC.

Referee 2 comment 3
Introduction: More details are needed in the introduction. This would be helpful for a reader that may not be well-versed in this topic. Furthermore, adding information about the importance of studying the associations that were examined in this study.

Answer to Referee 2, comment 3
The introduction has now been expanded as given below:

Introduction

The metabolic syndrome (MetS) is a cluster of metabolic risk factors, including obesity, hypertension, insulin resistance/hyperglycemia and dyslipidemia that has been shown to be associated with cardiovascular disease[6, 7]. There is now accumulating evidence that the MetS also may be an important risk factor for several specific cancers as well as overall cancer mortality [8]. A recent meta-analysis has reported an increased risk for liver, colorectal, bladder, pancreatic, breast and endometrial cancer related to the MetS [8].

There is strong epidemiological evidence for an association between obesity and an increased risk of EAC[9] and a decreased risk of ESCC[10]. Studies on However, knowledge on the risk of esophageal cancer in relation to other MetS components such
as hypertension and dyslipidemia is limited. Previous epidemiological studies have not demonstrated any clear evidence for an association between hyperglycemia and esophageal cancer overall, but a significant association in subanalysis of esophageal cancer with mortal outcome and esophageal cancer among men [11-13]. An association between blood lipids and esophageal cancer has been reported from one study that was not able to adjust for BMI or smoking habits [14]. It is noteworthy that all these studies share the methodological problem of using all esophageal cancer as endpoint. Considering the highly separate biological and epidemiological profile of EAC and ESCC[2], the lack of differentiation between EAC and ESCC significantly limits the scientific value of all these studies. [14][14] are lacking. Studies on the association between hypertension and EAC and ESCC are lacking.

The aim of the present study was to investigate the association between BMI...
Baseline characteristics of the Me-Can cohort including proportions for gender, age and BMI are presented in table 1. Mean follow-up time was 12 years. We have now included the following in the manuscript text:

Baseline characteristics for the Me-Can cohort and cases of EAC and ESCC are presented in table 1. Fifty percent of the participants were male and 50% were female, mean age at baseline was 44.0 years, mean BMI was 25.3, 27.7 % were current smokers, 27.4 % were former smokers and 44.6% were never-smokers. Mean time of follow-up was 12 years.

Referee 2 comment 7
Results: The presentation of the findings should be re-organized in a way that is easier for the reader to follow along with. Findings from the tables are not presented in a logical way, so the reader is left to flip through the tables to determine where cited values are shown and this is distracting.

Response to referee 2, comment 7
An epidemiological study of this kind generates large amounts of results and it is indeed a challenge to find a way to present data in a way that is clear, logical and easy to digest for the reader. Tables provide the most structured presentation and can be regarded as the core of the presentation of the results in this paper. The text in the results section complement the tables by presenting results per investigated metabolic factor instead of per analysis (which is the "red line" through the tables). This inevitably leads to references to several tables in every section and tables will reappear later down the text in coming section. We understand that this leads to some “flipping” through tables for the reader but our opinion is nevertheless that this is the most logical way to present the results in text. In order to give the presentation of the results a more clear structure, we have now added subheadings in the results section. In addition, the borderline significant finding regarding triglycerides as a continuous variable and ESCC risk has been added.

Referee 2 comment 8
Results (and Table 4): Please provide p-trend for findings in Table 4 and present those when the finding are discussed in the text.

Response to referee 2, comment 7
P-value for trend over BMI is presented in table 2. In table 4 different but overlapping BMI categories are tested (25-29.9, ≥25 and ≥30) and calculation of p-values for trends was therefore not possible. P-values for trends over ESH/ESC hypertension grade have been included in table 4.

Referee 2 comment 9
Discussion: The discussion should be reorganized for improved readability. For instance, the discussion of the strengths and weaknesses should be moved to the end of the discussion (just prior to the conclusions section). Overall the discussion doesn’t flow well and the implications of the study’s findings in the context of the literature could be done in a more scholarly manner.
Response to Referee 2, comment 9
The discussion on strengths and weaknesses has been moved according to the suggestion by the referee. Several changes and additional references to the literature have been added according to comments below. We hope that this has improved the flow of the discussion.

Referee 2 comment 10
Discussion: The subheadings should be removed entirely.

Response to Referee 2, comment 10
Subheadings have been removed.

Referee 2 comment 11
Discussion: On page 14, the location of the following in-text citation is inappropriate and should be revised from “An increased risk of esophageal cancer in general related to hypertension diagnosed below the age of 60 years was recently reported in a study from the Saskatchewan Health database, but we know of no studies to date, exploring the possible association between hypertension and ESCC or EAC [41]” to “An increased risk of esophageal cancer in general related to hypertension diagnosed below the age of 60 years was recently reported in a study from the Saskatchewan Health database [41], but we know of no studies to date, exploring the possible association between hypertension and ESCC or EAC.” In this section, a more scholarly discussion of the present study’s findings in the context of the literature is needed.

Response to referee 2, comment 11
The in-text citation has been rearranged as suggested. Please see “Response, referee 1, discretionary revisions” for the expansion of the discussion on this finding.

Referee 2 comment 12
Discussion: A more scholarly discussion of the present study’s findings, related to the association of serum glucose and EAC and ESCC, in the context of the literature is needed.

Response to referee 2, comment 11
We have expanded the discussion on this topic as given below:

An association between high blood glucose and an increased risk of cancer overall has been reported in several prospective studies [24, 44]. Proposed mechanisms for this association include a direct mitotic effect of insulin like growth factor and oxidative stress related to hyperglycemia [45]. We did not find any association between serum glucose and EAC or ESCC, and no significant association between glucose and ESCC except for when glucose was entered as a continuous variable. Previous studies on the association between esophageal cancer and serum glucose have been conflicting, with either demonstrating no association for overall esophageal cancer in most studies [24, 44, 46] but positive associations in only subgroups of hyperglycemic subjects (i.e. men with diabetes [46, 47]), or only for fatal esophageal cancer [24] or fatal esophageal cancer among men [44]). A limitation to all these studies is that there was no differentiation between EAC and ESCC. The association between diabetes and esophageal cancer has recently been investigated in a metaanalysis where an increased
risk was found among men but not women [48]. Subanalysis of three studies separating EAC from ESCC revealed that diabetes was associated with EAC [48]. However, all these studies have used all esophageal cancer as end-point and comparisons with our findings are therefore difficult.

**Referee 2 comment 13**
Discussion: A more scholarly discussion of the present study’s findings, related to the association of serum/plasma lipids and EAC and ESCC, in the context of the literature is needed. While the authors state that “there is no evidence for such an association in the literature,” other studies have examined similar associations and as such, they should be cited in this paper. Here are two citations that may be useful for this section: Wulaningsih W et al., J Cancer Epidemiol 2012;2012: 792034 (article ID), 10 pages and Sako A et al. Cancer Letters 2004;208:43-49, as well as some of the references cited by these authors.

**Response to referee 2, comment 13**
The paper by Sako et al referred by the referee above is a paper that investigate how serum lipids measured after diagnosis of esophageal cancer correlate with lymph node metastasis status. The paper does not investigate how serum lipids affect the risk of esophageal cancer and it is not possible to conclude if high serum lipids was a cause of a consequence of lymph node metastasis. We therefore consider this reference as less relevant for our current study and have not included this in the discussion. The interpretation of the paper by Wulaningsih et al is also difficult since there was no differentiation between EAC and ESCC, no adjustment for smoking and no adjustment for BMI. In other words, both smoking and obesity are highly likely confounders in the association between esophageal cancer in general and triglycerides and LDL/HDL ratio that the authors report. This suspicion is supported by our own findings in the present study, we found a statistically significant trend over triglyceride quintiles in crude analysis that was completely abolished when BMI was adjusted for. This reference is now discussed in the discussion section.

In relation to this comment, the following amendments have been done in the manuscript:

Well-designed studies on the association between blood lipids and different subtypes of esophageal cancer are lacking. A positive association between esophageal cancer and both triglycerides and low-density lipoprotein cholesterol/high-density lipoprotein cholesterol has been reported in a recent cohort study [47]. However, BMI and smoking was not adjusted for in that study. We observed a statistically significant trend over triglyceride quintiles and risk of EAC in crude analysis that disappeared when BMI was adjusted for indicating that BMI may have been a confounder in the above-mentioned study [47]. We did not find any association between triglycerides or cholesterol and the risk of EAC or ESCC. To the best of our knowledge, there is no evidence for such an association in the literature.

**Referee 2 comment 14**
Discussion: A more scholarly discussion of the present study’s findings, related to the
association of features of the metabolic syndrome and esophageal cancer, in the context of the literature is needed. A good citation for this section would be Ryan AM et al. Cancer Epidemiology 2011;35:309-319.

Response to referee 2, comment 14
This part of the discussion has been expanded as given below:

Two different causal links between obesity and EAC can be hypothesized. One possible mechanism is through and increased risk for gastroesophageal reflux. Obesity is associated with an increased risk of gastro-esophageal reflux[30] which in turn is associated with the development of intestinal metaplasia in the distal esophagus, i.e., Barrett’s esophagus[31], a pre-malignant condition associated with the risk of EAC[32]. Another possible mechanism for the association between obesity and EAC is through a hormonal and/or metabolic systemic disequilibrium related to the MetS[33]. The MetS has been demonstrated to be associated with several site-specific cancers, including liver, colorectal, breast, pancreatic, urinary bladder, and endometrial cancer[34]. The mechanisms for the association between the MetS and cancer are not fully characterized. Chronic low-grade inflammation, high levels of trophic hormones (i.e., insulin and insulin-like growth factor) or lifestyle-related factors related to the MetS have been proposed as putative mechanisms[34]. Overweight has been associated with a wide variety of other cancers where no mechanical link can be proposed[35] and some previous studies have also indicated that the association between BMI and EAC is independent of reflux symptoms[27].

In the present study, we found a statistically significant association between the surrogate score for the MetS and the risk of EAC. However, BMI was the only metabolic factor with a statistically significant association with the risk of EAC. Therefore, we consider that our findings are more in support of the theory of suggest that obesity leading to gastro-esophageal reflux and esophageal dysplasia may be the more important mechanism. Nevertheless, this does not exclude a role for metabolic state related to the MetS for the development of EAC. High leptin levels and low levels of high molecular weight adiponectin have been associated with an increased risk for progression from Barrett’s esophagus to EAC after adjustment for relevant other risk factors, including BMI[35]. Rather than an increased risk of cancer related to the metabolic state related to the MetS since BMI was the only metabolic factor with a statistically significant association with the risk of EAC. This hypothesis is in line with findings from other studies demonstrating that abdominal obesity is associated with EAC risk independently from BMI[37].

Minor essential revisions

Referee 2 comment 15
Tables 2 and 3: In the footnotes, correct “Se text” to “See text.”

Response to referee 2, comment 15
OK.

Discretionary revisions
Referee 2 comment 16
Methods (Statistical analysis): The authors should consider rewording the description of their interaction analysis to something similar to the following:
“Interactions between smoking and additional factors were tested by including cross-product terms in the regression models.”

Answer to referee 2, comment 16
This has been done.

Referee 2 comment 17
Results: On page 10, the last sentence, consider rewording “High BMI” to “Higher BMI.”

Answer to referee 2, comment 17
The sentence has been reworded according to the reviewers comment.

Referee 2 comment 18
Results: On page 11, first full paragraph, consider rewording “High mid BP” to “Higher mid BP.”

Answer to referee 2, comment 18
The sentence has been reworded according to the reviewers comment.