PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Alcohol consumption and mortality in patients with mild Alzheimer's disease: a prospective cohort study
AUTHORS	Berntsen, Sine; Kragstrup, Jakob; Siersma, Volkert; Waldemar, Gunhild; Waldorff, Frans

VERSION 1 - REVIEW

REVIEWER	Francesco Panza, MD, PhD Neurodegenerative Disease Unit, Department of Basic Medicine, Neuroscience, and Sense Organs, University of Bari Aldo Moro, Bari, Italy
REVIEW RETURNED	10-Mar-2015

GENERAL COMMENTS	Berntsen and colleagues, in this post-hoc analysis, found that in patients recently diagnosed with Alzheimer's disease (AD), those who had a moderate alcohol intake (two to three units per day) had a significantly lower risk of death compared with those who only had alcohol occasionally (one or less than one unit per day). Abstinence or high alcohol intake did not significantly affect mortality. While there is a series of studies on the association among alcohol consumption, AD, and late-life cognitive decline and on the relationship between alcohol and all-cause mortality, this is may be the first study investigating the association between alcohol consumption and mortality in AD patients. These findings should be in part explain a protective effect of moderate alcohol consumption against dementia and AD. Although the approach of this report was very interesting, which was often underestimated in others studies, some minor revisions should be considered before publication. 1. Introduction. Among meta-analyses and systematic reviews investigating the role of moderate alcohol use in late-life cognitive disorders the Authors should include some papers (Neuropsychiatr Dis Treat. 2011;7:465-84; Int J Geriatr Psychiatry. 2012 Dec;27:1218-38.). The Authors should include also some further introductive remarks of this issue. 2. Discussion. Possible mechanisms: Ritchie and colleagues evaluated the possible association between alcohol consumption and cognitive decline in old age [Age (Dordr). 2014 Jun;36(3):9638]. They found a significant gene × alcohol consumption interaction on lifetime cognitive change, suggesting that the effect of alcohol consumption on cognitive change may thus depend on genetic differences in the ability to metabolize alcohol. Individuals with higher genetic ability to process alcohol showed relative improvements in cognitive ability with more consumption, whereas those with low processing capacity showed a negative relationship between cognitive change and alcohol consumption with more

	consumption. The Authors should include this among possible mechanisms explaining the impact of alcohol consumption on mortality in AD patients. 3. Discussion. Limitations: alcohol consumption is often assessed only once (as in the present study), resulting in possible measurement error bias. The Authors should include also this as a possible limitation of their study, suggesting the use of an alcohol consumption measure obtained multiple times across the life course. A very recent study in which alcohol consumption was assessed 3 times in the 10 years preceding the first cognitive assessment was recently published (Alcohol consumption and cognitive decline in early old age. Sabia S, Elbaz A, Britton A, Bell S, Dugravot A, Shipley M, Kivimaki M, Singh-Manoux A. Neurology 2014;82:332-9). The Authors should consider to discuss also the findings of this report.

REVIEWER	Kirsten Mehlig Dept of Public Health and Community Medicine Section for Epidemiology and Social Medicine (EPSO) Sahlgrenska Academy, University of Gothenburg
REVIEW RETURNED	24-Mar-2015

GENERAL COMMENTS	Referee report for Alcohol consumption and mortality in patients with mild Alzheimer's disease (BMJ Open) Summary: this study investigates whether disease-protective health effects associated with moderate alcohol consumption observed in mentally healthy adults could be extended to patients with mild Alzheimer's disease. Major comments: Definition of the exposure: In this study, moderate alcohol consumption has been defined as 2-3 units / day but no definition of 'unit' is given. While absolute amounts for moderate drinking vary there is a consensus that the limits for women are about half the limits for men, based on biological differences in ethanol metabolism between the sexes. It is therefore necessary to present a sex-stratified analysis, in order to examine whether there is a minimum risk associated with a certain amount of ethanol intake in both sexes, and whether the protective amount differs between men and women. A limitation of this study is that the potentially moderate intake for women, ca. 1 unit / day, cannot be separated from < 1 unit / day. It could be that no minimum risk across ethanol categories is observable in women in this study. Eventually, a classification of ethanol into 3 categories (>0 and ≤ 1 unit/day, ≥ 2 units/day, and current abstainers) with the latter as reference could make sense in the female stratum. The problem of combining lifetime abstainers with former abusers into 'current abstainers' seems to be a minor one in this particular study. In the discussion, the authors state that adjustment for sex should be sufficient. This is not true because the differential effect of ethanol intake on men and women corresponds to the presence of an interaction term between each ethanol category and sex. This would be a way to account for the gender difference in the whole sample, and would be an alternative to stratified analysis. It could also be that gender differences are of minor importance at this old age but this would have to be explained. While it is recognized that at least 3 categories of intake are needed

	in order to test a non-linear risk curve, the number of observations, and of cases of death are very small in the categories other than the reference category, giving rise to large confidence intervals for corresponding effects (Table 2, Figure 1), a problem that would be even more important in stratified analysis. The rule of thumb that no more than 1/10 of the number of events in the minor outcome category (here, number of deaths) should be used for the number of predicting variables shows that this study is underpowered in view of the detailed definition of the exposure: with 56 deaths, the number of ethanol categories including gender interaction alone exceeds the recommended number of about 6 covariates, and the investigation of important confounders appears difficult. Stepwise selection of variables with ethanol categories forced into the model could be tested. At last, it is not clear that 'only at parties' is equivalent to '≤ 1 unit/day' as the amount is not specified in the former category. In addition, the pattern of intake does play a role wrt. health outcomes, i.e. occasional high intake or binge drinking is not equivalent to regular intake even if the average intake is the same. Subjects consuming alcohol 'only at parties' should be excluded at least in sensitivity analyses. Reverse causation: Reverse causation is a serious problem in cohort studies with short follow-up such as this one, and its consequences are well described by the authors (p. 8, 1st paragraph). How would the effect size change if all cases during the first year after baseline would be excluded from the analysis? If the protective effects of moderate alcohol consumption depend on incident death during the year after baseline exam the results should interpreted as coming from a cross-sectional study, and conclusions regarding causality should be toned down. A figure with Kaplan-Meier estimates of the survival function would be very helpful to illustrate the incidence of mortality in the different categories of ethanol intake, preferably by sex (see above). Such a figure would also show if the proportional hazard assumption is reasonable. It could replace Figure 1 that just repeats the information given in Table 2. Minor comments Please define the standard unit of ethanol intake used here. Was it explained to the caregiver as well? The question is not formulated that way (p. 3, last paragraph). Major limitations should include the small sample size (in relation to the detailed definition of the exposure), and the relatively coarse categories of ethanol intake that might not allow to single out moderate intake for women (see comments above). Awareness of disease: please add 'of disease' to Table 1, to ease understanding. Table 1: in view of the sample size, variables with 3 categories could be combined into 2 categories if meaningful, and a test of trend across categories of current ethanol intake could be applied that has more power than the chi square test, for instance, ever vs. never smoking ($p = 0.05$ for trend); CCI ≥ 1 vs. 0 ($p = 0.01$ for trend). Also, living alone vs. together ($p = 0.02$ for trend), and intervention vs. control ($p = 0.13$ for trend). On page 5 (statistical analysis), the question was raised whether 'a previously observed increased mortality in the intervention group could be attributed to differences in alcohol consumption'. This seems an important question, also in view of a weak trend of increasing allocation to the control group with higher ethanol intake, but it was never answered. Please comment on this question. What is meant by 'participants were randomized (1:1) to control …

	and intervention'? Was the intervention randomized within matched pairs? If so, which are the matching variables, and why does the analysis not take matching into account? If the accuracy of ethanol reporting is questionable if a primary caregiver does not live together with the patient, a sensitivity analysis could be performed that excludes patients living alone. In this way, one could investigate whether the protective effect of moderate intake is observed (disregarding significance) in the more homogeneous group of patients not living alone. At last, there could be effects of interaction between ethanol intake and drugs in this elderly sample of Alzheimer patients that should be discussed and taken into account if information is available.

REVIEWER	Nicolas Cherbuin Australian National University, Australia
REVIEW RETURNED	01-Apr-2015

GENERAL COMMENTS	This study investigated the association between informant-reported alcohol consumption of 321 individuals recently diagnosed with Alzheimer's disease and mortality risk over 36 months. Participants were individuals with mild AD or dementia with Lewy body taking part in the DAYSY study, a Danish intervention in which participants were randomised to control support during follow up or to control support plus DAISY intervention (multifaceted and semi-tailored counselling, support and education). Participants met DSM-IV and the NINDCS-ADRDA criteria and had MMSE > 20. Alcohol intake was assessed with a single question posed to informants with four possible answers (no alcohol, only at parties, one or less than one unit per day, two to three units per day or more than three units per day). Mortality data was obtained from The Danish Civil Registration System. Age, sex, smoking, household status, MMSE, quality of life, awareness and comorbidity were controlled for in analyses as well as RCT allocation group. The association between mortality and alcohol intake was assessed with Cox proportional hazard ratios. 53 participants (16.5%) died in the 36 months follow-up period. Moderate intake (2-3 units per day) was associated with a more than three-fold decreased risk of premature death compared to those consuming less than 1 unit/day. Those who consumed more than three units per day or who did not consume any alcohol did not differ from the other groups. These findings were interpreted as showing that alcohol intake has a protective effect in AD. This study investigates an important question as very little is known about the risks or benefits of alcohol intake in patients with dementia. The study design has substantial limitations but in the context of a dearth of evidence, the present findings should be considered with caution but should not be dismissed as they may provide valuable baseline information on which future research can be developed. Major comments: 1. The main weakness of this study is that alcohol intake is reported by informants after disease diagnosis with no premorbid measure available. It is therefore impossible to know if those who

	consume alcohol are those who are somewhat healthier and consequently are less likely to die in the follow-up period or whether the effect is really due to moderate alcohol intake in this stage of the disease. Although the authors discuss this possible effect they do so only after endorsing alcohol intake as protective (e.g. "To our knowledge we are the first to show the effect of alcohol on mortality in patients with a diagnosis of AD"). In the present context it is not clear that one alternative is more likely than the other and therefore they should be presented as equally probable. Moreover, since this is a correlational study it is not correct to suggest "an effect of alcohol", instead only reference to associations should be made. 2. No information is provided on informants' characteristics and on the reliability of their report on alcohol intake. Please, provide more details as it is not clear whether informants could be elderly and suffering from cognitive impairment themselves or other family members (e.g. children) who may not necessarily be fully aware of the alcohol intake of the person with mild dementia 3. On substantial limitation of the study is that the proportion of males and females is not the same in all groups and specifically is very different between the two groups between which the significant effect is demonstrated. This point should be highlighted and the possibility the effect presented is at least in part due to sexual dimorphism of the disease process, for which there is substantial evidence, should be discussed. Other comments: - the abstract should state that alcohol information was obtained through informants and not directly from individuals with mild dementia - Please clarify what is meant by unit of alcohol as this can be interpreted in varying ways Page 4, line 27: "...Scores ranged from 20-30..." this is inconsistent with data presented in table 1 which suggests that scores ranged from 20-26. Please clarify

VERSION 1 – AUTHOR RESPONSE

Reviewer Name Francesco Panza, MD, PhD
Institution and Country Neurodegenerative Disease Unit, Department of Basic Medicine, Neuroscience, and Sense Organs, University of Bari Aldo Moro, Bari, Italy
Please state any competing interests or state ‘None declared’: None declared

Berntsen and colleagues, in this post-hoc analysis, found that in patients recently diagnosed with Alzheimer’s disease (AD), those who had a moderate alcohol intake (two to three units per day) had a significantly lower risk of death compared with those who only had alcohol occasionally (one or less than one unit per day). Abstinence or high alcohol intake did not significantly affect mortality.
While there is a series of studies on the association among alcohol consumption, AD, and late-life cognitive decline and on the relationship between alcohol and all-cause mortality, this is may be the first study investigating the association between alcohol consumption and mortality in AD patients.

These findings should be in part explain a protective effect of moderate alcohol consumption against dementia and AD. Although the approach of this report was very interesting, which was often underestimated in others studies, some minor revisions should be considered before publication.

Introduction. Among meta-analyses and systematic reviews investigating the role of moderate alcohol use in late-life cognitive disorders the Authors should include some papers (Neuropsychiatr Dis Treat. 2011;7:465-84; Int J Geriatr Psychiatry. 2012 Dec;27:1218-38.). The Authors should include also some further introductive remarks of this issue.
Author reply:
We thank the reviewer for pointing our attention to these articles, which we agree are of relevance to our article. On Page 2 we have added the following:
"Systematic reviews and meta-analysis on the association between alcohol consumption and dementia have concluded that at present there is no indication that light-to-moderate alcohol consumption is harmful to dementia. On the contrary is has been stated that there is substantial evidence that light-to-moderate alcohol consumption reduces the risk of dementia and cognitive decline."

Discussion. Possible mechanisms: Ritchie and colleagues evaluated the possible association between alcohol consumption and cognitive decline in old age [Age (Dordr). 2014 Jun;36(3):9638]. They found a significant gene × alcohol consumption interaction on lifetime cognitive change, suggesting that the effect of alcohol consumption on cognitive change may thus depend on genetic differences in the ability to metabolize alcohol. Individuals with higher genetic ability to process alcohol showed relative improvements in cognitive ability with more consumption, whereas those with low processing capacity showed a negative relationship between cognitive change and alcohol consumption with more consumption. The Authors should include this among possible mechanisms explaining the impact of alcohol consumption on mortality in AD patients.
Author reply:
The article to which the reviewer refers has a very interesting point has been added to the discussion section of our article.
Page 7:
"It has been suggested that there are significant differences in subjects' abilities to metabolize alcohol. Genetic differences being a determining factor for alcohol having either a protective or a harmful effect on cognitive abilities in later life. Is should be considered whether this factor could be part of an explanation for the impact of alcohol on mortality in AD patients."

Discussion. Limitations: alcohol consumption is often assessed only once (as in the present study), resulting in possible measurement error bias. The Authors should include also this as a possible limitation of their study, suggesting the use of an alcohol consumption measure obtained multiple times across the life course. A very recent study in which alcohol consumption was assessed 3 times in the 10 years preceding the first cognitive assessment was recently published (Alcohol consumption and cognitive decline in early old age. Sabia S, Elbaz A, Britton A, Bell S, Dugravot A, Shipley M, Kivimaki M, Singh-Manoux A. Neurology 2014;82:332-9). The Authors should consider to discuss also the findings of this report.
Author reply:
We agree that this is an important limitation of our study and have added it to the discussion section.
Page 8:
"We assessed alcohol consumption only once at inclusion. It would have been more representative to obtain measurements of alcohol consumption multiple times throughout the participants' lives after the AD diagnosis; thus characterizing long-term alcohol consumption patterns for our AD patients and avoiding possible measurement errors."

Reviewer Name Kirsten Mehlig
Institution and Country Dept of Public Health and Community Medicine
Section for Epidemiology and Social Medicine (EPSO) Sahlgrenska Academy, University of Gothenburg Box 453 SE - 405 30 Gothenburg, Sweden
Please state any competing interests or state 'None declared': None declared

Summary: this study investigates whether disease-protective health effects associated with moderate alcohol consumption observed in mentally healthy adults could be extended to patients with mild Alzheimer's disease.
Major comments:
Definition of the exposure: In this study, moderate alcohol consumption has been defined as 2-3 units / day but no definition of 'unit' is given.
Author reply:
We thank the reviewer for pointing out this important lack of information in our article. We have now added the Danish unit of alcohol to the article.
Page 4:
"We used the Danish unit of alcohol. A Danish unit of alcohol: 12 g/ 15 ml of pure alcohol (a UK unit of alcohol is 10 ml of pure alcohol)."

While absolute amounts for moderate drinking vary there is a consensus that the limits for women are about half the limits for men, based on biological differences in ethanol metabolism between the sexes. It is therefore necessary to present a sex-stratified analysis, in order to examine whether there is a minimum risk associated with a certain amount of ethanol intake in both sexes, and whether the protective amount differs between men and women. A limitation of this study is that the potentially moderate intake for women, ca. 1 unit / day, cannot be separated from < 1 unit / day. It could be that no minimum risk across ethanol categories is observable in women in this study. Eventually, a classification of ethanol into 3 categories (>0 and ≤ 1 unit/day, ≥ 2 units/day, and current abstainers) with the latter as reference could make sense in the female stratum. The problem of combining lifetime abstainers with former abusers into 'current abstainers' seems to be a minor one in this particular study. In the discussion, the authors state that adjustment for sex should be sufficient. This is not true because the differential effect of ethanol intake on men and women corresponds to the presence of an interaction term between each ethanol category and sex. This would be a way to account for the gender difference in the whole sample, and would be an alternative to stratified analysis. It could also be that gender differences are of minor importance at this old age but this would have to be explained.
Author reply:
We thank the reviewer for this very well thought out comment. We completely understand the reviewers' point of view, however we have tested for an interaction between our categories of alcohol intake and sex in our model. This interaction has a p-value of p=0.9839 in the unadjusted model, and p=0.9529 in the adjusted model. Hence, the association between alcohol intake and mortality is similar for the two sexes and no stratification is needed. That being said - this is important information, and lacking in the previous version of the paper. Therefore, we added on page 5 to the statistical section: "A possible difference between sexes in the association of alcohol intake with mortality was tested by including an interaction term in the regression analyses.", and in the results section on page 6: "The interaction between sex and alcohol intake was not significant (P=0.9839 unadjusted, p=0.9529 adjusted)", and in the discussion section on page 9 : "The non-significant interaction term between sex and alcohol intake found in our analysis indicates that the association between alcohol intake and mortality is similar for the two sexes and it is not needed to stratify for sex in these analysis."

While it is recognized that at least 3 categories of intake are needed in order to test a non-linear risk curve, the number of observations, and of cases of death are very small in the categories other than the reference category, giving rise to large confidence intervals for corresponding effects (Table 2, Figure 1), a problem that would be even more important in stratified analysis. The rule of thumb that no more than 1/10 of the number of events in the minor outcome category (here, number of deaths) should be used for the number of predicting variables shows that this study is underpowered in view of the detailed definition of the exposure: with 56 deaths, the number of ethanol categories including gender interaction alone exceeds the recommended number of about 6 covariates, and the investigation of important confounders appears difficult. Stepwise selection of variables with ethanol categories forced into the model could be tested.
Author reply:
Once again we thank the reviewer for this very thorough comment. In our opinion the critical problem in the present paper is not so much lack of power (as may be induced by the inclusion of too many adjusting variables in the analyses), but possibly falsely concluding a significant association such as

the one found in the paper. The latter problem is not dependent on sample size, but on the 5% significance level chosen. We feel that we can reasonably conclude that there is decreased mortality for the 2-3 units per day class, even if some may consider the samplesize small.
The purpose of the rule of thumb mentioned is probably to avoid multicollinearity: the simultaneous inclusion of variables in a multivariable model that are very strongly correlated; this causes instable estimates (regardless of sample size). No associations in Table 1 witness of such very strong correlations with alcohol intake so that we do not think that this is a problem. To take away over fitting concerns, we performed a backwards stepwise model selection procedure as suggested by the reviewer, omitting sequentially variables (but not alcohol intake) with $p>0.05$ until all variables had $p<0.05$. This resulted in No alcohol HR=0.90 (0.32-2.53), 2-3 servings per day HR=0.29 (0.10-0.80), and More than 3 servings per day HR=1.77 (0.54-5.82) which mimic the original results so that we can conclude that over fitting is not a problem for our conclusion.

At last, it is not clear that 'only at parties' is equivalent to '≤ 1 unit/day' as the amount is not specified in the former category. In addition, the pattern of intake does play a role wrt. health outcomes, i.e. occasional high intake or binge drinking is not equivalent to regular intake even if the average intake is the same. Subjects consuming alcohol 'only at parties' should be excluded at least in sensitivity analyses.
Author reply:
This is a relevant comment. We tried omitting subjects with alcohol intake "only at parties". This resulted in No alcohol HR=1.23 (0.42-3.62), 2-3 servings per day HR=0.36 (0.12-1.10), and More than 3 servings per day HR=1.07 (0.22-5.27) which are similar results to the results from the non-truncated data.

Reverse causation: Reverse causation is a serious problem in cohort studies with short follow-up such as this one, and its consequences are well described by the authors (p. 8, 1st paragraph). How would the effect size change if all cases during the first year after baseline would be excluded from the analysis? If the protective effects of moderate alcohol consumption depend on incident death during the year after baseline exam the results should interpreted as coming from a cross-sectional study, and conclusions regarding causality should be toned down.
Author reply:
A very interesting thought. We tried to remove the first year of follow-up from the regressions analysis. This resulted in: No alcohol HR=0.46 (0.13-1.62), 2-3 servings per day HR=0.14 (0.040-0.51), and More than 3 servings per day HR=0.99 (0.24-4.06).

A figure with Kaplan-Meier estimates of the survival function would be very helpful to illustrate the incidence of mortality in the different categories of ethanol intake, preferably by sex (see above). Such a figure would also show if the proportional hazard assumption is reasonable. It could replace Figure 1 that just repeats the information given in Table 2.
Author reply:
We agree that a Kaplan-Meier estimate is normally a very helpful tool to illustrate incidence of mortality in different categories. Because of this we made a figure with Kaplan-Meier estimates in the beginning of our analysis in this study. Unfortunately we just did not think that the figure was particularly informative. We include the figure here in the review answer so that the reviewer may judge for herself if the paper would improve if the present Figure 2 was switched with (a version of) the below figure.

Minor comments

Please define the standard unit of ethanol intake used here. Was it explained to the caregiver as well? The question is not formulated that way (p. 3, last paragraph).
Author reply:
We thank the reviewer for pointing our attention to this lacking information in our article. We have on page 4 added:
"The Danish unit of alcohol was used in the study. A Danish unit of alcohol is 12 g/ 15 ml of pure alcohol. (A UK unit of alcohol is 10 ml of pure alcohol.)"

A unit was not specified in the questionnaire to the caregivers when assessing alcohol consumption. It is however considered common knowledge in Denmark that a unit is equivalent to one Danish pilsner (33cl), one glass of wine (12cl), one shot of schnaps (4cl) or one glass of brandy (8cl)."

Major limitations should include the small sample size (in relation to the detailed definition of the exposure), and the relatively coarse categories of ethanol intake that might not allow to single out moderate intake for women (see comments above).
Author reply:
Thanks to the reviewer for this relevant comment. Please refer to previous additional analysis indicating no gender differences.
Awareness of disease: please add 'of disease' to Table 1, to ease understanding.
Answer: We agree that this is important to ease understanding. Therefore " of disease" has been added to table 1.

Table 1: in view of the sample size, variables with 3 categories could be combined into 2 categories if meaningful, and a test of trend across categories of current ethanol intake could be applied that has more power than the chi square test, for instance, ever vs. never smoking ($p = 0.05$ for trend); CCI ≥ 1 vs. 0 ($p = 0.01$ for trend). Also, living alone vs. together ($p = 0.02$ for trend), and intervention vs. control ($p = 0.13$ for trend).
Author reply:
Thanks to the reviewer for these excellent suggestions on this matter. Our purpose with Table 1 is not so much the dissemination of results as it is an overview of the data. The p-values listed function as to get a quick overview of the associations shown in the table. Furthermore, as witnessed as the main result in the present paper, a monotone dose-response effect of alcohol intake is not expected, also not for most of the variables listed in Table 1, rather some U-formed association. In our opinion a test of trend would be misplaced in this case.

On page 5 (statistical analysis), the question was raised whether 'a previously observed increased mortality in the intervention group could be attributed to differences in alcohol consumption'. This seems an important question, also in view of a weak trend of increasing allocation to the control group with higher ethanol intake, but it was never answered. Please comment on this question.
Author reply:
We understand the reviewers' concerns on this matter. This sentence was added as an argument to include intervention group as an adjustment variable (since in principle a randomized treatment assignment cannot be a confounder for the present research question, or confounded by alcohol intake). But, no, the adjustment of the intervention effect for alcohol intake does not remove the increased mortality seen for the intervention group. To clarify this matter the sentence has been changed to: "Furthermore, the analysis was adjusted for RCT allocation group to rule out that a previously observed increased mortality in the intervention group could be attributed to differences in alcohol consumption" (page 5).

What is meant by 'participants were randomized (1:1) to control … and intervention'? Was the intervention randomized within matched pairs? If so, which are the matching variables, and why does the analysis not take matching into account?
Author reply:
We thank the reviewer for this comment. We understand how this is a bit unclear. What is meant is, that there were a similar number of patient and caregiver pairs in the two groups; there was no matching. We removed "(1:1)" so that there are no misunderstandings on this matter.

If the accuracy of ethanol reporting is questionable if a primary caregiver does not live together with the patient, a sensitivity analysis could be performed that excludes patients living alone. In this way, one could investigate whether the protective effect of moderate intake is observed (disregarding significance) in the more homogeneous group of patients not living alone.
Author reply:
It is a noteworthy point the reviewer has here. We found that there are 223 subjects not living alone. The results for the adjusted analysis was: No alcohol HR=1.26 (0.38-4.26), 2-3 servings per day HR=0.28 (0.083-0.96), and More than 3 servings per day HR=1.32 (0.28-6.14). This does not change our conclusion.

At last, there could be effects of interaction between ethanol intake and drugs in this elderly sample of Alzheimer patients that should be discussed and taken into account if information is available.
Author reply:

We agree with this comment. Unfortunately we were not able to adjust for interactions between drugs and ethanol. This is because we lack information on the participants medicine use. This is also mentioned as one of the things we did not adjust for in our analyses – on page 8:
“However there are a number of possible confounders that we did not adjust for. These include social status, BMI, *medication use* and genetic factors such as Apolipoprotein E (ApoE).”

Reviewer Name Nicolas Cherbuin
Institution and Country Australian National University, Australia
Please state any competing interests or state ‘None declared’: None declared

This study investigated the association between informant-reported alcohol consumption of 321 individuals recently diagnosed with Alzheimer's disease and mortality risk over 36 months. Participants were individuals with mild AD or dementia with Lewy body taking part in the DAYSY study, a Danish intervention in which participants were randomised to control support during follow up or to control support plus DAISY intervention (multifaceted and semi-tailored counselling, support and education). Participants met DSM-IV and the NINDCS-ADRDA criteria and had MMSE > 20. Alcohol intake was assessed with a single question posed to informants with four possible answers (no alcohol, only at parties, one or less than one unit per day, two to three units per day or more than three units per day). Mortality data was obtained from The Danish Civil Registration System. Age, sex, smoking, household status, MMSE, quality of life, awareness and comorbidity were controlled for in analyses as well as RCT allocation group. The association between mortality and alcohol intake was assessed with Cox proportional hazard ratios. 53 participants (16.5%) died in the 36 months follow-up period. Moderate intake (2-3 units per day) was associated with a more than three-fold decreased risk of premature death compared to those consuming less than 1 unit/day. Those who consumed more than three units per day or who did not consume any alcohol did not differ from the other groups. These findings were interpreted as showing that alcohol intake has a protective effect in AD.

This study investigates an important question as very little is known about the risks or benefits of alcohol intake in patients with dementia. The study design has substantial limitations but in the context of a dearth of evidence, the present findings should be considered with caution but should not be dismissed as they may provide valuable baseline information on which future research can be developed.

Major comments:

A:
The main weakness of this study is that alcohol intake is reported by informants after disease diagnosis with no premorbid measure available. It is therefore impossible to know if those who consume alcohol are those who are somewhat healthier and consequently are less likely to die in the follow-up period or whether the effect is really due to moderate alcohol intake in this stage of the disease. Although the authors discuss this possible effect they do so only after endorsing alcohol intake as protective (e.g. "To our knowledge we are the first to show the effect of alcohol on mortality in patients with a diagnosis of AD"). In the present context it is not clear that one alternative is more likely than the other and therefore they should be presented as equally probable.
B:
Moreover, since this is a correlational study it is not correct to suggest "an effect of alcohol", instead only reference to associations should be made.
A:
Author reply:
We acknowledge the argument presented by the reviewer and find it of great relevance. However, we have adjusted for premorbid conditions by means of Charlson’s Comorbidity Index, which is a commonly used measure for comorbidity. We have added a sentence regarding this in the discussion on page 8: “It is an obvious concern that perhaps subjects consuming alcohol moderately are generally healthier and consequently less likely to die in the follow-up period than those who do not consume alcohol. To rule this out we adjusted for pre-morbid conditions in our analysis by including Charlson’s comorbidity index as a possible confounder.”

B:
Author reply:
To accommodate the reviewers request on this matter, which we are in agreement with, we have changed “an effect of alcohol” to “association between alcohol and mortality..” on
Page 6:
“To our knowledge we are the first to show an association between alcohol and mortality in patients with a diagnosis of AD”.
“A protective association between alcohol and mortality was seen only in the group of patients who had two to three units per day”.
“There are several possible explanations for the protective association between moderate alcohol intake and mortality observed in our study:…”
“.. 3) the seemingly protective association may be caused by bias due to the fact that some patients with low intake may be in the terminal phase of their life.”
Page 8:
“This could be the reason for lack of power and therefor lack of association between alcohol and mortality in the groups “No alcohol” and “More than three units per day”. However the point estimates do not point towards an association.”
“Because units were not divided into types of alcohol we are unable to detect whether a possible protective association between moderate alcohol consumption and mortality is limited to one type of alcohol e.g. wine.”
Page 8:
“In patients with more severe disease, those with significant co-morbidity and patients living in a nursing home without a primary caregiver we might see a different association between alcohol and mortality.”
Page 9:
“The results of our study point towards a potential, positive association of moderate alcohol consumption on mortality in AD patients.”

No information is provided on informants' characteristics and on the reliability of their report on alcohol intake. Please, provide more details as it is not clear whether informants could be elderly and suffering from cognitive impairment themselves or other family members (e.g. children) who may not necessarily be fully aware of the alcohol intake of the person with mild dementia
Author reply:
Concerning this comment we kindly refer to previous additional analysis indicating no major differences in our result when conducting the analysis for spouses only.

On substantial limitation of the study is that the proportion of males and females is not the same in all groups and specifically is very different between the two groups between which the significant effect is demonstrated. This point should be highlighted and the possibility the effect presented is at least in part due to sexual dimorphism of the disease process, for which there is substantial evidence, should be discussed.
Author reply:
Concerning this comment we kindly refer to previous additional analysis indicating no gender differences.

Other comments:

the abstract should state that alcohol information was obtained through informants and not directly from individuals with mild dementia
Author reply:
The reviewer is right to suggest that this significant information should be included in the abstract. The following has been added to the abstract:
“Data regarding current daily alcohol consumption was obtained from the patient’s primary caregivers at inclusion.”

Please clarify what is meant by unit of alcohol as this can be interpreted in varying ways
Author reply:
Page 4:

We kindly refer to the previously added information on the definition on a unit alcohol.

Page 4, line 27: "...Scores ranged from 20-30..." this is inconsistent with data presented in table 1 which suggests that scores ranged from 20-26. Please clarify
Author reply:
We thank the reviewer for pointing our attention to this error in our manuscript. We have changed to section on page 4:
“MMSE was used to assess global cognitive functions. Scores ranged from 20-26 at inclusion, higher scores indicating better cognitive performance.”

VERSION 2 – REVIEW

REVIEWER	Francesco Panza, MD, PhD Neurodegenerative Disease Unit, Department of Basic Medicine, Neuroscience, and Sense Organs, University of Bari Aldo Moro, Bari, Italy
REVIEW RETURNED	08-Jun-2015

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.

REVIEWER	Kirsten Mehlig University of Gothenburg, Sweden
REVIEW RETURNED	02-Jul-2015

GENERAL COMMENTS	The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

REVIEWER	Nicolas Cherbuin Australian National University, Australia
REVIEW RETURNED	21-Jun-2015

GENERAL COMMENTS	This study investigated the association between alcohol intake and mortality risk in AD patients. Participants were 321 individuals who had participated in a psychological intervention, the DAISY study, taking place in Denmark. The intervention lasted 12 months with an additional 24 months follow-up producing overall a 3 year follow-up for the current investigation. The present study was not part of the original published protocol but was listed in an unpublished protocol. Original inclusion criteria were age >50, a consenting carer, probable AD or dementia with Lewy body diagnosed in past 12 months according to recognised clinical criteria , an MMSE >=20, no severe somatic or psychiatric comorbidities. Alcohol intake was assessed with a single question answered by primary carers based on which participants were classified as abstainers, one or less than one unit a day, 2-3 units per day, or more than 3 units per day (unit=12g/15ml alcohol). Mortality was assessed through the Danish Registration System. Analyses consisted of Cox regression analyses using both non-adjusted as well as adjusted models. Variables controlled for included age, sex, MMSE, quality of life, ADLs,

	smoking, a comorbidity index, household status and group allocation in the RCT). There were 25 individuals in the abstainer group, 227 in the <=1 unit/day, 57 in the 2-3 unit/day, and 12 in the >3 unit/day group.Groups were generally quite similar in age, MMSE, and other covariates but the proportion of females was lowest in 2-3 unit/day. 53 patients (16.5%) died in the 3 year follow-up. The main findings were that those in the 2-3 unit/day group were at lower risk of mortality than those in the <1 unit/day. In addition, abstainers and those in the >3 units/day did not significantly differ from those in the <=1 unit/day. The authors interpreted these findings as suggestive of a protective effect of alcohol on mortality risk in AD. This is an important clearly rationalised study. While posthoc, the design and methodology are generally adequate. The analyses are appropriate. The results are clearly presented. And the discussion and interpretation of findings is generally justified and defensible. I have relatively few concerns, however, a number of points need to be addressed in order to clarify important methodological/theoretical issues that have important bearings on the interpretation of the results. Major comments: 1. A clear hypothesis as to the expected association between alcohol intake and mortality is not unambiguously stated. At the end of the discussion the authors state that others have argued that alcohol may be harmful in AD but that they speculate that it may have a protective effect without communicating clearly what the hypothesis is. It would be better if this part of the manuscript was edited to read something like "We hypothesise that....". Alternatively, the authors may choose to state that they did not have a specific hypothesis and state why. 2. Somewhat related to the first point, there is no clear justification as to why the reference group in the analyses was the <=1 unit/day group. This choice may have been made based on sample size, however, based on the literature of ageing population studies it would be most likely predicted that abstainers would be at higher risk and consequently this group may have been logically selected as reference group. While selection of the reference group would not change the reported effects it is nevertheless important because it guides which effects are reported and how. This is particularly relevant here because only HRs are reported in relation to this reference group. A consequence of this is that the main reported findings are that those in the >=1 unit/day group are at higher risk than 2-3 unit/day group, but do not differ from the other two groups, thus giving the impression that abstaining and high intake may also be associated with increased risk although not significantly so. However, if the reference group chosen had been the abstainers, the reported results under a similar approach would have been a nil finding, as abstainers do not differ significantly from any other group. It is therefore important to clearly explain why a certain reference group was selected and to report not only the HRs in relation to the reference group but also those relating to 2-3 unit/day as this will show that abstainers or >3 unit/day do not differ from this group either. It can be argued that this can be seen in Figure 1 but it is such an important point central to question investigated here that it needs to be reported in detail in text and specifically discussed in the discussion.

	3. The fact that participants in the higher intake group appear to be at higher risk, although not significantly so, should be discussed in more detail as a number of previous studies have found little evidence of increased risk even in participants with much higher intake than presented here. 4. One confound that would probably benefit from further discussion is the possibility that carers characteristics (beyond accuracy of report) might influence the associations reported. Cognitive status as well as alcohol intake of carers may be particularly relevant as it may be more difficult for AD patients to consume alcohol if they carer are abstainers and impaired cognition in the carer may impact both capacity to report alcohol intake as well as ability to care and therefore could theoretically have an effect on the outcome investigated here. 5. It is particularly noteworthy that the female ratio is lowest in the 2-3 unit/day group which is also at lowest risk. This should be discussed in more detail. It is noted that sex was controlled for on some of the statistical models but it is doubtful that such statistical correction can fully control for such differences in group composition. This has prompted some to argue that analyses should be stratified by sex instead of controlling for this factor. 6. Similar to the previous point, comorbidities are higher in the group at lowest risk. To have a sense of the magnitude of this effect, the authors might consider adding supplementary analyses reporting on the risk associated with comorbidities. In any case this point should also be discussed more specifically. 7. It is somewhat surprising that education attainment (years of education) are not reported in table 1 and that education is not controlled for in the adjusted models. Education is known to be a major risk/protective factor for dementia and cognitive decline and is also associated with alcohol intake, levels of comorbidities and other important variables bearing on the question investigated here. Hence, this measure should be added to table 1 and included in adjusted analyses and any effect should be discussed in detail. Minor comments: Page 7, line 54: "A protective association between alcohol and mortality...." The word "protective" suggests a causative link here. Since correlational analyses cannot demonstrate such links it would be more appropriate to refer to "an association which is suggestive of a protective effect" as this clearly communicates the interpretative nature of the statement. Page 8, line 21: "....by the dementia" may read better with "....by dementia" Page 8, line 25: "...the caregiver was not entirely truthful about the amount of alcohol..." The word "truthful" suggests that caregivers may have deliberately lied. While this may be the case, it is more likely that caregivers may have had some bias in their reporting. It may therefore be more appropriate to replace with "...the caregiver was not entirely accurate about the amount of alcohol..." Method: While not directly relevant to this study, it would be useful to briefly state in the method what the aim of the DAISY study was

	(presumably slowing the disease process or improving coping) as it may give the reader some insight about the kind of participant willing to take part and their representativeness of the population of AD patients.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name Francesco Panza, MD, PhD
Institution and Country Neurodegenerative Disease Unit, Department of Basic Medicine,

Please leave your comments for the authors below The Authors have satisfactorily addressed previous concerns.

Author response:

Thank you very much for your kind response. We are glad, that we were able to address your concerns adequately.

Reviewer: 3

Reviewer Name Nicolas Cherbuin
Institution and Country Australian National University, Australia

Please leave your comments for the authors below This study investigated the association between alcohol intake and mortality risk in AD patients. Participants were 321 individuals who had participated in a psychological intervention, the DAISY study, taking place in Denmark. The intervention lasted 12 months with an additional 24 months follow-up producing overall a 3 year follow-up for the current investigation. The present study was not part of the original published protocol but was listed in an unpublished protocol. Original inclusion criteria were age >50, a consenting carer, probable AD or dementia with Lewy body diagnosed in past 12 months according to recognised clinical criteria , an MMSE >=20, no severe somatic or psychiatric comorbidities. Alcohol intake was assessed with a single question answered by primary carers based on which participants were classified as abstainers, one or less than one unit a day, 2-3 units per day, or more than 3 units per day (unit=12g/15ml alcohol). Mortality was assessed through the Danish Registration System. Analyses consisted of Cox regression analyses using both non-adjusted as well as adjusted models. Variables controlled for included age, sex, MMSE, quality of life, ADLs, smoking, a comorbidity index, household status and group allocation in the RCT). There were 25 individuals in the abstainer group, 227 in the <=1 unit/day, 57 in the 2-3 unit/day, and 12 in the >3 unit/day group.Groups were generally quite similar in age, MMSE, and other covariates but the proportion of females was lowest in 2-3 unit/day. 53 patients (16.5%) died in the 3 year follow-up. The main findings were that those in the 2-3 unit/day group were at lower risk of mortality than those in the <1 unit/day. In addition, abstainers and those in the >3 units/day did not significantly differ from those in the <=1 unit/day. The authors interpreted these findings as suggestive of a protective effect of alcohol on mortality risk in AD.

This is an important clearly rationalised study. While posthoc, the design and methodology are generally adequate. The analyses are appropriate. The results are clearly presented. And the discussion and interpretation of findings is generally justified and defensible. I have relatively few concerns, however, a number of points need to be addressed in order to clarify important methodological/theoretical issues that have important bearings on the interpretation of the results.

Author response:

We sincerely thank you for your positive remarks on our design, methodology, analyses and presentation of results. In the following we will address your concerns to our best effort.

Major comments:

1. A clear hypothesis as to the expected association between alcohol intake and mortality is not unambiguously stated. At the end of the discussion the authors state that others have argued that alcohol may be harmful in AD but that they speculate that it may have a protective effect without communicating clearly what the hypothesis is. It would be better if this part of the manuscript was edited to read something like "We hypothesise that....". Alternatively, the authors may choose to state that they did not have a specific hypothesis and state why.

Author response:

We attempted to address our main hypothesis in the Introduction: we wished to investigate whether evidence from population based studies regarding a positive association between moderate alcohol consumption and mortality could be transferred to an AD population.

To clarify this we have changed the last section in the Introduction concerning the aim of the study. “The aim of this study was to investigate whether the positive association between moderate alcohol intake and mortality shown in population-based studies on healthy subjects can be transferred to patients with mild AD”. (Page 3)

2. Somewhat related to the first point, there is no clear justification as to why the reference group in the analyses was the <=1 unit/day group. This choice may have been made based on sample size, however, based on the literature of ageing population studies it would be most likely predicted that abstainers would be at higher risk and consequently this group may have been logically selected as reference group. While selection of the reference group would not change the reported effects it is nevertheless important because it guides which effects are reported and how. This is particularly relevant here because only HRs are reported in relation to this reference group. A consequence of this is that the main reported findings are that those in the >=1 unit/day group are at higher risk than 2-3 unit/day group, but do not differ from the other two groups, thus giving the impression that abstaining and high intake may also be associated with increased risk although not significantly so. However, if the reference group chosen had been the abstainers, the reported results under a similar approach would have been a nil finding, as abstainers do not differ significantly from any other group. It is therefore important to clearly explain why a certain reference group was selected and to report not only the HRs in relation to the reference group but also those relating to 2-3 unit/day as this will show that abstainers or >3 unit/day do not differ from this group either. It can be argued that this can be seen in Figure 1 but it is such an important point central to question investigated here that it needs to be reported in detail in text and specifically discussed in the discussion.

Author response:

The <=1 unit/day group is by far the largest group; choosing another group as baseline would increase the uncertainty in the reported HR’s. This was a pragmatic choice. Furthermore the No Alcohol group may not be the natural reference group because it could consist of people that are sick and therefore not able to drink alcohol; this is stated in the discussion (“ the seemingly protective association may be caused by bias due to the fact that some patients with low intake may be in the terminal phase of their life.” (Page 7, 5th line))

We have done the analysis using 2-3 unit/day as baseline as these results cannot immediately be deducted from Figure 1; only the significant difference between <=1 unit/day and 2-3 unit/day is directly transferrable. From the results below it can be seen that risk is increased for all categories compared to the 2-3 unit/day category, but only significantly so for <=1 unit/day and >3 unit/day. This consolidates our conclusion.

We have included this table in the manuscript. Table 3.

Alcohol consumption	Number of deaths (percentage of total number of patients in the group)	HR unadjusted (95 %CI)	HR adjusted * (95 %CI)
No alcohol (n=25)	4 (16)	2.35 (0.59-9.40)	3.50 (0.84-14.62)
≤1 unit per day (n=227)	42 (18.5)	2.84 (1.02-7.93)	4.30 (1.47-12.57)
2-3 units per day (n=57)	4 (7.0)	1.0 (baseline)	1.0 (baseline)
More than 3 units per day (n=12)	3 (25.0)	4.02 (0.90-17.98)	6.50 (1.38-30.68)

3. The fact that participants in the higher intake group appear to be at higher risk, although not significantly so, should be discussed in more detail as a number of previous studies have found little evidence of increased risk even in participants with much higher intake than presented here.

Author response:

In our supplementary analysis using “Two to three units” as baseline we found significantly lower risk of death in moderate alcohol intake group than in the high intake group. We have now mentioned this in the discussion “When using “Two to three units per day” as reference group we found that those with a moderate alcohol consumption had significantly lower risk of mortality than subjects with high alcohol intake. This points in the direction that increased alcohol intake is only protective until a certain consumption level.” (page 8)

4. One confound that would probably benefit from further discussion is the possibility that carers characteristics (beyond accuracy of report) might influence the associations reported. Cognitive status as well as alcohol intake of carers may be particularly relevant as it may be more difficult for AD patients to consume alcohol if they carer are abstainers and impaired cognition in the carer may impact both capacity to report alcohol intake as well as ability to care and therefore could theoretically have an effect on the outcome investigated here.

Author response:

The following sentences have been added:

“However, there might still be some inaccuracies since we cannot rule out that some patients took more alcohol than reported by their primary caregivers, or that the primary caregiver was not entirely accurate about the amount of alcohol a patient consumed. Characteristics of the caregivers might

differentiate considerably between dyads (patient and primary caregiver). Cognitive abilities and the caregivers own alcohol consumption level are some of the things that are likely to have influenced both the actual amount of alcohol consumed by the patient and the amount reported." (page 9)

5. It is particularly noteworthy that the female ratio is lowest in the 2-3 unit/day group which is also at lowest risk. This should be discussed in more detail. It is noted that sex was controlled for on some of the statistical models but it is doubtful that such statistical correction can fully control for such differences in group composition. This has prompted some to argue that analyses should be stratified by sex instead of controlling for this factor.

Author response:
As requested we have done the analyses gender stratified. We have included a new table 4 with the requested analyses. In our analyses no differing effects were seen.

6. Similar to the previous point, comorbidities are higher in the group at lowest risk. To have a sense of the magnitude of this effect, the authors might consider adding supplementary analyses reporting on the risk associated with comorbidities. In any case this point should also be discussed more specifically

Author response:
As requested we have done the analyses stratified for comorbidity. We have included a new table 4 with the requested analyses. Again no differing effects were seen.

7. It is somewhat surprising that education attainment (years of education) are not reported in table 1 and that education is not controlled for in the adjusted models. Education is known to be a major risk/protective factor for dementia and cognitive decline and is also associated with alcohol intake, levels of comorbidities and other important variables bearing on the question investigated here. Hence, this measure should be added to table 1 and included in adjusted analyses and any effect should be discussed in detail.

Author response:

As requested we have included education in the analyses. This does not change the main results of our study. Please see Table 1, Table 2 and 3.

Minor comments:

Page 7, line 54: "A protective association between alcohol and mortality...." The word "protective" suggests a causative link here. Since correlational analyses cannot demonstrate such links it would be more appropriate to refer to "an association which is suggestive of a protective effect" as this clearly communicates the interpretative nature of the statement.

Author response:

The sentence has been changed as suggested.

Page 8, line 21: "....by the dementia" may read better with "....by dementia"

Authors response:
The sentence has been changed as suggested.

Page 8, line 25: "...the caregiver was not entirely truthful about the amount of alcohol..." The word "truthful" suggests that caregivers may have deliberately lied. While this may be the case, it is more

likely that caregivers may have had some bias in their reporting. It may therefore be more appropriate to replace with "...the caregiver was not entirely accurate about the amount of alcohol..."
Author response:

The sentence has been changed as suggested.

Method: While not directly relevant to this study, it would be useful to briefly state in the method what the aim of the DAISY study was (presumably slowing the disease process or improving coping) as it may give the reader some insight about the kind of participant willing to take part and their representativeness of the population of AD patients.

Author response:

We have added this:
“The aim of the original DAISY study was to assess the efficacy at 12 months of an early psychosocial counseling and support program for outpatients with mild Alzheimer’s disease and their primary care givers.” (page 3)

Reviewer:

Reviewer Name Kirsten Mehlig
Institution and Country University of Gothenburg, Sweden

Please leave your comments for the authors below thank you very much for your elaborate replies! there are a lot of new results in support of the main hypothesis that should appear in the manuscript rather than in the answer to the referee. my main and only concern left is that associations should always be presented in relevant subgroups, irrespective of significance (because power is necessarily reduced), but it is important to see whether the associations point into the same direction. here, the sex-difference is of particular interest, and results should be added. this is why I did not cross 'yes' in limitations of the study adequately discussed, but it is a problem that should be easily fixed.
more details are given in the attached file (in green).

While absolute amounts for moderate drinking vary there is a consensus that the limits for women are about half the limits for men, based on biological differences in ethanol metabolism between the sexes. It is therefore necessary to present a sex-stratified analysis, in order to examine whether there is a minimum risk associated with a certain amount of ethanol intake in both sexes, and whether the protective amount differs between men and women. A limitation of this study is that the potentially moderate intake for women, ca. 1 unit / day, cannot be separated from < 1 unit / day. It could be that no minimum risk across ethanol categories is observable in women in this study. Eventually, a classification of ethanol into 3 categories (>0 and ≤ 1 unit/day, ≥ 2 units/day, and current abstainers) with the latter as reference could make sense in the female stratum. The problem of combining lifetime abstainers with former abusers into ‘current abstainers’ seems to be a minor one in this particular study. In the discussion, the authors state that adjustment for sex should be sufficient. This is not true because the differential effect of ethanol intake on men and women corresponds to the presence of an interaction term between each ethanol category and sex. This would be a way to account for the gender difference in the whole sample, and would be an alternative to stratified analysis. It could also be that gender differences are of minor importance at this old age but this would have to be explained.

(Author first reply: We thank the reviewer for this very well thought out comment. We completely understand the reviewers’ point of view, however we have tested for an interaction between our categories of alcohol intake and sex in our model. This interaction has a p-value of p=0.9839 in the unadjusted model, and p=0.9529 in the adjusted model. Hence, the association between alcohol

intake and mortality is similar for the two sexes and no stratification is needed. That being said - this is important information, and lacking in the previous version of the paper. Therefore, we added on page 5 to the statistical section: "A possible difference between sexes in the association of alcohol intake with mortality was tested by including an interaction term in the regression analyses.", and in the results section on page 6: "The interaction between sex and alcohol intake was not significant (P=0.9839 unadjusted, p=0.9529 adjusted)", and in the discussion section on page 9 : "The non-significant interaction term between sex and alcohol intake found in our analysis indicates that the association between alcohol intake and mortality is similar for the two sexes and it is not needed to stratify for sex in these analysis.)

Referees reply: The distribution of subjects across ethanol categories is very inhomogeneous, with > 70% in the category of low alcohol consumption. It is interesting that no interaction between ethanol intake and sex was observed wrt. mortality (by the way, the p-value is just an estimate for a probability that a certain null hypothesis is true, and it is enough to say that p > 0.9). However, it is also questionable if there is enough power to observe such an interaction given only 7 events in the 21% of subjects with higher ethanol intake. Given the large gender-difference in ethanol intake (Table 1), and in mortality (not shown, though higher proportion of women in the study), and the well-known gender-differences in ethanol metabolism it is important to know the estimates in the subgroups of men and women, too (irrespective of significance). It is likely that the protective effect of intermediate intake is only seen in men because the ethanol categories are not ideal for women with no distinction between 1 unit and less than 1 unit/day (see my earlier comments). But this is a consequence of the fact that the study was not designed to investigate this particular hypothesis, and the problem should simply be described and added to the limitations.

Author response:

As requested we have done the analyses gender stratified. We have included a new table 4 with the requested analyses. In our analyses no differing effects were seen.

OBS: there is a misprint in Table 2, 'No alcohol (n=25) not (n=26)'

Author response:

Thank you for pointing our attention to this. The mistake has now been corrected.

While it is recognized that at least 3 categories of intake are needed in order to test a non-linear risk curve, the number of observations, and of cases of death are very small in the categories other than the reference category, giving rise to large confidence intervals for corresponding effects (Table 2, Figure 1), a problem that would be even more important in stratified analysis. The rule of thumb that no more than 1/10 of the number of events in the minor outcome category (here, number of deaths) should be used for the number of predicting variables shows that this study is underpowered in view of the detailed definition of the exposure: with 56 deaths, the number of ethanol categories including gender interaction alone exceeds the recommended number of about 6 covariates, and the investigation of important confounders appears difficult. Stepwise selection of variables with ethanol categories forced into the model could be tested.

(Authors first reply: Once again we thank the reviewer for this very thorough comment. In our opinion the critical problem in the present paper is not so much lack of power (as may be induced by the inclusion of too many adjusting variables in the analyses), but possibly falsely concluding a significant association such as the one found in the paper. The latter problem is not dependent on sample size, but on the 5% significance level chosen. We feel that we can reasonably conclude that there is decreased mortality for the 2-3 units per day class, even if some may consider the samplesize small. The purpose of the rule of thumb mentioned is probably to avoid multicollinearity: the simultaneous inclusion of variables in a multivariable model that are very strongly correlated; this causes instable

estimates (regardless of sample size). No associations in Table 1 witness of such very strong correlations with alcohol intake so that we do not think that this is a problem. To take away over fitting concerns, we performed a backwards stepwise model selection procedure as suggested by the reviewer, omitting sequentially variables (but not alcohol intake) with p>0.05 until all variables had p all variables had p <0.05. This resulted in No alcohol HR=0.90 (0.32-2.53), 2-3 servings per day HR=0.29 (0.10-0.80), and More than 3 servings per day

HR=1.77 (0.54-5.82) which mimic the original results so that we can conclude that over fitting is not problem for our conclusion.)

Referees reply:

The above result could be worth to include into the manuscript, together with the information whichvariable(s) were kept in the model.

Author response:

We have included our conclusion on the backwards, stepwise model selection procedure in the appendix.

At last, it is not clear that 'only at parties' is equivalent to '≤ 1 unit/day' as the amount is not specified in the former category. In addition, the pattern of intake does play a role wrt. health outcomes, i.e. occasional high intake or binge drinking is not equivalent to regular intake even if the average intake is the same.

Subjects consuming alcohol 'only at parties' should be excluded at least in sensitivity analyses.

(Authors first reply: This is a relevant comment. We tried omitting subjects with alcohol intake "only at parties". This resulted in No alcohol HR=1.23 (0.42-3.62), 2-3 servings per day HR=0.36 (0.12-1.10), and More than 3 servings per day HR=1.07 (0.22-5.27) which are similar results to the results from the non-truncated data.)

Referees reply:

Even this result could be included as a sensitivity analysis, with a remark regarding the changed effect estimate for high vs. low ethanol intake.

Author response:

We have included the analyses in the manuscript as requested. Kindly see Table 4.

Reverse causation: Reverse causation is a serious problem in cohort studies with short follow-up such as this one, and its consequences are well described by the authors (p. 8, 1st paragraph). How would the effect size change if all cases during the first year after baseline would be excluded from the analysis? If the protective effects of moderate alcohol consumption depend on incident death during the year after baseline exam the results should interpreted as coming from a cross-sectional study, and conclusions regarding causality should be toned down.

(Authors first reply: A very interesting thought. We tried to remove the first year of follow-up from the regressions analysis. This resulted in: No alcohol HR=0.46 (0.13-1.62), 2-3 servings per day HR=0.14 (0.040-0.51), and More than3 servings per day HR=0.99 (0.24-4.06).)

Referees reply:

This result should definitely be included as a sensitivity analysis as it strengthens the author's main

hypothesis, and helps to refute concern no. 3 (discussion p.8, lines 11-12). Numbers of events / at risk should be given, too, i.e. how many events were observed 2-3 years after baseline etc.

Author response:

We have included the analyses in the manuscript as requested. Kindly see Table 4.

A figure with Kaplan-Meier estimates of the survival function would be very helpful to illustrate the incidence of mortality in the different categories of ethanol intake, preferably by sex (see above). Such a figure would also show if the proportional hazard assumption is reasonable. It could replace Figure 1 that just repeats the information given in Table 2.

(Authors first reply: We agree that a Kaplan-Meier estimate is normally a very helpful tool to illustrate incidence of mortality in different categories. Because of this we made a figure with Kaplan-Meier estimates in the beginning of our analysis in this study. Unfortunately we just did not think that the figure was particularly informative. We include the figure here in the review answer so that the reviewer may judge for herself if the paper would improve if the present Figure 2 was switched with (a version of) the below figure.)

Referees reply: nice Figure showing that the proportional hazard (PPH) assumption is fulfilled (this should be shown using a log-cumulative hazard plot, but the non-crossing of lines in the survival plot is enough given the very small number of events in all but the reference category). Could be either added as a remark saying that the applicability of the Cox model was confirmed by a graphical check of the PPH assumption. Alternatively, the figure could be given because it also shows the relatively low overall mortality in patients

with mild AD, with 80% of patients still alive after 3 years.

Author response:

The Kaplain Meier plot has been added to the appendix with a remark on proportional hazard as suggested. The original Figure 1 has been removed from the manuscript.

If the accuracy of ethanol reporting is questionable if a primary caregiver does not live together with the patient, a sensitivity analysis could be performed that excludes patients living alone. In this way, one could investigate whether the protective effect of moderate intake is observed (disregarding significance) in the more homogeneous group of patients not living alone.

(Authors first reply: It is a noteworthy point the reviewer has here. We found that there are 223 subjects not living alone. The results for the adjusted analysis was: No alcohol HR=1.26 (0.38-4.26), 2-3 servings per day HR=0.28 (0.083-0.96), and More than 3 servings per day HR=1.32 (0.28-6.14). This does not change our conclusion.)

Referees reply: Again an interesting result supporting the robustness of the manuscripts main result that should be included as a sensitivity analysis, and referred to in the discussion.

Author response:

As suggested it has been added to the manuscript see Table 4.

VERSION 3 – REVIEW

REVIEWER	Kirsten Mehlig Section for Epidemiology and Social Medicine, Department of Public Health and Community Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg Sweden
REVIEW RETURNED	16-Sep-2015

GENERAL COMMENTS	The authors might add that the sample size was too small to properly investigate further group differences such as different associations between alcohol consumption and mortality in men and in women.

REVIEWER	Nicolas Cherbuin Australian National University
REVIEW RETURNED	03-Oct-2015

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.