PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Omega-3 Fatty Acid Supplement Use in the 45 and Up Study Cohort
AUTHORS	Lui, Chi-Wai; Adams, Jon; Sibbritt, David; Broom, Alex; Wardle, Jonathan

VERSION 1 - REVIEW

REVIEWER	Parker Magin Conjoint Senior Lecturer Discipline of General Practice University of Newcastle Australia I do not have any financial conflicts of interest. Other potential conflicts of interest: During the period 2003-8, Professor Adams was a co-supervisor of my PhD and a Co-investigator in two research projects. Subsequent to this, I have contributed two chapters co-written with Professor to Adams to books edited by Professor Adams.
REVIEW RETURNED	17-Nov-2012

GENERAL COMMENTS	The topic of the demographics of Omega-2 supplement use in Australia is an important one and this large community-based study is an appropriate means of initially exploring the issue. The rationale for the study is well-presented. The methodology is sound and the paper well-written. The findings (especially the high prevalence of omega-3 supplementation) are interesting and important and, overall, the authors' interpretations are reasonable. The paper is a significant contribution to the literature, but some specific points to consider are: While the paper cites a number of Cochrane reviews, for a number of diseases there are more recent or updated Cochrane reviews of omega-3 efficacy. Some of these could be cited. In the last paragraph of the Introduction, page 7 there is discussion of diet and healthy eating. This creates some confusion with the topic of the paper, Omega-3 supplementation. Omega-3 dietary content and supplementation are separate issues. A limitation of the paper not acknowledged is that there doesn't seem to have been linkage in the survey of Omega-3 supplement consumption and the condition for which it was being used. Thus the interpretation of the association of disease treatment status and Omega-3 consumption in the regression models must be guarded. The authors suggest a possible lack of access to Omega-3 supplements as a possible reason for participants in regional and remote regions being less likely to have used Omega-3 supplements. At face value this is unconvincing. As the authors state, previous Australian research has suggested CAM therapies are more likely to be used in rural regions. Especially as the most

	commonly-used supplement, it seems unlikely that poor access to Omega-3 supplements is a factor here.

REVIEWER	Professor Wendy H. Oddy Telethon Institute for child Health Research Centre for Child Health Research The University of Western Australia Western Australia, Australia
REVIEW RETURNED	10-Dec-2012

THE STUDY	Too many national reports for an international journal
GENERAL COMMENTS	Omega-3 Supplement Use: A secondary analysis of 266,848 Australians aged 45 years and older We know very little omega-3 consumption. The aim of this paper is to examine the use and users of omega-3 amongst a large sample of older Australians. A secondary analysis was made of data from the 45 and Up Study. What is 'secondary'? It is in the abstract under design. It's also in the title. This implies 'less than'. I would advise not to use the term, 'secondary'. Use of the term 'omega-3' – it ought to be omega-3 fatty acids. The title ought to be Omega-3 fatty acid Supplement Use…. An acronyms list would be useful i.e. GPs, CAM, etc. these should be spelled out the first time they are used. Is the referencing method correct for this journal? Under key messages on Page 5: Osteoarthritis, osteoporosis, high cholesterol, and anxiety and/or depression were (not are) positively associated with omega-3 use, while cancer and high blood pressure are negatively associated with the use of omega-3. Does this mean people with these conditions used n-3 more or that increased n-3 led to increases in these conditions? Is there reverse causality in action in this paper such that those with higher rates of conditions such as depression, anxiety, osteoarthritis and osteoporosis are more likely to take omega-3? Page 6 – First paragraph sentence is to long – break into tow. Second sentence could begin 'Research also shows the use of dietary supplements is common in many European countries – (give percentage here). Last paragraph on page 6 – insert references here following words '…cholesterol lowering effect'. There are a number of long sentences i.e. Page 3, first paragraph, first sentence –, on Page 6, 2nd paragraph. Page 7, 2nd paragraph beginning 'In recent years the international…nutrition and …' should be made into two sentences. Also sentence in that same paragraph beginning 'Furthermore, given the rise of ….' Not all long sentences have been high-lighted – these are just two examples. Re-write and break long sentences into two. On page 7, co-authors state that there is currently insufficient scientific evidence on the efficacy of omega-3 regarding improvement of …. Is this correct? There does seem to be a lot of literature on the topic of omega-3 fatty acids. Page 7 – last line – '…this paper reports…' in actual fact this paper 'describes' rather than reports. Final sentence on page 7 should present the aim of the manuscript. Page 9 – Were any other dietary measures or measures of dietary intake collected? This is because an increase in the amount of omega-3 could correlate to a better diet overall.

	Page 9 – paragraph 2, under statistical analyses, 3rd line – '…health status characteristics variables…' ought to be 'health characteristics.' What happened if they had 'ever' has any of these illnesses? Was this accounted for at all? Page 10 – in the description of variables used, please include the comparison category. For example, Use of omega-3 was also higher for those participants: residing in inner regional areas ($p<0.0001$) etc. Include comparison category – relative to outer regional areas? Having a trade etc. compared to what? Having an annual household income of …. Compared to what? Being widowed etc. compared to what? Page 10 – participants who reported being treated for cancer or did not report being treated for anxiety or depression were lower users of omega-3. How relevant is this analysis? We do not know if the cancer became before any use of supplements, or if those taking supplements stopped due to illness. Page 11 – if participant had cancer there use of omega-3 was reduced. Can this be mentioned and discussed in the discussion. Page 12, first sentence – insert as '…likely to use omega-3…' Page 13 – lines 6 to 10, there is too much 'local' detail here. Is this necessary as how relevant is this to other populations? Page 13 – what is CAM – include in the acronyms list. Page 13 – last line – is this research largely about the complexities of rural supplement use? Table 1 – some of the categories could be collapsed, for example age – 70 years to 80+ could possibly be collapsed. Table 2 – The percentage using alcohol does no5 look different although it is significantly different – why is this? The size of the sample makes it easier to see significant effects – some of the columns have very small percentages in them in Table 2. It is not clear how anything could be based on these results. It is not clear if taking omega-3 supplements have an impact on any of these disease outcomes – although this was not tested. It is not really clear about the aim of this manuscript. Table 3- is 'Insurance' a socioeconomic indicator? Are all these presented results in the multivariate model? Do the results in Table 3 mean that if someone had these conditions they were statistically more likely to take omega-3? Other comments It would have been interesting to see the reasons why people took omega-3 and if anyone prescribed it (i.e. health professional) It would have been interesting to see omega-3 intake from food for those on supplements compared to those not on supplements. Food intake was not captured so it is difficult to make generalisations to the findings. Aims – users of omega-3 are non-smokers, small alcohol drinkers and being treated for conditions associated with omega-3. Lower users are those with cancer and those not reporting anxiety. No statistics are given about cancer or high blood pressure in the cohort. Other studies on same topic have not been mentioned in the paper.

REVIEWER	Richard L Nahin Senior Advisor to the Director, National Center for Complementary and Alternative Medicine, National Institutes of Health, USA I have no competing interests.
REVIEW RETURNED	16-Jan-2013

THE STUDY	In the Introduction, the authors cite a 7-year old systematic review (Wang et al 2006) that probably overstates omega-3's beneficial effects on cardiovascular disease. More recent data or reviews should be cited - e.g., Kromhout et al Eur. Heart J. 2012; Rizos et al JAMA 2012. On page 8, there are differing values given for the number of participants (265,000 and 266,848) enrolled in the “45 and Up Study.” Please clarify. How many individuals had to be approached to achieve the final cohort (226500? 266848?)? How many individuals approached were ineligible (e.g., <45 years of age)? How many could not be contacted? How many individuals contacted and eligible actually refused participation? What was the final response rate in the “45 and Up Study”? A CONSORT style flow chart would be helpful. The authors make the statement that “In response to the large sample size and multiple comparisons, a p-value of <.005 was adopted for statistical significance." How was this value of <.005 derived. A Bonferroni correction based on the number of chi square analyses would suggest a corrected p-value of <0.0024, while a Hochberg correction would suggest a p-value of <.003. Also, correcting for the number of statistical tests does not also account for the extraordinarily large sample size and the potential for statistically significant, but meaningless differences (see below). An analysis of the absolute differences in use of omega 3’s by demographic characteristic is warranted. It is also not clear how the corrected p-value of <0.005 was applied for statistical testing. Was this ONLY used for the chi sq tests? Was this also used as the p-value necessary for a demographic variable to stay in the backwards regression? If not, it should have been. Also, the confidence intervals in the regression should be based on the same corrected p-value used for statistical testing – i.e., 99.5% CI should have been used in table 3. Without this correction, it is not at all clear what the observed odds ratios mean in relation to the chi square tests, and it is likely the current logistic regression is open to many false positive conclusions. Standard Errors need to be listed for the prevalence rates in tables 1 and 2. What statistical software was used for the analyses?
RESULTS & CONCLUSIONS	The authors appear to confuse statistical significance with meaningful differences. Given the huge sample size, it is not surprising that a difference of 1% lead to statistical significance in chi square tests (Table 1 and 2). However, no case is made that these small difference are meaningful from a public health or policy standpoint. Does it really matter that 10% of individuals using omega 3 supplements reported ‘fair/poor” quality of live versus 11% in those not using omega 3 supplements? Or that the small differences in the Place of Residence matter? Etc. Without discussion of what these small absolute differences mean, rather than of the logistic regression results that are driven primarily by the large sample size, the impact of the analyses appears minimal. In the Title, Abstract and Discussion, the authors present the data as

	if they represent all Australia, when in fact, the sample was from one state, New South Wales. This is a substantial limitation of the study and should be discussed as such. For instance, data from the Australian Bureau of statistics suggests that the population of New South Wales is older but better educated than other parts of Australia. How might these and other demographic differences impact on generalizability?
REPORTING & ETHICS	Inadequate description of the survey process and response rate.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Dr Parker Magin

1. While the paper cites a number of Cochrane reviews, for a number of diseases there are more recent or updated Cochrane reviews of omega-3 efficacy. Some of these could be cited.
We have updated the references as suggested to reflect the latest development in the evidence base. The following five reviews/meta-analyses have been added to the manuscript with the last two being Cochrane reviews: Rizos et al. 2012, Chowdhury et al. 2012, Kromhout et al. 2012, Sydenham et al. 2012, James et al. 2011.

2. In the last paragraph of the Introduction, page 7 there is discussion of diet and healthy eating. This creates some confusion with the topic of the paper, Omega-3 supplementation. Omega-3 dietary content and supplementation are separate issues.
We agree with the reviewer's comments. To avoid confusion we have deleted the discussion on diet and healthy eating from this paragraph.

3. A limitation of the paper not acknowledged is that there doesn't seem to have been linkage in the survey of Omega-3 supplement consumption and the condition for which it was being used. Thus the interpretation of the association of disease treatment status and Omega-3 consumption in the regression models must be guarded.
This limitation has now been added to the Discussion as follows:
"The interpretation of our findings is limited by the fact that the association between omega-3 fatty acid supplement consumption with particular health conditions does not necessarily imply that omega-3 fatty acid supplements have been used specifically for these conditions."

4. The authors suggest a possible lack of access to Omega-3 supplements as a possible reason for participants in regional and remote regions being less likely to have used Omega-3 supplements. At face value this is unconvincing. As the authors state, previous Australian research has suggested CAM therapies are more likely to be used in rural regions. Especially as the most commonly-used supplement, it seems unlikely that poor access to Omega-3 supplements is a factor here.
We acknowledge that this point may have been confusing as initially written. We have now deleted the 'lack of access' explanation and inserted a more nuanced interpretation of previous research. We have also suggested further investigation into the urban-rural use of CAM as follows:
"… the results from our study help add to the evidence-base and discussion of this important health service issue and highlight the need for further investigation into the complexities of regional variation in supplement use."

Reviewer 2: Prof Wendy H. Oddy

5. What is 'secondary'? It is in the abstract under design. It's also in the title. This implies 'less than'. I would advise not to use the term, 'secondary'.
We have now removed the word 'secondary' from both the title and abstract.

6. Use of the term 'omega-3' – it ought to be omega-3 fatty acids. The title ought to be Omega-3 fatty acid Supplement Use.

‘Fatty acid’ has now been added to the title as suggested.

7. An acronyms list would be useful i.e. GPs, CAM, etc. these should be spelled out the first time they are used.
As suggested, a glossary has now been added. We also checked to make sure the terms are written in full the first time they are used in the manuscript.

8. Is the referencing method correct for this journal?
We can confirm that the referencing method follows the requirement of BMJ Open, as per the published author instructions.

9. Under key messages on Page 5: Osteoarthritis, osteoporosis, high cholesterol, and anxiety and/or depression were (not are) positively associated with omega-3 use, while cancer and high blood pressure are negatively associated with the use of omega-3. Does this mean people with these conditions used n-3 more or that increased n-3 led to increases in these conditions?
The word “are” has been replaced with the word “were” in this section.
To avoid confusion regarding the use of positive and negative associations, we have re-written the point as follows:
“People with osteoarthritis, osteoporosis, high cholesterol, and anxiety and/or depression were more likely to use omega-3 supplements, while people with cancer and high blood pressure were less likely to use omega 3 supplements.”

10. Is there reverse causality in action in this paper such that those with higher rates of conditions such as depression, anxiety, osteoarthritis and osteoporosis are more likely to take omega-3?
We are confused by this comment. Firstly, as this is a cross-sectional study we are unable to determine causality, only associations. Secondly, the natural assumption of our findings is that if a person has a condition such as osteoarthritis that this would prompt them to use omega-3 fatty acid supplements. Reverse causality would imply that using omega-3 fatty acid supplements results in a person getting osteoarthritis, which does not seem plausible.

11. Page 6 – First paragraph sentence is to long – break into tow. Second sentence could begin 'Research also shows the use of dietary supplements is common in many European countries – (give percentage here).
The first sentence in the paragraph has now been broken into two, and the second sentence has been revised as follows:
“Research also shows the use of dietary supplements is common in European countries such as Denmark (66% of men and 51% of women) and the United Kingdom (48% of women and 36% of men).”

12. Last paragraph on page 6 – insert references here following words '…cholesterol lowering effect'.
References have now been added as suggested.

13. There are a number of long sentences i.e. Page 3, first paragraph, first sentence –, on Page 6, 2nd paragraph. Page 7, 2nd paragraph beginning 'In recent years the international…nutrition and …' should be made into two sentences. Also sentence in that same paragraph beginning 'Furthermore, given the rise of ….' Not all long sentences have been high-lighted – these are just two examples. Re-write and break long sentences into two.
We have now revised the sentences as suggested by the reviewer. We have also reviewed and made revisions throughout the manuscript regarding long sentences.

14. On page 7, co-authors state that there is currently insufficient scientific evidence on the efficacy of omega-3 regarding improvement of …. Is this correct? There does seem to be a lot of literature on the

topic of omega-3 fatty acids.
Please refer to point 1 above. We have amended this to say conflicting or insufficient evidence, as the evidence of effectiveness of omega-3 for these conditions remains equivocal. We have reviewed and updated the literature on the efficacy of omega-3 fatty acid supplements and we believe our remarks now reflect the evidence available.

15. Page 7 – last line – '…this paper reports…' in actual fact this paper 'describes' rather than reports.
We have now changed ‘reports’ to ‘describes’ as suggested.

16. Final sentence on page 7 should present the aim of the manuscript.
The last sentence has now been broken down into two, and added the aim as follows:
“In response, this paper describes the findings of the first study to examine the use of omega-3 fatty acid supplement use in Australia. It aims to provide analysis of the prevalence and characteristics of omega-3 fatty acid supplement use amongst a large sample of Australians (n=266,848) aged 45 years and older.”

17. Page 9 – Were any other dietary measures or measures of dietary intake collected? This is because an increase in the amount of omega-3 could correlate to a better diet overall.
We agree with the reviewer that this would be an interesting association to consider. The 45 and Up study does ask questions about dietary intake (e.g. how many times each week do you eat cheese) but these questions were unfortunately inadequate to provide a meaningful measure of dietary intake of omega-3 fatty acids.

We have included this point in the limitations paragraph in the Discussion.

18. Page 9 – paragraph 2, under statistical analyses, 3rd line – '…health status characteristics variables…' ought to be 'health characteristics.' What happened if they had 'ever' has any of these illnesses? Was this accounted for at all?
Although the questionnaire asked both ‘past 4 weeks’ and ‘ever’ questions related to illness, we based the analysis on ‘past 4 weeks’ for two reasons. First, the lists of illnesses were not consistent, with the ‘4 weeks’ list including arthritis, thyroid disorder, osteoporosis, and high cholesterol (none of these were included in the list of ‘ever’ illnesses). Second, there was concern about possible recall bias regarding the ‘ever’ questions, with some participants being diagnosed with an illness several decades ago.

As this is an important issue for readers to consider when interpreting our findings, we have added this point to the limitations paragraph in the Discussion.

19. Page 10 – in the description of variables used, please include the comparison category. For example, Use of omega-3 was also higher for those participants: residing in inner regional areas ($p<0.0001$) etc. Include comparison category – relative to outer regional areas? Having a trade etc. compared to what? Having an annual household income of …. Compared to what? Being widowed etc. compared to what?
As requested, we have now added the comparison categories in the description of tables 1 and 2.

20. Page 10 – participants who reported being treated for cancer or did not report being treated for anxiety or depression were lower users of omega-3. How relevant is this analysis? We do not know if the cancer became before any use of supplements, or if those taking supplements stopped due to illness.
As explained in points 9 and 10, we are unable to comment on the direction of causality using data of

a cross-sectional study.
We have included a discussion about this limitation (see point 3).

21. Page 11 – if participant had cancer their use of omega-3 was reduced. Can this be mentioned and discussed in the discussion.
The lower use of omega-3 by people with cancer was previously mentioned in the Discussion (4th paragraph), and we have made additions to this discussion as suggested.

22. Page 12, first sentence – insert as '…likely to use omega-3…'
We have updated the sentence as suggested.

23. Page 13 – lines 6 to 10, there is too much 'local' detail here. Is this necessary as how relevant is this to other populations?
The 'local' detail is important because it allows readers to properly comprehend the findings in our Australian sample. We have added a clarifying contextual statement.

24. Page 13 – what is CAM – include in the acronyms list.
Please refer to point 7 above. The term is now included in the Glossary.

25. Page 13 – last line – is this research largely about the complexities of rural supplement use?
We discuss and contextualise a number of our findings in the Discussion, and the rural complexity of supplement use is just one of the findings. In this sentence, we are simply trying to interpret the findings of our analyses.
To clarify this, we have now changed the sentence to read "… and results from our study help…."

26. Table 1 – some of the categories could be collapsed, for example age – 70 years to 80+ could possibly be collapsed.
Typically, collapsing categories is done because one or more categories have small frequencies and hence this might impact on statistical analyses. Generally, categories should not be collapsed if their ratios are in opposite directions. In the case of age in this study, there was no issue with regards to frequencies being too small and the ratios for the 70-79 (17% vs. 15%) and 80+ (9% vs. 11%) age groups are in opposite directions. As such, no additional collapsing of categories was considered to be appropriate.

27. Table 2 – The percentage using alcohol does not look different although it is significantly different – why is this?
The 0-6 drinks category percentages differ by 2 percent (64% and 62%) while the >21 drinks category also differs by 2 percent (6% and 8%). In terms of frequencies, these differences are: 55640 vs. 111542 for the 0-6 drinks category; and 5216 vs. 14392 for the >21 drinks category. Given that the chi-square tests is calculated as a function of cell frequencies, the statistically significant finding is most likely due to the study's very large sample size. This is why we set statistical significance at $p<0.001$ and also conducted a backward stepwise model building approach to determine only the most important of predictor variables. We have mentioned these issues in our discussion.

28. The size of the sample makes it easier to see significant effects – some of the columns have very small percentages in them in Table 2. It is not clear how anything could be based on these results. It is not clear if taking omega-3 supplements have an impact on any of these disease outcomes – although this was not tested. It is not really clear about the aim of this manuscript.
We agree that the very large sample size does make interpretation of bivariate analyses (tables 1 and 2) difficult because most associations appear significant (see response to point 27). However, it is important to note that not all associations were shown to be statistically significant. So our analyses did allow us to identify those variables not associated with omega 3 fatty acid supplement use. These

are useful findings. Further, regardless of the sample size issue, we cannot determine if “taking omega-3 fatty acid supplements have an impact on any of these disease outcomes” due to the cross-sectional study design.
Our aim was to identify the important predictors of omega 3 fatty acid supplement use. In total, 21 variables were considered to potentially be predictive of Omega 3 use (Tables 1 and 2). The final model (Table 3) contained 14 variables. So the model building process did eliminate 7 variables. Note that our discussion placed emphasis on Table 3 findings, rather than Tables 1 and 2.

29. Table 3- is 'Insurance' a socioeconomic indicator? Are all these presented results in the multivariate model? Do the results in Table 3 mean that if someone had these conditions they were statistically more likely to take omega-3?
Health insurance is often considered a pseudo measure of socio-economic status given that income has a significant bearing on whether or not a person can afford private health insurance.
In relation to the results in Table 3, if the odds ratio is above 1.0 then a person is more likely to take omega 3 fatty acid supplements. This is how results were explained in the Results section (when describing Table 3) and in the Discussion.

30. It would have been interesting to see the reasons why people took omega-3 and if anyone prescribed it (i.e. health professional)
We agree that it is important to understand the reasons behind the consumption/prescription of omega-3. As the 45 & Up survey did not collect this information, we are not able to provide an answer to this question, but we have referred to other research exploring this question in the Introduction and Conclusion.

31. It would have been interesting to see omega-3 intake from food for those on supplements compared to those not on supplements. Food intake was not captured so it is difficult to make generalisations to the findings.
See response to point 17.

32. Aims – users of omega-3 are non-smokers, small alcohol drinkers and being treated for conditions associated with omega-3. Lower users are those with cancer and those not reporting anxiety. No statistics are given about cancer or high blood pressure in the cohort.
We are confused by the reviewers comment as we have provided statistics for cancer and high blood pressure in the manuscript. The distribution of omega-3 use by people with and without cancer or high blood pressure is provided in Table 2. The odds ratios for cancer and high blood pressure are provided in Table 3 and the 4th paragraph in the Result section describes the odds ratios for both cancer and high blood pressure.

33. Other studies on same topic have not been mentioned in the paper.
Please refer to point 1. We have now updated the references to include the latest evidence on omega-3.

Reviewer 3: Dr Richard L Nahin
34. In the Introduction, the authors cite a 7-year old systematic review (Wang et al 2006) that probably overstates omega-3's beneficial effects on cardiovascular disease. More recent data or reviews should be cited - e.g., Kromhout et al Eur. Heart J. 2012; Rizos et al JAMA 2012.
Please refer to point 1. We have now updated the references and included the two studies as suggested.

35. On page 8, there are differing values given for the number of participants (265,000 and 266,848) enrolled in the '45 and Up Study.' Please clarify.
To avoid confusion we have replace “over 250,000” with the exact number of ‘266,848’.

36. What was the final response rate in the '45 and Up Study'?
In order to clarify this issue extra information has now been added to the manuscript:
“The overall response rate to the mailed invitations to join the study is estimated to be 17.9%, however, the exact response rate is difficult to specify as some people may not have received the invitation if their address details were incorrect in the Medicare Australia database.[20] The 45 and Up study sample has excellent heterogeneity and is reasonably representative of the (State of) New South Wales population; has a response rate comparable to similar studies internationally and in Australia; and is among the most representative large scale cohort studies in the world.[21]”

37. The authors make the statement that 'In response to the large sample size and multiple comparisons, a p-value of <.005 was adopted for statistical significance." How was this value of <.005 derived. A Bonferroni correction based on the number of chi square analyses would suggest a corrected p-value of <0.0024, while a Hochberg correction would suggest a p-value of <.003. Also, correcting for the number of statistical tests does not also account for the extraordinarily large sample size and the potential for statistically significant, but meaningless differences (see below). An analysis of the absolute differences in use of omega 3's by demographic characteristic is warranted.
We thank the reviewer for identifying this error. We actually set the statistical significance at the more conservative p<0.001. This has now been corrected in the manuscript. Note that all bivariate analyses shown to be statistically significant had a p-value that was <0.0001 (i.e. well below our set significance level).
We agree that there is a potential for meaningless differences to be statistically significant. This is why we conducted a model building exercise to produce the most parsimonious model that is predictive of omega 3 use (see response to points 27 and 28). As such, we do not see how an analysis of the absolute difference between observed and expected cell frequencies will assist in interpretation of findings.

38. It is also not clear how the corrected p-value of <0.005 was applied for statistical testing. Was this ONLY used for the chi sq tests? Was this also used as the p-value necessary for a demographic variable to stay in the backwards regression? If not, it should have been. Also, the confidence intervals in the regression should be based on the same corrected p-value used for statistical testing – i.e., 99.5% CI should have been used in table 3. Without this correction, it is not at all clear what the observed odds ratios mean in relation to the chi square tests, and it is likely the current logistic regression is open to many false positive conclusions.
A p-value of 0.001 was used in all statistical tests, including the logistic regression modelling (i.e. yes, a p-value < 0.001 was necessary for a variable to stay in the backwards regression). We have amended the statistical analyses text to make it clear that a p-value of 0.001 was used for all statistical tests.
We have amended Table 3 and relevant text, to include 99.9% CIs.

39. Standard Errors need to be listed for the prevalence rates in tables 1 and 2.
The standard errors for the prevalence rates have now been added to tables 1 and 2.

40. What statistical software was used for the analyses?
The sentence ‘All analyses were conducted using the statistical software SAS 9.2’ has now been added to the Method section.

41. The authors appear to confuse statistical significance with meaningful differences. Given the huge sample size, it is not surprising that a difference of 1% lead to statistical significance in chi square tests (Table 1 and 2). However, no case is made that these small difference are meaningful from a public health or policy standpoint. Does it really matter that 10% of individuals using omega 3 supplements reported ‘fair/poor’ quality of live versus 11% in those not using omega 3 supplements?

Or that the small differences in the Place of Residence matter? Etc. Without discussion of what these small absolute differences mean, rather than of the logistic regression results that are driven primarily by the large sample size, the impact of the analyses appears minimal.
We are aware of the distinction between statistical significance and meaningful difference in this study, and have discussed this in our interpretation of results. However, as no such definition of what a meaningful difference is for the comparisons made, we were forced to concentrate on statistical significance. As pointed out in our response to points 27 and 28, it is important to note that the statistical tests conducted did eliminate variables from the final model presented in Table 3, so the large sample size did not hinder our ability to determine those variables that are not associated with omege-3 use. Further, we are buoyed by the fact that our findings were largely supported by other literature.
To address the issue of the impact that the large sample size had on our findings, we have added a more comprehensive discussion of this in the limitations paragraph of the Discussion:
"Finally, as the statistical tests used in our analyses are influenced by sample size, the very large sample size in this study can make small difference appear to be significant. As such, readers need to take into account the absolute differences when interpreting the odds ratios."

42. In the Title, Abstract and Discussion, the authors present the data as if they represent all Australia, when in fact, the sample was from one state, New South Wales. This is a substantial limitation of the study and should be discussed as such. For instance, data from the Australian Bureau of statistics suggests that the population of New South Wales is older but better educated than other parts of Australia. How might these and other demographic differences impact on generalizability?
We have stated in the Abstract/Setting that the research setting is in New South Wales, Australia. This information is also repeated in the Method section when we describe the 45 and Up Study. To reduce confusion, we have now added 'living in the State of New South Wales' to the end of the last sentence of Abstract/Objective and to Key Message. We also highlighted this limitation at the end of the Discussion as follows:
"Given the sample of 45 and Up Study was drawn from the State of New South Wales, generalisation of the findings of this research to other parts of Australia should be treated with caution."

43. Inadequate description of the survey process and response rate.
We have now added greater detail of the survey process and response rate, as follows:

"… individuals aged 45 years and over and resident in New South Wales were randomly selected from the Medicare Australia database, which provides virtually complete coverage of the general population. Eligible individuals were mailed an invitation to take part, an information leaflet, the study questionnaire and consent form and a reply paid envelope (available at www.45andUp.org.au). Participants joined the 45 and Up study by completing the questionnaire and consent form and mailing them to the Study coordinating centre. The study over-sampled, by a factor of two, individuals aged 80 years and over and people resident in rural areas; all residents of remote areas were sampled. The 45 and Up Study sample included approximately 10% of the general population in the target age range. Recruitment began in February 2006 and the analyses reported in this paper relate to the 266,848 participants joining the study at the close of December, 2009. The overall response rate to the mailed invitations to join the study is estimated to be 17.9%, however, the exact response rate is difficult to specify as some people may not have received the invitation if their address details were incorrect in the Medicare Australia database.[20] The 45 and Up study sample has excellent heterogeneity and is reasonably representative of the (State of) New South Wales population; has a response rate comparable to similar studies internationally and in Australia; and is among the most representative large scale cohort studies in the world.[21]"

VERSION 2 – REVIEW

REVIEWER	Parker Magin Conjoint Senior Lecturer Discipline of General Practice University of Newcastle
REVIEW RETURNED	16-Feb-2013

GENERAL COMMENTS	The authors have fully addressed the issues raised in my previous review.

REVIEWER	Richard L. Nahin Senior Advisor to the Director National Center for Complementary and Alternative Medicne National Institutes of Health USA I have no competing interests with this study.
REVIEW RETURNED	21-Feb-2013

THE STUDY	The authors have thoughtfully responded to reviewer comments and concerns. In most cases, the additional information has strengthened and clarified the paper. However, in a few cases the additional information provided by the authors in either the revised manuscript or their Cover Letter to the journal editor have revealed other issues of concerns. These are describes as follows: First, in the revised manuscript the author state that the “45 and Up Study” have a response rate of approximately 17.9%. This low rate might impact on the generalizability of the current data if the non-responders are different from responders. Recognizing this possibility, the “45 and Up” investigators made the following statement in their first “methods” paper (International Journal of Epidemiology 2008; 37:941-947): “Although derived from the general population, the relatively low response rate means that the cohort is unlikely to be directly representative of the general population [of New South Wales].” The “45 and Up” investigators tried to determine if there were systematic differences between responders and nonresponders by comparing their data to the New South Wales Population Health Survey (PHS) administered by the NSW Department of Health. In a paper by Mealing et al (BMC Medical Research Methodology, 2010, 10:26) the “45 and Up” investigators found that there was overlap between the sample populations in these two surveys for some measured demographic and health status characteristics (e.g., sex, age, education, etc.) but not others (primary language, health insurance, smoking status, psychological distress, and diagnosis of several health conditions). These differences do suggest some amount of nonrandomness in whether a contacted individual did, indeed, respond to “45 and Up.” Furthermore, since some of the characteristics found noncongruent between “45 and Up” and PHS are in the final logistic regression model in the present study (and are therefore associated with the dependent variable, use of W3 Fa), I do think the authors should be cautious in generalizing their data to the whole New South Wales population, let alone all of Australia. The low response rate and possibility of nonresponse bias should be stated as a clear limitation of the study.

	Second, additions to the first three paragraphs of the RESULTS section indicate that the authors are misinterpreting (or overstating) their chi square analyses. The chi square test for homogeneity for Row X Column tables only provides a global assessment as to whether there is an association between the two variables described by the table, not about what drives this association. For instance, in table 1, the authors show that there is a highly significant association between use of W3 Fa and age. But rather than just state this fact in the RESULTS section, the authors say "Use of w3 FA supplements is highest among females compared to males ($p<0.0001$) and those aged 60-79 years compared to those of other ages ($p<0.0001$).' This p-value ($p<0.0001$) is for the global assessment of age vs. w3 FA use, and has no relevance as to whether those ages 60-79 used the supplement more than other age groups. The authors makes this kind of misstatement throughout the second and third paragraph of the RESULTS sections (e.g., for place of residence, education, income, marital status, smoking status, alcohol consumption, etc.). As such, these sections need to be drastically rewritten.
RESULTS & CONCLUSIONS	Rewriting the RESULTS section (above) will also make this section more in line with a clarification made by the authors in their Cover Letter (last paragraph of item 28); specifically that, in fact, these chi square analyzes were preformed to indentified significant associations between use of w3 FA and various demographic and health status characteristics, with these characteristics then being entered into the backward regression model. This use of chi square analysis is completely appropriate and should be clearly statement in the METHODS section. One final point. The authors have done a commendable job stating that the data from "45 and Up" are from New South Wales in many parts of the Abstract and manuscript. However, this clarification was not carried over to the manuscript title, or to the conclusions for the Abstract and manuscript. A more appropriate title would be along the lines of: "Omega-3 fatty acid supplement use: an analysis of 266,848 individuals living in New South Wales, Australia, aged 45 years and older," or even more accurately: "Omega-3 fatty acid supplement use in the 45 and Up cohort." These changes are particularly important given: 1) the poor response rate and real possibility of nonresponse bias; and 2) accuracy in data reporting. It is no more correct to imply (even if by omission) that the present data represent all of Australia, then it would be to present survey data from England as representing all of the United Kingdom, or data from the Kanto region representing all of Japan, or data from California as representing all of the United States.

VERSION 2 – AUTHOR RESPONSE

Reviewer: Dr Richard L. Nahin

1. Low response rate and possibility of nonresponse bias of the '45 and Up Study'.

In response to this comment we have provided additional description of the sample in the Methods, as follows:

"The 45 and Up study sample has excellent heterogeneity and - in comparison to the (State of) New South Wales Population Health Survey - is reasonably representative of the New South Wales population in terms of gender, age and education; although there were differences in terms of primary language, health insurance, smoking status, psychological distress, and diagnosis of some health conditions.[21]"

We have also clearly stated this limitation in the Discussion section, as follows:
"... and as the study sample has been shown to be not representative of the New South Wales population on a number of characteristics, caution should be made in generalising the findings to the New South Wales population."

2. Misinterpreting or overstating chi square analyses.
In response to this comment, we have drastically re-written the results section (pertaining to Tables 1 and 2) as suggested by the reviewer. We have also amended the relevant section of the Methods as follows:
"The chi-square tests were used to identify those variables to be included in the logistic regression model building. Logistic regression modelling, that commenced with all significant demographic and health characteristics (identified in the chi-square tests), was conducted using a backward stepwise method, to parsimoniously predict use of w3 FA supplements."

3. Clarification of 'representativeness' of the study in the manuscript title, the conclusions for the Abstract and manuscript.
As suggested, we have changed the manuscript title to "Omega-3 Fatty Acid Supplement Use in the 45 and Up Study Cohort". We have also added the following to the conclusion of the abstract "This study, analysing data from the 45 and Up Study cohort …" and the following to the conclusion of the manuscript "Our analysis of data from the 45 and Up Study cohort …."

VERSION 3 - REVIEW

REVIEWER	Richard L. Nahin Senior Advisor to the Director, National Center for Complementary and Alternative Medicine National Institutes of Health USA I have no competing interests.
REVIEW RETURNED	07-Mar-2013

THE STUDY	The authors now discuss the lack of representativeness of their data as a study limitation, and have also changed their title and conclusions accordingly.
GENERAL COMMENTS	The authors have done an excellent job responding to review concerns.