

## PEER REVIEW HISTORY

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### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Nutrient intake and brain biomarkers of Alzheimer's in at-risk cognitively normal individuals: a cross-sectional neuroimaging pilot study
<b>AUTHORS</b>	Mosconi, Lisa; Murray, John; Davies, Michelle; Williams, Schantel; Pirraglia, Elizabeth; Spector, Nicole; Tsui, Wai; Li, Yi; Butler, Tracy; Osorio, Ricardo; Glodzik, Lidia; Vallabhajosula, Shankar; McHugh, Pauline; Marmar, Charles; de Leon, Mony

### VERSION 1 - REVIEW

<b>REVIEWER</b>	Martha Clare Morris Rush University Medical Center
<b>REVIEW RETURNED</b>	11-Mar-2014

<b>GENERAL COMMENTS</b>	<p><b><u>Overall Comments</u></b></p> <p>This is a novel study that is an important contribution to the literature even given its cross-sectional design that limits interpretation of the findings and the small sample size that further limits analyses and interpretation. The authors need to highlight these limitations more in their discussion. The writing of the manuscript could be greatly improved. There is excessive use of acronyms throughout the manuscript that causes cumbersome reading. Many statements and descriptions are imprecisely presented (a few are detailed below). The discussion could be greatly improved by integrating their study findings with the large number of longitudinal, prospective studies and clinical trials on these dietary components and dementia. The authors should take note that their study is not an investigation of dietary patterns but of individual nutrients and therefore the literature on dietary patterns is not as relevant to the study findings.</p> <p><b><u>Abstract</u></b></p> <p>Line 50-51: The statement beginning with "These effects...." is too vaguely worded to interpret the meaning.</p> <p>Page 3, Line 8-11: The conclusion statement ignores the numerous longitudinal and incident studies of nutrients and food groups that have linked diet to brain health and the development of dementia.</p> <p>Page 4: Strengths and Limitations: An important limitation, particularly for conclusions about interaction effects, is the small sample size</p> <p>Page 8, lines 18-20 : It is not correct to use the references cited as</p>
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evidence of validation of the FFQ because they do not use the same FFQ and the Australian population has different diet behaviors even if it may have used the Willett 61-item FFQ.

### **Statistical Methods**

Page 9 Line 53-54: It is important to note whether the forward or backward selection approach was used to examine nutrient associations. The ideal analyses if the sample size was larger would be to analyze the nutrients simultaneously to take into account conjoint confounding among the model covariates.

Nutrient associations are often non-linearly related to outcomes and this possibility should be explored in the analyses, for example through analyzing indicator variables of quantiles of the nutrient.

### **Results**

Page 11 Line 20: It should be clearly stated in both text and tables what form of the nutrient variable is being described, e.g. total intake, food intake, or supplement intake. The findings for all 3 of these variable forms should be stated.

(Page 12, Lines 27-32: The findings for each nutrient may be best placed all together, that is, food form of the nutrient, total intake, supplement intake only)

Page 11, Lines 35-40: What is the difference in the meaning of these two sentences and what does “post-hoc examination” refer to specifically?

### **Discussion**

Beta carotene or vitamin A cannot factually be called “strong antioxidants”

Page 14, Lines 36-43: It is stated that “the present data is consistent with epi studies of dietary patterns that are protective against AD.” This statement is not correct since the analyses presented are not at all of dietary patterns but of individual nutrients. Also, 3 of the 4 studies cited are from the same study and the 4<sup>th</sup> study is not at all about AD so the statement and cited literature do not support the idea of consistency of findings that dietary patterns are protective against AD.

Figures are not clear but it appears as if the x-axis for nutrients are actually the residuals. The mean values of the nutrients should be added to the residuals to give meaning to the nutrient levels. The reader needs to know the range of nutrient level in the population to be able to interpret the study results.

Nutrient level is extremely important to the analysis, interpretation and reporting of results. The authors do not report associations here

	<p>for vitamin E, for example, and this may be because the nutrient intake levels are narrow and unlikely to show association, or that the distribution does not include the level below and above which association may be observed. Another example is folate. The authors find positive benefit for higher folate. The literature on dementia is suggestive that it is low to moderate folate levels (obtained through foods) that demonstrate positive findings; higher intake levels may be harmful particularly in individuals with low vit b12 status. For these reasons, the nutrient distributions for every nutrient analyzed must be very clearly presented so that findings can be interpreted and compared across studies.</p> <p>The study has important weaknesses that were not detailed in the discussion. These include the very small sample size that increases chance results (false negatives and false positives), limits the ability to fully adjust for confounders (the best analyses is simultaneous statistical control of potential confounders because of conjoint confounding that stepwise approaches do not capture) and decreases the likelihood of a wide distribution of nutrient intake to fully test potential associations. Vitamin B12 and vitamin D are two nutrients that are not well captured at all by dietary intake assessment. This is because b12 absorption is impaired by stomach conditions and medication use, and a significant component to vitamin D nutritional status is sun exposure and diseases and conditions. These issues have not been addressed in the discussion but cause suspicion that the findings for these nutrients may be spurious.</p>
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<b>REVIEWER</b>	Michael Fenech CSIRO, Australia
<b>REVIEW RETURNED</b>	28-Mar-2014

<b>GENERAL COMMENTS</b>	<p>Main comment:</p> <p>The study of Lisa Mosconi and colleagues provides important new data on the association of dietary factors on with brain measurements of amyloidosis and glucose metabolism. Although the study is based on a small sample of 49 subjects it was well-designed and yielded significant associations with dietary factors previously identified as being potentially protective or detrimental with regards to dementia risk. At best these results can only be considered as being preliminary even though they demonstrate the feasibility of the approach used and its efficacy. Therefore the title of the paper should be changed by indicating that this is in effect a pilot study.</p> <p>Other concerns:</p> <p>Throughout the text and in the abstract the term “metabolism” is used loosely when in fact FDG is only measuring glucose uptake and presumably its metabolism. The authors should change the text so that the term “glucose metabolism” is utilised each time and</p>
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	<p>replace “metabolism”. I found about at least four instances in the text where this change is required.</p> <p>Page 8 line 6. A reference for APOE genotyping should be provided.</p> <p>Page 10 line 31. Explain “dummy” variable</p> <p>Page 11 line 39. Which nutrients are the ones relating to the statement “significant associations between these nutrients and (glucose??) metabolism”?</p> <p>Page 12 line 21. Who exactly are “the other subjects”???</p> <p>Page 12 lines 40-42. It seems to me that folate could not possibly be from “fish” because the folate content of fish ranges from 0-15 microgram per 100 gram which is much less than that found in legumes and cruciferous vegetables (43-180 microgram per 100g). Furthermore 6 foods were mentioned but only 5 correlation coefficients are provided. Perhaps “fish” was erroneously mentioned.</p> <p>Page 13 line 8. Was PIB retention also negatively correlated with DHA?</p> <p>Page 13 line 53. Folic acid is a synthetic form of folate used in supplements and not the natural form. Replace “folic acid” with “folate”.</p>
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### VERSION 1 – AUTHOR RESPONSE

Reviewer #1

Q1. Abstract

(a) Line 50-51: The statement beginning with “These effects....” is too vaguely worded to interpret the meaning.

The statement has been revised to clarify that the associations of vitamin B12, vitamin D and  $\omega$ -3PUFA EPA with PiB retention were independent of gender, APOE and family history.

(b) Page 3, Line 8-11: The conclusion statement ignores the numerous longitudinal and incident studies of nutrients and food groups that have linked diet to brain health and the development of dementia.

The conclusion statement has been revised to better acknowledge the important contribution of previous studies to the field.

(c) Page 4: Strengths and Limitations: An important limitation, particularly for conclusions about interaction effects, is the small sample size

According to the Reviewer’s suggestions, we have included an additional bullet point to underline that our preliminary findings of interaction effects between nutrients and AD risk factors such as gender, APOE genotype and family history need to be replicated with larger samples.

Q2. Page 8, lines 18-20 : It is not correct to use the references cited as evidence of validation of the FFQ because they do not use the same FFQ and the Australian population has different diet behaviors even if it may have used the Willett 61-item FFQ.

The statement on page 8 has been revised.

Q3. Statistical Methods.

(a) Page 9 Line 53-54: It is important to note whether the forward or backward selection approach

was used to examine nutrient associations. The ideal analyses if the sample size was larger would be to analyze the nutrients simultaneously to take into account conjoint confounding among the model covariates.

We used both forward and backward stepwise procedures, as clarified on page 10.

Our understanding is that the difference between forward and backward regression is that in forward stepwise one keeps adding independent variables to the model if they significantly contribute to the model. In backward stepwise regression one starts with all of the variables in the model and in each step take out the variables which are not significantly contributing to the model. The problem with the forward approach is that one may miss out on variables which are only significant with the presence of another variable, while the problem with the backward approach is that the model may be over fit. Given the small sample, we used the forward approach as the primary method, especially as predictors (nutrients) were examined one by one for associations with biomarkers. However, results remained unchanged by using the backward regression, showing that the method did not affect the composition of the final model. Moreover, according to the Reviewer's suggestions, we have included a more detailed section about the limitations of the study, including the above statistical considerations, on page 18-19.

(b) Nutrient associations are often non-linearly related to outcomes and this possibility should be explored in the analyses, for example through analyzing indicator variables of quantiles of the nutrient.

According to the Reviewer's suggestion, we compared FDG and PiB measures according to the tertiles of the predefined 10 nutrients using analysis of variance. First, we created tertile nutrient groups using the age- and total caloric intake-adjusted residuals for each nutrient using SPSS. Second, we used GLM with post-hoc Tukey tests to test for biomarker differences across tertile groups, for each nutrient, including the same confounds as in the linear regression analyses. If the 3rd tertile is higher than the 2nd tertile, and the 2nd tertile is higher than the 1st tertile, this suggests a linear relationship. If instead the 1st or 3rd tertile is significantly different from the 2nd tertile, but they are not different from each other, this suggests a quadratic relationship.

On FDG-PET, there were no significant differences in glucose metabolism for any nutrient tertile groups as main effects. Nonetheless, we checked post-hoc Tukey tests as an exploratory analysis, which showed some differences at trend level across Beta-carotene, vitamin C and saturated fat tertiles. Specifically, for Beta-carotene, the 3rd tertile showed a trend towards higher glucose metabolism than the 1st tertile ( $p < 0.12$ ). For vitamin C, the 3rd tertile showed a trend towards higher glucose metabolism than the 2nd tertile ( $p < 0.09$ ). For saturated fats, the 3rd tertile group had lower glucose metabolism than the 1st tertile ( $p = 0.03$ ) and there were no significant differences with the 2nd tertile. These data are consistent with linear relationships.

On PiB-PET, there was a significant difference in PiB retention by vitamin B12 and vitamin C tertile group ( $p = 0.009$ ). For vitamin B12, the 3rd tertile showed lower PiB retention than the 1st and 2nd tertiles ( $p < 0.04$ ), suggesting a linear relationship. Similar effects were observed for vitamin D although only at a trend level ( $p = 0.09$ ). For vitamin C, the 2nd tertile showed higher PiB retention vs. 1st and 3rd tertiles ( $p < 0.01$ , respectively), suggesting a quadratic relationship.

As the only analysis suggesting a possible quadratic relationship between nutrients intake and biomarkers is the one focusing on vitamin C vs. PiB retention, and considering the small sample, our preference is to not include this analysis in the paper as it would only make it more difficult to follow without providing much more insight than the linear regression analysis. The issue of linear vs. quadratic relationships has been however included in the discussion on page 18-19.

#### Q4. Results

(a) Page 11 Line 20: It should be clearly stated in both text and tables what form of the nutrient variable is being described, e.g. total intake, food intake, or supplement intake. The findings for all 3 of these variable forms should be stated.

The results section has been revised according to the Reviewer's suggestion. We have added an

additional statement on page 8-9 detailing that only 41% of our subjects were taking supplements on a regular basis (mostly a multivitamin every day, for at least one year prior to PET), and that there were no significant associations between nutrients from supplements only and biomarkers in any ROIs (page 11 and 14). As such, our main analysis focused on nutrients from food sources, as clarified on page 8, 11 and 14.

(b) Page 11, Lines 35-40: What is the difference in the meaning of these two sentences and what does “post-hoc examination” refer to specifically?

The results section has been revised to better clarify the observed interaction effects.

#### Q5. Discussion

(a) Beta carotene or vitamin A cannot factually be called “strong antioxidants”

The statement on page 15 has been revised.

(b) Page 14, Lines 36-43: It is stated that “the present data is consistent with epi studies of dietary patterns that are protective against AD.” This statement is not correct since the analyses presented are not at all of dietary patterns but of individual nutrients. Also, 3 of the 4 studies cited are from the same study and the 4th study is not at all about AD so the statement and cited literature do not support the idea of consistency of findings that dietary patterns are protective against AD.

The statement on page 16 and the references have been revised according to the Reviewer’s suggestions.

(c) Figures are not clear but it appears as if the x-axis for nutrients are actually the residuals. The mean values of the nutrients should be added to the residuals to give meaning to the nutrient levels. The reader needs to know the range of nutrient level in the population to be able to interpret the study results.

Age and caloric intake-adjusted residuals are presented in the Figures, as specified in the legend to the Figures. According to the Reviewer’s suggestions, the mean values of the nutrients have been included in Table 1.

(d) Nutrient level is extremely important to the analysis, interpretation and reporting of results. The authors do not report associations here for vitamin E, for example, and this may be because the nutrient intake levels are narrow and unlikely to show association, or that the distribution does not include the level below and above which association may be observed. Another example is folate. The authors find positive benefit for higher folate. The literature on dementia is suggestive that it is low to moderate folate levels (obtained through foods) that demonstrate positive findings; higher intake levels may be harmful particularly in individuals with low vit b12 status. For these reasons, the nutrient distributions for every nutrient analyzed must be very clearly presented so that findings can be interpreted and compared across studies.

According to the Reviewer’s suggestions, the nutrient distributions for all nutrients analyzed have been included in Table 1. These important considerations have been included in discussion on page 19-20.

(e) The study has important weaknesses that were not detailed in the discussion. These include the very small sample size that increases chance results (false negatives and false positives), limits the ability to fully adjust for confounders (the best analyses is simultaneous statistical control of potential confounders because of conjoint confounding that stepwise approaches do not capture) and decreases the likelihood of a wide distribution of nutrient intake to fully test potential associations. Vitamin B12 and vitamin D are two nutrients that are not well captured at all by dietary intake assessment. This is because b12 absorption is impaired by stomach conditions and medication use, and a significant component to vitamin D nutritional status is sun exposure and diseases and conditions. These issues have not been addressed in the discussion but cause suspicion that the findings for these nutrients may be spurious.

According to the Reviewer's suggestions, we have included more details about the limitations of the study, including the above considerations, on page 18-20.

Finally, we have reduced the use of acronyms throughout the manuscript.

#### Reviewer #2

Q1. [...] At best these results can only be considered as being preliminary even though they demonstrate the feasibility of the approach used and its efficacy. Therefore the title of the paper should be changed by indicating that this is in effect a pilot study.

The title of the study has been revised according to the Reviewer's suggestion.

Q2. Throughout the text and in the abstract the term "metabolism" is used loosely when in fact FDG is only measuring glucose uptake and presumably its metabolism. The authors should change the text so that the term "glucose metabolism" is utilised each time and replace "metabolism".

According to the Reviewer's suggestion, the term "metabolism" has been replaced by "glucose metabolism" throughout the text.

Q3. Page 8 line 6. A reference for APOE genotyping should be provided.

A reference for APOE genotyping has been provided on page 7.

Q4. Page 10 line 31. Explain "dummy" variable

The word "dummy" has been replaced with "dichotomous" (page 10). Ethnicity was used as a dichotomous variable (White/non-Hispanic vs. other ethnic groups).

Q5. Page 11 line 39. Which nutrients are the ones relating to the statement "significant associations between these nutrients and (glucose??) metabolism"?

We apologize for the lack of clarity. The results section has been thoroughly revised to better describe the associations between specific nutrients and biomarkers.

Q6. Page 12 line 21. Who exactly are "the other subjects"???

The results section has been thoroughly revised to better describe the associations between specific nutrients and biomarkers as a function of risk factors for AD (page 12-13).

Q7. Page 12 lines 40-42. It seems to me that folate could not possibly be from "fish" because the folate content of fish ranges from 0-15 microgram per 100 gram which is much less than that found in legumes and cruciferous vegetables (43-180 microgram per 100g). Furthermore 6 foods were mentioned but only 5 correlation coefficients are provided. Perhaps "fish" was erroneously mentioned. We apologize for the misprint. "Fish" was erroneously mentioned.

Q8. Page 13 line 8. Was PIB retention also negatively correlated with DHA?

PIB retention was not significantly correlated with DHA or ALA, as specified on page 14.

Q9. Page 13 line 53. Folic acid is a synthetic form of folate used in supplements and not the natural form. Replace "folic acid" with "folate".

The text has been revised according to the Reviewer's suggestion.

**VERSION 2 – REVIEW**

<b>REVIEWER</b>	Martha Clare Morris Rush University Medical Center, Chicago, IL USA
<b>REVIEW RETURNED</b>	22-Apr-2014

<b>GENERAL COMMENTS</b>	<p>Page 38. The statement that “the sffq has been validated....” Is very misleading and should not be included. The FFQs used in some of these studies was not the 61 –item version used in this population and therefore cannot be presented as evidence of its validity in this population.</p> <p>Page 41: line 33. The phrase “Results remained substantially unchanged...” does not make sense and suggest removing the word, “substantially”.</p> <p>Page 45; lines 35-38- there is actually very little evidence to support an association between vitamin b12 and AD. Most of the evidence from prospective studies is null.</p> <p>Page 46 Lines 3-32 should be omitted as the study did not investigate the relation of food groups to imaging and the argument oversteps the data. The study was of nutrient relations and the literature comparison should be of nutrient associations.</p> <p>Page 49: line 15-17- the sentence on the vit E RCT should not be included given that it was not of food intake but vitamin supplements and the primary analyses here is of food intake. supplements are not the same as food for many reasons.</p> <p>Page 49; Lines 22-30: The phrase, “that low to moderate folate levels(obtained through foods) may be beneficial” is not correct as stated and can be deleted so that the sentence is as follows: “The literature indicates that whereas higher food intake of folate may be beneficial, high intake levels of folic acid (the synthetic form of folate) may be harmful, particularly in individuals with low B12 status.”</p> <p>Figures: The manuscript should not be published without adding the mean values of the nutrients to the residual so that the figures clearly present the range of nutrient level as this is so critically important to interpret the findings.</p> <p>Discussion section: The authors’ response to previous review on non-linear associations does not address the problem. Testing for non-linear associations should not be based on formal statistical significance tests as these are highly dependent on sample size. A non-significant finding between tertile values does not mean that the association is indeed linear. A limitation of the study that needs to be stated in the discussion is that the sample size is too small to detect non-linear associations that may be present for nutrients and the imaging measures.</p>
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**VERSION 2 – AUTHOR RESPONSE**

Reviewer #1

Q1. Page 38. The statement that “the sffq has been validated....” Is very misleading and should not be included. The FFQs used in some of these studies was not the 61 –item version used in this population and therefore cannot be presented as evidence of its validity in this population. According to the Reviewer’s comment, the statement on page 8 has been removed.

Q2. Page 41: line 33. The phrase “Results remained substantially unchanged...” does not make

sense and suggest removing the word, “substantially”.  
The word ‘substantially’ has been removed.

Q3. Page 45; lines 35-38- there is actually very little evidence to support an association between vitamin b12 and AD. Most of the evidence from prospective studies is null.  
The statement on page 16 has been revised.

Q4. Page 46 Lines 3-32 should be omitted as the study did not investigate the relation of food groups to imaging and the argument oversteps the data. The study was of nutrient relations and the literature comparison should be of nutrient associations.  
The misleading sentence on page 16 has been removed. We feel that mentioning previous investigations of dietary patterns in AD is justified, as future work is needed to translate nutrient/biomarkers associations into dietary recommendations. Our preference is to defer to the Editor the decision of whether or not to further reduce our discussion.

Q5. Page 49: line 15-17- the sentence on the vit E RCT should not be included given that it was not of food intake but vitamin supplements and the primary analyses here is of food intake. supplements are not the same as food for many reasons.  
The sentence on page 19 has been deleted.

Q6. Page 49; Lines 22-30: The phrase, “that low to moderate folate levels(obtained through foods) may be beneficial” is not correct as stated and can be deleted so that the sentence is as follows: “The literature indicates that whereas higher food intake of folate may be beneficial, high intake levels of folic acid (the synthetic form of folate) may be harmful, particularly in individuals with low B12 status.”  
The sentence on page 19 has been modified according to the Reviewer’s request.

Q7. Figures: The manuscript should not be published without adding the mean values of the nutrients to the residual so that the figures clearly present the range of nutrient level as this is so critically important to interpret the findings.  
The range of nutrient levels (minimum, mean, maximum) has been added to the figures.

Q8. Discussion section: The authors’ response to previous review on non-linear associations does not address the problem. Testing for non-linear associations should not be based on formal statistical significance tests as these are highly dependent on sample size. A non-significant finding between tertile values does not mean that the association is indeed linear. A limitation of the study that needs to be stated in the discussion is that the sample size is too small to detect non-linear associations that may be present for nutrients and the imaging measures.  
The statement on page 19 has been revised according to the Reviewer’s request.