Author's response to reviews

Title: Serum concentration of eicosapentaenoic acid is associated with cognitive function in patients with coronary artery disease

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Version: 2 Date: 2 October 2014

Author's response to reviews: see over
We thank the reviewers for their valuable comments and suggestions.

Comments by the reviewers are below, followed by our responses.

Referee 1:
Reviewer's report:
Discretionary Revisions
1. There doesn't appear to be any evaluation of correlations between serum fatty acids and measurements of cardiac function (BNP and LVEF). Testing potential correlations here could help to further illuminate the relationships between serum PUFA, MMSE scores, and cardiac functions.

We evaluated the correlations between serum fatty acid levels and measurements of cardiac function (brain natriuretic peptide [BNP] and left ventricular ejection fraction [LVEF]). We added a sentence in the Results (see below) and included two Supplemental Tables.

Results
Interestingly, both LVEF and BNP (parameters of cardiac function) were associated with serum levels of DHA and DHA/AA but not EPA or EPA/AA (Supplemental Tables 1, 2).

Supplemental Table 1
Pearson correlation analysis between left ventricular ejection fraction and serum levels of polyunsaturated fatty acids

<table>
<thead>
<tr>
<th>Variables</th>
<th>R</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHA</td>
<td>0.23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EPA</td>
<td>0.10</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>AA</td>
<td>0.08</td>
<td>0.35</td>
</tr>
<tr>
<td>DGLA</td>
<td>0.10</td>
<td>0.24</td>
</tr>
<tr>
<td>EPA/AA</td>
<td>0.06</td>
<td>0.45</td>
</tr>
<tr>
<td>DHA/AA</td>
<td>0.22</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

AA, arachidonic acid; DGLA, dihomogammalinolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid
### Supplemental Table 2

Pearson correlation analysis between brain natriuretic peptide levels and serum levels of polyunsaturated fatty acids

<table>
<thead>
<tr>
<th>Variables</th>
<th>R</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHA</td>
<td>−0.25</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EPA</td>
<td>−0.08</td>
<td>0.31</td>
</tr>
<tr>
<td>AA</td>
<td>−0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>DHLA</td>
<td>−0.22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EPA/AA</td>
<td>−0.02</td>
<td>0.77</td>
</tr>
<tr>
<td>DHA/AA</td>
<td>−0.24</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

AA, arachidonic acid; DGLA, dihomogammalinolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid

2. Was DPA (n-3) measured? If would be nice to see that included in Table 2.

We appreciate the reviewer’s suggestion; however, we did not measure DPA (n-3) in our study.

Consider using DGLA instead of DHLA (seems to be the more common abbreviation.)

We changed DHLA to DGLA throughout the manuscript, as suggested.
Major Compulsory Revisions

1. For the statistical analysis methods, please provide more details about the type of multiple regression analysis performed (e.g. stepwise, best subsets). Were any tests of collinearity amongst predictors evaluated? Were any non-linear associations evaluated? Were any model diagnostics evaluated (e.g. residuals vs. fits)?

We used multiple regression analysis of the standard least squares method. Collinearity amongst predictors and non-linear associations were not evaluated; however, residuals and residual vs. fit plots were examined to ensure homoscedasticity, as suggested. We added the following sentences to the Statistical analysis section of our manuscript.

Statistical analysis
Multiple regression analysis (standard least squares method) was used to assess the degrees of association between the CAD risk factor variables and the MMSE scores. Residuals and residual vs. fit plots were examined to ensure homoscedasticity.

2. The introduction/background section seems to focus specifically on EPA. Despite this relationship being a main finding of the study, it seems that the original investigational questions were broader. The measurements of cardiac function are not mentioned anywhere in the introduction. The introduction should be revised to reflect the study objectives and could include a hypothesis of what the authors anticipated would be the significant relationships (if appropriate), or a clarification that analyses were exploratory in nature.

Based on your suggestions, we revised the Introduction/Background section as follows:

Background
In addition, hypoxia/ischemia resulting from reduced cerebral blood flow due to cardiac dysfunction may be associated with dementia. However, it is currently unknown whether reduced serum levels of n-3 PUFAs are associated with cognitive impairment, and more specifically, which components of PUFAs are associated with cognitive function in CAD patients. Therefore, the aim of this study was to investigate the association between cognitive function and n-3 PUFA levels (including eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], dihomogammalinolenic acid [DGLA], and arachidonic acid [AA]) in CAD patients.
and to identify which components of PUFAs are associated with cognitive function in these patients. We hypothesized that decreased level of EPA would be associated with cognitive impairment in patients with CAD.

3. The concluding statement of the abstract that EPA is a potential biomarker of cognitive function does not seem warranted by the study results. A more appropriate conclusion would be that the correlation potentially lends further support to a role of dietary n-3 in preventing cognitive decline in people with CAD.

Based on your suggestion, we revised the concluding statement as follows.

**Conclusions:**
Serum EPA concentration is associated with cognitive function in patients with CAD, suggesting that a low serum EPA level is a risk factor for cognitive impairment independent of cardiac function, including left ventricular ejection fraction. This correlation potentially lends further support to a role of dietary n-3 PUFAs in preventing the cognitive decline in CAD patients.

4. In Tables 3 and 4, units are required for all continuous variables in order to interpret the beta coefficient. Please revise to include these.

We added the required units to Tables 3 and 4, as suggested

**Minor Essential Revisions**
1. Abstract methods: mention other variables examined (e.g. BNP and LVEF).

We revised the Methods section of the Abstract as suggested:

We retrospectively evaluated cognitive function with the mini-mental state examination (MMSE), serum levels of PUFAs (including eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], dihomogammalinolenic acid [DGLA], and arachidonic acid [AA]), cardiovascular risk factors (hypertension, dyslipidemia, diabetes mellitus, cerebrovascular disease, and history of current/previous smoking), and parameters of cardiac function (left ventricular ejection fraction [LVEF] and brain natriuretic peptide [BNP] levels) in 146 Japanese CAD patients.
2. Abstract results: provide R and p-value for each significant predictor.

We provided revised R and p-values for each significant predictor.

3. Throughout the manuscript, change “single regression” to Pearson correlation (more accurate because you discuss R rather than R-squared.

We replaced “single regression” with “Pearson correlation” throughout the manuscript, as suggested.

4. Abstract conclusions: mention LVEF findings, too.

We revised the Conclusions section of the Abstract as follows.

Serum EPA concentration is associated with cognitive function in patients with CAD, suggesting that a low serum EPA level is a risk factor for cognitive impairment independent of cardiac function including left ventricular ejection fraction.

5. Background, first paragraph: instead of “Therefore, preventing dementia might lead to suppression of cardiovascular events,” mention the possibility of strategies that prevent both CAD and dementia.

We revised the sentence as follows:

Therefore, the modification of these lifestyle-related problems could be strategies for coronary artery disease (CAD) and dementia prevention.

6. Background, end of first paragraph: is identification of residual and conventional risk factors such as hypertension, diabetes, and smoking the objective of your study? These are reported in the results but not discussed.

As the reviewer pointed out, the identification of residual risk factors (but not the identification of conventional risk factors) was an objective of the study. We revised the sentence as follows:
Therefore, the identification of residual risk factors is important for dementia prevention.

7. Background, end of second paragraph: revise to say “serum levels of n-3 PUFA ‘may be’ risk factors for both...”
8. Methods: change “casual plasma glucose” to “non-fasting plasma glucose.”
9. Methods: change “Since n-6 PUFAs, including AA, are ‘known’ to be pro-inflammatory” to “Since n-6 PUFAs, including AA, are ‘often considered’ to be pro-inflammatory”
10. Statistical analysis: change log-transferred to “log transformed” and Pearson’s single regression to Pearson’s correlation.

We revised 7. as “These studies indicate that a reduced serum level of n-3 PUFAs may be a risk factor for both CAD and cognitive impairment.”
All other sentences were revised as suggested above.

11. Discussion: clarify for reader that dividing EPA by AA did not increase predictive power of EPA alone. AA seemed to have no relationship with MMSE, while low levels of EPA were independently correlated.

We added the following sentence to the Discussion.

Discussion
AA levels showed no association with MMSE scores, while low EPA levels were independently correlated with MMSE scores. Therefore, EPA/AA did not increase the predictive power of EPA alone.

Referee 2:

In this study, the authors demonstrated that decreased serum levels of EPA and a reduced EPA/AA ratio are associated with cognitive impairment in patients with CAD, indicating that decreased EPA, rather than DHA, is a risk factor for development of cognitive impairment at this patient population.
I think this article is properly studied and well documented enough to be published on NUTRITION JOURNAL.
I would have some comments to recommend for improvement but all of them is as discretionary revisions.
1. It would be better to be discuss about the possibility of confoundings among LVEF, BNP and age because single association of LVEF or BNP with MMSE score was shown but both of them was not associated with MMSE score on multiple regression analysis.

BNP but not LVEF was associated with age in the study. Although there is a possibility of confounding between cardiac function and age, our results indicate that the MMSE score was associated with EPA levels independent of cardiac function. According to the reviewer’s suggestion, we added the following sentence:

In addition, the Pearson correlation analysis exhibited an association between MMSE scores and LVEF or BNP; however, multiple regression in an age-adjusted model showed no association. Although there is a possibility of confounding between cardiac function and age, our results indicate that MMSE scores were associated with EPA levels independent of cardiac function.

2. It also would be better to be discussed about anti-inflammatory effect of EPA or n-3 PUFA and the possibility of its contribution to the anti-dementia effect of them.

We added the following paragraph and references to discuss the anti-inflammatory effects of EPA:

It has been reported that vascular inflammation is associated with cognitive function [16]. EPA is known to have the ability to attenuate tumor necrosis factor-α-induced upregulation of vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and monocyte chemoattractant protein-1.[17] The anti-inflammatory effects of EPA might contribute to the prevention of cognitive impairment.