Author's response to reviews

Title: Ceramides predict verbal memory performance in coronary artery disease patients undertaking exercise: a prospective cohort pilot study

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Author's response to reviews: see over
October 15, 2013

Re: Ceramides predict verbal memory performance in coronary artery disease patients undertaking exercise: a prospective cohort pilot study (Research Article MS: 1106072860979390)

Dear Dr. Visser:

Thank you for your consideration of the aforementioned manuscript in *BMC Geriatrics*. We have addressed the reviewers’ comments as detailed in the attached itemized list. We hope that you will now find the manuscript acceptable for publication.

Please feel free to contact me should you have any concerns.

Sincerely,

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Reviewer Comments

Reviewer 1

1. *It is important to mention more prominently that ceramide species in the blood were measured.*

   We have emphasized that ceramide species were measured in plasma isolated from blood samples throughout the manuscript.

2. *Saleem et al. find a correlation between long ceramide 24:0 concentrations at baseline and age. It would be important to discuss this finding more in detail, since also other groups have observed that plasma ceramide concentration decreases with age (Gorska et al., 2002).*

   We thank the reviewer for their comment and agree that age is an important factor to consider. In the present study, we did see an inverse correlation with age among individuals with CAD, which is in line with Gorska et al., 2002. However, many other studies, mostly in animal models, suggest that ceramides increase with age (please see review of Cutler & Mattson, 2001). A co-author, Dr. Mielke, has been examining changes in ceramides with age in two separate cohorts (unpublished data). The changes in ceramides appear to differ by sex and depend on the carbon-chain length of ceramide. Men have higher levels of most ceramides in mid-life. However, overtime, women tend to increase whereas men remain flat or decrease. For ceramide C22:0, women do increase over the ages of 55-90 while men have a gradual decrease. In contrast, for ceramide C24:1, levels increase for both men and women with age, but women have a much faster increase and significantly higher levels after the age of 75. As there have been few epidemiological studies with large sample sizes examining ceramide levels in the population, and factors that affect them, there are many questions. Dr. Mielke’s research will help to clarify these questions. However, at this point we feel it is premature to discuss age in detail.

   We have added the following sentences discussing the association between age and ceramide concentrations in the Discussion section:

   “We observed an inverse correlation with age among individuals with CAD, which is in line with Gorska et al., 2002. However, many other studies, mostly in animal models, suggest that ceramides increase with age (please see review of Cutler & Mattson, 2001). The relationships of ceramides to age are complex and appear to depend on the species of ceramide and site of sampling (peripheral versus brain versus cerebrospinal fluid). As such, the exact relationships between age and ceramide concentrations remain to be determined. Epidemiological studies with large sample sizes examining ceramide levels in the population, and factors that affect them (e.g., gender, disease states) will be needed to address this question.”

3. *Since some ceramide species can be transported, it would be important to mention this in the discussion, and explain that recently ceramide transporter proteins (CERTs) have been identified in the blood and described to interact with proteins relevant to neurodegeneration in Alzheimer’s disease pathology (Mencarelli and Martinez et al. 2012) (Mencarelli et al.*
We have added the following sentence in the Discussion section:

“In addition, ceramide transporting proteins (CERTs) have been recently identified in the blood and shown to interact with proteins associated with dementia pathology such as serum amyloid P component (Mencarelli and Martinez et al. 2012) (Mencarelli et al. 2012). Thus, a variety of mechanisms may relate ceramide concentrations to cognitive decline.”

Reviewer 2

1. **How does the ceramide concentration at baseline compare to other studies?**

   Baseline concentrations of plasma ceramides in this study were similar to other reports using high performance liquid chromatography coupled electrospray ionization tandem mass spectrometry (LC/MS/MS) with quantitative measures of analyte area (counts per second; cps) (Mielke et al., 2010) (Mielke et al., 2011). Ceramide concentrations in this study could not be compared to studies utilizing different methodologies and quantitative measures.

   The following sentence was added in the Discussion section:

   “Baseline concentrations of plasma ceramides in this study are similar to other reports that have used LC/MS/MS with quantitative measures of analyte area (Mielke et al., 2010) (Mielke et al. 2011).”

2. **How does the memory z-score at baseline compare to other studies?**

   We have added the following sentence in the Discussion section:

   “Baseline participant memory scores were in the non-impaired range and consistent with other studies assessing memory impairment in healthy elderly (Mielke et al., 2010) and those with CAD (Swardfager et al., 2011) (Saleem et al., 2013); however, even subtle deficits in verbal memory performance are associated with poorer clinical outcomes in patients with CAD (Swardfager et al. 2011).”

3. **It cannot be taken for granted that improvement in memory is due to the physical exercise. The cognitive improvement could also be explained by training effects.**

   We have addressed this as a potential limitation in the Discussion section:

   “Practice effects may have contributed to the overall improvement in verbal memory; however, a one-year interval between testing would be expected to minimize such effects on tests of verbal memory (Bartels et al., 2010).”

4. **Any claim for the use of ceramides as ‘prognostic marker’ for cognitive risk in CAD is quite premature because the sample size was small and overlap considerable. The finding could be better interpreted as that increased ceramide levels are associated with (relatively) worse...**
cognitive outcomes, also seen in other studies in other type of patients.

We recognize that this is a pilot study and the claim for the use of ceramides as a ‘prognostic marker’ for cognitive risk is premature. We have amended the concluding sentence of the Abstract:

“Plasma ceramide concentrations should be further examined as potential predictors of cognitive response to exercise and worse cognitive outcomes in patients with CAD.”

The interpretation of the results has also been amended in the Conclusions section:

“These preliminary findings suggest that ceramides and other sphingolipid species should be explored as prospective cognitive biomarkers in patients with CAD.”

5. Table 1: replace ‘association with’ by ‘p-value’.

As requested, ‘association with’ has been change to ‘p-value’ in Table 1.

Formatting:
As requested, the full institutional names of the ethics committees that approved the study have been added to the Methods section. Figure 1 has also been cropped as requested.