Author's response to reviews

Title: Diet quality and hippocampal volume in humans

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Version: 3 Date: 4 August 2015

Author's response to reviews: see over
Diet quality and hippocampal volume in humans

Reviewer 1:

Thank you for the positive comments. We are in agreement and have included one part of your comments in the conclusion. Here we address further comments and questions:

1. It is unclear if dietary quality was used as a predictor of Yr4 hippocampal volume, or if only associations were examined between diet and overall volumes acquired at both time points. The abstract, introduction and discussion should clarify the exact longitudinal nature of research.

We have amended the text as suggested:

(Abtract)

The aim of this study was to examine the association between dietary patterns and hippocampal volume in humans, and assess whether diet was associated with differential rates of hippocampal atrophy over time.

End of results in abstract:

While hippocampal volume declined over time, there was no evidence that dietary patterns influenced this decline.

(Introduction)

Added new last sentence:

Using longitudinal data, we also investigated whether dietary patterns were associated with differential rates of atrophy over time.

(Methods)

Additional models tested for an interaction between each of the dietary factors and wave on hippocampal volume to evaluate whether diet was associated with differential atrophy over time.

(Results)

The test of diet as predictor of atrophy/preserving hippocampal volume is evident in statement:

There was no evidence of an interaction ... between each of the dietary factor scores and time (i.e., diet was not associated with differential atrophy: $\beta_{\text{prudent} \times \text{time}} = 20.8, se=24.4, p=0.40; \beta_{\text{western} \times \text{time}} = 27.2, se=28.8, p=0.34)$.

Discussion

Discussion of the longitudinal test is in 1st paragraph:

2. The use of two imaging protocols is not sufficiently addressed. Why were different protocols used for a longitudinal study, especially given that the same type of scanner was apparently available at both times. Adjustment for changes in ICV does not adequately control for the potential that different imaging
Protocol may have significantly altered hippocampal volume estimates, especially given that the ICV was derived using an automated process. The differing slice thickness and other acquisition parameters between the two time points may have altered the appearance of hippocampal and other anatomical boundaries—which could have substantially altered manual tracing, an effect which would not necessarily have been manifested in a system actually larger or smaller hippocampal volume between the two time points. As one expects the hippocampal volumes to decrease over time (in this older population), it is especially difficult to identify effects of protocol differences. Were any comparisons made between hippocampal tracings of individuals scanned with both protocols on the same day? Alternatively, mean hippocampal volumes could be compared between individuals who were age 64 at baseline with individuals who were age 64 at Yr4. Regarding ICV it would be helpful to include mean volumes at both time points in order to demonstrate any systematic bias or lack thereof in intracranial vault size. In addition to more fully addressing the problem of two imaging protocols, the authors should highlight this problem as a study limitation in their Discussion.

We thank this reviewer for giving us the opportunity to address these methodological issues. But please note that the methodology applied here has been scrutinized in detail in the following paper based on the same dataset and published in Neurology:


The scanning protocol was changed to optimize acquisition quality. We agree that where possible scanning parameters should be kept constant. After the first wave of assessment it was realized that improved image quality could be achieved by modifying the acquisition protocol. Moreover, in this study participants are scanned in bulk every 4 years and adjustments may also have addressed scanner drift. It is nowadays more common for participants to be scanned on multiple scanners on multiple sites with no ability to adjust for these factors due to small cell sizes (e.g. ADNI). In the present study all participants were scanned on the same scanner during a narrow time window at each wave. As such variance between individuals would not be affected by a systematic bias (as would be the case had participants been scanned on different scanners or with different protocols at a single time point). Consequently, the only likely consequence of change in scanning parameters is a possible effect on measurement accuracy. We address this question below but it is worth noting that the significant findings reported in this study do not relate to longitudinal change. As such this discussion is largely academic in the present context.

The following figure shows boxplots of the left and right hippocampal volumes after adjustment for ICV.
As suggested by Reviewer 1, we compared 62 individuals aged 64-65 at wave 1 to 50 individuals aged 64-65 at wave 2. A significant difference was detected between the two waves in left and right hippocampal volumes and ICV after adjustment for ICV. This may suggest that correction for ICV did not fully adjust for methodological differences or that somehow the individuals aged 64-65 at wave 1 differed significantly from individuals aged 64-65 at wave 2 in some unidentified ways. It is not possible to unambiguously answer this question. This point has been added to the limitation section as suggested.

"While we corrected our analyses for differences in ICV between waves 1 and 2, these corrections may only have partly controlled for differences in scanning protocols between waves. However, this is unlikely to have important implications for the findings presented as no significant effect was detected in relation to longitudinal change."

3. Figure 1 should identify N for each diet group given that many participants likely did not fit into any of the three categories (e.g., those +1SD on prudent diet but average on Western).

Fig 1 illustrates the predicted values derived from the final statistical model and not based on measures from specific respondents. Because the figures are derived from the model, it is not accurate to say that the estimates are based on a specific n. Rather, we have
included a description in the results section that provides further explanation of the estimates and note the proportion of respondents who would be classified below/above this specific point based on their scores derived by combining prudent and western diet scores.

"These estimates are derived from the final statistical model and not based on measures from specific respondents. Overall, the data suggests that 6.6% and 8.2% of respondents would have scores on a combined western and prudent dietary factor that would have them classified with good or poor diets respectively."

4. How did the hippocampal atrophy—in either absolute term or % change—compare with population norms? This information is important in terms of generalizability and because of the problem of two imaging protocols, identified above.

We recently conducted a systematic review with meta-analysis on this topic, which also included volumetric changes between waves 1 and 2 in this dataset; this indicated that while % change was in the upper bound in this sample it overlapped with a number of other studies. Moreover, meta-regression indicated that shorter length of follow-up was a significant factor explaining this difference. It should also be noted that while many studies used broad age ranges (e.g. 20-80 years), the fact that the PATH cohort has a narrow 4-year age range may also have contributed to this difference. This article is referenced.


5. It would be helpful for authors to briefly address issues in comparing human with non-human studies. For example, a high sucrose diet is not an accurate approximation of a western diet, and human trials of vitamin supplementation (e.g., alpha-tocopherol) have failed to show benefits that would be expected based on animal studies.

There have now been a multitude of studies that have used actual ‘junk food’ (cafeteria diets) in experimental models examining the impact on BDNF and other brain systems and a sentence clarifying this has now been added to the introduction (with references).

Vitamin supplementation is a very different beast to dietary change – supplementation is not equivalent to diet and your point is well taken. A sentence addressing this particular point has now been included and further clarification of animal versus human data made.

The conclusion now also includes a section relating the findings to the implications for humans and reinforcing the focus on whole of diet.

6. Did authors consider handedness as a covariate? This factor is of potential importance given the laterality of findings.

Post-hoc analysis showed handedness was not associated with (left or right) hippocampal volume, and its inclusion in the final model did not change the results observed on key covariates.
Reviewer 2.

1. In the background, it is stated that hippocampal volume is reduced in adults with depression [17], while antidepressants increase neurogenesis [18, 19]. Is the inference that hippocampal volume is increased? Ref 18 is in the rat and 19 is a short review. Please provide clarification.

We have added another reference comprising a meta-analysis of the literature in humans, establishing that BDNF levels are increased following antidepressant treatment and reference to a study suggesting that antidepressant treatment can increase hippocampal volumes in humans. We have also made the point that BDNF appears to mediate the impact of antidepressants on depression in humans and that these levels are detrimentally affected in models of Western diet.

2. There is an interesting difference in those subjects of the larger group who were included in the study, in terms of being less likely to report heart problems or poor self-rated health, being married and their participation in regular exercise. Can the authors comment on this?

The difference observed suggest that the included participants were slightly better off in terms of health and health behaviours. This would likely attenuate the strength of the observed relationships. However, it is important to note that while representativeness is of critical importance when generating prevalence data, it is of marginal importance when examining associations between exposures and outcomes.

http://ije.oxfordjournals.org/content/42/4/1018.full
(*Commentary: Representativeness is usually not necessary and often should be avoided)

3. Food frequency was completed at baseline—is it possible that there was a greater shift (eg increased energy density, or poorer food quality) in diet in some of the participants which may have affected their hippocampal volume? Perhaps some comment could be added. The authors have already commented regarding the fairly short time window between the two scans but do not really address whether it is possible that behaviour may have altered (or that this has not been surveyed).

The reviewer raises a relevant point and we have now addressed this issue in the limitations section of the discussion:

"Finally, we assumed stability of dietary intakes over the four-year interval; however, there may have been some variability that we were unable to monitor."

4. Is the reduction in hippocampal volume seen over the 4-year window commensurate with that observed in other studies?

Please refer to response to Reviewer 1.

5. In Figure 1 predicted hippocampal volumes are shown across poor, average and good diet quality groups—how many subjects appear in each group?
Other:
To better illustrate the overall association between diet and left hippocampus volume, we modelled.

Thank you, this change has been made.

Please complete reference 54.

This has been updated.

Perhaps Figure 1 could be redrawn using a graphics program to improve quality.

We have now provided this in excel format and updated the manuscript with this image, which has improved the quality.