PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Vascular Biomarkers to Predict Response to Exercise in Alzheimer's Disease: The Study Protocol
AUTHORS	Li, Danni; Thomas, Robin; Tsai, Michael; Li, Ling; Vock, David; Gremiel, Susan; Yu, Fang

VERSION 1 - REVIEW

REVIEWER	Dr Nicolas Farina Brighton and Sussex Medical School, UK
REVIEW RETURNED	04-Apr-2016

GENERAL COMMENTS	The purpose of the protocol to determine whether n-3 fatty acids effect the cognitive response to an exercise intervention in AD. The results will be used to generate power calculations for further large-scale studies. This is a very interesting protocol that is embedded within the well-designed FIT-AD trial. That being said, I feel there are some changes that could improve the quality of the manuscript. Pg 2. The first sentence of the abstract needs to be refined. • Throughout the manuscript there is no description about the potential differences between aerobic and anaerobic exercise on AD. Please remove "Aerobic" from the first sentence. • The statement "promising treatment" is too broad. Exercise could have a benefit to a number of elements commonly associated with Alzheimer's disease (e.g. depression, functioning). The focus of this protocol is cognition and therefore the abstract should reflect this. Pg 4. I agree that generally speaking both exercise and n-3 fatty acid supplementation has yet to be established as effective. However, please be more specific to which outcomes you are referring to (presumably cognition). Pg 4. A single RCT that is 10 years old is cited [9] for the effects of n-3 fatty acids supplementation, please include either more RCTS, or recent review/systematic review instead. Pg 4 and 5. Where possible remove evidence from healthy older adult populations (e.g. citations 10, 11, 12) and instead include evidence from people with dementia. This may not be possible in all cases, but it is important to reference studies within an AD sample due to pathological differences. For example, there is evidence that lower DHA levels are associated with lower MMSE in AD (Wang et al., 2008; Nutritional biomarkers in Alzheimer's disease: the association between carotenoids, n-3 fatty acids, and dementia severity). Pg 4 and 5. The introduction would benefit from briefly describing that exercise has several mechanisms which could potentially improve cognition, but that you particularly interested in its interaction with n-3 fatty acids. Pg 6. It would be useful if a brief description of the sham exercise condition was described in text.

	Pg 6. “The details of the exercise intervention have been published elsewhere” - place citation next to this statement. Pg 7. Please describe what your definition of a mild-to-moderate AD is. Pg 14. The final paragraph is very broad, and needs to be related back to the study. Please describe why slowing cognitive decline in AD is important and what is the implications if n-3 are found to predict the cognitive responses of exercise interventions in AD (e.g. n-3 supplementation). Pg 14. Please reference evidence that personalised treatments can improve QOL of people with AD. General points • It would be useful to know at what stage the FIT-AD trial is (e.g. Date started and the number of participants recruited) so to give a clearer overview of the timeline in which this study fits. • The ADAS-Cog is used as the only measure of cognition. It would be worthwhile acknowledging that this is a global measure of cognition, and may not detect cognitive changes that a limited to specific cognitive domains. • It should be highlighted that the results from this trial may be limited to the intervention type, and may not reflect other forms (e.g. anaerobic) or difference intensity exercises. • In addition, there would be value in stating that the findings would be limited to AD with mild-moderate severity only.

REVIEWER	Prashanthi Vemuri Mayo Clinic Rochester, MN, USA
REVIEW RETURNED	17-May-2016

GENERAL COMMENTS	There is no clear indication if imaging biomarkers will be additionally considered. It is stated in the methods that hippocampal volume is measured in the FIT-AD trial.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

The purpose of the protocol to determine whether n-3 fatty acids effect the cognitive response to an exercise intervention in AD. The results will be used to generate power calculations for further large-scale studies. This is a very interesting protocol that is embedded within the well-designed FIT-AD trial. That being said, I feel there are some changes that could improve the quality of the manuscript.

Pg 2. The first sentence of the abstract needs to be refined.
• Throughout the manuscript there is no description about the potential differences between aerobic and anaerobic exercise on AD. Please remove “Aerobic” from the first sentence.

Response: we have removed “Aerobic” from the first sentence. We have also revised the three sentences that follow the first one to highlight early on in the paper that aerobic exercise is the intervention being tested:

“Exercise interventions are a promising treatment for improving cognition in persons with Alzheimer’s disease. This is similar to Alzheimer’s disease pharmacotherapies in which only 18% to 48% of treated patients demonstrate improvement in cognition. Aerobic exercise interventions positively

affect brain structure and function through biologically sound pathways. However, an under-studied mechanism of aerobic exercise's effects is n-3 fatty acids in plasma."

• The statement "promising treatment" is too broad. Exercise could have a benefit to a number of elements commonly associated with Alzheimer's disease (e.g. depression, functioning). The focus of this protocol is cognition and therefore the abstract should reflect this.

Response: we agree with the comment and have added "in improving cognition" in the first sentence (please see the above) to reflect the focus of this study on cognition. Furthermore, we have described other positive benefits of exercise (please see the above).

Pg 4. I agree that generally speaking both exercise and n-3 fatty acid supplementation has yet to be established as effective. However, please be more specific to which outcomes you are referring to (presumably cognition).

Response: we agree with the comment and have specified outcome "in improving cognition" (please see the above).

Pg 4. A single RCT that is 10 years old is cited [9] for the effects of n-3 fatty acids supplementation, please include either more RCTS, or recent review/systematic review instead.

Response: We have deleted the sentence on the effects of n-3 fatty acids supplementation.

Pg 4 and 5. Where possible remove evidence from healthy older adult populations (e.g. citations 10, 11, 12) and instead include evidence from people with dementia. This may not be possible in all cases, but it is important to reference studies within an AD sample due to pathological differences. For example, there is evidence that lower DHA levels are associated with lower MMSE in AD (Wang et al., 2008; Nutritional biomarkers in Alzheimer's disease: the association between carotenoids, n-3 fatty acids, and dementia severity).

Response: we have introduced two sentences describing the evidence on healthy older adults and on people with AD, separately, as follows:

"Lower levels of n-3 fatty acids in red blood cells, especially docosahexaenoic acid [DHA], were associated with cognitive impairment even in persons free of clinical dementia.5 Lower levels of plasma n-3 fatty acids were lower in AD6 and were associated with lower Mini Mental Status Exam (MMSE) scores in individuals with AD.7"

References:

5. Tan Z, Harris W, Beiser A, et al. Red blood cell omega-3 fatty acid levels and markers of accelerated brain aging. Neurology 2012;78(9):658-64.
6. Conquer JA, Tierney MC, Zecevic J, et al. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. Lipids 2000;35(12):1305-12.
7. Wang W, Shinto L, Connor WE, et al. Nutritional biomarkers in Alzheimer's disease: the association between carotenoids, n-3 fatty acids, and dementia severity. Journal of Alzheimer's Disease 2008;13(1):31-38.

Pg 4 and 5. The introduction would benefit from briefly describing that exercise has several mechanisms which could potentially improve cognition, but that you particularly interested in its interaction with n-3 fatty acids.

Response: we have added the following sentences at the beginning of the last paragraph of the Introduction:

"Aerobic exercise interventions positively affect brain structure and function through biologically sound pathways. However, an under-studied mechanism of aerobic exercise's effects is n-3 fatty acids in plasma."

Pg 6. It would be useful if a brief description of the sham exercise condition was described in text.

Response: we have added the following sentences in the Design subsection of the Methods section as follows:

"Stretching exercise is used in the FIT-AD Trial as sham exercise with intensity not exceeding 20% of the heart rate reserve or Rating of Perceived Exertion (RPE) of 8, and includes primarily seated movements and static stretches.8"

Pg 6. "The details of the exercise intervention have been published elsewhere" - place citation next to this statement.

Response: we have added reference 27 next to this statement.

Pg 7. Please describe what your definition of a mild-to-moderate AD is.

Response: our definition of mild to moderate dementia is a score of 15-26 on the MMSE. We have clarified our definition in the Design subsection of the Methods section as follows:

"We will use a cohort design to recruit 25 subjects enrolled in the FIT-AD Trial from both the cycling intervention and sham exercise groups. The FIT-AD Trial is a pilot randomized controlled trial that investigates the effects of a 6-month, individualized, moderate-intensity cycling intervention on cognition and hippocampal volume in community-dwelling older adults with mild-to-moderate AD (defined by Mini Mental State Score [MMSE] score of 15-16).27"

Pg 14. The final paragraph is very broad, and needs to be related back to the study. Please describe why slowing cognitive decline in AD is important and what is the implications if n-3 are found to predict the cognitive responses of exercise interventions in AD (e.g. n-3 supplementation).

Response: we agree with the comment and have revised discussion in the last paragraph:

"If plasma n-3 fatty acids were found to predict cognitive responses of aerobic exercise interventions in AD, n-3 supplementation may be recommended to enhance the pro-cognitive effects of exercise, further slowing down cognitive decline in patients with AD. Cognitive decline and associated physical

function impairment in AD is the leading cause of nursing home placement.34 35 Interventions directed to slowing down cognitive and other related functional impairment, and hence nursing home placement will not only improve quality of life of patients with AD, but also save billions in formal care. 36 Personalized treatment has revolutionized therapies for cancer. 37 There is good evidence to support the value of personalized interventions to address behavioral and psychological symptoms in people with dementia and to improve their quality of life. 38 39 Our pilot study will generate the knowledge to inform future studies, which will be critical for developing effective, personalized exercise treatments in AD, which will likely maximize resources, reduce healthcare costs, and improve treatment efficacy and quality of life for patients with AD."

Pg 14. Please reference evidence that personalised treatments can improve QOL of people with AD.

Response: we have included references 38 and 39 that personalized treatment can improve QOL of people with AD in the last paragraph.

References

38. Testad I, Corbett A, Aarsland D, et al. The value of personalized psychosocial interventions to address behavioral and psychological symptoms in people with dementia living in care home settings: a systematic review. International psychogeriatrics 2014;26(07):1083-98.
39. Bredesen DE, Amos EC, Canick J, et al. Reversal of cognitive decline in Alzheimer's disease. Aging 2016;8(6).

General points

• It would be useful to know at what stage the FIT-AD trial is (e.g. Date started and the number of participants recruited) so to give a clearer overview of the timeline in which this study fits.

Response: we have added a sentence in the Design subsection of the Methods section as follows:

"The FIT-AD trial began recruitment in March 2014 and enrolled its first subject on 6/3/2014. The FIT-AD trial met its enrollment goals of 24 and 22 subjects for years 1 and 2, respectively, and began its year 3 enrollments on 5/1/2016 and had enrolled 2 subjects of the 22 subjects by 5/30/2016."

• The ADAS-Cog is used as the only measure of cognition. It would be worthwhile acknowledging that this is a global measure of cognition, and may not detect cognitive changes that a limited to specific cognitive domains.

Response: we have discussed this as one of limitations in second to last paragraph in the Discussion section as follows. Moreover, because the FIT-AD Trial will collect cognitive measures in specific domains, including attention, processing speed, executive function, memory and language, which may be used in hypothesis generating analyses in this study.

"There are three limitations to our study. First, we chose global cognition measured by the ADAS-Cog as the outcome in this study because the ADAS-Cog is the FDA-approved cognitive measure for drug RCTs and will allow us to compare our results to other published studies. However, this global measure may not detect cognitive changes in specific cognitive domains that have differential responses to aerobic exercise intervention. The FIT-AD Trial will collect cognitive measures of specific

domains, including attention, processing speed, executive function, memory and language,27 which may be used in hypothesis generating analyses in this study. Second, results from the FIT-AD Trial may be limited to aerobic exercise, and may not reflect on other forms of exercise (e.g., anaerobic) or interventions with different intensity or duration. Lastly, results from the study will be limited to older adults with mild-to-moderate AD only and are not generalizable to individuals with severe AD or early-onset, familial AD."

• It should be highlighted that the results from this trial may be limited to the intervention type, and may not reflect other forms (e.g. anaerobic) or difference intensity exercises.

Response: we have discussed this as one of limitations in second to last paragraph in the Discussion section (please see the above).

• In addition, there would be value in stating that the findings would be limited to AD with mild-moderate severity only.

Response: we have discussed this as one of limitations in second to last paragraph in the Discussion section (please see the above).

Reviewer 2

There is no clear indication if imaging biomarkers will be additionally considered. It is stated in the methods that hippocampal volume is measured in the FIT-AD trial.

Response: we will not include imaging outcomes in this pilot study. We have included a statement in the Method and Analysis section of the Abstract as follows:

"Global cognition as measured by the Alzheimer's Disease Assessment Scale-Cognition (ADAS-Cog) at baseline, 3, 6, 9, and 12 months, will be used as the main outcome in this pilot study."