Reviewer's report

**Title:** A randomized controlled trial investigating the impact of different modes of vitamin B12 supplementation over subclinical vitamin B12 deficiency on sensory, motor and cognitive function among older people living in Santiago, Chile. A study protocol [ISRCTN 02694183].

**Version:** 1  **Date:** 23 May 2011

**Reviewer:** Alan Dangour

**Reviewer's report:**

This is an interesting and innovative study that builds on the significant experience of this group. Little is currently known in this field and successful completion of this study would be an important scientific advance.

It is very important that trial protocols are published and I fully support the intentions of the authors. At present the protocol requires some improvement and below I provide a list of suggested changes. Two major compulsory revisions are needed. First, the authors must fully integrate the cluster nature of their trial into the protocol and secondly, the authors must be much clearer about their primary and secondary outcomes.

**Title**
1. “over subclinical vitamin B12 deficiency” is not needed in the title. The word “cluster” should be in the title.

**Abstract**
2. As this is a cluster trial – you should state the number of clusters in the abstract before the number of people recruited
3. Primary outcomes – you list far too many primary outcomes (see later).

**Background**
4. Para 3 sentence 2 – “There are presently no randomised…” you should cite the OPEN study (Dangour et al. Nutr. J 2011)
5. You do not provide a definition of “deficiency”- and then you say in paragraph 4 that “CBL deficiency… ranges from 7-51% depending on cut-off values” – so the definition of deficiency is clearly important and should be discussed in more detail.
6. Primary aim – it is not at all clear why you are providing the supplementation in two forms – is the primary aim to determine the effect of supplementation on various biochemical and physiological outcomes – or is it to determine whether there is a difference in effect of supplementation depending on the route of administration? Or is it both? You must make it much more clear why you are conducting this study – it would also be helpful if you could describe somewhere...
in your paper why you think it is necessary to provide the supplement via two different routes.

Design and methodology
7. You do not mention the word “cluster” in this section!

Study hypotheses
8. I would urge you to think very carefully about these hypotheses. In my opinion you can test hypothesis 1 (an association) in a cross-sectional study. Hypothesis 2 cannot be tested via this RCT as “poor cognition, nerve conduction and sensory functional loss” could be caused by numerous factors other than the “CBL intake provided by PACAM”. Hypothesis 3 is very confusing – are you conducting this complicated and probably expensive RCT to see whether different routes of B12 supplementation can “correct subclinical CBL deficiency equally well” or to see if B12 supplementation will “improve …cognitive and sensory status, and nerve conduction…”? It’s not clear to me from reading the protocol why you are conducting this trial.

Criteria for Cluster inclusion
9. You say that you have a “convenience sample of 15 health centres” and in Figure 1 you say that the “Health centres [were] randomly selected from 94 in Santiago” – which is it?

Selection of participants from health centres
10. “all will be active participants in the PACAM program” – how will you define “active” and how will this be measured?

Participant exclusion criteria
11. Sentence 1 - How will the existence of “medical conditions” be assessed? Will this be self-reported information?

Recruitment
12. Paragraph 1 last sentence – “The remaining health centre was kept…” By my calculations there are 6 remaining health centres i.e. 21-15 = 6

13. Paragraph 2 – I don’t understand why the trial number given to each participant will be used “to identify the supply of fortified food and tablets to be indicated for each participant”. Surely the food/tablets for each participant will depend on which cluster they belong to.

14. Paragraph 2 – here for the first time you inform the reader that it is the milk that will be fortified (not the food supplement). Why is this? Some rationale for this decision will be helpful. [Probably best to put this in the section on “Intervention”].

15. Paragraph 3 – “Forty individuals will be randomly selected from each arm”. This is very confusing and it is not clear why this is happening. Are you saying that the neurophysiological testing will only take place on 120 participants in total i.e. 8 participants from each cluster – what is the rationale for doing this – and
would it be better to discuss this when you talk about your sample size requirements?

Recruitment of participants
16. Sentence 3 “the research team will check the absence of exclusion criteria” – how will this be done?

17. Sentence 6 “Potential participants will be those with an MMSE score of less than 20 (out of a maximum of 30)”. Is this correct? From the next few sentences it seems to me that you mean “with an MMSE score of more than 24 out of 30”.

18. Paragraph 1 last sentence “Eligible participants are defined as those with fasting blood glucose…” – what are these various cut-offs based on? Some references would help.

Intervention
19. Please provide a rationale for why you are fortifying the milk and not the dried food mix.

20. Please use the term “adherence” not “compliance” throughout the paper.

21. Your definition of adherence is collection of 50% of the food and disappearance of 67% of the pills. Some rationale for this difference would be helpful.

Outcome measures
22. You should make a clear distinction between indicators of study adherence, primary, secondary and exploratory outcomes.

23. I would propose that “Serum CBL status” is a measure of study adherence and is not a primary study outcome.

24. At present you list of nerve conduction outcomes, MMSE and haematological measures measured at baseline 4, 9 and 18 months as primary outcomes. This is not correct. You must provide 1 or 2 clearly specified primary outcomes for the study and these should be the ones on which you have powered your study.

25. You also have lots and lots of secondary outcomes – do you really believe that you might influence all of these outcomes with your intervention? Maybe it would be good to define a short list of secondary outcomes and then list the others as exploratory outcomes. It would be good to know how you decided on your list of cognitive function outcomes – do they measure different cognitive domains?

Sample size
26. This is a cluster randomised trial and so your definition of sample size should be based on 2 things, first the number of clusters per arm and then the number of individuals required per cluster. Please re-write your sample size section with your sample size calculations more clearly defined first by cluster and then by individuals per cluster.

Trial monitoring
27. Please use “adherence” rather than “compliance”. The sentence “Besides home visits with the same objective will be perform in 25% of subjects in each study arm” does not make sense.

Assessment at 4, 9 and 18 months
28. Some rationale for these repeat measures is needed.

Data analysis
29. More information is needed on how the multiple time-outcomes will be presented and analysed.

Conclusion
30. I do not think that the study is designed to test “the hypothesis that unrecognised subclinical CBL deficiency is associated with impaired cognition…” This hypothesis could be tested in a cross-sectional design.

Figure 2
31. This is a cluster trial – please provide information on clusters in the flow chart.

English
32. The English needs improvement in places. For example (and I stopped after page 2):
33. Abstract – last sentence – “CBL intakes that might potentially may contribute in preserving…” delete “may”.
34. Background – para 1 sentence 2 – “Both are involve in one carbon” – what does “Both” refer to and it should be “are involved in one carbon”
35. Para 1 sentence 5 – “CBL administration is widely used by intramuscular injection” – sense?

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I am a work colleague of Drs. Sanchez, Albala, Lera and Uauy and that I am the principal investigator of a similar trial - the OPEN study, the protocol of which was recently published in Nutrition Journal (Dangour et al. Nutr J. 2011 Mar 11;10:22). As this report present the protocol of a study with which I have not been involved, I do not consider this a conflict of interest for my role as a
reviewer.