PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	EFFECT OF OMEGA 3 FATTY ACID SUPPLEMENTATION ON ENDOTHELIAL FUNCTION, ENDOGENOUS FIBRINOLYSIS AND PLATELET ACTIVATION IN PATIENTS WITH A PREVIOUS MYOCARDIAL INFARCTION: A RANDOMISED CONTROLLED TRIAL
AUTHORS	Din, Jehangir; Sarma, Jaydeep; Harding, Scott; Lyall, Karin; Newby, David; Flapan, Andrew

VERSION 1 - REVIEW

REVIEWER	Philip C Calder Professor of Nutritional Immunology University of Southampton United Kingdom
REVIEW RETURNED	25-Apr-2013

GENERAL COMMENTS	This manuscript describes findings from a fairly small trial (n=20) of omega-3 fats in post-MI patients. Outcomes relate to endothelial function. The dose of omega-3 is about 1.8 g/day and they were given for 6 weeks in a cross-over design. Placebo was olive oil. No effects of omega-3 were seen. The manuscript is very well written. The main limitation is the small size of the study. The negative findings are well discussed. Specific comments: 1. Introduction, line 3 of text. Delete "any". 2. Figures 1A and 1B should be deleted and the data aded to Table 2. 3. Text and Table 2. "arachidonic" NOT "arachadonic". 4. Table 3 should be deleted and the results descibed as "data not shown". 5. Page 11, last line. Do you mean "acetylcholine or substance P". 6. Table 5 should be deleted. 7. Table 2. What comparison does the P value relate to? 8. Was there any carry-over of fatty acids into the placebo phase when Omacor was given first?

REVIEWER	Dr Andrew Hinde Head of Division of Social Statistics and Demography University of Southampton SOUTHAMPTON SO17 1BJ
REVIEW RETURNED	03-May-2013

THE STUDY	It is a minor point, but the sample size is rather small, so some

	readers might consider the failure to find statistically significant effects might be simply the result of the small size of the study. I am not one of thee readers: I am happy that any statistically significant effects that might be uncovered with a larger sample would be very modest. Nevertheless, the authors might consider adding a brief mention of the small size of the sample and its possible implications in the discussions section.
REPORTING & ETHICS	There was no CONSORT or equivalent checklist. The statement about ethics is as follows: '[a]ll subjects gave written informed consent and the study was undertaken with the approval of the local research ethics committee and in accordance with the Declaration of Helsinki'. This seemed vague and did not convince me. I searched for the trial on www.clinicaltrials.gov to no avail.
GENERAL COMMENTS	This seems pretty sound methodologically. My main concerns are the ethical clearance reporting - which does matter these days, and the lack of any comment on the small sample size.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

*This manuscript describes findings from a fairly small trial (n=20) of omega-3 fats in post-MI patients. Outcomes relate to endothelial function. The dose of omega-3 is about 1.8 g/day and they were given for 6 weeks in a cross-over design. Placebo was olive oil. No effects of omega-3 were seen. The manuscript is very well written. The main limitation is the small size of the study. The negative findings are well discussed.

We have added the following section to the Discussion to address concerns about the sample size [Page 16]:

“Our study has potential limitations that should be acknowledged. First, the sample size is relatively small which raises the possibility of a type II error due to lack of power. However, the sample size was based on separate power calculations for the vascular function and the platelet monocyte studies, and we have previously detected modest changes in these outcome measures with similar sample sizes.17,18 Although it is possible we lacked power to detect very small changes, we believe the study had sufficient power to detect any clinically relevant effects of omega-3 fatty acids.”

*Specific comments:
1. Introduction, line 3 of text. Delete "any".

We have deleted “any” as suggested.

2. Figures 1A and 1B should be deleted and the data added to Table 2.

Figures 1A and B have been deleted and the data added to Table 2.

3. Text and Table 2. "arachidonic" NOT "arachadonic".

We have amended the spelling in the text and in Table 2.

4. Table 3 should be deleted and the results descibed as "data not shown".

Table 3 has been deleted and the results described as suggested.

5. Page 11, last line. Do you mean "acetylcholine or substance P".

We have amended the wording as suggested.

6. Table 5 should be deleted.

As suggested, we have deleted Table 5.

7. Table 2. What comparison does the P value relate to?

The P value relates to repeated measures 1-way ANOVA comparing the difference between the three means. We have amended the table to include both the EPA and DHA data, and clarify P values for individual comparisons as well as all means.

8. Was there any carry-over of fatty acids into the placebo phase when Omacor was given first?

We had incorporated a 4 week washout between study arms to avoid any carry over effects. We measured fatty acids at baseline and at the end of each treatment phase and did not detect any carry-over of fatty acids after 6 weeks of placebo in the group who had Omacor first [Table below]. As we did not measure fatty acids at the beginning of the second treatment stage we cannot fully exclude the possibility of some carry over into the early part of this phase. However, we feel any such effect would be modest and unlikely to change our results.

Comparison of EPA and DHA at baseline and after placebo 1st or placebo 2nd (after Omacor+washout)

	Baseline	Placebo 1st	Placebo 2nd (after Omacor+washout)	P Value
EPA, %	2.14±0.3	1.63±0.13	1.87±0.22	0.3
DHA, %	4.89±0.31	4.39±0.32	4.31±0.42	0.5

Mean±SEM. Data analysed using 1-way ANOVA. P=NS for all individual comparisons (baseline vs placebo 1st, baseline vs placebo 2nd, placebo 1st vs placebo 2nd) for both EPA and DHA.

We have added the following line to the results section to clarify this point [Page 12]:

“We did not detect any carry-over of EPA or DHA after 6 weeks of placebo in the group who had omega-3 fatty acids first (data not shown).”

We have also added the following passage to the discussion section to acknowledge this potential limitation [Page 16]:

“…as we did not measure fatty acids at the beginning of the second treatment stage we cannot fully exclude the possibility of some carry-over of EPA or DHA into the early placebo phase in the group receiving omega-3 fatty acids first. However, we feel any such effect would be modest and unlikely to alter the study outcomes.”

Reviewer 2

*It is a minor point, but the sample size is rather small, so some readers might consider the failure to find statistically significant effects might be simply the result of the small size of the study. I am not

one of thee readers: I am happy that any statistically significant effects that might be uncovered with a larger sample would be very modest. Nevertheless, the authors might consider adding a brief mention of the small size of the sample and its possible implications in the discussions section.

As documented above, we have added the following section to the Discussion to address concerns about the sample size [Page 16]:

“Our study has potential limitations that should be acknowledged. First, the sample size is relatively small which raises the possibility of a type II error due to lack of power. However, the sample size was based on separate power calculations for the vascular function and the platelet monocyte studies, and we have previously detected modest changes in these outcome measures with similar sample sizes.17,18 Although it is possible we lacked power to detect very small changes, we believe the study had sufficient power to detect any clinically relevant effects of omega-3 fatty acids.”

*There was no CONSORT or equivalent checklist. The statement about ethics is as follows: '[a]ll subjects gave written informed consent and the study was undertaken with the approval of the local research ethics committee and in accordance with the Declaration of Helsinki'. This seemed vague and did not convince me. I searched for the trial on www.clinicaltrials.gov to no avail.

As outlined above, we have retrospectively registered the study with clinical trials.gov (ClinicalTrials.gov Identifier: NCT01888211). The study was started before registration of studies looking at vascular biology endpoints was deemed mandatory, which is why it was not registered earlier.

The study was approved by the local ethics committee (approval number LREC/2003/8/13; South East Scotland Research Ethics Committee 2). This is part of the NHS Health Research Authority National Research Ethics Service. These details are included on the clinical trials.gov entry, including contact details for the committee coordinator (Joyce Clearie; Joyce.Clearie@nhslothian.scot.nhs.uk). We will be happy to provide any further information if necessary.