

Author's response to reviews

Title: A randomised cross-over trial in healthy adults indicating improved absorption of omega-3 fatty acids by pre-emulsification.

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Author's response to reviews: see over

Nutrition Journal Editorial Team,

Please find below details of the changes made to our manuscript MS: 8956042921119935

The changes are firstly presented in the form of response to the referees detailing all of the points that were raised in their reviews. In addition, all of the amendments have been documented in point-to-point order to facilitate an assessment of the changes that have been made overall.

If you need anything further please do not hesitate to contact me.

Yours Sincerely,
Nigel Plummer

NUTRITION JOURNAL EDITORIAL

November 2006

MS: 8956042921119935

A randomised cross-over trial in healthy adults assessing the effect of emulsification on fatty acid and triacylglycerol absorption from an omega-3 rich oil mixture.

Iveta Garaiova, Irina A Guschina, Sue F Plummer, James Tang, Duolao Wang and Nigel T Plummer

Reviewer: Seher Khan

Major Compulsory Revisions

[1] Page 4 Background "However even with supplementation it is often difficult to reach the intake required to elicit a nutritional benefit and this may be due to unpalatability of liquid fish oils or inconvenience and cost implications of the consumption of sufficient fish oil capsules. A more palatable version of fish oils presented as emulsions may offer a route to improved compliance in supplementation." Difficult to reach intake and improved compliance-these claims require references

2] Many studies have been reported with emulsified fatty acids, the authors should mention about them and also state clearly why this study was undertaken.

3] Reasons for taking blood at intervals of 1.5, 3, 4.5, 6, 7.5 and 9 hour after ingestion of oil/emulsion?

5] What standards were used for GC?

6] In Figure legends 2 Fatty acids are not mentioned (page 16)

- (1) "Sufficient dietary intake" has been removed and reference to recommended daily/weekly intakes of fish oil for UK is found in Background p3 line 6
- (2) The background has been amended to provide more detail of the thinking behind the study. Although there are many more publications available on absorption of fatty acids, the focus here has been to compare absorption of oils and emulsified oils specifically
- (3) Methods: Blood Specimen Collection p6 line 1 has been amended to explain sampling regime imposed
- (4) ?

- (5) Internal standard was pentadecanoate (Methods Analysis of Total Fatty Acids p7 line 8)
- (6) Figure 2 has been re-titled and all abbreviations are detailed in the legend

Reviewer: Huiling Mu

Reviewer's report:

General

In this paper, the absorption of fatty acids in the form of oil or emulsion was investigated in a single meal study, fast absorption of n-3 PUFA was observed from the emulsion. It has been documented previously that fine emulsions can result in improved efficiency of lipases because larger surface area makes the lipids more accessible to hydrolysis by lipases. Even though the topic is not new, this study provides further documentation about importance of the physical properties of ingested fat, especially for the n-3 PUFA. The discussion was also properly based on the results and literatures. However, more information should be provided for the method section, regarding the subjects such as average \pm SD for age. More details about the diet should be provided, such as energy% of fat in the diet, how the meals were prepared regarding the 30 ml oil mixture or the emulsion, which oils were used for the oil blend.

The title needs to be more specific. For instance it could be "Improved absorption of omega-3 fatty acids in healthy adults by pre-emulsification".

The importance of this study should be addressed clearly in the background section.

Major Compulsory Revisions

The authors should check the calculation and units for lipid data. They are very different from values observed normally in human plasma.

To determine patient's BMI-related health risk, BMI between 27 and 30 means moderate health risk. BMI 25-29.9 is defined as grade 1 obesity (moderate overweight) by WHO. The authors need to give the average \pm SD for BMI. If the BMI values were too high, they also need to point out why they chose these subjects.

Minor Essential Revisions

Background

The emulsified droplets in the stomach contain diacylglycerols instead of monoacylglycerols because of the selectivity of pre-duodenal lipases.

Methods

1. Which kind of on-column injection was used? Normally on-column injection needs to start with low temperature (cold trap) and the initial column temperature should be lower than the boiling point of the solvent. Therefore I wonder how the chromatograms look like when the initial temperature was 170C.
2. Which internal standard was used? It should be mentioned in the method.
3. How long time was used for the acid-catalyzed methylation?
4. More information about the diet should be provided as mentioned previously.
5. Were equal amount of rice or pasta provided for the participants and how much?

Results

1. Table 1, which fatty acids are included in "others"?
2. Figure 1, the y-axis should be ?TAG. Similarly the y-axis for figure 2 and 3 should be correctly labelled.
3. SEM should be shown in the figure 3.
4. Figure 2 and figure 3 should be in the same scale in order to avoid misunderstanding.
5. Figure 3 B shows that the level of linoleic, oleic and palmitic acids was lower than the baseline level at the end of the experiment. Were the differences significant? Can you explain the effect?
6. The figure text and symbols should be larger and readable.

Discussion

The contribution of endogenous fatty acids should be considered.

General:

- The Methods section has been expanded to include the details of composition and quantity of food included in the Methods Study Design and Diet p5 and p6
- Average BMI has been calculated Methods Subjects p5 line 3
- The title of the paper has been amended as recommended
- The Background has been amended to provide more detail of the thinking behind the study.

Major

- The calculations for the lipid data have been carefully checked and typing errors have been found for the TAG values in Table 2 and Figure 1. The TAG units have been corrected from $\mu\text{mol}/\text{dl}$ to $\mu\text{mol}/\text{l}$
- The BMI has been calculated for the subjects participating in the study and has been included in the Methods Subjects p5 line 3. The average BMI is approx 25 (grade 1obesity moderately overweight). The population included in the study were healthy individuals (none with long term health problems) based in South Wales considered to provide a representative group. In the recent Welsh Health Survey (2004) it was determined that 54% of adults are classified as overweight or obese. (A UK wide market research survey at the same time identified the average UK BMI at 29.1)

Minor

Background

- (1) Diacylglycerols included on p4 line 5

Methods

- (1) On column injection was included in error and has been amended (Methods Analysis of Total Fatty Acids from Plasma p7)
- (2) Internal standard was pentadecanoate (Methods Analysis of Total Fatty Acids from Plasma p7 line8)

- (3) Methylation process was acid catalysed for 2h at 70°C (Methods Analysis of Total Fatty Acids from Plasma p7 line1)
- (4) Details on composition of the diet have been included in Study Design and Diet p5-6
- (5) Equal amounts of pasta/rice were provided (Methods Study Design and Diet p6)

Results

- (1) Table 1 has been amended with more detail
- (2) The axes on the figures have been amended
- (3) Figure 3 has been amended with SEM included
- (4) The scales have been standardised for Figures 2 and 3
- (5) The dropping of fatty acid levels below the baseline is addressed in the Discussion p12 line16
- (6) It would appear that the pdf version of the Figures/Tables created by the journal were printing out very small. We have enlarged our figures and altered their presentation as far as possible in line with the requirements of the journal.

Discussion

The contribution of endogenous fatty acids is considered in the Discussion p11 line 21

Reviewer: Philip C Calder

Reviewer's report:

General

This is a manuscript reporting postprandial changes in total TAG, total NEFA and individual fatty acids following consumption of a single meal providing omega-3 fatty acids in an unemulsified or an emulsified form. Emulsification increased the postprandial TAG response and the appearance of EPA and DHA in plasma. This is interpreted as indicating improved bioavailability of these fatty acids from the emulsified oil. This may be so, but there are other interpretations such as impaired clearance and this is not recognised. Nor do the authors recognise that an enhanced postprandial TAG response is now seen as a cardiovascular risk factor. Thus emulsification could lead to a pro-atherogenic post prandial response. In general I think that the study is an interesting one and is clearly timely from the point of view of industry. It appears to have been carefully done. The question posed by the authors is new and it is well defined. The methods used are appropriate and fairly well described; sufficient details are provided to replicate the work. The data appear sound and well controlled. Discussion and conclusions could be improved. The title and abstract are accurate. The writing is generally acceptable.

Major Compulsory Revisions:

1. When the authors use a term like "increased significantly", such as in the Results section of the abstract, do they mean "was higher than" compared to the other group or do they mean increased significantly over time within a group. This is not really clear and needs attention throughout the manuscript.
2. Last two words of the abstract suggest that "fish oils" are "fatty acids". They aren't - they contain fatty acids.

3. Background, line 5. Should state 200-500 mg per day.
4. Background. Does not mention pancreatic lipase.
5. What does "sufficient dietary intake of omega-3 fatty acids may be difficult to achieve" actually mean? What is sufficient? Please make this a sensible, scientific statement.
6. Methods. It is essential that the total carbohydrate, fat, protein and energy contents of the two meals are given.
7. How was intake of boiled rice, pasta and fruit controlled?
8. AUC does not represent "absorption of TAG or fatty acid" as stated on page 7. It represents the integration of absorption and clearance.
9. Page 7, 1st line of results. It is not characteristics that are reported in Table 2 but total plasma fatty acid concentrations.
10. Top of page 8. Has "poor absorption" of omega-3 PUFAs been reported before. It would seem that that would be the starting point for this study. If previous studies report high absorption, how could emulsification improve this? On the other hand if absorption is poor
11. Dietary TAG are normally emulsified in the intestine. Is this process not efficient or effective?
12. Page 9, line 9. But the "nature of the test meal" is controlled here.
13. I found the figures rather small and difficult to see. They will need to be improved for publication.

General

The general comments made by the reviewer have been considered and changes have been made to the manuscript to accommodate the suggestions made – particularly with regard to the impaired clearance.

- [1] Attention has been paid to the comment regarding "increased significantly" and amendments have been made to the manuscript where appropriate.
- [2] Abstract Conclusions amended to "pre-emulsification of fish oils may be a useful means of boosting absorption of these beneficial fatty acids" Background p3 line4
- [3] WHO recommendation is that one portion of fish should provide between 200 – 500 mg of EPA/DHA and the UK recommendation is for 2 portions of fish per week or >200 mg omega-3 PUFAs/day Background p3 line6
- [4] A discussion on pancreatic lipase has been included in Background p4 line5
- [5] "Sufficient dietary intake" has been amended to refer to recommended daily/weekly intakes of fish oil for UK Background p3
- [6] & [7] Details of composition and quantity of food have been included in the Methods Study Design and Diet p5 and p6
- [8] AUC has been corrected to "integration of absorption and clearance" Statistical Analysis p7 line3
- [9] First line of results has been amended to exclude "characteristics" p8
- [10] "Poor absorption" of the omega-3 PUFAs was a "relative" expression for this study - comparison of the results of the two groups. Amendments have been made to clarify this statement p8/9
- [11] As for [10], the study does not intend to imply that dietary TAGs are not effectively emulsified in the intestine but that there is a difference in the response between the two treatment groups.

[12] Quite different absorption profiles have been found depending on the nature of the test meal and these differences have been included in the discussion – primarily to support the dietary regime imposed for this study but also to illustrate the effects that have (or have not) been observed if the meal composition is altered.

[13] It would appear that the pdf version of the Figures/Tables created by the journal were printing out very small. We have enlarged our figures and altered their presentation as far as possible in line with the requirements of the journal.

Summary of manuscript MS: 8956042921119935 revision

Page 1

New Title

Page 2

No change

Page 3

Conclusion: ‘particularly fish oils’ deleted

Background: Line 6 – ‘or take > 200 mg/day of omega-3 PUFAs’ added into the sentence

Line 8 – the sentence begin with ‘In fact’ more detailed

Line 12 – the second paragraph from the end of Background Section begin with ‘Sufficient dietary ...’ moved here and been rewritten.

Page 4

Line 5 – 2-monoacylglycerols replaced by diacylglycerols

Line 5 – the sentence begin with ‘Further emulsification’ in more details

Line 19 – new sentence ‘There have been ...’

Line 23 – ‘may be improved’ changed to ‘improve’

Line 24 – the sentence ‘This study ..’ amended.

Page 5

Method - Subjects: Line 2 – mean age \pm SD added

Line 3 – mean BMI \pm SD added

Study design and diet: Line 4 and 5 – mixtures of oils in details

Page 6

Lines 2-7 – test meal in more details

Blood specimen collections: First sentence includes a reasons for taking blood at mentioned time intervals

Page 7

Analysis of total fatty acids from plasma: before page 6

Line 1 – 2 h added

Line 3 – an on - column deleted

Line 7 – the sentence begin with ‘The fatty acid esters ...’ include pentadecanoate as internal standard.

Statistical analysis: Line 3 – the sentence ‘AUC represents the extent of absorption of a triacylglycerol or fatty acid’ replaced by ‘AUC represents the integration of absorption and clearance of a triacylglycerol or fatty acid’.

Page 8

Results: before the chapter started on page 7

Line 1 – first paragraph amended.

Line 5 – ‘The rate and extent’ replaced by ‘The rate and total extent’

Line 7 – ‘significantly lower extent of absorption’ replaced by ‘significantly lower total absorption’

Line 7 – ‘AUC’ added into the sentence

Line 8 – ‘or ratio between’ replaced by ‘with the ratio between’

Lines 10-15 – Paragraph amended.

Line 18 – the sentence ‘...and that higher levels of PUFAs were detected for the emulsion group’ replaced by ‘...and that increased total absorption of PUFAs, as determined by AUC, was observed for the emulsion group’

Page 9

Line 2 – ‘when compared with the oil group’ added into the sentence.

Discussion: before the chapter started on page 8

Lines 1-9 – the sentences amended.

Line 10 – paragraph moved from the beginning of chapter of the previous version and amended.

Line 13 – new sentence begins with ‘Conversely...’ and new reference 14 added.

Line 17 – new sentence begins with ‘Under these conditions ..’ added.

Page 10

Line 3 – sentence amended.

Line 6 – the beginning of the sentence ‘A number of factors’ replaced by ‘Several factors have been identified that’ and the paragraph amended.

Line 22 – new addition to sentence ‘The fish oil used...’

Line 23 – new sentence begins with ‘It is interesting..’

Page 11

Line 6 – part of the sentence ‘and this could account for the lower recovery of fatty acids observed with the fish oil’ replaced by ‘and this could account for the lower levels of omega-3 fatty acids with the non-emulsified oil group’

Line 8 – ‘the rate and extent’ added into the sentence

Lines 21-25 – new paragraph added

Page 12

Line 1 – first sentence amended and split into two sentences

Lines 9-15 – two new sentences added

Line 16 – the beginning of the sentence ‘There was a ...’ replaced by ‘We observed a ...’

Line 19 – the sentence begins with ‘In support ...’ amended.

Line 22 – new sentence ‘Moreover, ...’ and new reference added

Line 24 - the beginning of the sentence ‘In this study’ replaced by ‘In our study’

Page 13

Line 2 – new sentence ‘The effect of ..’ added

Conclusions: before the chapter started on page 11

Competing interests: before the chapter started on page 11

Page 14

Authors’ contributions: before the chapter started on page 12

Acknowledgements: before the chapter started on page 12

References: before the chapter started on page 12

Page 15 - 16

Reference No:

Previous version	Revised version
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5	4
14	12
22	13
	14 – new
12	15
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17	19
18	20

Page 17

Reference No:

Previous version	Revised version
19	21
20	22
21	23
	24 – new
23	25
	26 – new
24	deleted

Page 18

Figures: before the chapter started on page 16

Figure 1: new title

Figure 2: new title and fatty acids mentioned in the legend

Figure 3: new title

Page 19

before page 17

Table 1: new title

Table amended; 'others' in more details

Page 20

before page 18

Table 2: new title

$\mu\text{mol/dl}$ corrected to $\mu\text{mol/l}$ in the legend (typing error)

Page 21

before page 19

Table 3: new title

PUFA – polyunsaturated fatty acid added into the legend