Reviewer's report

Title: Effect of n-3 polyunsaturated fatty acid on gene expression of the critical enzymes involved in homocysteine metabolism

Version: 1 Date: 17 December 2010

Reviewer: Mark Bouwens

Reviewer's report:

This study describes the effects of 3 types of omega-3 fatty acids on gene expression of several essential enzymes involved in homocysteine metabolism. The authors show data for 6 enzymes; MTHFR, MAT, CSE, SAHH, MTR and CBS, of which only the first three respond to in vitro exposure to the fatty acids EPA, DHA and ALA.

General remarks:

Although the findings presented might be interesting for those with closely related research interests, the data shown in the manuscript are limited and the details often lacking.

English can be approved significantly. I advise the authors to have the paper reviewed by a native English speaker.

Use of references is extensive, but sometimes seems not very effective. This should be critically reviewed by the authors.

The discussion is extensive, several parts can be greatly reduced in amount of text.

• Major Compulsory Revisions

- Methods should be given in more detail and clarified at several points. Some immediate questions that I had were;
  o Were antibiotics added to the culture medium and which concentrations?
  o What was the diluting agent for the fatty acids? (EtOH?)
  o Did controls also contain this diluting agent?
  o Was cell viability tested and what were the results after 48 hours of incubation with the polyunsaturated fatty acids and diluting agent?
  o Was RNA quantified and were all samples comparable in used amount for cDNA synthesis?

- Figure 2 has no error bars, while it is described that there were 3 replicated in three experiments and that each gene was duplicated twice. What is the n shown in this figure?

- An important discussion points is lacking and could be addressed in the discussion section; does the amount of RNA of an enzyme correlate with it
activity and functioning?
- The data represented are limited. It would greatly improve this manuscript if data was collected regarding:
  o Enzyme activity
  o Protein expression
  o SAM concentrations
  o Potential transcription factors involved, using a more extensive in silico analysis tool, as started in the discussion.
- Page 10, line 19-21; clarify this statement, why is this likely and are there no other possible mechanisms. Use references to argument.

• Minor Essential Revisions
- Table 3 is unnecessary and should be deleted.
- Abstract, methods; sentences are not proper English. Rewrite.
- Abstract, line 15; three groups have not be described before.
- Abstract line 22-23; Delete last sentence.
- Page 3, line 20-22, this needs a proper reference.
- Page 4, line 9; phospholipids abbreviation has not been explained yet.
- Page 4, line 19; …one report.. = paper from authors themselves. In other words, you suggested…..
- Page 5, line 16; must be ‘one ml containing 1x10^5 cells’
- Page 5-6, line 22-1; delete sentence ‘The phospholipids….Gas chromatography’
- Page 6, line 5; rpm should be converted to rcf
- Page 6, line 6; which solvents were used?
- Same line; describe TLC.
- Page 6, line 11; this line seems to indicate that the fatty acids were solved in ethanol and that the control does contain ethanol. Make clear in methods.
- Page 6, line 14; reference used is not specific, use other
- Page 8, line 7-9; text is not detailed enough, clarify
- Page 9, line 5; delete ‘firstly’ is there is no ‘second’
- Page 10, line 7; 12 gram per what time unit?
- Page 13, line 9-11; expand on this and use as results
- Page 13, line 12; I agree that further investigation is warrented, but the authors should make the first attempt by increasing the amount of data presented here.
- Table 1, last gene; I assume beta-actin is intended, but both name and accession number are incorrect (accession number is for mouse 18S gene). Correct this.
• Discretionary Revisions

- It is unnecessary to describe the complete comparative Ct method (page 7, line 3-18), this has been done before and often better. You can refer to previous publications.

- Can you give arguments for the choice for 150 microMolar of PUFA, while plasma concentrations of these fatty acids are far lower (more in range of 10 uM)

**Level of interest:** An article of limited interest

**Quality of written English:** Not suitable for publication unless extensively edited

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

**Declaration of competing interests:**

I declare that I have no competing interests