Relative validation of a minimally invasive method of assessing the intake of long-chain omega-3 fatty acids

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Introduction
Despite having the ability for in vivo synthesis of long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA), evidence suggests that our capacity for synthesis is limited¹. As a consequence, we need to rely on dietary sources of LC n-3 PUFA’s, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

EPA and DHA are key nutrients for development² and the moderation of inflammatory responses implicated in the mechanism for cardiovascular disease³.

Very little data is available on current UK intakes of EPA and DHA. Methodology to comprehensively establish intakes of EPA and DHA involves analysis of food intake over a period of at least fourteen days⁴. This is due to the fact that sources of EPA and DHA in the diet are limited to very few, infrequently consumed foods, including oily fish and some specially fortified products such as milk, eggs and yogurts.

There is also a paucity of data about how intakes relate to the status of LC n-3 PUFA’s in the body. Establishing LC n-3 PUFA status usually involves invasive methodology to samples lipid levels in various fractions of the blood and plasma⁵.

Developing methodology for quick and reliable assessment of DHA and EPA intakes has many valuable applications in public health research and is also likely to facilitate the investigation of vulnerable, hard to reach populations. Combining such methodology with a minimally invasive assessment of status allows the potential impact of health education initiatives to be assessed.

Project Outcomes
Twenty subjects were recruited to the study (16 females) with an average age of 30.4 years (range 19 – 47 years). Seventeen of the participants (85%) were considered fish-eaters.

Average intakes of EPA, DHA and total LC n-3 PUFA (EPA + DHA) as assessed by the FFQ and the 14 day diet record are presented in Table 1.

Table 1: Average intakes of EPA, DHA and total LC n-3 PUFA

<table>
<thead>
<tr>
<th>Assessment method</th>
<th>EPA (g/day)</th>
<th>DHA (g/day)</th>
<th>LC n-3 PUFA (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFQ</td>
<td>0.16 ± 0.13</td>
<td>0.23 ± 0.21</td>
<td>0.40 ± 0.34</td>
</tr>
<tr>
<td>14 day diet record</td>
<td>0.15 ± 0.13</td>
<td>0.27 ± 0.23</td>
<td>0.42 ± 0.35</td>
</tr>
</tbody>
</table>

Despite mean intakes of LC n-3 PUFA being close to current UK recommendations of 0.45mg/d (SACN, 2004), only 7 participants (35%) met or exceeded recommended intakes by either method. Only 5 of these participants, however, were assessed as meeting recommended intakes by both methods of assessment.

Good agreement between FFQ and diet record
Bland Altman analysis showed good agreement between the FFQ and the 14-day diet records in assessing intakes of EPA (Fig. 1) and DHA (Fig. 2). For EPA all mean differences were within the limits of agreement, and the results indicated that the FFQ underestimated intakes by a mean of 10mg/d (Fig. 1).

![Fig 1: Bland-Altman plot showing the level of agreement between the FFQ and diet record results for average daily intake of EPA (g/d)](image)

Study Design

The aim of this study was to validate a minimally invasive method of assessing dietary intakes and correlate this with whole blood levels of LC n-3 PUFA.

Intakes of EPA and DHA were assessed using:
- a targeted food frequency questionnaire (FFQ), which had previously been developed for use in healthy adults⁶-⁷.
- a 14-day diet record of all foods and beverages consumed

Whole blood fatty acid levels were assessed using the Rapid Omega Test⁸, which involves collecting a finger prick blood sample on a Whatman 903® neonatal std card and subsequent analysis of fatty methyl esters by gas-liquid chromatography. This technique has previously been validated against red blood cell polar lipid fatty acids⁹.

**Study design**

Day 1

14-day diet record

FFQ

Rapid Omega

Intakes were assessed against UK recommendations⁶. Levels of agreement between intake assessment methods for EPA, DHA and total n-3 PUFA were investigated using Bland-Altman analysis. Correlation between whole blood levels, measured using the Rapid Omega test, and dietary intakes were also investigated.
**Good agreement between FFQ and diet record**

For DHA the majority of mean differences were within the limits of agreement, except for one participant, and the results indicated that the FFQ overestimated intakes by a mean of 30mg/d (Fig. 2).

**Correlation between FFQ intake and whole blood fatty acid levels**

Significant correlation was shown between intakes of EPA (Fig. 4) and DHA (Fig. 5), assessed using the FFQ, against whole blood levels (% total fatty acids).

**Conclusions**

- Intakes of LC n-3 PUFA in 65% of this adult population were below current UK recommendations, which causes concern in relation to the risk of cardiovascular disease.
- The targeted FFQ is a valid method for estimating dietary intakes of EPA and DHA, which is quick to complete.
- Intakes of EPA and DHA correlated with whole blood percentage fatty acid levels measured from a finger prick blood sample using the Rapid Omega test.
- The FFQ and Rapid Omega test provide minimally invasive methods of measuring intakes of LC n-3 PUFA and the corresponding whole blood fatty acid status.
- In combination, these methods could be employed to assess LC n-3 PUFA intakes in a wide range of population groups in response to health education initiatives.

**References:**

1 Brenna JT, Salem N, Sinclair AJ and Cunnane SC; ISSFAL 2009
2 Innis SM 2007.
3 Giugliani D, Ceriello A and Esposito K 2006
4 Andersen LF, Solvoll K, Johannson LR, et al. 1999
5 Hodge AM, Simpson JA, Gibson RA et al. 2007
6 Milrhead L 2010 unpublished data
7 Shillinglaw E 2010 unpublished data
8 Bell JG, MacKinlay EE, Dick JR et al 2009, ‘in press’
9 Scientific Advisory Committee for Nutrition 2004