DEVELOPMENT OF A ROBUST DRIED BLOOD SPOT METHOD FOR THE EVALUATION OF N-3 FATTY ACID STATUS OF INDIVIDUALS

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Abstract

Increased consumption of n-3 long chain polyunsaturated fatty acids (LCPUFA) is associated with higher n-3 LCPUFA status in the circulation, which has in turn been associated with a number of health benefits in humans (Calder et al. 2006; Makrides et al. 2009; Einvik et al. 2010).

The conventional approach to assay n-3 fatty acid status in humans involves invasive venous blood collection and expensive, time consuming multi-step processes as that limit its application in large-scare clinical trials and routine population screening (Risé et al. 2007). Recently, efforts have been made to adopt the dried blood spot (DBS) as a quick, inexpensive and minimally invasive alternative for the measurement of fatty acid status in humans (Marangoni et al. 2004). However, the existing DBS approaches have had only limited success in providing an accurate tool for the measurement of n-3 LCPUFA status in humans. This has been due to the presence of fatty acid contaminants in blood collection papers which are released during sample processing (Nishio et al. 1986; Ichihara et al. 2002), and the failure to prevent significant oxidative loss of the n-3 LCPUFA in DBS sample during transportation and storage (Min et al. 2011; Bell et al. 2011).

This thesis aimed to develop a novel DBS technique which would overcome these limitations and enable the technology to be used for the accurate evaluation of n-3 LCPUFA status in human subjects. Firstly, a wide range of potential collection matrices and lab consumables were tested to determine which contained the lowest contaminant levels. A range of antioxidants and chelating agents were then tested with DBS in order to identity the optimal combination of these factors for protecting the
LCPUFA in DBS from oxidation. The protection system which provided optimal protection consisted of a combination of an antioxidant and a chelating agent applied to silica gel coated blood collection paper, and this resulted in more than 90% of the original n-3 LCPUFA content (expressed as a weight percentage in blood total lipids) in the DBS being retained following 2 months of storage at room temperature (20-25°C). This system (termed “PUFAcoat”) represents a significant improvement in LCPUFA stability in DBS compared with previously reported standard DBS protection systems. For example, the standard Fluka system (Fluka blood collection kit) uses a single antioxidant (butylated hydroxytoluene, BHT) as protectant, and normal chromatography paper as a collection paper which retains only ~60% of the n-3 LCPUFA content in the applied DBS over the same time period (Min et al. 2011).

To explore the mechanisms underlying the protective effect of the “PUFAcoat” and to improve my understanding of the processes causing the rapid breakdown of LCPUFA in DBS, a novel in vitro system (comprising an oil blend on collection paper) was developed. Using this model I established that iron-induced oxidation was the principle driver of the rapid loss of the n-3 LCPUFA absorbed on the blood collection paper, and that iron chelating agent in the “PUFAcoat” system eliminated this process by binding the irons in the DBS samples.

The clinical validity of the “PUFAcoat” system was established in a human study that compared the fatty acid spectrum obtained from my DBS method (using capillary blood) with those obtained by traditional analytical techniques (using venous blood fractions). This study demonstrated strong correlations in fatty acid status between my DBS method and conventional measurements, which indicate the potential of use of
my DBS method as an appropriate alternative to conventional assessments. Moreover, this clinical study showed that the n-3 LCPUFA status obtained using my DBS method reflected the habitual dietary n-3 fatty acid intakes of the study population.

This thesis is the first report of a protection system that is capable of stabilising the n-3 LCPUFAs in human DBS samples over 2 months storage at room temperature. Thus, my newly developed DBS method offers a significant improvement in the useability and reliability of the DBS technique for assessing n-3 LCPUFA status in humans. My DBS method has significant potential for use in large-scale clinical testing and population based screening diagnostics which focused on the role of n-3 fatty acid status in human health.
Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>arachidonic acid</td>
</tr>
<tr>
<td>ALA</td>
<td>alpha-linolenic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BHA</td>
<td>butylated hydrazoxyanisol</td>
</tr>
<tr>
<td>BHT</td>
<td>butylated hydroxytoluene</td>
</tr>
<tr>
<td>CHD</td>
<td>coronary heart disease</td>
</tr>
<tr>
<td>DBS</td>
<td>dried blood spot</td>
</tr>
<tr>
<td>DHA</td>
<td>docosahexaenoic acid</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPA</td>
<td>docosapentaenoic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene diamine tetraacetic acid</td>
</tr>
<tr>
<td>EPA</td>
<td>eicosapentaenoic acid</td>
</tr>
<tr>
<td>FAMEs</td>
<td>fatty acid methyl esters</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>LA</td>
<td>linoleic acid</td>
</tr>
<tr>
<td>LCPUFA</td>
<td>long chain polyunsaturated fatty acids</td>
</tr>
<tr>
<td>MUFA</td>
<td>monounsaturated fatty acids</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acids</td>
</tr>
<tr>
<td>RA</td>
<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>RCT</td>
<td>randomized controlled trial</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SFA</td>
<td>saturated fatty acids</td>
</tr>
<tr>
<td>TBHQ</td>
<td>tert-Butylhydroquinone</td>
</tr>
<tr>
<td>TDM</td>
<td>Therapeutic drug monitoring</td>
</tr>
<tr>
<td>TG</td>
<td>triglycerides</td>
</tr>
<tr>
<td>TLC</td>
<td>thin-layer chromatography</td>
</tr>
<tr>
<td>TLE</td>
<td>total lipid extract</td>
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