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 LCUFA 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. Morever, 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.

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List of Abbreviations

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