Increasing fish consumption in women of child-bearing age: an evaluation of risks and benefits

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List of Abbreviations

AA	Arachidonic acid
ABS	Australian Bureau of Statistics
AHP	Allied Health Professional
AHRQ	Agency for Healthcare Research and Quality
AI	Adequate intakes
ALA	Alpha-linolenic acid
ALSPAC	Avon Longitudinal Study of Parents and Children
ALSWH	Australian Longitudinal Study on Women's Health
AMDR	Acceptable macronutrient distribution ranges
AQ	AsureQuality Limited
BDI	Beck Depression Inventory
BMI	Body mass index
BSID	Bayley Scales of Infant Development-Mental Development
CLAMSDQ	Clinical Linguistic and Auditory Milestone Scale Developmental Quotients
CRP	C-reactive protein
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CURF	Confidentialised Unit Record Files
CVFAS	Cold vapour atomic fluorescence spectroscopy
DHA	Docosahexaenoic acid
DOMInO	DHA to Optimize Mother Infant Outcome
DNBC	Danish National Birth Cohort
DPA	Docosapentaenoic acid
DQES	Dietary Questionnaire for Epidemiological Studies
EAR	Estimated average requirement
EEG	Electroencephalogram
EPA	Eicosapentaenoic acid
EPC	Evidence-based Practice Center

EPDS	Edinburgh Postnatal Depression Scale
ERG	Electroretinogram
FRDC	Fisheries Research & Development Corporation
FSANZ	Food Standards Australia New Zealand
GEE	Generalised estimating equations
HDL	High-density lipoprotein
HPLC	High-performance liquid chromatography
ICER	Incremental cost effectiveness ratio
IgE	Immunoglobulin E
IOM	The Institute of Medicine of the National Academies of Science
IUGR	Intrauterine growth retardation
LCn3PUFA	Long chain n-3 polyunsaturated fatty acids
LOR	Limit of Reporting
MADRS	Montgomery-Asberg Depression Rating Scale
MAR	Minimal angle of resolution
MDI	Mental Developmental Index
MeHg	Methyl mercury
NATA	National Association of Testing Authorities
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council
NMI	National Measurement Institute
NNS	National Nutrition Survey
NRV	Nutrient reference values
NUHEAL	Nutraceuticals for a Healthier Life
PAL	Physical activity level
PCR	Polymerase chain reaction
PDI	Psychomotor Development Index
PIH	Pregnancy-induced hypertension

PUFA	Polyunsaturated fatty acids
RCT	Randomised controlled trials
RDI	Recommended dietary intake
RMIT	Royal Melbourne Institute of Technology
RPD	Relative percentage difference
SARDI	South Australian Research and Development Institute
SCORAD	SCORing Atopic Dermatitis
SDT	Suggested dietary target
SiPS	Salmon in Pregnancy Study
SLS	Sodium lauryl sulphate
SNP	Single nucleotide polymorphisms
SPME	Solid phase microextraction
THg	Total mercury
UL	Upper level of intake
VEP	Visual evoked potential
WHO	World Health Organization

Summary

Epidemiological studies have consistently demonstrated positive association of infant neurodevelopment with maternal fish consumption, mostly attributed to the abundance of long chain n-3 polyunsaturated fatty acids (LCn3PUFA) in fish. However, fish consumption by Australian women is overall less than optimal. Secondary analysis of nationally conducted surveys [1995 National Nutrition Survey and the Australian Longitudinal Study on Women's Health (ALSWH) in 2003 and 2009] demonstrated that less than half of Australian women of child-bearing age would consume fish at least twice a week as recommended by the Australian Dietary Guidelines. Of concern was the even lower fish intake in women who were pregnant or had recently given birth when compared with other women in the ALSWH surveys. This observation suggests that women may consume less fish whilst pregnant for fear of potential contaminants that might be present in fish.

A dietary modelling exercise based on the food consumption pattern from the Australian Dietary Guidelines confirms that one would need to consume three serves of oily or high LCn3PUFA fish per week to meet the suggested dietary target of 430 mg of LCn3PUFA for women as recommended by the National Health and Medical Research Council.

In order to ascertain the levels of LCn3PUFA and other nutrients present in Australian fish/fish products, several commonly consumed fish/fish products selected for use in the ensuing randomised trial were analysed following standardised procedure. Mercury contents of these fish study foods were also tested and found to be relatively low (range: 1.1 µg–7.0 µg/100 g).

To assess the acceptability of a diet that included more fish and its effects on biological parameters, a single-blinded randomised controlled eight-week trial was conducted in healthy women aged 18–50 years who normally consumed no more than one oily fish meal per week. The higher fish diet included four serves per week of a variety of fresh and convenience fish products (including canned and frozen, oily and non-oily) and were provided to the participants. The control group was asked to maintain their usual lower fish/higher meat diet and participants were provided with four serves of beef, chicken or deli-meat per week. After eight weeks,

significant increases in mean eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and methyl mercury levels were observed in the intervention group when compared to the control group. Although blood mercury level did rise with increased fish intake, it was still at a level accepted as safe. The median acceptability score for both diets was the same suggesting no difference in diet acceptance.

A cost-effectiveness study conducted post-trial demonstrated that including fish in a diet is an economical means to obtain LCn3PUFA. To obtain equal amount of DHA, it would have cost sixty times more if consuming the meat study food compared to the fish study food.

In conclusion, it has been demonstrated that consuming a variety of fish and fish products several times a week is an acceptable and cost-effective means of improving LCn3PUFA status without causing detrimental increases in mercury levels, provided low-mercury containing fish are consumed. Women of child-bearing age are advised to consume high-LCn3PUFA but low-mercury containing fish as part of a healthy diet.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Lily L H Chan

Presentations

Poster presentation

Chan, L, Grieger, JA, Miller, Cobiac, L 2010, 'Simulation of fish intake in women of child-bearing age', poster presented to the 2010 Annual Scientific Meeting of the Nutrition Society of Australia, Perth, Australia, 30 November–3 December.

Oral presentation

Chan, L, Grieger, JA, Cobiac, L 2010, 'Fish and seafood intake in Australian women of child-bearing age', abstract presented to the International Seafood & Health Conference, Melbourne, Australia, 8–10 November.

Chan, L, Miller, M, Thompson, C, Midgley, J, Cobiac, L 2011, 'Assessment of the effects and acceptability of a higher fish diet in women of child-bearing age' abstract presented to the 35th Annual Scientific Meeting of the Nutrition Society of Australia and New Zealand, Queenstown, New Zealand, 30 November–2 December.

Oral presentation at the Flinders Clinical and Molecular Medicine Cluster Seminar Series, Flinders University, 25 August 2010 and 3 August 2011.

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I would also like to thank the Australian Seafood Cooperative Research Centre (CRC) for funding the project. In addition, the CRC also provided opportunities and programmes to assist with the development of various skills and insight into the industry.

Many thanks also go to Simplot Australia, another sponsor of our project, and its staff. Dr Jocelyn Midgley, Ms Sasha King and Ms Crystal Yam have all been very helpful and provided valuable comments on the draft reports.

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I am indebted to Dr Kathryn Burdon and her staff and students in the Department of Ophthalmology (Flinders University), and Professor Robert Gibson and his staff at the Fatty Acid Laboratory (Adelaide University) for allowing me to work in their laboratories and to learn from them. I also appreciate the help from Professor Julie Ratcliffe who guided me through the cost-effective analysis.

The Australian Bureau of Statistics and the PSA Committee of Australian Longitudinal Study on Women's health have both kindly provided survey data for the secondary analysis of fish consumption in Australian women.

Professional editor, Dr Gaye Wilson, provided copyediting and proofreading services, according to the guidelines laid out in the university-endorsed national guidelines, 'The editing of research theses by professional editors'. The integrity of the final submitted thesis remains my responsibility.

And last but not least, I would like to thank my family, in particular, my mother who has accompanied me all through this journey in search of knowledge and making sure that I have adequate rest and nutrition.

Overview and thesis structure

Nutrition in women of child-bearing age is important for their own health as well as the wellbeing of their offspring. A well-balanced diet from a wide variety of nutritious foods is required to provide the nutrients the body needs. Fish consumption provides many nutrients and is the major source of the long chain n-3 polyunsaturated fatty acids (LCn3PUFA) in the diet, a nutrient that has gained interest in the last 20 to 30 years in many areas, including the areas of cardiovascular and infant health. However, dietary intakes of fish in Australian women are often reported to be less than optimal. The focus of this project is therefore to examine the current fish consumption pattern in Australian women of child-bearing age, to conduct nutrient profiling of a diet that includes more fish, and to assess its acceptability and effects on the blood levels of fatty acids, total mercury (THg), methyl mercury (MeHg), selenium, C-Reactive protein, lipids and iron. Also an analysis is undertaken to determine the cost-effectiveness of a higher fish diet in terms of obtaining adequate amounts of DHA from the diet.

Chapter 1 outlines the rationale of the thesis, including project aims and objectives.

Chapter 2 examines the available evidence of LCn3PUFA on maternal and infant outcomes. As many studies involving LCn3PUFA are conducted with the administration of fish oil rather than fish *per se*, the second part of Chapter 2 summarises studies that are specifically related to fish intakes.

Chapter 3 examines the fish consumption pattern in Australian women of child-bearing age. Intake data from the 1995 National Nutrition Survey and the more recent Australian Longitudinal Study on Women's Health are described here.

Chapter 4 describes the process where simulated diets were generated to represent two dietary patterns. The two diets differed in the amount of fish included but both followed the recommendations of the Australian Dietary Guidelines. The nutrient profiles of these two theoretical dietary patterns are compared. The dietary modelling exercise was used to inform the intervention diet implemented in the randomised controlled clinical trial conducted subsequently.

In preparation for the randomised controlled clinical trial, the fish and fish products selected for use in the trial were analysed for their nutrient composition as well as selected heavy metals such as THg, MeHg, cadmium, and lead. Details of the analytical procedures are described in Chapter 5.

Details of the randomised controlled trial comparing the acceptability and effects of two diets differing in the amount of fish are described in Chapter 6.

A cost-effective analysis of a higher fish diet based on the data from the randomised controlled trial was conducted and is reported in Chapter 7.

Chapter 8 is the concluding chapter, where findings from the project are summarised and implications for future practice and research are discussed.

This project is funded by the Australian Seafood Cooperative Research Centre (CRC). The funding covered all expenses related to the project and included a stipend paid to the candidate. All fish and fish products, both for analytical purpose and as study food provided to participants in the trial, were provided by Simplot Australia free of charge. Table 1 lists the tasks related to this project and key personnel/organisation responsible for them.

Table 1. Project tasks

Tasks	Key personnel / Organisation responsible
Overall	
• Project inception, funding application and overall management	Professor Lynne Cobiac (candidate's principal supervisor and the project's principal investigator)
Fish composition analysis	
• Proximates, fatty acids, minerals, heavy metals (except mercury or methyl mercury), vitamin A & E	AsureQuality Limited (fee paying service)
• Mercury and methyl mercury	Hill Laboratories (fee paying service)
• Vitamin D	National Measurement Institute (fee paying service)
Randomised controlled trial	
• Recruitment and coordination of the trial	Candidate
• Clinical assessment – height, weight and blood pressure	Research nurse or trained phlebotomist blinded to the study group allocation
• Clinical assessment – body composition by dual energy X-ray absorptiometry (DXA)	Trained DXA operator blinded to the study group allocation
• Dietary assessment – 3-day weighed-food record data entry and analysis	Candidate
• Laboratory assessment – determination of single nucleotide polymorphisms (SNP) in blood	Candidate (under the supervision of Dr Kathryn Burdon, Department of Ophthalmology, Flinders University)
• Laboratory assessment – determination of fatty acids level in blood	Candidate (under the supervision of the staff at the Fatty Acid Lab, Waite Campus, University of Adelaide)
• Laboratory assessment – blood lipids, iron status and haemoglobin	Healthscope Pathology, Wayville, South Australia (fee paying service)
• Laboratory assessment – mercury, methyl mercury and selenium levels in blood	Brooks Rand Lab, Seattle, USA (fee paying service)
• Laboratory assessment – blood C-reactive protein level (high sensitivity)	CSIRO, Adelaide, South Australia (fee paying service)
Statistical analyses	
• All statistical analyses	Candidate

Chapter 1 Project rationale, aims and objectives

1.1 Rationale

Consumers are advised to eat more fish for a range of health benefits, including for growth and development, protection against heart disease, and lowering of plasma triglycerides. However, there are some caveats in these recommendations for some sub-groups of the population, such as those women who are pregnant or who wish to become pregnant. This in general relates to the level of MeHg present in fish.

Higher maternal fish intake during pregnancy has been shown to be associated with better childhood developmental outcomes, longer gestation, higher birth weights (Cohen et al. 2005; Oken et al. 2008a) and improved maternal and adult outcomes such as improved mental health, reduction of cardiovascular risk factors and inflammation. The long chain n-3 polyunsaturated fatty acids (LCn3PUFA) docosahexaenoic acid (DHA) provided by consuming fish in particular appears essential for neurocognitive development of the developing foetus. It has been suggested that pregnant and lactating women should aim to achieve at least 200 mg DHA/day although intakes of up to 1 g DHA per day (or close to 3 g/d of total LCn3PUFA) have been used in randomised clinical trials without adverse effects (Koletzko et al. 2007). However, one of the potential problems with recommending increases in the consumption of fish to pregnant women to achieve higher LCn3PUFA intakes relates to the levels of MeHg in fish, especially those of the larger predatory species.

It could be argued that LCn3PUFA can be achieved with the consumption of fish oil supplements, thereby avoiding the ingestion of other contaminants contained in fish. However, fish also provide other dietary compounds that are not found in fish oils but may also contribute to both infant and maternal health, such as selenium, iodine, vitamin D, and zinc. Dietary interventions with whole fish can introduce other potential health benefits (e.g. cardiovascular) beyond that of the LCn3PUFA (Cobiac et al. 1991). Based on this it may be desirable to eat more fish, rather than take fish oil supplements to obtain the benefits from a wider range of nutrients than just LCn3PUFA.

In the US, the general guidelines for pregnancy are to consume 340 g (or two serves) of fish per week. In Australia, the recommended serving size of fish is 100 g cooked (or 115 g raw) fish fillet according to the Australian Dietary Guidelines released in 2013. The recommended amount in the US of 340 g per week is therefore closer to three and a half serves here in Australia. In Australia, the current dietary advice from Food Standards Australia New Zealand (FSANZ) for pregnant women is that 2–3 serves of most fish can be safely eaten each week, but to limit the intakes of orange roughy (Sea Perch), catfish shark, swordfish, marlin or broadbill to once a week or fortnight for the purposes of avoiding contaminants that may be damaging to the sensitive developing foetus (FSANZ 2011a).

The net outcome of this communiqué may result in fewer women of child-bearing age, those who are pregnant or wish to become pregnant, consuming fish, or more women consuming inadequate amounts. It is highly important that this key group of women during pregnancy consumes enough seafood to ensure that the developing foetus obtains adequate levels of DHA. It is unclear what type, culturally acceptable and sustainable pattern of available Australian fish would provide the recommended average intake of at least 200 mg DHA/day in approximately 2–4 serves per week. It is understood that the limitation on the number of fish serves is to reduce the risk of being exposed to potential contaminants. It may be that Australian fish can be consumed by women of child-bearing age in greater or lesser amounts compared to the fish available and consumed in other countries. Comprehensive and current information on the LCn3PUFA, in particular DHA, content and of the compositional profile of Australian fish is vital to provide information to women that is based on evidence for the Australian setting.

1.2 Aims and objectives

Several aims and objectives are aligned with this thesis, which are listed below.

Aim 1: To provide an up-to-date review of the benefits of fish and LCn3PUFA intakes in relation to maternal and infant health.

Scientific databases such as Medline and Web of Science were searched to identify studies that had examined fish/LCn3PUFA and maternal and/or infant health. Results were summarised and presented in tables.

Aim 2: To assess the current level of fish consumption in Australian women of child-bearing age.

Studies conducted nationally that have assessed fish intakes in Australian women were examined to quantify the amount and frequency of fish consumption. Two such studies were identified and analysed.

Aim 3: To develop a healthy Australian seafood dietary pattern to achieve sufficient intakes of LCn3PUFA.

Dietary modelling was undertaken to determine the number of serves of fish required to achieve the recommended intake of LCn3PUFA. The nutrient profile of a diet consistent with the recommendations of the Australian Dietary Guidelines and with higher fish content was compared to the nutrient reference values.

Aim 4: To add to the existing database of compositional profile of fish products.

Several commercially available and commonly consumed fish and fish products were analysed to ascertain their nutrient profiles. Their THg and MeHg contents were also analysed.

Aim 5: To assess the acceptability and effects of a diet that is higher in fish.

A single-blinded randomised controlled trial was conducted to assess the acceptability and effects of a diet that was higher in fish when compared to a typical Australian diet, which was generally lower in fish but higher in meat. An analysis was also conducted to determine the cost-effectiveness of a higher fish diet in terms of obtaining adequate amounts of DHA from the diet.

Chapter 2 Literature review — Long chain n-3 polyunsaturated fatty acids and fish intakes on maternal and infant outcomes

2.1 Long chain n-3 polyunsaturated fatty acids (LCn3PUFA) and maternal & infant outcomes

Introduction

It is well known that maternal nutrition has an impact on the foetus and the subsequent growth and development of the child. Several Cochrane Systematic Reviews have highlighted the importance of macro- and micronutrient intake during pregnancy as well as the pre-conception period (Kramer & Kakuma 2003; Haider & Bhutta 2006; De-Regil et al. 2010). The LCn3PUFA are essential fatty acids that have received a great deal of attention since the publication of an epidemiology survey 30 years ago reporting a lower frequency or absence of acute myocardial infarction, diabetes mellitus, thyrotoxicosis, bronchial asthma, multiple sclerosis and psoriasis in Greenlanders who consumed a higher amount of fatty fish when compared to the West-European populations (Kromann & Green 1980). Consumption of LCn3PUFA has also been shown to be beneficial in prolonging gestation, reducing the risk of pre-term delivery and is associated with positive infant neurodevelopmental outcomes (Cetin & Koletzko 2008). The purpose of this literature review is to summarise the current understanding of the benefits associated with LCn3PUFA intake in women during pregnancy and lactation in terms of maternal and infant outcomes.

Methodology

In 2005, a systematic review of the significance of LCn3PUFA for maternal and child health was published (Lewin et al. 2005). This work was conducted by the University of Ottawa Evidence-based Practice Center (EPC), under contract to the Agency for Healthcare Research and Quality (AHRQ) in the United States. For this reason, the electronic database Medline® was searched from January 2003 to March 2013 with the aim of identifying studies conducted subsequent to the comprehensive Lewin et al. (2005) review. This literature review will focus on randomised

controlled trials (RCTs) identified by this search, those included in Lewin et al. (2005) and other available systematic reviews, as data generated from these study designs provide more reliable estimates of effects. Included in this review were RCTs designed to study the effects of LCn3PUFA supplementation in women during pregnancy and/or lactation on length of gestation, pre-term or post-term delivery rate, incidence of pregnancy-induced hypertension, pre-eclampsia, eclampsia, perinatal depression, foetal development, as well as the growth, neurological development, visual function and atopic disease of the offsprings. LCn3PUFA supplements could be in the form of fish oil, LCn3PUFA enriched functional food or algal oil. Excluded were studies that involved LCn3PUFA supplementation in the infants rather than the mothers. Quality of each trial was assessed according to the Cochrane Collaboration's tool for assessing risk of bias (Higgins & Green S. (eds.) 2011). Search terms used included omega-3, n-3 fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), maternal, pregnancy, and breast feeding. The detailed search strategy can be found in Appendix 1.

Results

Thirty-six RCTs were identified that investigated the relationship of LCn3PUFA supplementation during pregnancy and/or lactation and various maternal and infant health outcomes. The majority of the interventions included the use of fish oils. Several RCTs used LCn3PUFA-containing functional foods (e.g. DHA enriched eggs or cereal bars) but only one RCT used oily fish as an intervention. Average daily intervention doses varied from as low as 100 mg of EPA+DHA through to 4.95 g of EPA+DHA combined. Studies are described in Appendix 2. Since the publication of the comprehensive systematic review by Lewin et al. in 2005, two Cochrane Systematic Reviews (Makrides, Duley & Olsen 2006; Delgado-Noguera, Calvache & Bonfill Cosp 2010) and 16 other systematic reviews, some including meta-analyses, have been conducted in an attempt to consolidate the available evidence relating to LCn3PUFA supplementation (Szajewska, Horvath & Koletzko 2006; Eilander et al. 2007; Horvath, Koletzko & Szajewska 2007; Dziechciarz, Horvath & Szajewska 2010; Jans, Giltay & Van der Does 2010; Muhlhausler, Gibson & Makrides 2010; Klemens, Berman & Mozurkewich 2011; Kremmyda et al. 2011; Salvig & Lamont 2011; Wojcicki & Heyman 2011; Campoy et al. 2012; Imhoff-Kunsch et al. 2012; Larqué et al. 2012; Lo et al. 2012; Rodríguez et al. 2012;

Gould, Smithers & Makrides 2013). A summary of risk of bias assessment for each included study can be found in Appendix 3. Findings from individual trials, systematic reviews or meta-analyses are summarised below.

2.1.1 Pregnancy outcome and maternal health

Proposed mechanisms of effects

LCn3PUFA is involved with the production of eicosanoids and are precursors of prostaglandins (PG) 3-series, prostacyclins (PGI) 3-series , thromboxane (TX) 3-series and leukotrienes (LT) 5-series. The productions of these eicosanoids are in direct competition with the metabolism of the LCn6PUFA and suppress the production of the pro-inflammatory PG 2-series, platelet aggregator and vasoconstrictor TX 2-series, and inducer of inflammation LT 4-series (Simopoulos 1991) (see Figure 2.1). LCn3PUFA is thus said to have anti-inflammatory, antithrombotic and vasodilation properties.

The average length of gestation is 280 days or 40 weeks after the onset of the woman's last menstrual period. Babies born alive before 37 weeks of pregnancy are classified as pre-term. Pre-term births can be further sub-categorised to moderate to late pre-term (born between 32 and <37 weeks), very pre-term (born between 28 and <32 weeks) and extremely pre-term (born <28 weeks). Globally, it was estimated that around 14% of childhood deaths (younger than 5 years) in 2010 were related to pre-term birth complications (Liu et al. 2012). The proposed mechanism for the LCn3PUFA to exert an effect on the length of gestation is their ability to inhibit uterine production of prostaglandins, $PGF_{2\alpha}$ and PGE_2 which are mediators of uterine contractions and cervical ripening (Olsen et al. 1986). Adding LCn3PUFA to the diet may help to correct the imbalance between TXA_2 and PGI_2 production found in women with pre-eclampsia (Walsh 1985). Studies have shown that higher DHA levels in mothers' milk and greater seafood consumption were associated with lower prevalence rates of postpartum depression (Hibbeln 2002; Golding et al. 2009). LCn3PUFA have been shown to affect receptors and neurotransmitter implicated in depression (Rees, Austin & Parker 2008).

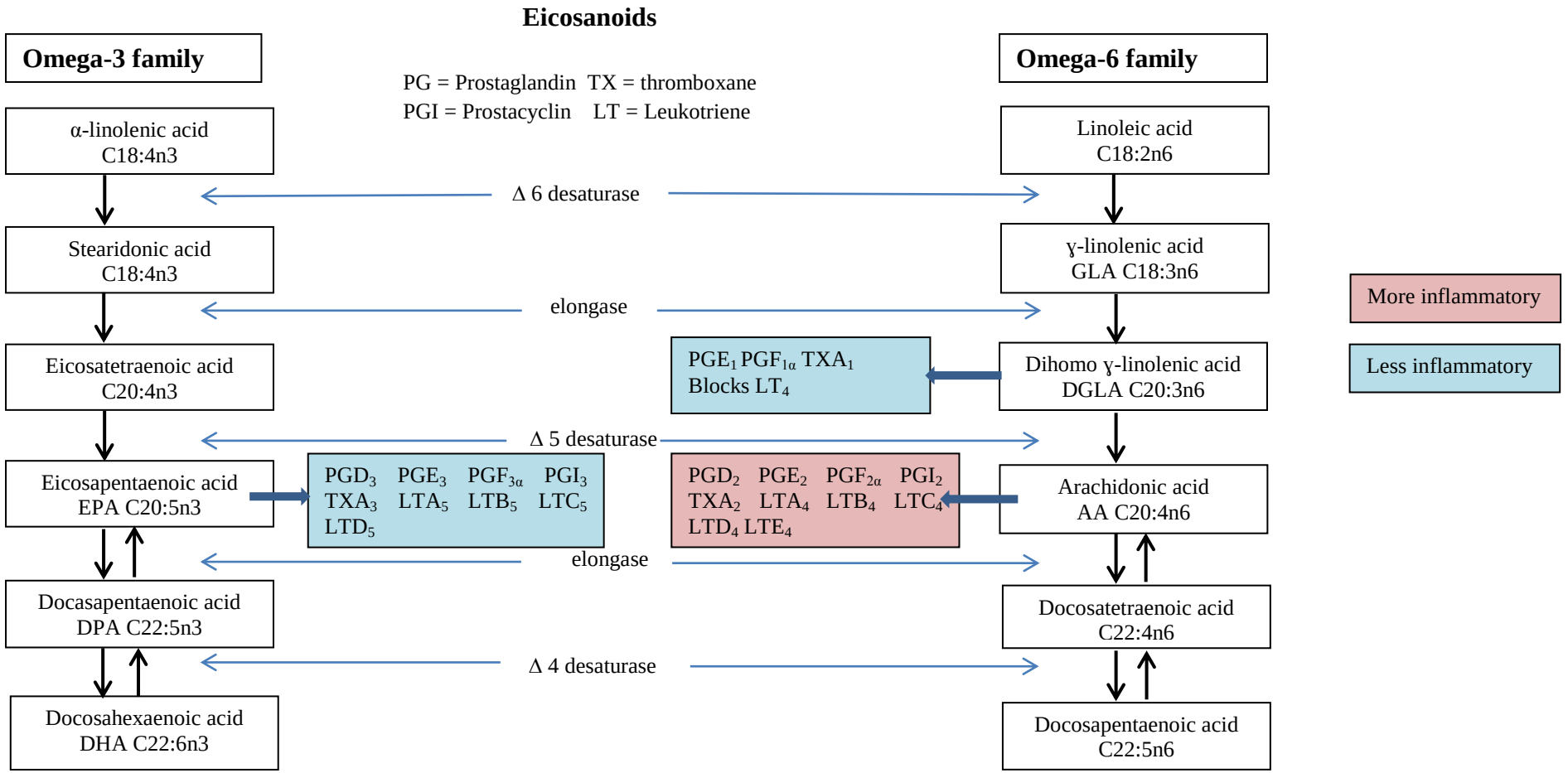

Figure 2.1 Essential fatty acid production and metabolism to form eicosanoids

2.1.1.1 Length of gestation, pre-term or post-term delivery rate

Results from individual trials

Length of gestation

Twenty-five RCTs reported the length of gestation as either a primary or secondary outcome. Eight of these trials (4 studies of low risk and 4 studies of high risk of bias) demonstrated a significant increase in the duration of pregnancy in the supplemented group when compared with the control group while the remaining 17 studies (7 low risk, 8 high risk and 2 with unclear risk of bias) could detect no statistically significant differences (Table 2.1). The largest difference in the duration of pregnancy was seen in the trial by Olsen et al. (2000). In that trial, which included women who had previously experienced pre-term delivery (before 259 days of gestation, EARL-PD trial), daily supplementation of 1.3 g of EPA and 0.9 g of DHA from 20-week gestation onwards resulted in a mean pregnancy duration of 269.2 days (SD, 19.7; n=108) compared to 260.7 days (SD, 29.5; n=120) in the control group. No study has recorded a significant reduction in pregnancy duration with LCn3PUFA supplementation.

Pre-term delivery rate

Sixteen RCTs reported on the incidence of pre-term birth (<37 weeks gestation) with possible results from three trials (2 studies with low risk and 1 with high risk of bias) (Table 2.1). Olsen et al. (2000) observed a significant reduction in the recurrence of pre-term birth events in the supplemented group of their EARL-PD trial (Odds Ratio [OR], 0.54; 95% Confidence Interval [CI], 0.30–0.98; p<.05, n=228). Smuts et al. (2003a), who used high-DHA eggs as their dietary intervention, also reported fewer pre-term deliveries (5%) in the intervention group when compared with those who were provided with ordinary egg (25%) and those who had low egg intake (26%). However, the sample size was small in this study (n=37) and no p-value for this outcome was reported. The same research group conducted another larger trial (n=350) using a similar study design but with only two groups, high-DHA eggs *vs*. ordinary eggs. In this later trial, no difference in the incidence of pre-term delivery was shown although there was an increase in duration of gestation of 6.0 days (SD,

2.3) in the high-DHA egg group when compared with the ordinary egg group after controlling for maternal body mass index (BMI) and the number of prior pregnancies (Smuts et al. 2003b). In another much larger trial (n=2399), the DHA to Optimize Mother Infant Outcome (DOMInO) study by Makrides et al. (2010), supplementation of 800 mg of DHA and 100 mg of EPA per day did not influence the occurrence of total pre-term birth (5.60% in the supplemented group *vs*. 7.34% in the non-supplemented group; Adjusted Relative Risk [RR], 0.77; 95% CI, 0.56–1.05; p=.09). However, the study did find that there were fewer early pre-term births (<34 weeks gestation) in the supplemented group when compared with the control group (1.09% *vs*. 2.25%; Adjusted RR, 0.49; 95% CI, 0.25–0.94; p=.03). No statistically significant differences in the rate of pre-term births were seen in the remaining 12 RCTs.

Post-term delivery rates

Four studies reported on post-term delivery rates (>294 days gestation). The study by Olsen et al. (1992) (n=533) and Hauner et al. (2012) (n=208) did not observe any significant difference in post-term delivery rate with supplementation. However, Olsen et al. (2000) demonstrated an increase in post-term delivery rates when the results of the six individual trials of different populations were combined (3.3% in the supplemented group *vs*. 1.4% in the non-supplemented group; OR, 2.44; 95% CI, 1.20–4.97; p=.01). Makrides et al. (2010), in their DOMInO trial also observed more post-term births requiring obstetric intervention in the DHA group when compared with the control group (17.59% *vs*. 13.72%; Adjusted RR, 1.28; 95% CI, 1.06–1.54; p=.01).

Results from systematic reviews and meta-analysis

Length of gestation

A Cochrane systematic review by Makrides et al. (2006) reported a significant increase in mean gestation of 2.6 days in women supplemented with LCn3PUFA during pregnancy compared to the non-supplemented group (weighted mean difference [WMD], 2.55 days; 95% Confidence Interval [CI], 1.03–4.07 days). This was based on a meta-analysis of three RCTs (Olsen et al. 1992; Olsen et al. 2000; Smuts et al. 2003b) totalling 1621 women. When these women were classified into

low/moderate and high-risk pregnancy groups, results still favoured the LCn3PUFA supplemented group (WMD, 2.23 days; 95% CI, 0.67–3.80 days; 1393 women of low/moderate risk; WMD, 8.50 days; 95% CI, 2.05–14.95 days; 228 women of high-risk).

Another meta-analysis of LCn3PUFA supplementation on pregnancy outcomes in women with low-risk pregnancies was performed by Szajewska et al. (2006) and supported the view by Makrides et al. (2006). LCn3PUFA supplementation was associated with a significantly longer duration of pregnancy (WMD 1.57 days; 95% CI, 0.35–2.78 days). This meta-analysis included six RCTs (Olsen et al. 1992; Helland et al. 2001; Smuts et al. 2003a; Malcolm et al. 2003b; Smuts et al. 2003b; Sanjurjo et al. 2004) totalling 1278 women. The same authors further explored the effect of LCn3PUFA supplementation in women with high-risk pregnancies (Horvath, Koletzko & Szajewska 2007) and found no evidence that supplementation influenced the duration of pregnancy.

Salvig & Lamont published a systematic review in 2011 and also examined the effect of LCn3PUFA supplementation on gestational age. Combined results of three RCTs (Olsen et al. 1992; Olsen et al. 2000; Smuts et al. 2003b) suggest that gestational age is 4.51 days (95% Cl, 2.26–6.76) longer with supplementation.

Conversely, Imhoff-Kunsch et al. (2012) found no such effect in their meta-analysis of eight RCTs (Olsen et al. 1992; Olsen et al. 2000; Smuts et al. 2003b; Dunstan et al. 2004; Sanjurjo et al. 2004; Judge, Harel & Lammi-Keefe 2007b; Bergmann et al. 2008; Ramakrishnan et al. 2010a) totalling 2802 women (WMD, 0.87 day; 95% CI, -0.11–1.84 days).

The systematic review by Larqué et al. (2012) examined nine RCTs (Olsen et al. 1992; Helland et al. 2001; Smuts et al. 2003b; Knudsen et al. 2006; Krauss-Etschmann et al. 2007; Bergmann et al. 2008; Innis & Friesen 2008; Makrides et al. 2010; Ramakrishnan et al. 2010a) and arrived at the conclusion that although LCn3PUFA supplementation during pregnancy has a moderate effect in prolonging gestation, it is not enough to be included as a general recommendation in order to avoid pre-term deliveries.

Pre-term delivery rates

The increases in pregnancy duration with LCn3PUFA supplementation do not always translate into reductions in pre-term delivery rates. In Lewin et al. (2005), a meta-analysis of eight RCTs involving 1574 women was performed comparing intake of EPA and DHA versus control for the incidence of pre-term delivery. No significant difference was found (OR 0.88; 95% CI, 0.62–1.25) compared to the control groups. Meta-analysis of two other trials involving a total of 328 women comparing intake of DHA versus control also demonstrated no significant difference between supplemented and non-supplemented groups (OR, 0.53; 95% CI, 0.13–2.29).

In Makrides et al. (2006), no significant reduction in the risk of pre-term delivery was detected when all women were considered (RR, 0.92 with supplementation; 95% CI, 0.79–1.07; five RCTs, 1916 women) and when women were classified into low/moderate risk (RR, 0.95 with supplementation; 95% CI, 0.80–1.13; three RCTs, 1393 women) and high-risk pregnancy groups (RR, 0.82 with supplementation; 95% CI, 0.60–1.12; three RCTs, 523 women). However, women in the supplemented group did have a lower risk of having early pre-term delivery of <34 weeks gestation (RR, 0.69 with supplementation; 95% CI 0.49–0.99, p=.044; two RCTs, 860 women). Makrides et al. (2006) also performed a meta-analysis on the incidence of post-term delivery and observed no significant difference between supplemented and non-supplemented women (RR, 1.68 with supplementation; 95% CI, 0.77–3.66; p=.19; two RCTs, 1970 women). However, the authors commented that there were insufficient data to provide a reliable conclusion.

Szajewska et al. (2006) also found no significant difference in the percentage of pre-term delivery between the supplemented and the control group from three RCTs, totalling 861 women (RR, 0.67 with supplementation; 95% CI, 0.41–1.10). Similar to the results of Makrides et al. (2010), Horvath et al. (2007) found no significant difference in pre-term delivery in the group of women with high-risk pregnancies from three RCTs (RR, 0.82 with supplementation; 95% CI, 0.60–1.12, 523 women) but did show a reduction in early pre-term delivery (RR, 0.39 with supplementation; 95% CI, 0.18–0.84; two RCTs, 291 women).

A meta-analysis of three RCTs (Olsen et al. 1992; Olsen et al. 2000; Smuts et al. 2003b) by Salvig & Lamont (2011) suggests a protective effect with LCn3PUFA supplementation for both pre-term birth (RR, 0.61; 95% Cl, 0.40–0.93) and early pre-term birth (RR, 0.32; 95% Cl, 0.09–0.95).

The result of the more recent systematic review by Imhoff-Kunsch et al. (2012) is also in agreement with that of Makrides et al. (2010) and Horvath et al. (2007). The meta-analysis from nine RCTs involving 6505 women showed that the difference in the risk of pre-term birth was non-significant between the LCn3PUFA-supplemented group and the non-supplemented group (RR, 0.09; 95% CI, 0.82–1.01). In relation to early pre-term birth, the risk was shown to be significantly lower in the supplemented group from a meta-analysis of five RCTs involving 4343 women (RR, 0.74; 95% CI, 0.58–0.94). One of the trials included in both of these two meta-analyses (Harper et al. 2010) was conducted in a group of women who were also receiving 17 α-hydroxyprogesterone caproate, a drug previously shown to reduce the rate of recurrent pre-term delivery among women who had a history of spontaneous pre-term delivery (Meis et al. 2003).

Post-term delivery rates

A meta-analysis of two RCTs (Olsen et al. 1992; Olsen et al. 2000) that had reported data for the risk of prolonged gestation beyond 42 weeks was conducted by Makrides et al. (2006) and showed no significant difference between supplemented and control groups (RR, 1.68; 95% CI, 0.77–3.66, 1970 women). The authors concluded that no reliable conclusion could be made due to insufficient data.

Conclusion

Overall, most systematic reviews agreed that although LCn3PUFA supplementation during pregnancy slightly increases the length of gestation by 1.6–4.5 days, it does not reduce the risk of pre-term delivery. However, there is some evidence for a lower risk of early pre-term delivery.

Table 2.1 RCTs with pregnancy outcomes (length of gestation, pre-term and post-term delivery rates)

Trial ID	Reference / Location	Intervention dose per day (n=number in analysis); duration	Control (n=number in analysis)	Outcomes (compared with control group)		
				Length of gestation	Pre-term delivery rate (<259 days)	Post-term delivery rate (>294 days)
2	Olsen et al. 1992 / Denmark	1.28 g of EPA & 0.92 g of DHA (n=266); from 30 week gestation to delivery	Olive oil capsules (n=136) OR No supplement (n=131)	↑*** in fish oil group cf. olive oil ↔ between fish oil and no supplement group	↔ between the three groups	↔ between the three groups
4	Bulstra-Ramakers et al. 1994 / Netherlands	3 g of EPA, DHA also present but dose NR (n=32); from 12–14 week gestation to delivery	Coconut oil capsules (n=31)	NR	↔	NR
5	Onwude et al. 1995 / United Kingdom	1.62 g of EPA & 1.08 g of DHA (n=113); from 19–26 week to 38 week gestation	Air-filled capsules (n=119)	↔	↔	NR
8	Olsen et al. 2000/ 9 European countries (Earl-PD)	1.28 g of EPA & 0.92 g of DHA (n=108); from ~20 week gestation to delivery	Olive oil capsules (n=120)	↑*	↓*	↑* when all six trials (Trial IDs 8–13) were combined
9	Olsen et al. 2000/ 9 European countries (Earl-IUGR)	1.28 g of EPA & 0.92 g of DHA (n=131); from ~20 week gestation to delivery	Olive oil capsules (n=132)	↑*	↔	↑* when all six trials (Trial IDs 8–13) were combined

Trial ID	Reference / Location	Intervention dose per day (n=number in analysis); duration	Control (n=number in analysis)	Outcomes (compared with control group)		
				Length of gestation	Pre-term delivery rate (<259 days)	Post-term delivery rate (>294 days)
10	Olsen et al. 2000/ 9 European countries (Earl-PIH)	1.28 g of EPA & 0.92 g of DHA (n=167); from ~20 week gestation to delivery	Olive oil capsules (n=183)	↔	↔	↑* when all six trials (Trial IDs 8–13) were combined
11	Olsen et al. 2000/ 9 European countries (Twins)	1.28 g of EPA & 0.92 g of DHA (n=286); from ~20 week gestation to delivery	Olive oil capsules (n=283)	↔	↔	↑* when all six trials (Trial IDs 8–13) were combined
12	Olsen et al. 2000/ 9 European countries (Threat-PE)	2.88 g of EPA & 2.07 g of DHA (n=42); from ~33 week gestation to delivery	Olive oil capsules (n=34)	↔	↔	↑* when all six trials (Trial IDs 8–13) were combined
13	Olsen et al. 2000/ 9 European countries (Susp-IUGR)	2.88 g of EPA & 2.07 g of DHA (n=36); from ~33 week gestation to delivery	Olive oil capsules (n=27)	↑*	↔	↑* when all six trials (Trial IDs 8–13) were combined
14	Helland et al. 2001/ Norway	0.80 g of EPA, 1.18 g of DHA & 0.03 g of AA (n=175); from 17–19 week gestation to 3 months after delivery	Corn oil (n=166)	↔	↔	NR
15	Smuts et al. 2003a/ United States	High-DHA eggs providing 184 mg DHA (n=18); from 24–28 week gestation to delivery	Regular eggs providing 35 mg DHA (n=19) **OR** Low egg intake, 11 mg DHA from egg (n=16)	↑ (p=NR)	↓ (p=NR)	NR

Trial ID	Reference / Location	Intervention dose per day (n=number in analysis); duration	Control (n=number in analysis)	Outcomes (compared with control group)		
				Length of gestation	Pre-term delivery rate (<259 days)	Post-term delivery rate (>294 days)
16	Smuts et al. 2003b/ United States	High-DHA eggs providing 146 mg DHA (n=142); from 24–28 week gestation to delivery	Regular eggs providing 32 mg DHA (n=149)	↑**	↔	NR
17	Malcolm et al. 2003a/ United Kingdom	200 mg DHA (n=31); from 15 week gestation to delivery	Sunflower oil placebo capsules (n=29)	↔	NR	NR
19	Dunstan et al. 2004/ Australia	1.11 g of EPA & 2.2 4g of DHA (n=40); from 20 week gestation to delivery	Olive oil capsules (n=43)	↔	NR	NR
20	Sanjurjo et al. 2004/ Spain	40 mg EPA & 200 mg DHA (n=8); from 26–27 week gestation to delivery	Placebo dietary formula (n=8)	↔	NR	NR
22	Krauss-Etschmann et al. 2007/ Germany, Hungary & Spain (NUHEAL)	150 mg of EPA & 500 mg of DHA (n=69) OR 400 µg of folic acid (n=65) OR 150 mg of EPA, 500 mg of DHA & 400 µg of folic acid (n=64); from 22 week gestation to delivery	Placebo milk-based supplement (n=72)	↔	NR	NR

Trial ID	Reference / Location	Intervention dose per day (n=number in analysis); duration	Control (n=number in analysis)	Outcomes (compared with control group)		
				Length of gestation	Pre-term delivery rate (<259 days)	Post-term delivery rate (>294 days)
23	Knudsen et al. 2006/ Denmark	0.1 g EPA+DHA (n=374) OR 0.3 g EPA+DHA (n=370) OR 0.7 g EPA+DHA (n=367) OR 1.4 g EPA+DHA (n=358) OR 2.8 g EPA+DHA (n=373) OR 2.2 g of ALA (n=369); from 17–27 week gestation to expected date of delivery	No treatment (n=748)	↔	NR	NR
24	Tofail et al. 2006/ Bangladesh	1.8 g of EPA & 1.2 g of DHA (n=125); from 25 week gestation to delivery	Soy oil capsules (n=124)	↔	↔	NR
25	Bergmann et al. 2008/ Germany	Basic supplement + 4.5 g of fructo-oligosaccharide + 200 mg of DHA (n=43); from 21 week gestation to 3 months after delivery	Basis supplement (n=37) **OR** Basis supplement + 4.5 g of fructo-oligosaccharide (n=36)	↔	NR	NR
26	Judge et al. 2012/ United States	214 mg of DHA (n=22); from 24 week gestation to delivery	Placebo cereal bars with corn oil (n=25)	↔	NR	NR

Trial ID	Reference / Location	Intervention dose per day (n=number in analysis); duration	Control (n=number in analysis)	Outcomes (compared with control group)		
				Length of gestation	Pre-term delivery rate (<259 days)	Post-term delivery rate (>294 days)
31	van Goor et al. 2010/ Netherlands	220 mg of DHA (n=42) **OR** 220 mg of DHA & 220 mg of AA (n=41); from 14–20 week gestation to 3 months after delivery	Soy bean oil capsules (n=36)	↔	NR	NR
32	Furuhjelm et al. 2009/ Sweden	1.6 g of EPA & 1.1 g of DHA (n=52); from 25 week gestation to 3–4 months after delivery	Soy oil capsules (n=65)	↔	NR	NR
33	Makrides et al. 2010/ Australia	100 mg of EPA & 800 mg of DHA (n=1197); from ~22 week gestation to delivery	Vegetable oil capsules (n=1202)	↑ (p=.05)	↓* (in the number of very pre-term birth, i.e. <34 weeks' gestation)	↑*
34	Ramakrishnan et al. 2010a/ Mexico	400 mg of algal DHA (n=486); from 18–22 week gestation to delivery	Placebo capsules containing corn-soy oil blend (n=484)	↔	↔	NR
35	Miles et al. 2011/ United Kingdom	2 x 150 g salmon portions per week resulting in median daily intake (from total diet) of 134 mg of EPA & 269 mg of DHA	Usual diet consisting of <2 portions per month of oily fish, resulting in median daily intake (from total diet) of 12 mg of EPA &	↔	NR	NR

Trial ID	Reference / Location	Intervention dose per day (n=number in analysis); duration	Control (n=number in analysis)	Outcomes (compared with control group)		
				Length of gestation	**Pre-term delivery rate (<259 days)**	**Post-term delivery rate (>294 days)**
		(n=53); from 20 week gestation to delivery	20mg of DHA (n=54)			
36	Hauner et al. 2012/ Germany (INFAT)	180 mg of EPA & 1020 mg of DHA, concomitant reduction of AA intake to ~90mg per day; n-6:n-3 PUFA ratio ~3.5:1 (n=92); from 15 week gestation to 4 months after delivery	Healthy balanced diet and to refrain from taking fish oil or DHA supplements; n-6:n-3 PUFA ratio ~7:1 (n=96)	↑***	↔	↔

* = p<.05; ** = p<.01; *** = p<.005; NR = not reported; ↑ = increase/higher; ↓ = decrease/lower; ↔ = no significant difference; cf. = compared with

2.1.1.2 Incidence of pregnancy-induced hypertension, pre-eclampsia and eclampsia

Results from individual trials

Eleven RCTs were identified that reported the effect of LCn3PUFA supplementation on pregnancy-induced hypertension (PIH), pre-eclampsia and/or eclampsia (Table 2.2). Of the nine studies that have blood pressure as an outcome, none demonstrated a statistically significant effect on blood pressure or the incidence of PIH with LCn3PUFA supplementation. In one of these studies, D'Almeida et al. (1992) (high risk of bias) showed that the group supplemented with one gram of magnesium had the lowest number of cases of PIH (2 out of 50) when compared to the GLA+EPA+DHA group (9 out of 50) and the control group (13/50) but the p-value was not reported.

Pre-eclampsia, a condition of hypertension accompanied by proteinuria and often oedema that occurs during pregnancy, was reported in nine RCTs. D'Almeida et al. (1992) demonstrated a statistically significant effect on the reduction of pre-eclampsia incidence in the group supplemented with GLA+EPA+DHA and the group supplemented with magnesium when compared to the control group using olive oil (p=.0005). No significant effect was demonstrated in the remaining eight RCTs. Eclampsia, a life threatening condition characterised by the appearance of seizures usually in a patient who has developed pre-eclampsia, was reported in two RCTs. In the D'Almeida study, none of the women in the intervention groups (GLA+EPA+DHA or magnesium supplemented) developed eclampsia, but there were three cases in the control group. In Smuts et al. (2003b), data for pre-eclampsia and eclampsia were combined with five cases (out of 142) reported in the high-DHA egg group and 10 cases (out of 149) in the regular egg group, p>.05. As many of these RCTs had small sample sizes and the observed number of cases of pre-eclampsia and eclampsia was low, most studies were not powered to detect a meaningful clinical difference in these outcomes. However, even in the large supplementation trial of Makrides et al. (2010) (low risk of bias), no beneficial effect was found with LCn3PUFA supplementation.

Results from systematic reviews and meta-analysis

Lewin et al. (2005) reviewed eight RCTs published between 1992 and 2003 (totalling 2335 pregnant women) on the effect of LCn3PUFA supplementation to the incidence of PIH, and/or pre-eclampsia and/or eclampsia (D'Almeida et al. 1992; Laivuori et al. 1993; Bulstra-Ramakers, Huisjes & Visser 1995; Onwude et al. 1995; Salvig, Olsen & Secher 1996; Olsen et al. 2000; Smuts et al. 2003b) (Table 2.2). Meta-analysis by Lewin of two (Onwude et al. 1995; Olsen et al. 2000) out of the eight trials comparing intake of EPA+DHA *vs*. control demonstrated no significant difference in incidence of PIH between the groups (OR, 1.07; 95% CI, 0.75–1.51).

Makrides et al. (2006) reported no significant difference in the incidence of PIH from five RCTs (D'Almeida et al. 1992; Bulstra-Ramakers, Huisjes & Visser 1995; Onwude et al. 1995; Salvig, Olsen & Secher 1996; Olsen et al. 2000) (RR, 1.09 with supplementation; 95% CI, 0.90–1.33; 1831 women), pre-eclampsia from four RCTs (D'Almeida et al. 1992; Onwude et al. 1995; Salvig, Olsen & Secher 1996; Olsen et al. 2000) (RR, 0.86 with supplementation; 95% CI, 0.59–1.27; 1683 women) or eclampsia from one RCT (D'Almeida et al. 1992) (RR, 0.14 with supplementation; 95% CI, 0.01–2.70; 100 women) following marine fatty acids supplementation when compared to control. No significant difference was seen in the incidence of pre-eclampsia when women were classified into low/moderate (RR, 1.01 with supplementation; 95% CI, 0.52–1.98, three RCTs, 1130 women) or high-risk pregnancy groups (RR, 0.80 with supplementation; 95% CI, 0.50–1.29; two RCTs, 553 women).

Szajewska et al. (2006) found no significant difference in the rate of pre-eclampsia or eclampsia between supplemented and non-supplemented women with low pregnancy risk from two RCTs (Smuts et al. 2003a; Smuts et al. 2003b) (RR, 0.73; 95% CI, 0.22–2.37; 328 women). No significant difference was observed in the incidence of PIH from three RCTs (Bulstra-Ramakers, Huisjes & Visser 1995; Onwude et al. 1995; Olsen et al. 2000) (RR, 1.06; 95% CI, 0.87–1.29; 645 women) or pre-eclampsia from one RCT (Olsen et al. 2000) (RR, 0.72; 95% CI, 0.35–1.49; p=.37; 321 women) between supplemented and non-supplemented women with high pregnancy risk either (Horvath, Koletzko & Szajewska 2007).

The systematic review by Imhoff-Kunsch et al. (2012) included the same studies as in Makrides et al. (2006) when performing their meta-analysis on PIH and pre-eclampsia and therefore, similarly, no significant difference between supplemented and non-supplemented groups was found.

Conclusion

Overall, based on these systematic reviews and meta-analyses, no significant effect was demonstrated with LCn3PUFA supplementation in the prevention of PIH, pre-eclampsia or eclampsia, regardless of whether the pregnancy was considered high or low-risk.

Table 2.2 RCTs with pregnancy outcomes (pregnancy-induced hypertension, pre-eclampsia, eclampsia)

Trial ID	Reference/ Location	Intervention dosage (n=number in analysis)	Control (n=number in analysis)	Outcomes		
				Pregnancy-Induced Hypertension (cases/total)	Pre-eclampsia	Eclampsia
1	D'Almeida et al. 1992/ Angola	0.30 g of GLA, 0.14 g of EPA & 0.08 g of DHA (n=50) **OR** 1.0 g of Magnesium (n=50); from within first four months of pregnancy to delivery	Olive oil capsules as placebo (n=50)	GLA+EPA+DHA (9/50) Magnesium (2/50) Control (13/50) ↓ in incidence in Magnesium group (p=NR)	GLA+EPA+DHA (2/50) Magnesium (2/50) Control (5/50) ↓*** in GLA+EPA+DHA and Magnesium group cf. control	GLA+EPA+DHA (0/50) Magnesium (0/50) Control (3/50) ↓ in incidence in GLA+EPA+DHA and Magnesium group (p=NR)
2	Salvig et al. 1996/ Denmark	1.28 g of EPA & 0.92 g of DHA (n=266); from 30 week gestation to delivery	Olive oil capsules (n=136) OR No supplement (n=131)	EPA+DHA (8/266) Control (Olive oil) (5/136) Control (No oil) (2/131) ↔ in the increase in BP between the 3 groups	EPA+DHA (0/266) Control (Olive oil) (4/136) Control (No oil) (1/131)	NR
3	Laivuori et al. 1993/ Finland	1.80 g of EPA & 1.20 g of DHA (n=3) **OR** 3.75 g of LA & 0.45 g of GLA (n=4); from 26–36 week gestation to delivery	Maize oil/corn oil (n=5)	↔ in changes in blood pressure between the 3 groups of pre-eclamptic women	↔ in clinical symptoms between the 3 groups of pre-eclamptic women	NR
4	Bulstra-Ramakers et al. 1994/ Netherlands	3 g of EPA, DHA also present but dose NR (n=32); from 12–14 week gestation to delivery	Coconut oil capsules (n=31)	EPA (12/32) Control (7/31) ↔	NR	NR

Trial ID	Reference/ Location	Intervention dosage (n=number in analysis)	Control (n=number in analysis)	Outcomes		
				Pregnancy-Induced Hypertension (cases/total)	Pre-eclampsia	Eclampsia
5	Onwude et al. 1995/ United Kingdom	1.62 g of EPA & 1.08 g of DHA (n=113); from 19–26 week to 38 week gestation	Air-filled capsules (n=119)	EPA+DHA (38/113) Control (35/119) ↔	EPA+DHA (15/113) Control (18/119) ↔	NR
10	Olsen et al. 2000/ 9 European countries (Earl-PIH)	1.28 g of EPA & 0.9 g of DHA (n=152–167); from ~20 week gestation to delivery	Olive oil capsules (n=169–183)	EPA+DHA (55/167) Control (61/183) ↔	EPA+DHA (11/152) Control (17/169) ↔	NR
11	Olsen et al. 2000/ 9 European countries (Twins)	1.28 g of EPA & 0.92 g of DHA (n=246–274); from ~20 week gestation to delivery	Olive oil capsules (n=251–279)	EPA+DHA (38/274) Control (29/279) ↔	EPA+DHA (14/246) Control (6/251) ↔	NR
15	Smuts et al. 2003a/ United States	High-DHA eggs providing 184 mg DHA (n=18); from 24–28 week gestation to delivery	Regular eggs providing 35mg DHA (n=19) **OR** Low egg intake, 11 mg DHA from egg (n=16)	NR	High-DHA eggs (1/18) Regular eggs group (0/19) Low egg (0/16)	NR
16	Smuts 2003b et al. / United States	High-DHA eggs providing 146mg DHA (n=142); from 24–28 week gestation to delivery	Regular eggs providing 32 mg DHA (n=149)	NR	Pre-eclampsia/eclampsia combined High-DHA eggs (5/142) Regular eggs (10/149) ↔	Pre-eclampsia/eclampsia combined High-DHA eggs (5/142) Regular eggs (10/149) ↔

Trial ID	Reference/ Location	Intervention dosage (n=number in analysis)	Control (n=number in analysis)	Outcomes		
				Pregnancy-Induced Hypertension (cases/total)	Pre-eclampsia	Eclampsia
19	Barden et al. 2006/ Australia	1.11 g of EPA & 2.24 g of DHA (n=40); from 20 week gestation to delivery	Olive oil capsules (n=43)	↔ in BP between groups during or after pregnancy	NR	NR
33	Zhou et al. 2012/ Australia	100 mg of EPA + 800 mg of DHA (n=1197); from ~22 week gestation to delivery	Vegetable oil capsules (blend of rapeseed, sunflower and palm oil) (n=1202)	EPA+DHA (98/1197) Control (107/1202) ↔ in incidence	EPA+DHA (60/1197) Control (58/1202) ↔ in incidence	NR

* = p<.05; ** = p<.01; *** = p<.005; NR = not reported; ↑ = increase/higher; ↓ = decrease/lower; ↔ = no significant difference; cf. = compared with

2.1.1.3 Perinatal depression

Results from individual trials

Three RCTs were identified that were conducted in women with depressive disorder during the perinatal period (varying from 12 weeks gestation up to six months postpartum) and had used doses ranging from 2.0 g to 3.4 g per day of LCn3PUFA (EPA+DHA), with the intervention lasting between six and eight weeks (Freeman et al. 2008; Rees, Austin & Parker 2008; Su et al. 2008) (Table 2.3). Whilst all three RCTs resulted in symptom improvement with reductions in depression scores (Hamilton Rating Scale for Depression [HAM-D], Edinburgh Postnatal Depression Scale [EPDS], Beck Depression Inventory [BDI] or Montgomery-Asberg Depression Rating Scale [MADRS]), only one trial with high risk of bias demonstrated significant differences between intervention and the control group (Su et al. 2008).

Five other RCTs conducted in healthy pregnant women also assessed mood status following LCn3PUFA supplementation (Llorente et al. 2003; Krauss-Etschmann et al. 2007; Doornbos et al. 2009; Mattes et al. 2009; Makrides et al. 2010) (Table 2.3) and involved a total of 2958 women. Daily dosage used varied from 200 mg of DHA to 3.35 g EPA and DHA combined. Three studies commenced supplementation from around 20–22 weeks gestation and ceased at delivery (Krauss-Etschmann et al. 2007; Mattes et al. 2009; Makrides et al. 2010). One study supplemented for four months after delivery (Llorente et al. 2003) while another study commenced at 14–20 week gestation and continued for three months after delivery (Doornbos et al. 2009). No significant differences were observed between intervention and control groups in terms of depression scores (BDI, EPDS, Structured Clinical Interview for DSM-IV: Clinical Version [SCID-CV], blue questionnaire scores), proportion of women with EPDS >12, information processing scores or indices of sleep quality.

Results from systematic reviews and meta-analysis

Jans et al. (2010) included seven studies (Llorente et al. 2003; Krauss-Etschmann et al. 2007; Freeman et al. 2008; Rees, Austin & Parker 2008; Su et al. 2008; Doornbos et al. 2009; Mattes et al. 2009) in their meta-analysis of the effect of LCn3PUFA supplementation on mood and depression in pregnant or postpartum women. The pooled effect size on all contrasts was non-significant (Standardised difference in

means, -0.03; 95% CI, -0.18–0.13, p=.76, 612 women) and therefore indicated no or only a small decrease in perinatal depression with LCn3PUFA supplementation. However, when only the three trials conducted in depressed patients were considered, the pooled effect size showed some effectiveness, although still not statistically significant (Standardised difference in means, 0.17; 95% CI, -0.21–0.55). The authors therefore suggested that the benefits of LCn3PUFA supplementation might be restricted to the depressed population. Due to limitations of the individual studies as well as the heterogeneity of the studies included in the meta-analysis, the authors concluded that it may be too early to draw conclusions, although available data at the time demonstrated no beneficial effect of LCn3PUFA supplementation on perinatal depression.

Another systematic review by Wojcicki and Heyman (2011) evaluated the evidence from 10 studies (three prospective longitudinal cohorts, five RCTs, two pilot trials). Six of these studies found no association between LCn3PUFA and perinatal depression (Llorente et al. 2003; Marangell et al. 2004; Browne, Scott & Silvers 2006; Freeman et al. 2008; Rees, Austin & Parker 2008; Doornbos et al. 2009), two studies demonstrated beneficial effects (Freeman et al. 2006; Su et al. 2008) and two studies had mixed results (Golding et al. 2009; Strom et al. 2009). Again, due to the heterogeneity of the included studies, interpretation of the results was difficult. The authors suggest future RCTs to commence supplementation early in pregnancy before DHA demands peak and with dosage close to 2 g per day of EPA+DHA in combination with n-6 fatty acids.

Larqué et al. (2012) examined the evidence from seven studies (Freeman et al. 2006; Freeman et al. 2008; Rees, Austin & Parker 2008; Su et al. 2008; Doornbos et al. 2009; Freeman & Davis 2010; Makrides et al. 2010) and one meta-analysis (Jans, Giltay & Van der Does 2010). Again the authors were unable to achieve a final conclusion due to the various limitations present in the studies, although findings thus far suggest no observable effects.

Conclusion

Overall, currently available evidence does not support the benefits of LCn3PUFA in preventing perinatal depression in healthy pregnant women. However, whether

LCn3PUFA supplementation could be an option in reducing depressive symptoms in depressed pregnant women remained to be tested.

Table 2.3 RCTs with maternal health as outcome (perinatal depression)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes (compared with control)
18	Llorente et al. 2003/ United States	~200 mg of DHA for 4 months after delivery (Prophylactic trial)	Soy & corn oil capsule	↔ in depression scores as measured by: • BDI at baseline, 3 weeks, 2 months or 4 months after delivery (n=89) • EPDS at 18 months (n=63) • SCID-CV at 18 months (n=49) ↔ in information processing scores as measured by the Stroop Interference Test after 4 months of supplementation (n=27)
19	Matte et al. 2009/ Australia	1.11 g of EPA & 2.24 g of DHA from 20 week gestation to delivery (Prophylactic trial)	Olive oil capsules	↔ in maternal BDI scores between the two groups prior to or post supplementation (n=75) ↔ in improvement between the two groups observed in the group with BDI≥10 (n=16)
22	Krauss-Etschmann et al. 2007/ 3 European countries	150 mg of EPA + 500 mg of DHA **OR** 400 µg of folic acid **OR** 150 mg of EPA + 500 mg of DHA + 400 µg of folic acid, from 22 week gestation to delivery (Prophylactic trial)	Placebo milk-based supplement	↔ in EPDS scores at delivery (n=270)
27	Su et al. 2008/ Taiwan	2.2 g of EPA & 1.2 g of DHA for 8 weeks during pregnancy (Treatment trial)	Olive oil capsules	↓* in HAM-D, EPDS and BDI scores ↑* response rate in intervention group as defined by the change of Hamilton Rating Scale for Depression score at weeks 6 and 8 (n=24–36) ↑ remission rate as defined by HAM-D ≤7 at weeks 4, 6 and 8 but not significantly different statistically
28	Freeman et al. 2008/ US	1.1 g of EPA & 0.8 g of DHA for 8 weeks; supportive psychotherapy was also provided (Treatment trial)	Corn oil capsules with small amount of fish oil added; Supportive psychotherapy was also provided	↔ in EPDS or HAM-D scores at baseline or over the trial period (n=51) ↓*** in EPDS and HAM-D scores in both intervention and control groups cf. baseline
29	Rees et al. 2008/ Australia	0.4 g of EPA & 1.6 g of DHA for 6 weeks (Treatment trial)	Sunola oil as placebo	↔ in EPDS, HAM-D or MADRS scores at baseline or over the trial period (n=26) ↓*** in EPDS, HAM-D and MADRS scores in both intervention and control groups cf. baseline

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes (compared with control)
31	Doornbos et al. 2009/ Netherlands	220 mg DHA **OR** 220 mg each of DHA+AA from 14–20 week gestation to 3 months after delivery (Prophylactic trial)	Soy bean oil	↔ in depression scores between groups as measured by: • EPDS or changes in EPDS at week 36 of pregnancy (n=111) and 6 weeks postpartum (n=100) • Blue questionnaire scores (n=60) ↔ in EPDS scores between week 36 of pregnancy and 6 weeks postpartum for any group ↔ in sleep quality between groups as measured by the duration of efficient sleep and sleep efficiency [total time of real (effective) sleep / the total time attempted sleep x 100%] at week 36 of pregnancy (n=101) and 4 week postpartum (n=92) ↔ in sleep quality over time
33	Makrides et al. 2010/ Australia	100 mg of EPA + 800 mg of DHA from ~22 week gestation to delivery (Prophylactic trial)	Vegetable oil capsules (blend of rapeseed, sunflower and palm oil)	↔ in percentage of women reporting high levels of depressive symptoms (EPDS score >12) at 6 week or 6 month postpartum (n=2399) ↔ in the percentage of women with new medical diagnosis for depression or a diagnosis requiring treatment during study period

* = p<.05; ** = p<.01; *** = p<.005; NR = not reported; ↑ = increase/higher; ↓ = decrease/lower; ↔ = no significant difference; cf. = compared with;

BDI = Beck Depression Inventory; EPDS = Edinburgh Postnatal Depression Scale; SCID-CV = Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Axis I Disorders – Clinician Version; HAM-D = Hamilton Rating Scale for Depression; MADRS = Montgomery-Asberg Depression Rating Scale

2.1.2 Child health and development

Proposed mechanisms of effects

The suggestion that increase in maternal LCn3PUFA intake may lead to increased birth weight of the offspring could be explained by two reasons. Firstly, this increase in birth weight could be secondary to the prolonged gestation. Secondly, there may be increased foetal growth rate resulting from improved placental blood flow due to reduced blood viscosity. Studies have shown that LCn6PUFA are potent promoters of adipogenesis and therefore it has been hypothesised that a reduction in n6:n3 fatty acids ratio during fat cell development may limit adipose tissue growth and prevent obesity later in life (Ailhaud & Guesnet 2004). DHA are most abundance in the brain grey matter and retina phospholipids and is generally regarded as essential for proper neurological and visual functioning (Neuringer & Connor 1986). Altered fatty acids supply can lead to altered composition of the immune cell phospholipids, which in turn leads to changes in immune cell function and immune response (Calder 2008). Incorporation of increased amounts of the LCn3PUFA EPA and DHA into cell membranes has been shown to reduce production of pro-inflammatory eicosanoid mediators such as prostaglandin E_2 (PGE_2) from LCn6PUFA, arachidonic acid (AA) (Calder 2008). Metabolism of EPA and DHA has also recently been shown to give rise to the E- and D- series of resolvins, which have potent anti-inflammatory and inflammation resolving properties (Calder 2009). Thus it is plausible that LCn3PUFA or food containing high LCn3PUFA content, such as fish, may be beneficial to the prevention or treatment of atopic disease where inflammation plays a major role.

2.1.2.1 Foetal development (Anthropometry at birth)

Results from individual trials

Twenty-one trials have reported on at least one of the following outcomes: birth weight, birth length, head circumference, incidence of low birth weight, or recurrence/incidence of intrauterine growth retardation (IUGR) (Table 2.4).

No significant difference was observed in birth weight in 14 (5 studies of low risk, 7 of high risk and 2 of unclear risk of bias) of the 19 RCTs examined (Olsen et al.

1992; Onwude et al. 1995; Olsen et al. 2000; Helland et al. 2001; Malcolm et al. 2003a; Smuts et al. 2003b; Dunstan et al. 2004; Sanjurjo et al. 2004; Tofail et al. 2006; Bergmann et al. 2007; Krauss-Etschmann et al. 2007; Judge, Harel & Lammi-Keefe 2007b; Innis & Friesen 2008). Significant reduction in birth weight was observed in one study conducted in women who had IUGR in an earlier pregnancy (Olsen et al. 2000). Four studies (3 low risk and 1 high risk of bias) demonstrated increases in birth weight (Olsen et al. 2000; Smuts et al. 2003a; Makrides et al. 2010; Ramakrishnan et al. 2010a) although the effect was only evident amongst primigravid women in one of these studies and one study did not report the p-value. In the study by Makrides et al. (2010) (n=2399), the difference in birth weight was no longer significant between the supplemented and non-supplemented groups when birth weights were corrected for gestational age and sex, thereby indicating that group differences were mainly a function of gestational age at birth.

Similarly, the majority of the RCTs reported non-significant differences in birth length (10 out of 13 RCTs) and head circumference (nine out of 11 RCTs). Two studies reported an increase (Smuts et al. 2003a; Smuts et al. 2003b) and one study reported a significant decrease (Bergmann et al. 2007) in birth length in the supplemented groups. Two studies reported increases in head circumference with supplementation (Smuts et al. 2003a; Ramakrishnan et al. 2010a). The study by Smuts et al. (2003a) did not report the p-value, while the effect of increased head circumference with supplementation was observed in primigravid women in the study by Ramakrishnan et al. (2010a).

Nine studies have reported incidence of low birth weight. Six of the nine studies reported no significant differences in incidence, while three studies demonstrated a reduction in incidence with supplementation (D'Almeida et al. 1992; Smuts et al. 2003a; Makrides et al. 2010).

Only six studies out of the 21 RCTs have reported the incidence of IUGR. The majority of studies (five out of six studies) demonstrated no effect with supplementation while one study showed a reduction in the prevalence rate of IUGR (14% control *vs.* 7.1% DHA supplemented) when only primigravid women were included (Ramakrishnan et al. 2010a).

Results from systematic reviews and meta-analysis

Lewin et al. (2005) reviewed 11 RCTs that had birth weight as an outcome. Two trials (Olsen et al. 2000; Smuts et al. 2003a) demonstrated a significant increase in birth weight in the LCn3PUFA supplemented group, although one study (Smuts et al. 2003a) did not report the p-value (Table 2.4). One trial (Olsen et al. 2000) had the opposite effect and reported a lower mean birth weight when compared to the olive oil control arm. No significant difference in mean birth weight was observed in the remaining eight trials (Olsen et al. 1992; Onwude et al. 1995; Olsen et al. 2000; Helland et al. 2001; Malcolm et al. 2003a; Smuts et al. 2003b; Dunstan et al. 2004). Data from two of these eleven trials were used to conduct a meta-analysis and no significant difference was observed in mean birth weight between supplemented and non-supplemented groups (WMD, -61.51g; 95% CI, -256.21–133.18g; p=.54). Recurrence of IUGR did not differ in the three trials that had investigated the effect of LCn3PUFA in women with a history of IUGR in previous pregnancies (Table 2.4). A meta-analysis of these three trials (Bulstra-Ramakers, Huisjes & Visser 1995; Onwude et al. 1995; Olsen et al. 2000) demonstrated no significant difference in the incidence of IUGR (OR, 1.14; 95% CI, 0.79–1.64; p=.48). Incidence of low birth weight did not differ in five trials (Bulstra-Ramakers, Huisjes & Visser 1995; Olsen et al. 2000; Smuts et al. 2003b) but was lower in the intervention group in two trials, p-values not reported (D'Almeida et al. 1992; Smuts et al. 2003a). Birth length was not significantly different between supplemented and non-supplemented groups in four trials (Olsen et al. 1992; Helland et al. 2001; Malcolm et al. 2003a; Dunstan et al. 2004), and was higher in two trials (Smuts et al. 2003a; Smuts et al. 2003b), although no p-value was reported in one (Smuts et al. 2003a). An increase in head circumference was observed only in one trial with a small sample size (n=37) with no p-value (Smuts et al. 2003a); no significant difference was observed in the other four trials that had reported head circumference at birth (Helland et al. 2001; Malcolm et al. 2003a; Smuts et al. 2003b; Dunstan et al. 2004).

Makrides et al. (2006) reported in a meta-analysis a significant increase in birth weight (WMD, 47.24 g; 95% CI, 1.05–93.44; p=.045; three RCTs, 2440 infants) and birth length (WMD, 0.48 cm; 95% CI, 0.13–0.83; p=.008; two RCTs, 824 infants) in the supplemented group (Table 2.4). This increase in birth weight was only evident in women with low-risk pregnancy (WMD, 55.79 g; 95% CI, 4.83–106.74 g; p=.032;

three RCTs, 1946 infants) but not in high-risk pregnancy (WMD, 12.11 g; 95% CI, −97.34–121.56 g; p=.83; one RCT, 494 women). There appears no difference, however in the incidence of small-for-gestational age (SGA) (RR, 1.13; 95% CI, 0.96–1.34; p=.15; one RCT, 1374 infants) or low birth weight (RR, 1.00; 95% CI, 0.88–1.12; p=.94; five RCTs, 2302 infants). No significant difference was seen when women were classified into low/moderate or high-risk pregnancy groups.

Szajewska et al. (2006) also reported no significant increase in birth weight (WMD, 53.97 g; 95% CI, -3.11–111.04g ; p=.06; six RCTs, 1278 infants) or birth length (WMD, 0.23 cm; 95% CI, -0.04–0.50 cm; p=.09; five RCTs, 1262 infants) in infants born to women with low-risk pregnancy following n-3 supplementation (Table 2.4). A significant increase in head circumference however was demonstrated in infants born to women who, during pregnancy, were supplemented with LCn3PUFA (WMD, 0.26 cm; 95% CI, 0.02–0.49 cm; p=.03; four RCTs, 729 infants) (Table 2.4). In the meta-analysis of women with high-low pregnancies, Horvath et al. (2007) observed that LCn3PUFA supplementation did not result in significant reduction in the incidence of IUGR (RR, 1.03; 95% CI, 0.73–1.47; p=.85; two RCTs, 295 infants) or low birth weight in the newborn (RR, 1.03; 95% CI, 0.71–1.51; p=.87; two RCTs, 494 infants).

A meta-analysis of four RCTs was conducted by Salvig & Lamont (2011) and suggested that the mean birth weight of children born to women who received LCn3PUFA supplementation during pregnancy was higher by 71.42 g (95% CI, 4.73–138.12, p<.05) than those women who received no supplementation.

.Imhoff-Kunsch et al. (2012) published their systematic review on the effect of LCn3PUFA intake during pregnancy on maternal, infant and child health outcomes. Several meta-analyses were conducted that demonstrated a significant increase in birth weight with supplementation (WMD, 42.22 g; 95% CI, 14.76–69.68) but no difference in birth length (WMD, 0.27 cm; 95% CI, -0.13–0.67) nor head circumference (WMD, -0.21 cm; 95% CI, -0.84–0.42). There was also no difference between supplemented and non-supplemented groups in the risk of low birth weight (RR, 0.92; 95% CI, 0.83–1.02) or risk of IUGR (RR, 1.06; 95% CI, 0.2–1.21).

Based on the evidence from eight RCTs and several previously published meta-analyses, Larqué et al. (2012) concluded that although there was a moderate effect on

higher birth weight with LCn3PUFA supplementation, the use of supplementation to reduce low birth weight or IUGR is still controversial but remains a possibility.

Conclusion

LCn3PUFA supplementation during pregnancy is likely to increase birth weight of the newborn secondary to the increase in length of gestation. However, there is not enough evidence to support a recommendation of LCn3PUFA supplementation as a means to reduce the risk of low birth weight or IUGR.

Table 2.4 RCTs with child health at birth as outcomes (Birth weight, birth length, head circumference, low birth weight, IUGR)

Trial ID	Reference/ Location	Intervention dosage (n=number in analysis)	Control (n=number in analysis)	Outcomes (compared with control group)				
				Birth Weight	Birth Length	Head Circumfer-ence	Incidence of LBW	Recurrence/ Incidence of IUGR
1[a,b,f]	D'Almeida et al. 1992/ Angola	0.30 g of GLA, 0.14 g of EPA & 0.08 g of DHA (n=50)	Olive oil capsules as placebo (n=50)	NR	NR	NR	↓ (p=NR) (Weight <2000 g)	NR
2[a,b,c,e,f,g]	Olsen 1992/ Denmark	1.28 g of EPA & 0.92 g of DHA (n=266)	Olive oil capsules (n=136) OR No supplement (n=131)	↔ between the 3 groups	↔ between the 3 groups	NR	NR	NR
4[a,b,d,f]	Bulstra-Ramakers 1994/ Netherlands	3 g of EPA, DHA also present but dose NR (n=32)	Coconut oil capsules (n=31)	NR	NR	NR	↔	↔
5[a,b,d,f]	Onwude 1995/ UK	1.62 g of EPA & 1.08 g of DHA (n=113)	Air-filled capsules (n=119)	↔	NR	NR	NR	↔
8[a,b,d,e,f]	Olsen 2000/ 9 European countries (Earl-PD)	1.28 g of EPA & 0.92 g of DHA (n=108)	Olive oil capsules (n=118)	↑*	NR	NR	↔	NR
9[a,b,d,e,f]	Olsen 2000/ 9 European countries (Earl-IUGR)	1.28 g of EPA & 0.92 g of DHA (n=131-135)	Olive oil capsules (n=132-133)	↓*	NR	NR	↔	↔

Trial ID	Reference/ Location	Intervention dosage (n=number in analysis)	Control (n=number in analysis)	Outcomes (compared with control group)				
				Birth Weight	Birth Length	Head Circumfer-ence	Incidence of LBW	Recurrence/ Incidence of IUGR
11[a,b,f]	Olsen 2000/ 9 European countries (Twins)	1.28 g of EPA & 0.92 g of DHA (n=554–556 infants)	Olive oil capsules (n=566–557 infants)	↔	NR	NR	↔	↔
13[a,f]	Olsen 2000/ 9 European countries (Susp-IUGR)	2.88 g of EPA & 2.07 g of DHA (n=34)	Olive oil capsules (n=26)	↔	NR	NR	NR	NR
14[a,c,g]	Helland 2001/ Norway	0.80 g of EPA, 1.18 g of DHA & 0.03 g of AA (n=175)	Corn oil (n=166)	↔	↔	↔	NR	NR
15[a,c]	Smuts 2003a/ US	High-DHA eggs providing 184 mg DHA (n=18)	Regular eggs providing 35 mg DHA (n=19)	↑ (p=NR)	↑ (p=NR)	↑ (p=NR)	↓ (p=NR)	NR
16[a,b,c,e,f,g]	Smuts 2003b/ US	High-DHA eggs providing 146 mg DHA (n=142)	Regular eggs providing 32 mg DHA (n=149)	↔	↑*	↔	↔	NR
17[a,c]	Malcolm 2003a/ UK	200 mg DHA (n=31)	Sunflower oil placebo capsules (n=29)	↔	↔	↔	NR	NR
19[a,f]	Dunstan 2004/ Australia	1.11 g of EPA & 2.24 g of DHA (n=40)	Olive oil capsules (n=43)	↔	↔	↔	NR	NR

Trial ID	Reference/ Location	Intervention dosage (n=number in analysis)	Control (n=number in analysis)	Outcomes (compared with control group)				
				Birth Weight	Birth Length	Head Circumfer-ence	Incidence of LBW	Recurrence/ Incidence of IUGR
20[c,f]	Sanjurjo 2004/ Spain	40 mg EPA & 200 mg DHA (n=8)	Placebo dietary formula (n=8)	↔	NR	NR	NR	NR
22[g]	Krauss-Etschmann 2007; Germany, Hungary & Spain (NUHEAL)	150 mg of EPA & 500 mg of DHA (n=69) OR 400 µg of folic acid (n=65) OR 150 mg of EPA, 500 mg of DHA & 400 µg of folic acid (n=64)	Placebo (n=72)	↔	↔	↔	NR	NR
24	Tofail 2006/ Bangladesh	1.8 g of EPA & 1.2 g of DHA (n=125)	Soy oil capsules (n=124)	↔	↔	↔	NR	NR
25[f,g]	Bergmann 2007/ Germany	Basic supplement + 4.5 g of fructo-oligosaccharide + 200 mg of DHA (n=43)	Basic supplement +/- 4.5 g of fructo-oligosaccharide (n=74)	↔	↓*	↔	NR	NR
26[f]	Judge 2007b(Courville et al. 2011)(Courville et al. 2011)/ US	DHA-containing cereal-based bar providing on average 214 mg of DHA per day (n=16)	Placebo cereal-based bar containing corn oil (n=14)	↔	↔	↔	NR	NR

Trial ID	Reference/ Location	Intervention dosage (n=number in analysis)	Control (n=number in analysis)	Outcomes (compared with control group)				
				Birth Weight	Birth Length	Head Circumfer-ence	Incidence of LBW	Recurrence/ Incidence of IUGR
30[g]	Innis & Friesen 2008/ Canada	400 mg of algal DHA	Corn oil/soy bean oil	↔	↔	NR	NR	NR
33[f,g]	Makrides 2010/ Australia	Maternal intake of DHA-rich fish oil concentrate, providing 800 mg/day of DHA and 100 mg/day of EPA (n=1197)	Vegetable oil capsules (blend of rapeseed, sunflower and palm oil) (n=1202)	↑ ***	↔	↔	↓*	↔
34[f,g]	Ramakrishnan 2010a/ Mexico	Maternal intake of 400 mg of algal DHA (n=487)	Placebo capsules containing olive oil (n=486)	All women ↔ Primigravidae ↑*	All women ↔ Primigravidae ↔	All women ↔ Primigravidae ↑*	All women ↔ Primigravidae ↔	All women ↔ Primigravidae ↓*

* = p<.05; ** = p<.01; *** = p<.005; NR = not reported;↑ = increase/higher, ↓ = decrease/lower, ↔ = no significant difference; cf. = compared with

[a] = studies reviewed in Lewin et al. 2005; [b] = studies reviewed in Makrides et al. 2006; [c] = studies reviewed in Szajewska et al. 2006; [d] = studies reviewed in Horvath et al. 2007; [e] = studies reviewed in Salvig & Lamont 2011; [f] = studies included in meta-analysis by Imhoff-Kunsch et al. 2012; [g] = studies reviewed in Larqué et al. 2012

2.1.2.2 Growth pattern

Results from individual trials

Ten trials have been found assessing maternal LCn3PUFA supplementation during pregnancy and/or lactation on the physical growth pattern of their offspring (Table 2.5). Three trials involved supplementation during pregnancy (Malcolm et al. 2003b; Tofail et al. 2006; Dunstan et al. 2008; Stein et al. 2011), three trials commenced supplementation after delivery and continued for three to four months (Jensen et al. 1999; Jensen et al. 2005; Lauritzen et al. 2005a) and two trials commenced supplementation at 15–24 week gestation and continued until 3–4 months after delivery (Helland et al. 2001; Bergmann et al. 2007; Hauner et al. 2012).

Supplementation during pregnancy:

No significant difference was observed in growth pattern (with respect to weight, height and head circumference) when assessed at three or seven months (Malcolm et al. 2003b), 10 months (Tofail et al. 2006), 18 months (Stein et al. 2011) or 2.5 years of age (Dunstan et al. 2008) between intervention (ranging from 0.2 g DHA to 1.8 g EPA+1.2 g DHA) and the control group.

Maternal supplementation during lactation:

No significant difference was observed in weight or height of children between intervention (ranging from 0.2 g DHA to 0.4 g EPA+0.9 g DHA) and the control group when assessed at various time points up to 2.5 years of age (Jensen et al. 1999; Jensen et al. 2005; Lauritzen et al. 2005a). A significant increase (0.68 cm) in head circumference was observed by Lauritzen et al. (2005a) in the intervention group when assessed at 2.5 years but not at birth, two, four or nine months of age, while Jensen et al. (1999; 2005) found no significant difference. Lauritzen et al. (2005a) also observed a significant increase in waist circumference (1.54 cm ± 0.63; p=.017) and BMI (0.65 ± 0.28; p=.022) in the intervention group at 2.5 years when compared to the control group. However, the difference in BMI was no longer apparent when re-assessed at seven years of age (Asserhoj et al. 2009). Blood pressure (diastolic, systolic and mean arterial pressure) in children in the two arms of the study did not differ at 2.5 years (Larnkjaer et al. 2006) but an increase in systolic blood pressure

(3.8 mm Hg ± 1.7; p<.05) and mean arterial pressure (2.8 mm Hg ± 1.3; p<.05) was observed in the fish oil group when compared to the olive oil control group.

Maternal supplementation during pregnancy and lactation:

Helland et al. (2001) observed no significant difference in weight, length or head circumference when assessed at six weeks, three, six, nine or twelve months after birth between the intervention and control groups. Hauner et al. (2012) also found no significant difference in these parameters between supplemented and non-supplemented groups when assessed at six weeks, four months or twelve months. Bergmann et al. (2007) however, observed a lower weight and BMI (p-value not reported) in the DHA supplemented group when compared to control when assessed at 21 months of age.

Results from systematic reviews and meta-analysis

Two RCTs were identified by Lewin et al. (2005) that addressed the question of growth pattern outcomes, but neither study showed any significant difference between the supplemented and non-supplemented groups.

A Cochrane Review by Delgado-Noguera et al. (2010) concluded that there was no significant difference between supplemented or non-supplemented groups in regard to the child's weight whether it was assessed as short term (less than 12 months; WMD, 0.24 kg; 96% CI, -0.07 to 0.55; 712 participants), medium term (12–24 months; WMD, -0.56 kg; 95% CI, -0.64–0.48; 117 participants) or long term (beyond 24 months; WMD, 0.22; 95% CI, -0.13–0.57; 834 participants). For child's length, the analysis favoured the control group long term (WMD, -0.75 cm; 95% CI,-1.38–-0.12; 834 participants). For head circumference, significantly larger circumference was reported in the supplemented group both at medium term (WMD, 0.70 cm; 95% CI, 0.56–0.84; 117 participants) and long term (WMD, 0.69 cm; 95% CI, 0.35–1.02; 244 participants).

A systematic review by Muhlhausler et al. (Muhlhausler, Gibson & Makrides 2010) that included three human trials showed contrasting results, thus no confident conclusion could be made. One study reported a significant reduction in BMI z-score in infants of mothers who were supplemented with LCn3PUFA during pregnancy and lactation while another study found no effect. The remaining trials where there

was supplementation during lactation only reported a significant increase in BMI and waist circumference at 2.5 years of age in the supplemented group (Lauritzen et al. 2005a). However, this difference was no longer evident when these children were followed up at seven years (Asserhoj et al. 2009).

Similarly, no conclusion could be made in the systematic review by Rodríguez et al. (2012) due to inconsistent results and heterogeneity of the study designs.

A systematic review published in 2012 by Campoy et al. concluded that there was no effect of prenatal or postnatal LCn3PUFA supplementation on physical growth. In all the studies reviewed, most showed no significant difference between supplemented or non-supplemented groups except for one study by Bergmann et al. (2007) where babies from supplemented mothers had a lower BMI at 21 months.

Conclusion

Overall, based on available evidence, supplementing mothers with LCn3PUFA during pregnancy and/or lactation appears to have no effect on physical growth pattern in their children.

Table 2.5 RCTs with child health as outcomes (physical growth pattern)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
7[a]	Jensen 1999/ US	Maternal daily intake of 0.20–0.25 g of DHA as either algal DHA OR Refined high-DHA fish oil during lactation	Placebo	↔ in weight, length, head circumference, triceps skinfold when assessed at 4 and 8 months after birth (n=126)
14[a,b,c,d,e]	Helland 2001, Helland 2008/ Norway	Maternal daily intake of 0.80 g of EPA, 1.18 g of DHA & 0.03 g of AA during pregnancy & lactation	Corn oil	↔ in weight, length or head circumference when assessed at 6 weeks, and at 3, 6, 9 and 12 months after birth (n=288) No significant correlations between umbilical plasma phospholipid concentrations of fatty acids or ratio of n-3/n-6 fatty acids and the children's BMI at 7 years of age (n=143)
17[e]	Malcolm 2003b/ UK	Maternal daily intake of 200 mg DHA during pregnancy	Sunflower oil placebo capsules	↔ in weight, length or head circumference when assessed at ~3 months (n=55) and at 7 months after birth (n=55)
18[e]	Jensen 2005, Jensen 2010/ UK	Maternal daily intake of ~200mg of DHA during lactation	Soy & corn oil capsule	↔ in weight, length or head circumference when assessed at 4, 8, 12, 18, 24, 30 or 60 months of age (n=119–160)
19[e]	Dunstan 2008/ Australia	Maternal daily intake of 1.11 g of EPA & 2.24 g of DHA during pregnancy	Olive oil capsules	↔ in weight, length or head circumference when assessed at 2.5 years of age (n=64)
21[b,c,d]	Lauritzen 2005a, Larnkjaer 2006, Asserhoj 2009/ Denmark	Maternal daily intake of 0.6 g EPA & 0.8 g DHA during lactation	Olive oil	↔ in weight at birth (n=122), at 2 months (n=104), at 4 months (n=100), at 9 months (n=100) and at 2.5 years of age (n=72) ↔ in length/height at birth (n=122), at 2 months (n=103), at 4 months (n=98), at 9 months (n=100) and at 2.5 years of age (n=70) ↑* in head circumference in fish oil group at 2.5 years (n=71) but not at birth (n=110), at 2 months (n=100), at 4 months (n=91) or at 9 months of age (n=97) ↑* in BMI at 2.5 years of age (n=70) but no longer significant difference when assessed at 7 years of age (n=64) ↑* in waist circumference at 2.5 years of age (68) ↔ in diastolic blood pressure at 2.5 years (n=57) or 7 years of age (n=64) ↔ in systolic blood pressure at 2.5 years (n=57) but ↑* at 7 years of age (n=64) ↔ in mean arterial pressure at 2.5 years (n=56) but ↑* at 7 years of age (n=64)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
24[e]	Tofail 2006/ Bangladesh	Maternal daily intake of 1.8 g of EPA & 1.2 g of DHA during pregnancy	Soy oil capsules	↔ in weight for height z-score, weight for age z-score, height-for-age z-score or head circumference when assessed at 10 months after birth (n=249)
25[b,c,d,e]	Bergmann 2007/ Germany	Basic supplement + 4.5 g of fructo-oligosaccharide + 200 mg of DHA during pregnancy and lactation	Basic supplement +/- 4.5g of fructo-oligosaccharide	↓ (p=NR) in weight and BMI in DHA group at 21 months cf. control
34[e]	Stein 2011/ Mexico	Maternal intake of 400 mg of algal DHA (n=487) from 18–22 week gestation to delivery (n=369)	Placebo capsules containing corn-soy oil blend (n=370)	At 18 months, results by treatment group and controlling for maternal height and age and sex of child: ↔ in weight, length and head circumference Results by treatment group and gravidity interaction: ↑* in length and length-for-age z-score in children born to primagravid women only
36[d]	Hauner 2012/ Germany	180 mg of EPA & 1020 mg of DHA, concomitant reduction of AA intake to ~90 mg per day; n-6:n-3 PUFA ratio ~3.5:1 (n=92); from 15 week gestation to 4 months after delivery	Healthy balanced diet and to refrain from taking fish oil or DHA supplements; n-6:n-3 PUFA ratio ~7:1 (n=96)	↔ in weight, length, head circumference at 6 week, 4 months or 12 months between groups

n=number in analysis, treatment and control groups combined; * = p<.05; ** = p<.01; *** = p<.005; NR = not reported; ↑ = increase/higher, ↓ = decrease/lower, ↔ = no significant difference; cf. = compared with

[a] = RCTs reviewed by Lewin et al. 2005; [b] = studies reviewed in Delgado-Noguera et al. 2010; [c] = studies reviewed in Muhlhausler et al. 2010; [d] = RCTs reviewed by Rodríguez et al. 2012; [e] = studies reviewed by Campoy et al. 2012

2.1.2.3 Neurological development

Twelve trials have been found assessing maternal LCn3PUFA supplementation during pregnancy and/or lactation on neurological development of their offspring (Table 2.6). Six trials involved supplementation during pregnancy only (Tofail et al. 2006; Judge, Harel & Lammi-Keefe 2007a; Dunstan et al. 2008; Makrides et al. 2010; Ramakrishnan et al. 2010b; Escolano-Margarit et al. 2011), three trials commenced supplementation after delivery and continued for three to four months (Gibson, Neumann & Makrides 1997; Jensen et al. 2005; Lauritzen et al. 2005b) and three trials commenced supplementation at between 14 to 25 weeks gestation and continued until three months after delivery (Helland et al. 2001; Karlsson et al. 2010; van Goor et al. 2011).

Supplementation during pregnancy:

Dunstan et al. (2008) examined the cognitive development of children born to a group of women who had received fish oil supplementation (1.1 g EPA and 2.2 g DHA per day) during pregnancy. At 2.5 years of age, no significant difference was observed in developmental quotients (except for a higher score for eye and hand coordination in the fish oil groups), behaviour or linguistic development between the intervention and control groups. Similarly, the Nutraceuticals for a Healthier Life (NUHEAL) study conducted across three European countries did not show any significant difference with maternal supplementation of 0.15 g EPA and 0.5 g DHA, when compared to those not supplemented with EPA+DHA, in terms of neurological optimality score, fluency score or incidence of minor neurological dysfunction when the children were assessed at four or 5.5 years of age (Escolano-Margarit et al. 2011). However, the authors managed to demonstrate the odds of children with maximal neurological optimality score increased with increasing cord blood DHA levels. There was also no significant difference in the Kaufman Assessment Battery for Children (K-ABC) scores between the EPA+DHA supplemented and non-supplemented groups when the children were assessed at 6.5 years. Tofail et al. (2006) found no significant difference in Bayley Scales of Infant Development-Mental Development Index (BSID Mental Developmental Index (MDI)), Psychomotor Development Index (PDI) or behaviour rating in children when assessed at ten months of age after maternal supplementation of LCn3PUFA (1.8 g

EPA & 1.2 g DHA per day). Judge et al. (2007a) observed no difference in cognitive functioning at nine months of age as assessed by the Fagan Test of Infant Intelligence but found a significant increase in problem-solving ability in the DHA supplemented group (214 mg DHA per day) when the Infant Planning Test was applied. In the study by Makrides et al. (2010), maternal supplementation of 800 mg per day of DHA during the second half of the pregnancy did not result in improved cognitive and language development in their offspring when assessed at 18 months of age. Ramakrishnan et al. (2010b) also concluded from their study that prenatal DHA supplementation (400 mg per day of DHA) did not improve global development scores.

Maternal supplementation during lactation:

Gibson et al. (1997), using increasing doses of DHA (0 g–1.3 g), demonstrated a positive association between infant erythrocyte DHA status and BSID MDI at one year of age but this association was lost when re-assessed at two years. No association was found between infant erythrocyte DHA status and BSID PDI at either one or two years of age (Gibson, Neumann & Makrides 1997). Jensen et al. (2005) found no significant difference in BSID MDI but observed a significant increase in BSID PDI when assessed at 30 months in the group of children whose mothers received 200 mg DHA supplementation. These children also performed significantly better on a test of sustained attention when assessed at five years of age (2010). Lauritzen et al. (2005b) observed no significant difference in problem-solving ability between the intervention and control groups (0.4 g EPA+0.9 g DHA *vs*. olive oil) when infants were assessed at nine months of age. However when infants were analysed separately based on gender, a positive effect was observed in girls but not boys as assessed by the Infant Planning Test. A negative effect in vocabulary comprehension at one year and sentence complexity at two years of age was seen in boys from the supplemented groups (0.4 g EPA + 0.9 g DHA) but not in girls. When these children were re-assessed at seven years of age, no group differences were observed with respect to the speed of information processing score, the Stroop score for working memory and inhibitory control or the Strengths and Difficulties Questionnaire scores, except a significantly lower prosocial score was observed with maternal fish oil supplementation in boys only (Cheatham et al. 2011).

Maternal supplementation during pregnancy and lactation:

Following maternal LCn3PUFA supplementation, Helland et al. (2001) did not observe a significant difference in electroencephalogram (EEG) maturity scores measured at one day or three months after birth when compared to the control group. There was also no significant difference in cognitive functioning when assessed at six or nine months of age. The Mental Processing Composite score was significantly higher in the intervention group when children were assessed at four years of age (Helland et al. 2003) but was no longer significantly different when assessed at seven years of age (Helland et al. 2008). Van Goor et al. conducted a study comparing the effect of supplementation of DHA alone (220 mg per day) *vs.* DHA + AA (220 mg each per day) *vs.* control (soy bean oil) during pregnancy and lactation. No difference in neurological development was observed when the children were assessed at two weeks (van Goor et al. 2010) or 18 months (van Goor et al. 2011), although a transient increase in the incidence of mildly abnormal general movement was observed when the children were assessed at three months of age (van Goor et al. 2010). In another study, pregnant women were supplemented with either 1.6 g EPA and 1.1 g DHA per day or a placebo from 25 weeks' gestation and during 3.5 months of lactation (Karlsson et al. 2010). Improved performance on visuospatial tasks and executive tasks involving behavioural inhibition was observed in the children born to the mothers of the EPA+DHA supplemented group when assessed at 46 months of age. However, neuropsychological tasks assessing language and memory did not show significant differences between groups (Karlsson et al. 2010).

Results from systematic reviews and meta-analysis

The two RCTs identified by Lewin et al. (2005) (one involving supplementation during pregnancy and one during lactation) did not show any significant difference in neurological outcomes as evaluated by EEG (Helland et al. 2001) or PDI of the BSID (Gibson, Neumann & Makrides 1997).

Five RCTs were assessed in a systematic review by Eilander et al. (2007) that had cognitive development as outcomes. The authors concluded that there is suggestive evidence for a beneficial effect of DHA supplementation during pregnancy and lactation or lactation only on mental and cognitive development.

Six RCTs were identified in the systematic review by Dziechciarz et al. (2010) that had neurological outcomes. Based on the results from these RCTs, the authors concluded that no clear and consistent benefit of maternal LCn3PUFA supplementation during pregnancy and/or lactation could be demonstrated in terms of neurodevelopment, although potential benefit could not be excluded.

In the systematic review by Larqué et al. (2012), six RCTs (eight publications) were examined on the effects of cognitive function from LCn3PUFA supplementation during pregnancy and/or lactation. While all six studies were of RCT design, the results in one study were analysed based on high or low maternal DHA level at delivery rather than the original intervention *vs.* control group (Colombo et al. 2004). The authors suggested that although not all studies reported improvements with supplementation, there appeared to be a positive relationship between maternal or cord blood DHA level and cognitive skills in the children, particularly in children with low DHA levels.

Four independent RCTs (six publications) were considered in the systematic review by Lo et al. (2012) for assessing the effect of LCn3PUFA supplementation on neurodevelopment. Results were inconsistent and the available evidence does not support supplementing all expecting mothers for improvement of infant neurodevelopment, although it is important to maintain a healthy diet that provides sufficient LCn3PUFA.

Of the eight RCTs considered by Campoy et al. (2012) for neurodevelopment, five trials involved supplementation of LCn3PUFA during pregnancy, one during pregnancy and lactation and two during lactation only. Evidence from this systematic review does not demonstrate consistent benefits in neurodevelopment, short or long term, with LCn3PUFA supplementation during pregnancy and/or lactation. However, some RCTs did suggest that prenatal DHA status might have positive effects on neurodevelopmental and behaviour outcomes.

Gould et al. (2013) examined nine RCTs that involved maternal LCn3PUFA supplementation during pregnancy only or during pregnancy and lactation that had neurodevelopment outcomes. Meta-analysis of these trials demonstrated no difference between maternal LCn3PUFA supplementation *vs.* control on cognitive, motor or language development in their offspring. The only exception was a positive

finding of increased developmental standard score in the supplemented group when compared to the non-supplemented group in children 2–5 years of age (WMD, 3.92 points; 95% CI, 0.77–7.08; n=156; p=.01).

In the Cochrane Review by Delgado-Noguera et al. (2010), four RCTs were examined for the effects of maternal LCn3PUFA supplementation on children's neurodevelopment. No significant difference was found in terms of language development, intelligence or problem-solving ability, psychomotor development or motor development. Only one study demonstrated an improvement in child attention with supplementation. The authors therefore concluded that maternal LCn3PUFA supplementation during pregnancy and/or lactation did not appear to improve children's neurodevelopment.

Conclusion

On balance, with more weighted evidence from the systematic reviews, LCn3PUFA supplementation during pregnancy and/or lactation did not appear to improve children's neurodevelopment however adequate intake must be maintained. Several studies did demonstrate a positive relationship between maternal/infant DHA status and better cognitive outcomes.

Table 2.6 RCTs with child health as outcomes (neurological development)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
6[a,b,h]	Gibson 1997/ Australia	Maternal daily intake of varying doses of DHA during lactation: 0.2 g of DHA; 0.4 g of DHA; 0.9 g of DHA; 1.3 g of DHA	Placebo containing 0 g of DHA	Positive association between infant erythrocyte DHA status and BSID MDI at 1 year of age (n=51) but the association was lost at 2 years old (n=49) No association found between infant erythrocyte DHA status and BSID PDI at either 1 or 2 years of age
14[a,b,c,d,e,f,g,h]	Helland 2001, 2003, 2008/ Norway	Maternal daily intake of 0.80 g of EPA, 1.18 g of DHA & 0.03 g of AA during pregnancy & lactation	Corn oil	↔ in EEG maturity scores at 1 day (n=148) and at 3 months after birth (n=122) ↔ in cognitive functioning as assessed by Fagan Test of Infant Intelligence at 6 months (n=262) and 9 months after birth (n=245) ↑* in Mental Processing Composite of the K-ABC tested at 4 years of age (n=84) but ↔ at 7 years of age (n=143)
18[b,c,f,h]	Jensen 2005, 2010/ UK	Maternal daily intake of ~200mg of DHA during lactation	Soy & corn oil capsule	↔ in Gesell Gross Motor Inventory, CAT or CLAMSDQ at 12 months (n=162) and at 30 months of age (n=147) ↔ in BSID-II MDI but ↑** in BSID-II PDI when assessed at 30 months (n=133) ↔ K-ABC (Hand movement), McCarthy (Leg coordination) Purdue Pegboard Test (Dominant hand and Non-dominant hand), Developmental test of visual-motor integration, Wechsler Primary and Preschool Scale of Intelligence but ↑**on the Sustained Attention subtest of the Leiter International Performance Scale-Revised at 5 years of age (n=119)
19[c,d,e,f,g]	Dunstan 2008/ Australia	Maternal daily intake of 1.11 g of EPA & 2.24 g of DHA during pregnancy	Olive oil capsules	↔ in cognitive outcomes at 2.5 years of age as measured by: • Griffiths Mental Development Scales (except higher score for eye and hand coordination in fish oil group) (n=72) • Peabody Picture Vocabulary Test IIIA (n=70) • Child Behaviour Checklist 1.5–5 years (n=72) • Language Development Survey (n=49–51)
21[b,c,f,h]	Lauritzen 2005b, Cheatham 2011/ Denmark	Maternal daily intake of 0.4 g EPA & 0.9 g DHA during lactation	Olive oil	↔ in motor function at 9 months after birth (n=100) Positive effect (p=.024) in problem solving ability in girls (n=35) but not boys (n=51) in fish oil group as assessed by Infant Planning Test at 9 months after birth

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
				Negative effect in vocabulary comprehension at 1 year in boys (n=52) but not girls (n=37) when compared with olive oil group At 7 years of age: ↔ between groups in speed of processing score, Stroop scores or Strengths and Difficulties Questionnaire scores except a ↓* in prosocial score was observed in the supplemented group when only boys were included in the analysis (n=64)
22[f,g]	Escolano-Margarit 2011, Campoy 2011/Germany, Hungary & Spain (NUHEAL)	Milk-based supplement providing 150 mg of EPA & 500 mg of DHA OR 400 µg of folic acid OR 150 mg of EPA, 500 mg of DHA & 400 µg of folic acid from 22 week gestation to delivery (Infant formula provided for 6 months if needed)	Placebo milk-based supplement	↔ in terms of neurological optimality score, fluency score or incidence of minor neurological dysfunction at the ages of 4 (n=167) or 5.5 years (n=148) between groups ↔ in K-ABC scores (tests designed to evaluate intelligence and achievement) at 6.5 years of age between groups (n=154)
24[b,c,d,e,f,g]	Tofail 2006/ Bangladesh	Maternal daily intake of 1.8 g of EPA & 1.2 g of DHA during pregnancy	Soy oil capsules:	↔ BSID-II MDI & PDI and behaviour ratings when assessed at 10 months after birth (n=249)
26[c,d,e,f,g]	Judge 2007a / US	Maternal daily intake of 214 mg of DHA during pregnancy	Placebo	↑ in problem solving ability in DHA supplemented group as assessed by the Infant Planning Test at 9 months of age (n=29) ↔ in cognitive functioning as assessed by Fagan Test of Infant Intelligence at 9 months of age (n=30)
31[g]	van Goor 2010, van Goor 2011/ Netherlands	220 mg DHA OR 220 mg each of DHA + AA; from 14–20 week gestation to 3 months	Soy bean oil	↔ in the distribution of neonatal neurological classification as well as the median neurological optimality score between groups when assessed at 2 weeks after birth (n=119)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
		after delivery		↑* rate of mildly abnormal general movements in DHA only supplemented group when compared with DHA + AA group or control group when assessed at 12 weeks after birth (n=119) ↔ in terms of neurological optimality score, fluency score, prevalence of simple and complex minor neurological dysfunction and the Dutch version of BSID-II MDI and PDI scores between groups (n=114) when assessed at 18 months
32[g]	Karlsson 2010/ Sweden	Fish oil capsules providing 1.6 g EPA+1.1 g DHA; from 25 week gestation to 3–4 months after delivery	Soy oil capsules (2.5 g LA, n-6)	At 46 months (n=NR), ↓* in errors in the executive task and visuospatial block design task ↔ in memory and language
33[d,f,g]	Makrides 2010/ Australia	Maternal intake of DHA-rich fish oil concentrate, providing 800 mg/day of DHA and 100 mg/day of EPA; from ~22 week gestation to delivery	Vegetable oil capsules (blend of rapeseed, sunflower and palm oil)	↔ in terms of mean cognitive scores, mean language scores, motor development, social-emotional behaviour and adaptive behaviour between groups overall as assessed by the Bayley Scales of Infant and Toddler Development, Third Edition at 18 months (n=726) However, mean language score and adaptive behaviour score were significantly lower in girls in the supplemented group than their counterparts in the control group
34[g]	Ramakrishnan 2010b/ Mexico	Maternal intake of 400 mg of algal DHA (n=487) from 18–22 week gestation to delivery (n=369)	Placebo capsules containing corn-soy oil blend (n=370)	↔ in development when assessed using the Spanish version of BSID-II at 18 months of age

n = number in analysis, treatment and control groups combined; * = p<.05, ** = p<.01; *** = p<.005; NR = not reported; ↑ = increase/higher, ↓ = decrease/lower, ↔ = no significant difference; cf. = compared with; BSID = Bayley Scales of Infant Development; CAT = Clinical Adaptive Test; CLAMSDQ = Clinical Linguistic and Auditory Milestone Scale Developmental Quotients; EEG = Electroencephalography; K-ABC = Kaufman Assessment Battery for Children; MDI = Mental Development Index; PDI = Psychomotor Development Index

[a] = studies reviewed in Lewin et al. 2005; [b] = RCTs reviewed in Eilander et al. 2007; [c] = RCTs reviewed in Dziechciarz et al. 2010; [d] = RCTs reviewed in Larqué et al. 2012; [e] = RCTs reviewed in Lo et al. 2012; [f] = RCTs reviewed in Campoy et al. 2012; [g] = RCTs reviewed in Gould et al. 2013; [h] = Delgado-Noguera et al. 2010

2.1.2.4 Visual function

Ten trials assessed the effect of maternal LCn3PUFA supplementation during pregnancy and/or lactation on visual function of their offspring (Table 2.7). Six trials involved supplementation during pregnancy (Malcolm et al. 2003a; Decsi, Campoy & Koletzko 2005; Jensen et al. 2005; Judge, Harel & Lammi-Keefe 2007b; Innis & Friesen 2008; Makrides et al. 2010; Smithers, Gibson & Makrides 2011; Stein et al. 2012). The remaining four trials commenced supplementation after delivery and continued for 3–4 months (Gibson, Neumann & Makrides 1997; Jensen et al. 1999; Jensen et al. 2005; Lauritzen et al. 2005b).

Supplementation during pregnancy:

Malcolm et al. (2003a) did not observe any significant difference in visual function in the offspring following maternal fish oil supplementation (200 mg DHA per day) as assessed by sceptic electroretinogram at birth or flash and pattern-reversal visual evoked potential (VEP) latency at three and seven months of age (Malcolm et al. 2003b). However, their results suggested there was a positive association between infant DHA status and visual development. Judge et al. (2007b) observed a significant difference in visual acuity (Teller acuity card) between the DHA supplemented group (214 mg DHA per day) and control group when the infants were assessed at four months of age (DHA *vs.* control; 3.7 ± 1.3 cycles/degree *vs.* 3.2 ± 1.3 cycles/degree; n=30) but this effect was lost when infants were re-assessed at six months (DHA *vs.* control; 5.9 ± 1.2 cycles/degree *vs.* 5.4 ± 1.3 cycles/degree; n=26). In the study by Innis and Friesen (2008) where healthy pregnant women were randomly supplemented with either 400 mg per day of DHA or a soybean/corn oil placebo, no significant difference was observed in visual acuity scores (Teller acuity card) in their children when assessed at 60 days (DHA *vs.* placebo; 2.60 ± 0.63 cycles/degree *vs.* 2.42 ± 0.50 cycles/degree; p=.30; n=135). However, the authors observed that a higher proportion of girls in the DHA group had visual acuity scores above the mean for girls when compared to those in the placebo group (p=.048). This effect was not seen in boys (p=.07). The NUHEAL study was conducted to investigate the effect of LCn3PUFA and folic acid supplementation. Healthy pregnant women were randomly assigned to receive either 500 mg of DHA, 500 mg of DHA+400 µg of folic acid, 400 µg of folic acid alone or placebo. After

delivery, mothers were encouraged to breastfeed their children but were provided with infant formulas for six months if needed. The two DHA groups received standard infant formulas with 0.5% of total fatty acids as DHA and 0.4% as AA whereas the folic acid only and placebo groups received standard infant formulas free of DHA and AA (Escolano-Margarit et al. 2011). Preliminary results published in 2005 from 109 children demonstrated no significant difference in latencies, amplitudes and minimum angle of resolution between the four supplemented groups. When the two DHA groups were combined and compared with the combined non-DHA groups, a significant difference was observed in the minimum angle of resolution (DHA *vs.* non-DHA; 1.4'± 7.7' *vs.* 3.0'± 4.9'; $p<.05$) (Broekaert et al. 2005). A follow-up study conducted in a subset of the DOMInO cohort demonstrated no significant difference in sweep VEP acuity between the LCn3PUFA supplemented group (100 mg EPA + 800 mg DHA per day) and control group (vegetable oil) (LCn3PUFA *vs.* control; 8.37 ± 2.11 cycles per degree *vs.* 8.55 ± 1.86 cycles per degree; p=.55; n=182) when the infants were assessed at four months of age (Smithers, Gibson & Makrides 2011). Similarly, in the study by Stein et al. (2012), no significant difference in VEP latencies or amplitude was observed between the offspring of DHA supplemented women (400 mg per day) and control women (corn/soy oil).

Maternal supplementation during lactation:

Gibson et al. (1997), using increasing doses of DHA (0 g to 1.3 g per day), observed no significant difference in VEP acuity among dietary groups when infants were assessed at three and four months after birth. Similarly, no significant difference in visual acuity was evident in the study by Lauritzen et al. (2004) after fish oil supplementation when infants were assessed at four and eight months of age. Lauritzen et al. (2004) however did demonstrate that infants with higher red blood cell levels of LCn3PUFA had better visual acuity at four months of age, thus suggesting that LCn3PUFA might influence visual maturation. Jensen et al. (1999) randomised breastfeeding women into three study groups where one group received ~200–250 mg of DHA per day as algal DHA, another group received ~200–250mg DHA as refined high-DHA fish oil and a third group acted as control. No significant differences were observed among groups in terms of VEP latency, sweep VEP acuity or Teller Card acuity when infants were assessed at 120 or 240 days. Transient VEP

amplitude was significantly lower in infants in the algal DHA supplement group when compared to the other two groups at 120 days but this effect was not evident when re-assessed at 240 days. The same research group later conducted a similar study but this time included only two groups, 200 mg of algal DHA *vs.* placebo (soy/corn oils) (Jensen et al. 2005). Similar to the previous study, no significant differences were observed between the supplemented group and non-supplemented groups in terms of visual acuity as measured by Teller Acuity Card procedure and sweep VEP at four and eight months of age. Transient VEP amplitude again was significantly lower in the DHA supplemented group at both four and eight months when compared with the control group but this difference was no longer significant when children were re-assessed at five years of age (Jensen et al. 2010).

Results from systematic reviews and meta-analysis

Lewin et al. (2005) reviewed three RCTs (one involving LCn3PUFA supplementation during pregnancy and two during lactation) and none detected significant differences in visual function between supplemented and non-supplemented groups.

Eilander et al. (2007) concluded in their systematic review that there is currently no supporting evidence for a beneficial effect on visual development with DHA supplementation during pregnancy and/or lactation. Supplementation studies identified in this review included one RCT conducted in pregnant women and three RCTs in lactating women but none has found significant effects of DHA supplementation on any of the indicators of visual development assessed. However, since the incorporation of DHA and AA in the developing brain is particularly high in the prenatal period, the authors suggested that theoretically supplementation during pregnancy would potentially have the largest impact on visual development of infants but supplementation studies in pregnant women are limited.

A Cochrane Review by Delgado-Noguera included three RCTs involving LCn3PUFA supplementation in breastfeeding mothers in their meta-analysis of children's visual acuity. No significant difference was observed between supplemented and non-supplemented groups (Standardised mean difference, -0.06; 95% CI, -0.25–0.14; three trials; n=401). The authors concluded that, based on the

limited evidence available, maternal LCn3PUFA supplementation during lactation did not appear to improve children's visual acuity and more evidence is needed.

Dziechciarz et al. (2010) reviewed the evidence from three study populations where healthy women were supplemented with LCn3PUFA during pregnancy and two study populations where supplementation took place during the first four months of lactation. The authors concluded that although no clear and consistent benefit of maternal LCn3PUFA on visual acuity of the offspring could be demonstrated, potential benefit could not be excluded and more studies were needed.

Three studies (two study populations) were examined by Lo et al. (2012) in their systematic review of the effectiveness of LCn3PUFA supplementation in pregnant women on visual outcomes of their offspring. One study found a significant difference in visual acuity at four months but this effect was no longer evident at six months. The other two studies conducted in the same study cohort also showed no significant differences between supplemented and non-supplemented groups, but a correlation between infant DHA status and visual development was suggested instead. The authors concluded that, based on currently available evidence, a recommendation to supplement all pregnant women with LCn3PUFA for visual development in their offspring could not be supported due to inconsistent findings.

Campoy et al. (2012) examined the evidence from four RCTs and two RCTs where women were supplemented with LCn3PUFA during pregnancy and during lactation respectively. The authors concluded that clear and consistent short- or long-term benefits of LCn3PUFA supplementation could not be demonstrated from the available evidence. In addition, the interpretation of study findings was made more difficult with different studies using different assessment methods and testing at different ages.

The findings of six RCTs involving LCn3PUFA during pregnancy were reviewed in the systematic review by Gould et al. (2013). The majority of the trials observed no significant differences between supplemented and non-supplemented groups, and those showing improvement were found to have some methodological limitations. The authors therefore concluded that more research would be needed to clarify the effect of LCn3PUFA supplementation on visual development.

Conclusion

Overall, due to the limited number of trials and differing methods of assessment and at differing times, no conclusion can be made regarding the benefits of LCn3PUFA supplementation during pregnancy and/or lactation on visual development. More evidence would be required to support a recommendation of LCn3PUFA supplementation in expecting and breastfeeding mothers.

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Table 2.7 RCTs with child health as outcomes (visual function)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
6[a,b,c]	Gibson 1997/ Australia	Maternal daily intake of varying doses of DHA during lactation: 0.2 g of DHA; 0.4 g of DHA; 0.9 g of DHA; 1.3 g of DHA	Placebo containing 0 g of DHA	VEP acuity: ↔ among dietary groups when assessed at 3 (n=26) and 4 months after birth (n=36)
7[a]	Jensen 1999/ US	Maternal daily intake of 0.20–0.25 g of DHA as either algal DHA OR Refined high-DHA fish oil during lactation	Placebo	VEP acuity (by sweep VEP and Teller Acuity Card): ↔ when assessed at 4 and 8 months after birth (n=126)
17[a,b,d,e,f]	Malcolm 2003a, 2003b/ UK	Maternal daily intake of 200 mg DHA during pregnancy	Sunflower oil placebo capsules	Retinal development: ↔ as assessed by scotopic electroretinogram (ERG) at birth (ERG intensity series: n=41; Maximum combined ERG: n=44) Flash VEP latency or pattern-reversal VEP latency: ↔ when assessed at birth, ~3 and 7 months after birth (n=55)
18[b,c,d,e]	Jensen 2005, Jensen 2010/ UK	Maternal daily intake of ~200mg of DHA during lactation	Soy & corn oil capsule	Sweep VEP acuity: ↔ when assessed at 4 months after birth (n=160) Teller Card acuity: ↔ when assessed at 4 months (n=147) and at 8 months after birth (n=147) VEP latency, VEP amplitude, Sweep VEP acuity, Bailey Lovie Acuity – right eye and left eye all ↔ when assessed at 5 years after birth (n=119)
21[b,c,d,e]	Lauritzen 2004/ Denmark	Maternal daily intake of 0.4 g EPA & 0.9 g DHA during lactation	Olive oil	Sweep VEP acuity: ↔ when assessed at 2 months (n=88) or at 4 months of age (n=97)
22[f]	Broekaert 2005/Germany, Hungary & Spain (NUHEAL)	Milk-based supplement providing 150 mg of EPA & 500 mg of DHA OR 400 µg of folic acid OR 150 mg of EPA, 500 mg of	Placebo milk-based supplement	At 8 weeks after birth (n=109) ↔ VEP latencies, amplitudes or minimal angle of resolution between the 4 groups Significantly smaller MAR in DHA supplemented groups cf. non-DHA supplemented groups (i.e. combined into 2 groups)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
		DHA & 400 µg of folic acid from 22 week gestation to delivery (Infant formula provided for 6 months if needed)		
26[d,e,f]	Judge et al. 2007b/ US	Maternal daily intake of 214 mg of DHA during pregnancy	Placebo	Teller acuity card: ↑* when assessed at 4 months (n=30) but ↔ at 6 months of age (n=26)
30[d,e,f]	Innis & Friesen 2008/ Canada	Maternal daily intake of ~400 mg of algal DHA from 16 week gestation to delivery	Corn-soybean oil blend	Teller acuity card: ↔ when assessed at 60 days of age (n=135)
33[e,f]	Smithers et al. 2011/ Australia	Maternal intake of DHA-rich fish oil concentrate, providing 800 mg/day of DHA and 100 mg/day of EPA, from ~22 week gestation to delivery	Vegetable oil capsules (blend of rapeseed, sunflower and palm oil)	↔ in VEP acuity or latency at 4 months of age (n=182)
34[f]	Stein 2012/ Mexico	Maternal intake of 400 mg of algal DHA from 18–22 week gestation to delivery	Placebo capsules containing corn-soy oil blend	↔ in VEP (amplitude and latencies) at 3 months (n=679) and 6 months (n=817) of age

n = number in analysis, treatment and control groups combined; * = p<.05; ** = p<.01; *** = p<.005; NR = not reported; ↑ = increase/higher, ↓ = decrease/lower, ↔ = no significant difference; cf. = compared with; MAR = Minimal angle of resolution; VEP = Visual Evoked Potential

[a] = RCTs reviewed in Lewin et al. 2005; [b] = RCTs reviewed in Eilander et al. 2007; [c] = RCTs reviewed in Delgado-Noguera et al. 2010; [d] = RCTs reviewed in Dziechciarz et al. 2012; [e] = RCTs reviewed in Campoy et al. 2012; [f] = RCTs reviewed in Gould et al. 2013

2.1.2.5 Atopic disease

Seven RCTs were identified that had assessed immunological and/or clinical outcomes in offspring following supplementation of LCn3PUFA during pregnancy and/or lactation (Table 2.8).

Results from individual trials

Supplementation during pregnancy:

Five RCTs (Dunstan et al. 2003; Krauss-Etschmann et al. 2008; Olsen et al. 2008; Noakes et al. 2012; Palmer et al. 2012) involved LCn3PUFA supplementation during pregnancy had clinical and/or immunological outcomes. Dunstan et al. (2003) conducted an RCT in a group of healthy pregnant women who had a history of allergic rhinitis or asthma. The authors reported that their children were less likely to have a positive skin prick test to egg at one year of age (OR, 0.34; 95% CI, 0.11–1.02; p=.055) following maternal fish oil supplementation from 20 weeks gestation and until delivery, although this trend did not reach statistical significance. In addition, atopic infants born to women in the fish oil group experienced less severe symptoms (OR, 0.09; 95% CI, 0.01–0.94; p=.045). It is worth noting that the authors stated that the study was not originally designed to examine clinical outcomes and that larger studies would need to be conducted to confirm this protective effect and to address long term outcomes. Olsen et al. (2008) matched the original cohort of women who participated in a six-week trial of either fish oil, olive oil or no supplement during pregnancy to the National Patient Registry for any confirmed asthma, atopic dermatitis and allergic rhinitis diagnosed in their children from birth to 16 years old (n=528). The hazard rate of asthma was reduced by 63% (95% CI, 8–85%; p=.03) and allergic asthma by 87% (95% CI, 40–97%; p=.01) in the fish oil group when compared with the olive oil group. Hazard rate reduction was also observed when asthma diagnosis was expanded to include atopic dermatitis and allergic rhinitis. An allergy follow-up study was conducted by Palmer et al. (2012) in a subset of the DOMInO cohort where participating pregnant women were supplemented with either fish oil capsules (100 mg EPA + 800 mg DHA per day) or vegetable oil capsules (n=706). Women were eligible for this follow-up study if the unborn baby had a mother, father or sibling with a history of medically diagnosed allergic disease. After adjusting for centre, parity, maternal history of allergic disease

and the gender of the infants, no significant differences were observed in the percentages of infants with allergic disease with sensitisation (Adjusted RR, 0.70; 95% CI, 0.45–1.09, p=.12) or without sensitisation (Adjusted RR, 1.10; 95% CI, 0.79–1.55, p=.57). The percentage of infants with eczema with sensitisation was lower in the supplemented group, however this difference did not reach statistical significance after adjustment (Adjusted RR, 0.64; 95% CI, 0.40–1.03, p=.06). There was a statistically significant lower percentage of infants who had egg sensitisation (with or without allergic disease) in the supplemented group (Adjusted RR, 0.62; 95% CI, 0.41–0.93; p=.02). In the study by Noakes et al. (2012), pregnant women were randomly assigned to either consume two 150 g portions of salmon each week or to continue with their usual diet of low oily fish consumption from 20 week gestation to delivery. Women were recruited only if their unborn baby was at risk of atopy (i.e. one or more first-degree relatives affected by atopy, asthma or allergy). When the infants were assessed at six months of age, no significant differences were observed between the salmon and control groups in terms of clinical outcomes (including incidence and severity of atopic dermatitis, incidence of wheeze, chest infection, pneumonia/bronchiolitis, itchy skin, dry skin and the rates of sensitisation) (n=86). There was, however, an attenuation of neonatal interleukin-10 (IL-10) production with the salmon intervention, the significance of which in relation to the risk of developing atopy and allergic disease was not yet clear. Krauss-Etschmann et al. (2008) have to-date only reported on immunological but not clinical outcomes from their NUHEAL study where pregnant women were supplemented with 0.15 g EPA+0.5 g DHA or 0.15 g EPA+0.5 g DHA+400 µg folic acid or 400 µg folic acid alone or placebo from 22 week gestation to delivery. The study demonstrated that EPA and DHA supplementation resulted in decreased mRNA levels of Th2 inflammatory cytokines in cord blood (CCR4, IL-4 and IL-13).

Maternal supplementation during lactation:

Lauritzen et al. (2005c) supplemented breastfeeding women with habitual low fish intake for four months after the birth of their children with either 1.5 g of LCn3PUFA per day or olive oil (in muesli bars or cookies or as capsules). A third group of breastfeeding women with high fish intake was included for comparison. There was no significant difference in the percentage of children who were reported to have a diagnosis of eczema, wheezing or food allergy at 2.5 years of age (n=91).

The authors acknowledged that the study was not powered or designed for atopy. In terms of immunological outcome, the median production of lipopolysaccharide-induced interferon γ (IFN-γ) in the fish oil group was found to be fourfold higher than that in the olive oil group (p=.034), which suggested a faster maturation of the immune system in children with maternal LCn3PUFA supplementation.

Maternal supplementation during pregnancy and lactation:

Furuhjelm et al. (2009) conducted an RCT in a group of pregnant women who had been affected by allergy themselves or had a husband or previous child with allergies. Participants were supplemented with either 1.6 g EPA + 1.1 g DHA per day or with placebo (soy oil) from 25 weeks gestation to three to four months of breastfeeding. When assessing the resultant offspring at 12 months of age, there was a lower prevalence of IgE-associated eczema (p<.05) and food allergy (p=.01) in the fish oil group compared to the placebo group (n=117).

Results from systematic reviews and meta-analysis

Three systematic reviews were found to have examined the effects of LCn3PUFA supplementation during pregnancy and/or lactation on infant allergic disease (Klemens, Berman & Mozurkewich 2011; Kremmyda et al. 2011; Larqué et al. 2012). Incidentally, the same five RCTs were considered in these reviews and therefore a similar conclusion was produced. Overall, available evidence suggests that maternal LCn3PUFA supplementation is associated with immunological changes in cord blood and clinical effects of reduced sensitisation to common allergens, reduced prevalence and severity of atopic dermatitis, and reduced childhood asthma. However, more long-term studies are required to confirm the benefits and to inform recommendations. Protective clinical effects are mostly seen in studies where supplementation was initiated during pregnancy. It is therefore suggested that LCn3PUFA supplementation may be more effective in the prenatal period during programming of the foetus and before disease is established.

Conclusion

Overall, there is promising evidence to suggest that maternal LCn3PUFA supplementation, particularly when initiated during pregnancy, influences allergic

biomarkers in children and therefore has a protective effect in preventing the development of allergic diseases. However, more studies investigating the timing and the dose of supplementation are required before definite conclusions can be made.

Table 2.8 RCTs with child health as outcomes (atopic disease)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
2 [a,b,c]	Olsen 2008/ Denmark	1.28 g of EPA & 0.92 g of DHA (n=263) during pregnancy	Olive oil capsules (n=136) OR No supplement (n=129)	↓* in hazard rate of all types of asthma (allergic and non-allergic) in the offspring during first 16 years of life following maternal fish oil supplementation when cf. olive oil ↔ between fish oil and no supplement
19 [a,b,c]	Dunstan 2003/ Australia	Maternal daily intake of 1.11 g of EPA & 2.24 g of DHA during pregnancy	Olive oil capsules	At 12 months of age: Occurrence of allergic disease symptoms (n=83) and positive skin prick test (n=72) was lower in fish oil group but did not reach statistical significance In infants with atopic dermatitis, those in fish oil group were less severely affected as assessed by SCORAD index (n=31)
21 [a,b,c]	Lauritzen 2005c/Denmark	Maternal daily intake of fish oil muesli bars, cookies or capsules providing in total 1.5 g of LCn3PUFA (0.4 g of EPA & 0.9 g of DHA) for 4 months after delivery	Olive oil muesli bars, cookies or capsules	No difference in the percentage of children reported to have a diagnosis of eczema, wheezing or food allergy between groups when followed up at 2.5 years of age (n=91)
22 [a,b,c]	Krauss-Etschmann 2008/ Germany, Hungary & Spain (NUHEAL)	Milk-based supplement providing 150 mg of EPA & 500 mg of DHA OR 400 µg of folic acid OR 150 mg of EPA, 500 mg of DHA & 400 µg of folic acid from 22 week gestation to delivery	Placebo milk-based supplement	In cord blood, EPA+DHA supplementation ± folic acid showed ↓*** mRNA levels of CCR4, IL-13, IL-4 but ↔ mRNA level of IFN-γ when cf. placebo (n=195)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
32 [a,b,c]	Furuhjelm 2009/ Sweden	1.6 g EPA+1.1 g DHA from 25 week gestation to 3–4 months after delivery	Soy oil	Prevalence of food allergies at 12 months was lower in supplemented group than in the control group (2% *vs*. 15%, p=.01) Incidence of IgE related eczema was lower in supplemented group than in the control groups both at 6 months (8% *vs*. 20%, p=.06) and at 12 months (8% *vs*. 24%, p=.02)
33	Palmer 2012/ Australia (DOMInO)	Maternal intake of DHA-rich fish oil concentrate, providing 800 mg/day of DHA and 100 mg/day of EPA	Vegetable oil capsules (blend of rapeseed, sunflower and palm oil)	At 1 year of age (n=706) and after adjusting for centre, parity, maternal history of allergic disease and infant gender: ↔ in the overall percentage of infants with IgE-associated allergic disease (eczema or food allergy) or allergic disease without sensitisation between groups, p=.12 and p=.57 respectively Trend ↓ in percentage of infants with atopic eczema (with sensitisation), p=.06 ↔ in percentage of infants with food allergy (with sensitisation), p=.93 ↓* in percentage of infants sensitised to egg (with or without allergic disease) in the supplemented group
35	Noakes 2012/ UK (SiPS)	2 x 150 g salmon portions per week resulting in median daily intake (from total diet) of 134 mg of EPA and 269 mg of DHA (n=53); from 20 week gestation to delivery	Usual diet consisting of <2 portions per month of oily fish, resulting in median daily intake (from total diet) of 12 mg of EPA & 20 mg of DHA (n=54)	At 6 months of age (n=86): No difference in the percentage of infants who had atopic dermatitis, wheeze, pneumonia/bronchiolitis, chest infections, itchy/dry skin or positive skin prick test No difference in the severity of atopic dermatitis as rated by SCORAD index,

n = number in analysis, treatment and control groups combined; * = p<.05; ** = p<.01; *** = p<.005; NR = not reported; ↑ = increase/higher, ↓ = decrease/lower, ↔ = no significant difference; cf. = compared with; SCORAD = SCORing Atopic Dermatitis; IgE = Immunoglobulin E; [a] = RCTs reviewed in Klemens et al. 2011; [b] = RCTs reviewed in Kremmyda et al. 2011; [c] = RCTs reviewed in Larqué et al. 2012

2.1.3 Conclusion

Based on the results of the described systematic reviews, meta-analyses and RCTs, there is good evidence that intake of LCn3PUFA can prolong gestation by about two days, although this does not translate to a reduction in pre-term (<37 weeks gestation) delivery rate. However, there is suggestion that LCn3PUFA supplementation may reduce the rate of early pre-term (<34 weeks gestation) delivery (Makrides, Duley & Olsen 2006). On balance, birth weight does not appear to be affected by supplementation of LCn3PUFA. It appears that LCn3PUFA did not influence visual acuity in these RCTs conducted with healthy term babies.

While some studies have demonstrated a positive effect on infant neurological development with LCn3PUFA intake initially, the association was no longer evident when re-assessed in later years. It is possible that the positive effect might have been diluted with other external factors since birth, such as socio-economic factors.

LCn3PUFA appears to have some beneficial effect on the risk of developing allergic disease in infants and for the prevention or treatment of maternal perinatal depression. However limited studies were included in this review.

2.2 Fish intakes and maternal & infant outcomes

A Medline search using the following key words: 'fish not fish oil' and 'maternal or infant' and 'benefit or risk' and 'study' returned 420 articles of which around 50 studies could be identified as relevant to the study question – what is the evidence of maternal fish consumption in relation to maternal and infant outcomes? More studies were identified from the references of some of these studies as well as from a systematic review conducted by the National Health and Medical Research Council (NHMRC 2012) aimed to determine the association of health outcomes and food groups consumed (including fish) by pregnant and breastfeeding women. Whilst there have been many randomised trials examining the effects of LCn3PUFA supplementation in the form of fish oil as shown in the earlier section of this chapter, evidence related to fish consumption and health outcomes for mothers and infants per se have largely emerged from observational studies and those from RCTs are scarce.

2.2.1 Evidence based on randomised controlled trials

Only two trials were identified that had used fish intake as interventions. The Salmon in Pregnancy Study (SiPS) conducted in the UK, also reviewed in Section 2.1, was the first RCT conducted in a group of pregnant women (n=123) who had a family history of atopy, allergy or asthma with fish as intervention. The hypothesis was that increased consumption of oily fish during pregnancy (from 20 weeks gestation to delivery) could prevent the development of atopic disease in their children. Results from this trial showed that although weekly consumption of 2 x 150g portions of farmed salmon improved the EPA and DHA status of the mothers and foetus (Miles et al. 2011) and modified some of the immune responses in the neonates, there were no difference in clinical outcomes of atopic sensitisation or incidence and severity of atopic dermatitis in the infants at 6 months of age when compared to the control group who maintained a low oily fish diet (Noakes et al. 2012). Another intervention trial was a non-randomised, controlled, multicentre intervention study conducted in Norway (Prevention of Allergy among Children in Trondheim (PACT) study) for the prevention of childhood allergy and included advice of daily intake of cod liver oil and twice weekly intake of oily fish during pregnancy as part of the intervention (Storro et al. 2010). Other components of the intervention also included reduced

indoor dampness and reduced tobacco exposure and as such it would be difficult to tease out the effect of increased fish consumption on the outcome of interest. Nevertheless, a significant lower incidence in parental-reported diagnosed asthma (OR, 0.72; 95% CI, 0.55–0.93; p=.01) and a lower risk of using asthma medication during the last 12 months (OR, 0.75; 95% CI, 0.58–0.96; p=.02) were observed in the intervention group (Dotterud et al. 2013).

2.2.2 Evidence based on seminal and/or large scale observational studies

2.2.2.1 The New Zealand Study

Women who had given birth to a child in hospitals from the northern part of the North Island in New Zealand were first invited in 1977 to take part in the study (Kjellström et al. 1986). Women who agreed to take part filled in a questionnaire about their diet and some environmental exposures during the pregnancy. In addition, a bundle of scalp hair was collected from each mother for the analysis of mercury level by cold vapour atomic absorption spectrophotometry technique. Of the 11 000 mothers and their children who participated, 935 mothers were identified as eating fish more than three times per week during the pregnancy. Of the 935 mothers, 73 had hair mercury concentrations above 6 mg Hg/kg (corresponding to the World Health Organization (WHO) maximum recommended mercury intake at the time) and were defined as the high mercury dose group. The new born child of each woman from the high mercury dose group was referred to as high mercury child. When the children were four years old, each high mercury child was matched with another reference child regarding maternal ethnic group, maternal age, hospital where the child was born and birthdate but none of these reference mothers ate fish more than once per week. Thirty-one matched pairs were successfully interviewed and assessed. Using the Denver Developmental Screening Test as the assessment tool, children from the high mercury group had a significantly higher prevalence of abnormal or questionable performance compared to their matched peers (χ^2=8.1, df=1, p<.005, 2-tailed) and the odds ratio was calculated to be 6.5 (Kjellström et al. 1986).

When the children were six years old, each high mercury child was matched with three other 'control' children based on maternal and child characteristics and also on maternal hair mercury (Kjellström et al. 1989). One group of control children had maternal hair mercury in the range of 3–6 mg/kg. Another group of control children had maternal hair mercury in the range of 0–3 mg/kg and mothers who consumed fish more than three times a week. A third group of control children had maternal hair mercury also in the range of 0–3 mg/kg but their mother consumed fish no more than three times a week. A total of 238 children (including 57 complete groups of matching children) were tested with several psychological, behavioural, and scholastic tests. Results demonstrated an association between prenatal MeHg exposure in fish and a decreased performance in these tests. An average hair mercury level during pregnancy of 13–15 mg/kg (corresponding to a monthly peak hair mercury of 25 mg/kg) was found to be associated with decreased test performance and statistically non-significant reduction in test performance was observed in the group with maternal hair mercury levels in the range of 6–10 mg/kg.

2.2.2.2 The Faroe Islands Study

The Faroe Islands birth cohort was recruited from three Faroese hospitals from March 1986 through to December 1987 (Grandjean et al. 1992). Umbilical cord blood from 1023 infants and maternal hair sample were analysed. Frequent ingestion of whale meat dinners, and to a lesser extent, fish consumption during pregnancy was associated with high mercury concentrations in cord blood and hair. Follow-up neurobehavioral assessments of these children were conducted at aged seven (n=917) and fourteen years (n=860). Whilst physical examination and neurophysiological testing did not show any mercury-related abnormalities during follow-up assessments at aged seven years, decreased neuropsychological functions were associated with higher umbilical cord blood mercury concentration, an indicator of prenatal MeHg exposure (Grandjean et al. 1997). Multiple regression analysis with adjustment for covariates showed decrease in performance in psychomotor function (Neurobehavioral Evaluation System 2 (NES2) Finger Tapping with preferred hand – Beta=-1.10, p=.05), attention (NES2 Continuous Performance Test log of total missed responses – Beta= 0.12, p=.02; NES2 Continuous Performance Test average reaction time – Beta=40.3, p=.001; Wechsler Intelligence Scale for Children Digit

Spans – Beta= -.0.27, p=.05), memory function (California Verbal Learning Test Short-term reproduction – Beta=-0.57, p=.02; California Verbal Learning Test Long-term reproduction – Beta=-0.55, p=.05), and language (Boston Naming Test no cues – Beta=-1.77, p=.0003; Boston Naming Test with cues Beta=-1.91, p=.0001). Similar results were obtained when these children were re-assessed at aged 14 years suggesting cognitive deficits from prenatal MeHg exposure might be permanent (Debes et al. 2006). At 14 years, higher cord blood concentrations were associated with lower finger tapping scores (p=.033), NES2 Continuous Performance Test average reaction time (p=.018), and Boston Naming Test with cues (p=.048). Postnatal MeHg exposure, as determined by children's hair collected at seven and fourteen years, appeared to be lesser of a risk predictor (Grandjean et al. 1997; Debes et al. 2006). The effects of mercury exposure therefore might be more prominent during foetal development stage.

2.2.2.3 The Seychelles Child Development Study

The Republic of Seychelles was chosen as the study site because of the high fish consumption in the Seychellois. The specific aim of the study was to establish if there was a relationship between foetal exposure to MeHg from maternal dietary fish and the development of their offspring in early childhood. (Marsh et al. 1995). The study was conducted in two phases; a cross-sectional pilot study which started in 1987 (n=789 mother-infant pairs) and a double-blind prospective longitudinal main study which started in 1989 (n=740 mother-infants pairs). The purpose of the pilot study was to guide the design of the main study. Prenatal MeHg exposure was estimated by measuring THg in maternal hair collected during enrolment when the infants were six months old. Assuming a growth rate of 1.1 cm/month, a segment of maternal hair representing growth during pregnancy was analysed for mercury.

For the main study, follow up of children were initially scheduled at 6.5, 19, 29, and 66 months of age, but was further extended to include assessment at 9 and 17 years. The median mercury level for the 740 mothers was 5.9 µg/g hair during pregnancy with an interquartile range of 6.0 µg/g (Myers et al. 1995). At 6.5 months, these children were administered the Fagan Test of Infant Intelligence (for testing of visual recognition memory and visual attention), the Denver Developmental Screening Test-Revised (for measures of personal-social, fine motor adaptive, language, and

gross motor development) as well as undergoing neurological examination by an experienced paediatric neurologist. No association between maternal hair mercury levels and adverse neurodevelopmental outcomes could be found.

At the 19-month assessment interviews, the primary care-givers were asked to recall their child's age at walking and at talking (n=738) (Myers et al. 1997). No associations were observed between prenatal MeHg exposure and these developmental milestones except for the age of walking in male (p=.043). However, when the five statistical outliers were excluded from the analysis, the association was no longer was significant (p=.16). Children were evaluated with the Bayley Scales of Infant Development, a measure of motor, language and cognitive development, at this interview and again at 29 months (n=736) (Davidson et al. 1995). No mercury-related effects were observed with the MDI (as analysed by multiple regression with adjustment for covariates) nor the PDI (as analysed by logistic regression) at either time point. The additional BSID Infant Behaviour Record conducted at 29 months, however, did show a significantly lower activity level in males (partial R^2 value for mercury=1.3, p=.0004) but not in females (partial R^2 value for mercury=0.005, p=.87) as maternal hair mercury level increased. The authors cautioned the interpretation of this finding as many factors could have influenced the children's activity levels.

When the children were at 66 months, a battery of tests were administered that included the General Cognitive Index of the McCarthy Scales of Children's Abilities (for cognitive ability), the Preschool Language Scale (for expressive and receptive language ability), the Letter and Word Recognition (for reading ability) and the Applied Problems (for mathematics ability) of the Woodcock-Johnson Tests of Achievement, the Bender Gestalt test (for visual-spatial ability), and the Child Behaviour Checklist (for the child's social and adaptive behaviour) (Davidson et al. 1998). Multiple regression analysis showed no negative associated between prenatal MeHg exposure with outcome scores, but other factors such as home environment, socio-economic status of the family, caregiver's IQ and the child's gender were shown to be related to development.

The results of tests conducted at 9 years (Myers et al. 2003) and 17 years of age (Davidson et al. 2011), as with earlier reports, showed no consistent adverse

associations between developmental/ behavioural outcomes and prenatal MeHg exposure. For some neurodevelopmental endpoints, performance even improved with increasing prenatal MeHg exposure (e.g. reduction in total trials to complete the Cambridge Neuropsychological Test Automated Battery Intra-Extra Dimensional Shift Set and improved Woodcock-Johnson Test of Scholastic Achievement-II Calculation scores) (Davidson et al. 2011). As one would not expect prenatal MeHg exposure to produce favourably effects, the authors therefore suggested that prenatal MeHg was most likely a surrogate marker for fish consumption and the improved performance reflects the influence of the LCn3PUFA in fish.

2.2.2.4 The Avon Longitudinal Study of Parents and Children (ALSPAC study)

This UK prospective cohort study enrolled more than 14 000 pregnant women between 1991 and 1992 and was designed to assess environmental factors (including diet) during and after pregnancy that might influence the development and growth of their offspring. All newborns were followed and information obtained regarding their health and development. Data for maternal food consumption were obtained when the mothers were at 32 weeks' gestation by self-completed food frequency questionnaire. Analysis of the 7421 mother and child pairs by Daniels et al. (2004) demonstrated that maternal fish intake during pregnancy was associated with higher mean developmental scores when the children were assessed by MacArthur Communicative Development Inventory at 15 months of age and the Denver Developmental Screening Test at 18 months. Hibbeln et al. (2007) further showed that maternal seafood intake of less than 340g per week was associated with increased risk of sub-optimal outcomes in their children for verbal intelligence quotient, prosocial behaviour, fine motor, communication and social development scores when children were assessed at various time points up to eight years of age. Another finding recently published from this study indicated that there was a significant increase in the odds of pregnant women experiencing high anxiety symptoms at 32 weeks of gestation in those who rarely or never consumed dark and oily fish when compared with those who consumed once to three times a week or more (Vaz et al. 2013).

2.2.2.5 Danish National Birth Cohort Study (DNBC)

The DNBC enrolled just over 100 000 pregnant women from 1996 to 2002 to investigate the various exposures from conception to early childhood and their impact on health later in life (Olsen et al. 2001). Maternal dietary assessment was conducted at 25 weeks' gestation via self-completed semi-quantitative food frequency questionnaire. Analysis of the 44 824 mother-infant pairs showed high intake of fish was inversely related to foetal growth (Halldorsson et al. 2007). In the highest consumption group (total fish intake >60 g/day), when compared to the low consumption group (total fish intake ≤5 g/day), the difference in birth weight was -25.2 g (95% CI, -47.4– -3.0), birth length was -0.08 cm (95% CI, -0.18–0.02), and head circumference was -0.11 cm (95% CI, -0.18– -0.03) after adjustment. On closer examination, these differences were driven by the consumption of fatty fish and no association was observed for lean fish consumption. The authors suggested that this observation of an inverse relationship with fatty fish consumption might be due to contamination of fatty fish by persistent organic pollutants. Child developmental milestones were assessed by interview with mothers at 6 and 18 months. Information were available from 25 446 mother-child pairs and higher maternal fish intake was shown to be associated with higher child developmental scores at 18 months (OR, 1.29; 95% CI, 1.20–1.38 for the highest versus the lowest quintile of fish intake, after adjusting for parental and child characteristics and duration of breastfeeding) (Oken et al. 2008a). High (*vs*. no) maternal fish intake during pregnancy was found to be protective against both early and ever asthma in seven year-old children according to Maslova et al. (2013). A total of 21 293 and 17 399 children from the DNBC were followed at 18 months and seven years of age respectively. After adjusting for potential confounders, an association for doctor-diagnosed asthma at 18 months and fish consumption was found (zero *vs*. high frequency intake: OR, 1.30; 95% CI, 1.05–1.63, p=.02). Similarly, at seven years of age, children whose mothers never consumed fish during pregnancy were more likely to have an asthma diagnosis (zero *vs*. high frequency intake: OR, 1.46; 95% CI, 0.99–2.13; p=.05) and been prescribed asthma medications (zero *vs*. high frequency intake: OR, 1.37; 95% CI. 1.10–1.71; p=.01). No association between fish intake and risk of postpartum depression hospital admission was found in the analysis of 54 202 women from this DNBC (intake of 0–3 g/day *vs*. >30 g/day of

fish: Adjusted OR, 0.82; 95% CI, 0.42–1.64) (Strom et al. 2009). In terms of postpartum depression prescription, low fish consumption was associated with higher risk (intake of 0–3 g/day *vs.* >30 g/day of fish: Adjusted OR, 1.46; 95% CI, 1.12–1.90).

2.2.2.6 Project Viva

Project Viva, a National Institutes of Health funded project in the US, followed just over 2000 women and their offspring from 1999 and suggested that higher fish intake was associated with reduced risk for pre-eclampsia (Oken et al. 2007), higher infant cognition at 6 months of age (assessed for visual recognition memory) (Oken et al. 2005) and better child cognitive test performance at 3 years of age (assessed using Peabody Picture Vocabulary Test and Wide-Range Assessment of Visual Motor Abilities) (Oken et al. 2008b). Frequency of fish consumption during pregnancy was shown not to be associated with length of gestation or risk of pre-term birth in the study; in fact there was a trend towards an inverse association between fish consumption and birth weight and foetal growth, although the harmful effect of this slightly reduced foetal growth was expected to be small (Oken et al. 2004).

2.2.2.7 Generation R study

Generation R study is a population-based prospective cohort study conducted in Rotterdam, Netherlands, to identify early environmental and genetics causes of normal and abnormal growth, development, and health (Jaddoe et al. 2010). Women with a delivery date between April 2002 and January 2006 were eligible to participate. In total, 9778 women and 9745 infants were enrolled in the study. Assessments were conducted in early, mid- and late pregnancy and the birth cohort would be followed until young adulthood. The fathers were also assessed once during their partner's pregnancy. Amongst other data collection, a semi-quantitative food frequency questionnaire was administered at enrolment with the aim of capturing the dietary intake within the first trimester of pregnancy. Analysis of 3380 mother-infant pairs in this cohort showed no consistent associations between consumption of total fish, lean fish or fatty fish with foetal growth characteristics during the second or third trimesters or at birth, after adjusting for potential confounders (Heppe et al. 2011). Leermakers et al. (2013) examined the dietary of

2796 mothers during pregnancy and similarly observed no consistent associations of maternal total fish, lean fish or fatty fish consumption with wheezing of their child up to four years of age. An association, however, was found between maternal fatty (but not total or lean) fish intake of 35–69 g/week and risks of eczema in their offspring (OR, 1.17; 95% CI, 1.00–1.38) and yet the highest category of fatty fish consumption (>70 g/week) showed no such association (OR, 1.06; 95% CI, 0.88–1.38).

2.2.3 Considerations by Australian and overseas health organisations

2.2.3.1 The National and Medical Research Council

In 2008, the NHMRC commissioned the conduction of literature reviews on food-diet-health-disease inter-relationships to support the revision of the Australian Dietary Guidelines. Whilst evidence statements can be made regarding the association of cardiovascular disease, dementia in older adults, depression, age-related macular degeneration, stroke, and several cancer types with fish consumption, there was inadequate evidence base to create an evidence statement related to infant cognition and fish consumption.

2.2.3.2 The Institute of Medicine of the National Academies of Science (IOM)

In the report released by the IOM, Seafood Choices: Balancing benefits and risks (Nesheim & Yaktine (eds) 2006), scientific evidence of health benefits associated with nutrients in seafood and health risks associated with seafood consumption was examined. The Committee responsible for this task, however, found that it was not feasible to formulate a quantitative benefit-risk assessment and balancing and therefore produced a qualitative scientific benefit-risk analysis and balancing of the benefits and risks of seafood consumption. The Committee identified four population groups for which evidence supports conclusions about their benefits or risks from eating seafood. The four populations groups are (1) Females who are or may become pregnant or who are breast-feeding (2) Children up to 12 years of age (3) Healthy adolescent and adult males and females (who will not become pregnant) and (4)

Adult males and females who are at risk of coronary heart disease. For the group of interest in this thesis, i.e. females who are or may become pregnant or who are breast-feeding, it was concluded that there may be benefit from consuming seafood, especially those with relatively higher concentrations of EPA and DHA. These women can safely consume 12 ounces (or about 340 g) of cooked seafood per week (up to 6 ounces or about 170 g per week if consuming albacore tuna) and should also avoid large predatory fish.

2.2.3.3 Food and Agricultural Organization of the United Nation (FAO) and the World Health Organization (WHO)

A published report by the FAO and WHO (FAO & WHO 2011) quantified the relationship between maternal DHA consumption and neurodevelopment based on the findings of two of the above mentioned studies, the ALSPAC study and the Project Viva study. The report suggested that an average IQ gain of 4.0 points was associated with each 100mg of DHA intake per day with 5.8 IQ points being the maximum potential IQ gain from maternal DHA consumption. The conclusion was that the benefits of LCn3PUFA from fish outweigh the risks of MeHg, and under most circumstances, maternal fish consumption lowers the risk of sub-optimal neurodevelopment in their offspring.

2.2.4 Conclusion

Fish is one of those foods that contains both beneficial nutrients (e.g. LCn3PUFA) and harmful contaminants (e.g. MeHg). Maternal intake of LCn3PUFA have been shown to produce positive neurodevelopment in their offspring while prenatal MeHg exposure demonstrated negative effects. The net health effect in fish consumption will therefore depend on the composition of the fish consumed and it is still unclear at which point when the benefits of LCn3PUFA outweighs the risks of MeHg exposure. The concurrent recommendation is therefore to include fish as part of a healthy diet, and in particular for women of child-bearing age, to consume fish that is high in LCn3PUFA and low in mercury.

2.3 Final conclusion

Based on the findings of all RCTs and systematic reviews combined, it appears that Ln3PUFA supplementation during pregnancy and/or lactation have limited effects on the maternal and infant outcomes discussed other than prolonging gestation by a few days and the subsequent higher birth weights. Other potential benefits although plausible had inconsistent findings. The inconsistency could in part explained by the heterogeneity of the study designs, with varying doses, different length and time of supplementation, different background diets of the participants, and different assessment tools applied. In terms of the effect of higher fish intake, very few RCTs have been conducted and the majority of the evidence were from observational or epidemiological studies. Also lacking in the literature were studies conducted in women prior to conception. It could be that the window of opportunity may have already been missed if supplementation were to commence after the first trimester. Although LCn3PUFA at this stage cannot be routinely recommended as a supplement during pregnancy, adequate intake from diets should be advocated as they are essential for normal, optimal development of the infants.

Chapter 3 Fish consumption in Australian women of child-bearing age

According to the Food and Agriculture Organization of the United Nations (FAO) (FAOSTAT 2012), the supply of fish and seafood available for human consumption in Australia has nearly doubled from 13.0 kg/capita/year in 1970 to 24.9 kg/capita/year in 2009 (Figure 3.1). These apparent consumption data represent the difference between the sources (commercial production + import + opening stock) and utilisation (export + fed to livestock + wastage + closing stock), divided by the population during that period.

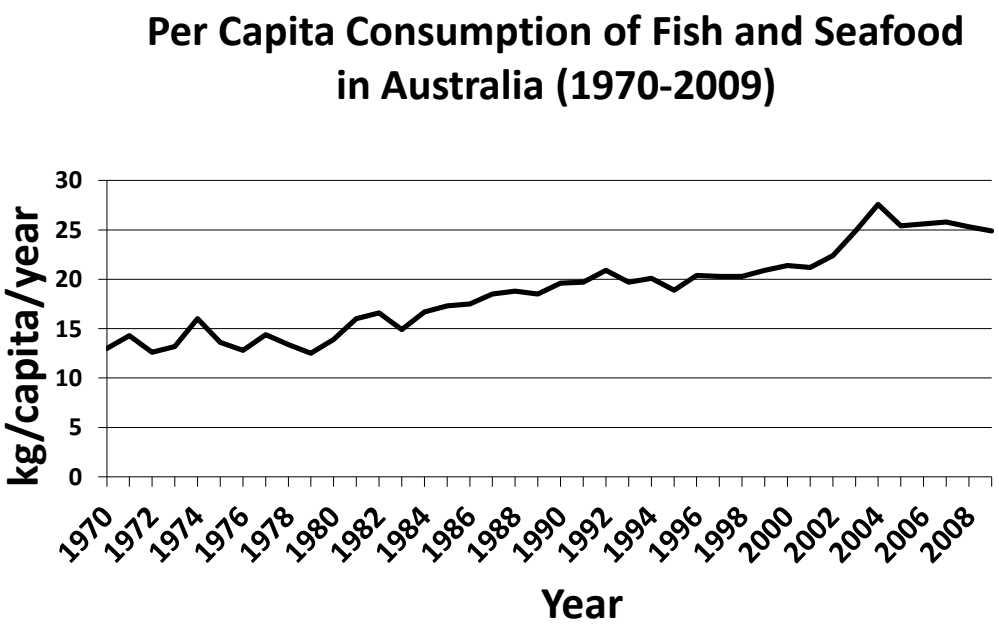


Figure 3.1 Per capita consumption of fish and seafood in Australia 1970–2009 according to the Food and Agriculture Organization of the United Nations (FAO) ***Source: FAOSTAT***

The per capita consumption of 24.9 kg/capita/year in 2009 for Australia was just above the median amongst the developed countries in the North American,[1] Asian and Oceanic and the European Union regions. The Portuguese and Japanese are high

[1] No data available for Greenland and Saint Pierre and Miquelon.
[2] The terminology 'extra food' has been replaced by 'discretionary choices' in the new Australian

consumers of fish and seafood at 61.1 and 56.6 kg/capita/year respectively (Figure 3.2).

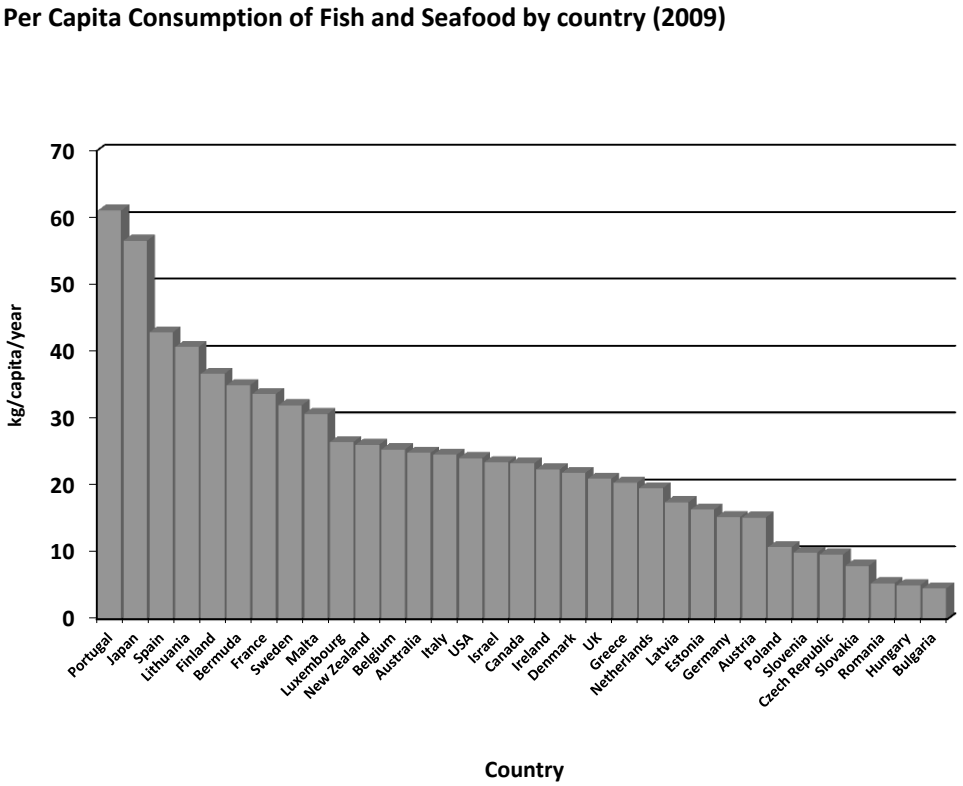


Figure 3.2 Per capita consumption of fish and seafood of developed countries within the North American, Asian and Oceanic and the European Union regions in 2009 according to the Food and Agriculture Organization of the United Nations (FAO) ***Source: FAOSTAT***

However, per capita 'consumption' represents 'food available for consumption' which is not the same as 'food intake'. Per capita 'consumption' data are calculated for the population as a whole but do not provide information on food consumption of subpopulations. They are useful for demonstrating trends and changes in food availability. 'Food intake' data are generally obtained from food records, food recalls or food frequency questionnaires administered to individuals. The Australian Dietary Guidelines released in February 2013 recommend that adult Australians and older children (who eat foods from animal sources) consume two serves of fish per week where a standard serve of fish is equivalent to 100 g cooked (or 115 g raw) fish fillet or one small can of fish (NHMRC 2013a). This recommendation holds true for pregnant women, except pregnant women are advised to also follow the guidance

from FSANZ in limiting their consumption of particular fish species as previously described in Section 1.1. In this chapter, the aim is to quantify the fish intake in Australian women of child-bearing age and to compare adherence to intake recommendations. Fish intake data were obtained from two studies of national population samples, the 1995 National Nutrition Survey (NNS) and the Australian Longitudinal Study on Women's Health (ALSWH). Although the 1995 NNS could be seen as out-dated, it is still the latest and most comprehensive national intake data available for use at this point. Using data from ALSWH allows examination of intake of a longitudinal nature.

3.1 Data from 1995 National Nutrition Survey (NNS)

3.1.1 1995 NNS Survey and analysis methods

The 1995 NNS was conducted between February 1995 and March 1996, and collected information on food and beverage intake, physical measurements, food-related habits and attitudes, and average food consumption over the previous months in a representative sample of Australians (McLennan & Podger 1998). This was a joint project between the then Commonwealth Department of Health and Family Services (now Department of Health) and the Australian Bureau of Statistics (ABS). Participants were interviewed at their own homes. Data on food and beverage intakes were collected via 24-hour recalls. Also included in the survey was a series of food-related questions that provided additional information on eating habits, barriers to dietary change and food security. Participants aged 12 years and over were left with a food frequency questionnaire for them to mail back when completed. A second 24-hour recall was conducted with 10% of the participants. Overall, information was obtained from 13 858 persons aged two years and over who agreed to take part in the survey. Data from both the first 24-hour recalls and food frequency questionnaire will be presented here as 24-hour recalls provide a snapshot of the population intakes on the day of the survey whereas food frequency questionnaires give a better estimation of usual intakes.

In the following analysis, only data from women aged 18–49 years were included, representing women of child-bearing age. Fish consumption data were estimated

from the 24-hour recall provided on the first interview as well as the food frequency questionnaire. Estimates were weighted according to the appropriate sample weighting factors provided in the dataset to account for the person's probability of being selected into the sample in order to provide a national estimate (ABS 2001). In order to calculate the combined frequency of all fish consumption (i.e. combining canned fish, steamed/baked/grilled fish and fried fish), individual frequency responses were converted into weekly equivalent frequencies in the following manner: 'Never or less than once a month' = 0.13; '1–3 times per month' = 0.50; 'Once per week' = 1.00; '2–4 times per week' = 3.00; '5–6 times per week' = 5.50; 'Once per day' = 7.00; '2–3 times per day' = 17.50; '4–5 times per day' = 31.50; '6 or more times per day' = 42.00. Individual frequency responses were then added up to give the frequency of consumption of all fish types.

3.1.2 1995 NNS Survey results

There were 3576 women aged 18–49 years who took part in the 1995 NNS and provided food and beverage intake data by 24-hour recall. Of these women, 2649 completed a usable food frequency questionnaire and provided answers to all three questions relating to fish intakes (i.e. canned, steamed/baked/grilled and fried fish).

Results from 24-hour recall

In 1995, 17.9% or approximately 1 in 5 Australian women of child-bearing age reported eating fish or seafood on the day of the survey (Table 3.1).

Table 3.1 Proportion of women (18–49 years) consuming fish and seafood products and dishes on the day of the 1995 National Nutrition Survey by 24-hour recall (n=3576)

Fish and seafood products and dishes sub-groups	Proportion of persons consuming (%)
Fin fish (excluding canned)	4.1
Crustacea and molluscs (excluding canned)	3.2
Other sea and freshwater foods	0.0
Packed (canned and bottled) fish and seafood	4.3
Fish and seafood products	5.7
Mixed dishes with fish or seafood as major component	2.9
Any fish or seafood products and dishes	17.9

The mean intake of all fish and seafood products and dishes combined was estimated to be 22.6 g per day or about 158 g per week (Table 3.2).

Table 3.2 Mean daily intake of fish and seafood products and dishes on the day of the 1995 National Nutrition Survey by 24-hour recall (n=3576)

Fish and seafood products and dishes sub-groups	Mean grams (s.d.) per person
Fin fish (excluding canned)	4.4 (27.0)
Crustacea and molluscs (excluding canned)	2.5 (19.1)
Other sea and freshwater foods	0.0 (0.2)
Packed (canned and bottled) fish and seafood	2.9 (17.3)
Fish and seafood products	5.5 (29.6)
Mixed dishes with fish or seafood as major component	7.3 (56.5)
All fish and seafood products and dishes in total	**22.6 (73.6)**

Since over 80% of the participants did not report eating any fish or seafood on the day of the survey, the median fish and seafood intake for the total population was 0 g/day. Table 3.3 shows the median intake of different categories of fish and seafood amongst consumers (n range 1–609). The median intake of all fish and seafood products and dishes combined was estimated to be 90.0 g per day.

Table 3.3 Median daily intake of fish and seafood products and dishes amongst consumers on the day of the 1995 National Nutrition Survey by 24 hour recall

Fish and seafood products and dishes sub-groups (n within sub-groups)	Median grams (IQR) per consumer
Fin fish (excluding canned) (n=133)	82.5 (47.5–141.7)
Crustacea and molluscs (excluding canned) (n=99)	48.0 (26.2–100.0)
Other sea and freshwater foods (n=1)	13.8 (13.8–13.8)
Packed (canned and bottled) fish and seafood (n=155)	51.3 (33.6–90.0)
Fish and seafood products (n=210)	75.00 (46.0–135.0)
Mixed dishes with fish or seafood as major component (n=94)	209.2 (105.0–326.0)
All fish and seafood products and dishes in total (n=609)	**90.0 (48.0–157.5)**

Results from food frequency questionnaire

Table 3.4 shows the proportion of women who consumed fish twice a week or more as reported in the food frequency questionnaire. When all fish types were combined, around 25% of women of child-bearing age consumed some fish twice a week or more.

Table 3.4 Proportion of women (18–49 years) who consumed fish twice a week or more as reported in the 1995 National Nutrition Survey Food Frequency Questionnaire (n=2649)

Types of fish	Frequencies of consumption		
	Less than once a week (%)	At least once a week but less than twice a week (%)	Twice a week or more (%)
Canned tuna, salmon, sardines	76.0	18.1	5.9
Fish, steamed, baked, grilled	78.1	17.1	4.8
Fish, fried	89.7	7.9	2.4
All fish types combined	**36.4**	**38.5**	**25.2**

3.2 Data from the Australian Longitudinal Study on Women's Health (ALSWH)

3.2.1 ALSWH Survey and analyses methods

The ALSWH is a collaborative project conducted by staff and investigators at The University of Newcastle and The University of Queensland with funding from the Australian Government Department of Health and Ageing (details are available from the Women's Health Australia's web site <http://www.alswh.org.au/>). The ALSWH is a longitudinal survey of over 40 000 women in three cohorts who were aged 18–23 (young), 45–50 (mid-age) and 70–75 (old) when surveys began in 1996 and agreed to participate in the project for 20 years. Participants were randomly selected from the Medicare database, with oversampling of women from rural and remote areas. Each age cohort was surveyed once every three years via surveys sent in the mail. The project assesses physical and emotional health, use of health services, health behaviours and risk factors, time use, socio-demographic factors, life stages and key events.

In the following analysis, the cohort of interest was the group of 14 247 women who were aged 18–23 years when they first took part in Survey 1 in 1996. They were surveyed again in 2000 (Survey 2), 2003 (Survey 3), 2006 (Survey 4), 2009 (Survey 5) and 2012 (Survey 6) with one final survey, which is beyond the scope for inclusion in this thesis, scheduled for 2015 (Survey 7).

In both Survey 3 and Survey 5, the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2) was included as a survey item. The DQES v2 assessed usual eating habits over the past 12 months (Cancer Council Victoria 2012). It comprised a food list of 74 items with 10 frequency response options ranging from 'never' to '3 or more times per day'. It also had three photographs of scaled portions of four foods (potato, vegetables, steak and casserole) so that participants could indicate the average amount eaten at main meals of each food. Responses to these portion size questions were then used to calculate a single portion size factor for the individual, which was in turn used for estimating the amount of all foods consumed. Frequency responses were converted to daily equivalent frequency scores in the following manner: 'Never' = 0.00; 'Less than once per month' = 0.02; '1–3 times per month' = 0.07; 'Once per week' = 0.14; 'Twice per week' = 0.28; '3–4 times per week' = 0.50; '5–6 times per week' = 0.78; 'Once per day' = 1.00; 'Twice per day' = 2.00; '3 or more times more day' = 3.00. Weekly frequency scores were obtained by multiplying the daily equivalent frequency scores by 7. Similarly, the estimated daily intake amounts were multiplied by 7 in order to convert to weekly amounts. Note that this frequency conversion procedure varies from the one used for the 1995 NNS in the previous section due to differing response options.

Fish consumption patterns were estimated from these food frequency questionnaires administered in Survey 3 (2003) and Survey 5 (2009). Respondents were asked how often they ate, on average, (i) fish, steamed, grilled or baked; (ii) fish, fried (include take-away); and (iii) fish, tinned (salmon, tuna, sardines etc.) during the previous 12 months. As the survey provided information in relation to the respondents' pregnancy status, fish intakes were also examined in terms of pregnancy status namely (1) pregnant at the time of completing the survey; (2) trying to conceive; (3) had given birth during the previous 12 months; or (4) all other women who did not fit into categories 1–3. Excluded from the analyses were those women whose pregnancy status could not be identified or could fall into more than one category. In addition

those women with calculated energy intake <4.5 or >20.0 MJ/day were also excluded as these energy values were considered biologically improbable (Meltzer et al. 2008). This approach of categorising women into their pregnancy status was adopted from the study by Hure et al. (2009) who examined the diet quality of women who took part in Survey 3.

Descriptive statistics were weighted by the area of residence, weighting factors to adjust for the initial oversampling in rural and remote areas. Inferential statistics using the method of generalised estimating equations (GEE) were used to explore if fish consumption changed with time, adjusting for energy intake and area of residence (urban/rural/remote). Further analysis was conducted to investigate if fish consumption was associated with pregnancy status or not, adjusting for energy intake, area of residence (urban/rural/remote), household income ($699 per week or less/$700–$1499 per week/$1500 per week or more), education (no formal or Year 10/Year 12/trade or apprenticeship/university degree or higher) and living with children (yes/no). The GEE procedure was used as it takes into account correlations within each participant in longitudinal studies.

3.2.2 ALSWH Survey results

Results from Survey 3 (2003) and Survey 5 (2009) food frequency questionnaire

Table 3.5 The number of women included and excluded from analysis

Categories	**2003**	**2009**
Included in analysis	**7477**	**6785**
Pregnant	599	669
Trying to conceive	452	580
Given birth during previous 12 months	829	1104
Other	5597	4432
Excluded from analysis	**1604**	**1415**
Pregnancy status could not be identified	111	56
Could be classified into more than one category	72	109
Calculated energy value <4.5 or >20.0 MJ/day	1416	1250
No food frequency data available	5	0
Total number of women who took part in survey	**9081**	**8200**

Ninety-two and a half (92.5%) and 93.6% of the respondents indicated that they had consumed some fish in the previous 12 months when surveyed in 2003 (Survey 3) and 2009 (Survey 5) respectively. Table 3.6 and Table 3.7 show the mean and median fish intake weekly frequency scores obtained from the respective surveys.

Table 3.6 Mean weekly intake frequency scores of fish in the young cohort of the Australian Longitudinal Study on Women's Health who consumed fish twice a week or more in 2003 and 2009 as estimated by the Dietary Questionnaire for Epidemiological Studies (Version 2)

Types of fish	Mean weekly frequency scores (s.d.)	
	Survey 3 (2003)	Survey 5 (2009)
Fish, tinned (salmon, tuna, sardines etc.)	0.9 (1.3)	1.1 (1.5)
Fish, steamed, baked or grilled	0.8 (1.0)	0.9 (1.1)
Fish, fried (include take-away)	0.3 (0.5)	0.3 (0.5)
All fish types combined	**2.0 (2.0)**	**2.3 (2.3)**

Table 3.7 Median weekly intake frequency scores of fish in the young cohort of the Australian Longitudinal Study on Women's Health who consumed fish twice a week or more in 2003 and 2009 as estimated by the Dietary Questionnaire for Epidemiological Studies (Version 2)

Types of fish	Median weekly frequency scores (IQR)	
	Survey 3 (2003)	Survey 5 (2009)
Fish, tinned (salmon, tuna, sardines etc.)	0.5 (0.1–1.0)	0.5 (0.1–2.0)
Fish, steamed, baked or grilled	0.5 (0.1–1.0)	0.5 (0.5–1.0)
Fish, fried (include take-away)	0.1 (0.0–0.5)	0.1 (0.0–0.5)
All fish types combined	**1.5 (0.8–2.6)**	**2.0 (1.0–2.9)**

Table 3.8 and Table 3.9 show the mean and median daily intake (of the total population, i.e. including consumers and non-consumers) of different types of fish estimated from Survey 3 and Survey 5 respectively.

Table 3.8 Mean daily intake of fish products in the young cohort of the Australian Longitudinal Study on Women's Health in 2003 and 2009 as estimated by the Dietary Questionnaire for Epidemiological Studies (Version 2)

Fish sub-groups	Mean grams (s.d.) per person	
	2003 (n=7477)	2009 (n=6785)
Fish, tinned (salmon, tuna, sardines etc.)	11.2 (17.6)	13.0 (18.3)
Fish, steamed, baked or grilled	15.2 (20.6)	17.0 (20.1)
Fish, fried (include take-away)	6.0 (10.7)	5.6 (9.3)
All fish types combined	**32.4 (36.1)**	**35.6 (35.3)**

Table 3.9 Median daily intake of fish products in the young cohort of the Australian Longitudinal Study on Women's Health in 2003 and 2009 as estimated by the Dietary Questionnaire for Epidemiological Studies (Version 2)

Fish sub-groups	Median grams (IQR) per person	
	2003 (n=7477)	2009 (n=6785)
Fish, tinned (salmon, tuna, sardines etc.)	5.7 (1.4–13.9)	6.9 (1.6–16.4)
Fish, steamed, baked or grilled	9.9 (2.9–19.1)	12.2 (5.1–21.2)
Fish, fried (include take-away)	2.8 (0.0–8.2)	2.6 (0.0–7.7)
All fish types combined	**23.3 (11.0–42.5)**	**27.1 (14.6–46.1)**

Overall, analysis showed that there was an increase in the proportion of the women who consumed fish (in any amount) (OR=1.19, p<.001), who consumed fish at least twice each week (OR=1.39, p<.001) and who consumed 200 g or more of fish each week (OR=1.30, p<.001) from Survey 3 to Survey 5 (Table 3.10), adjusted for energy intake and area of residence.

Table 3.10 Fish consumption pattern in the young cohort of the Australian Longitudinal Study on Women's Health in 2003 and 2009 as estimated by the Dietary Questionnaire for Epidemiological Studies (Version 2)

Consumption characteristics	N (%)*	
	Survey 3 (2003)	Survey 5 (2009)
Number of participants who reported having consumed some fish in the previous 12 months	6912 (92.5%)	6364 (93.6%)
Number of participants who reported usual fish intake of twice a week or more	2552 (35.5%)	2870 (42.7%)
Number of participants who reported usual fish intake of 200 g or more each week	3007 (41%)	3193 (47.2%)
Total number of participants	**7477**	**6785**

* N are actual numbers, percentages are weighted by the 'area of residence' to adjust for the initial oversampling of women in rural and remote areas, hence the percentages above shown do not necessarily correspond to percentages obtained by direct calculation of N values.

To further explore if pregnancy status affects fish intake, the mean daily fish intake was compared amongst the four different pregnancy status groups and the results are shown in Figure 3.3.

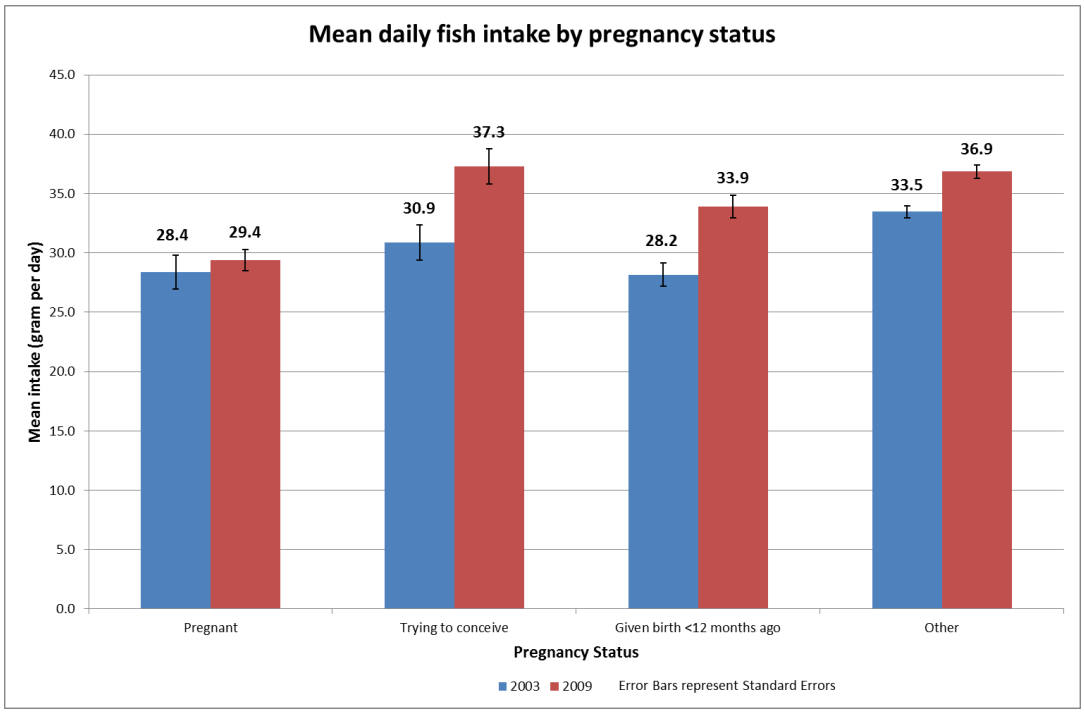


Figure 3.3 Mean fish daily intake in 2003 and 2009 by pregnancy status in the young cohort of the Australian Longitudinal Study on Women's Health as estimated by the Dietary Questionnaire for Epidemiological Studies (Version 2)

Compared to ‘other women’, women who were pregnant (OR=0.64, p<.001) or had given birth <12 months ago (OR=0.80, p<.001) at the time of survey were less likely to consume ≥200 g of fish/week, adjusted for energy intake, area of residence (urban/rural/remote), household income ($699 per week or less/$700–$1499 per week/$1500 per week or more), education (no formal or Year 10/Year 12/trade or apprenticeship/university degree or higher) and living with children (yes/no).

3.3 Discussion

Analysis of the 1995 NNS showed that the mean daily intake of fish and seafood products in women of child-bearing age (18–49 years) was 22.6 g per day. This equates to about 158 g or about one and a half serves per week if using the serving size suggested by the Australian Dietary Guidelines 2013, which is less than the recommendation of two serves per week. If intakes were to compare with dietary guidelines at the time (i.e. Dietary Guidelines for Australians 1992), adherence might be even lower as the then guidelines suggested a weekly intake of two to three serves of fish although no serving size was included in the guidelines (NH&MRC 1992). When examining the results from the food frequency questionnaire aimed to estimate usual food intake, only about a quarter of the women were consuming fish (excluding seafood) at least twice a week on a regular basis. One might argue that intake inadequacy cannot be inferred from the results of this food frequency questionnaire as no quantity was specified. Respondents could be consuming in larger amounts per eating occasion, for example, having one serve of 200 g once a week instead of one serve of 100 g twice week. However, this is unlikely to be case as the median daily intake among consumers from the 24-hour food recall in this same survey was estimated to be around 90 g.

When reviewing the ALSWH data, the mean daily intake of fish was estimated to be 32.4 g (or 227 g per week) and 35.6 g (or 249 g per week) in 2003 and 2009 respectively. If again using 100 g as the serving size, this equates to 2–2.5 serves per week and appears to meet the recommendation of the Australian Dietary Guidelines 2013 of 200 g per week. However, the proportion of women consuming 200 g of fish or more each week was estimated to be only around 41% and 47% in 2003 and 2009 respectively.

If these intakes were compared to the higher Heart Foundation recommendations of 300–450 g of oily fish per week for the benefit of cardio-protection, then fish consumption in Australian women would be seen as even less adequate. Price, accessibility and availability of good quality fish, lack of preparation skills, preference of family members may all contribute to the low consumption of fish and seafood in Australia (McManus et al. 2007; Neale et al. 2012).

Although current fish consumption is less than ideal, there appears to be an upward trend with time. Fish intake appears to have doubled from two decades ago. The estimated mean daily intake of fish and seafood products in the 1983 National Dietary Survey of Adults was 17 g/day in women 25–34 years and 18 g/day in women 35–44 years of age (Commonwealth Department of Health 1986). This was increased to 22.6 g/day in women aged 18–49 years in the 1995 NNS as shown on page 91. The ALSWH data in 2003 suggested a mean daily fish (excluding seafood) intake of 32.4 g when women were aged 24–31 years, and further increased to 35.6 g in 2009 for women aged 30–37 years. The Victorian Health Monitor, a state-wide survey conducted between 2009 and 2010, estimated a mean daily intake of fish and seafood of 32.6 g per day in women aged 18–75 years (Department of Health 2012). However, these results need to be interpreted with caution as the methodology used in these surveys was not always the same and the reporting age groups also differed. Nevertheless, this observation does correlate with the increased fish consumption data as reported by the FAO.

It is also noteworthy to observe a difference in fish consumption with pregnancy status. In ALSWH, women who were pregnant or had given birth less than 12 months before reported lower mean daily intakes when compared with ‘other women’. One explanation could be the concern of contaminants in fish products, with MeHg being one of the most well-known. In the study by Lando et al. (2012) evaluating the awareness of mercury in food and fish consumption levels among pregnant, postpartum and control (non-pregnant/non-postpartum) women of child-bearing age, pregnant and postpartum women ate less fish than the control group by 26.9 g and 13.3 g per week respectively. Incomplete information and/or the ability to remember which fish types were better to eat during pregnancy is likely to cause pregnant women to reduce or avoid fish just to err on the side of caution

(Bloomingdale et al. 2010) and therefore they may miss out on health benefits to themselves and their offspring.

The strengths of both studies include the large sample size and for the ALSWH, the longitudinal nature of the study design. As with all studies that involves dietary assessment, assessment tools selected may be a limitation for the study. For the 1995 NNS, dietary assessment included both 24-hour recall and food frequency questionnaires whereas the ALSWH included food frequency questionnaires only. A single 24-hour recall does not provide information on usual intake for an individual but is adequate as an estimate of the usual intake distribution within a population. However, 24-hour recall relies on the ability of the participants to remember the food consumed in the past 24 hours and therefore subjected to bias. Food frequency questionnaires assess usual intake by asking about the frequency of specific food or food groups that are eaten from a list. Again biases could be caused by errors in memory and the perception of intake frequencies.

In summary, according to available data, fish consumption in Australian women of child-bearing age appears to be sub-optimal and not meeting intake recommendations. Recent data from the ALSWH suggests that less than 50% of child-bearing women would consume ≥200 g of fish each week or eat fish at least twice a week. In addition, pregnant women and those who had recently given birth were shown to have a lower fish intake compared to other women. This could have public health implications, as adequate fish consumption may confer nutritional benefits to both the mother and her child. The results from the recently conducted National Nutrition and Physical Activity Survey (2011–13) would provide the most up-to-date dietary patterns of Australians, including the consumption of fish and fish products. These results are expected to be released in the first quarter of 2014. It would be useful to examine if the trend in fish consumption in women of child-bearing age has changed further in the last 4–5 years.

Chapter 4 Dietary Modelling—Comparing nutrient profiles of diets of differing fish and seafood contents

4.1 Background

Fish are a good source of protein and many other nutrients such as LCn3PUFA, EPA, DHA, selenium, iodine, and zinc. Studies have shown that adequate intake of DHA during pregnancy is essential to foetal brain and visual development. There is also increasing evidence supporting that pre-conception nutrition is important for fertility and optimal birth outcomes (Ramakrishnan et al. 2012) and therefore women of child-bearing age should maintain a nutritious diet in preparing for pregnancy.

The Australian Dietary Guidelines 2013 recommend that two serves of fish be included each week in the diet, as health benefits of fish may be seen with the consumption of 1.4–2.8 serves (140–280 g) of fish per week for adults (NHMRC 2013b). According to the Nutrient Reference Values (NRVs) defined by the NHMRC, the level of LCn3PUFA intake considered to be adequate is 160 mg per day for adult men and 90 mg per day for adult women. These references of adequate intakes (AI) are based on gender-specific median population intakes in Australia who seemingly had no apparent essential fatty acid deficiency, and therefore do not necessarily represent optimal intakes. The suggested dietary target (SDT), equivalent to the 90th centile of the Australian/New Zealand population, is set at 610 mg for men and 430 mg for women for the reduction of chronic disease risk (NHMRC 2006). This SDT is closer to the Heart Foundation's recommendation of daily intake of 500 mg EPA+DHA in order to lower the risk of coronary heart disease in Australian adults (Colquhuon et al. 2008). The Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition 2011 recommends that adult males, non-pregnant/non-lactating adult females consume 250 mg per day of EPA and DHA combined. For adult pregnant and lactating females, the minimum intake recommended is 300 mg per day of EPA and DHA combined, of which at least 200 mg per day should be DHA. The consensus statements developed by the Perinatal Lipid Intake Working Group in Europe also recommend a minimum intake of 200 mg of DHA per day in pregnant and lactating women (Koletzko et al. 2007).

Currently, no intake recommendation for DHA on its own exists for non-pregnant/non-lactating women.

In Australia, fish and seafood consumption and subsequently LCn3PUFA are generally considered low when compared to these recommendations. Analysis of the 1995 NNS indicated that fish and seafood products and dishes intake in women aged 19 and over was on average 22.6 g per day (McLennan & Podger 1999) and intake of LCn3PUFA was 195 mg per day (EPA: 60 mg; DPA: 52 mg; DHA: 83 mg) (Howe et al. 2006). Fish and seafood intake when including only women of child-bearing age (18–49 years) was the same at 22.6 g per day, as previously shown in Chapter 3. The most frequently consumed fish and seafood in 1995 were tuna and prawn, which are usually classified as low to medium sources of LCn3PUFA.

The aim of this modelling exercise was to demonstrate the number of serves of fish and seafood required to meet LCn3PUFA intake recommendations based on, firstly, varied types of fish and seafood that are commonly consumed (Model 1) and secondly, including only fish with a higher LCn3PUFA content (Model 2). Using the Australian Dietary Guidelines Sample Daily Food Patterns for Adults as food group intake recommendations (NHMRC 2013a), the nutrient profile between a low fish/seafood diet (Model 3) was compared with one with higher fish/seafood content (Model 4) by simulated dietary modelling. The result of this dietary modelling exercise also helped to inform the intervention diet used in the ensuing randomised controlled clinical trial (Chapter 6).

4.2 Method

Model 1: Estimation of the number of serves of fish and seafood required to meet EPA+DHA (250 mg) and LCn3PUFA (430 mg) intake recommendations based on commonly consumed fish and seafood

Establishing fish consumption patterns of Australian women (Step 1)

The Confidentialised Unit Record Files (CURFs) of the 1995 NNS were obtained from the ABS. This provided information regarding the types of fish and seafood consumed by Australians and their frequencies of consumption. As only women of child-bearing age were of interest, data from women aged 19–49 years were used for

this modelling exercise. Note that this population was slightly different to that analysed in Chapter 3 in that 18-year-old women were also excluded. The reason for excluding 18-year-olds was to match the age bands used in the NRVs (19–30 years and 31–50 years).

Defining fatty acids content of fish and seafood (Step 2)

The EPA and DHA content of fish and seafood identified in Step 1 was obtained from the Royal Melbourne Institute of Technology (RMIT) fatty acids database available in the FoodWorks software (Version: 6.0.2562) and exported to an MS Excel spreadsheet. The RMIT database was used as it has more complete fatty acids data on fish and seafood than NUTTAB 2010.

Defining serving sizes of foods (Step 3)

The serving sizes of foods were based on the recommendations of the Australian Dietary Guidelines. The suggested serving size for fish is 100 g cooked fish, or about 115 g if raw. The serving size for canned fish is one small tin which typically equates to around 70 g of fish or seafood.

Simulation (Step 4)

Risk Solver Premium V9.0.4.0 program (Frontline Systems, Inc.), an optimisation and simulation software program using MS Excel as the interface, was used to randomly select a fish or seafood product starting from one product and gradually increasing by one product at a time until intake recommendations for DHA and EPA+DHA were reached. Based on the popularity of the different types of fish and seafood as demonstrated from the 1995 NNS data, selection probability factors were assigned to each fish and seafood type. As such, those fish or seafood that were more commonly consumed, for example, canned tuna and prawns, had a proportionally higher chance of being selected. This random selection process was repeated 1000 times (known as trials) and the average nutrient intake value of all 1000 trials was calculated.

Model 2: Manipulation of fish type (by selecting fish with higher LCn3PUFA content) to meet intake recommendations with fewer fish serves

Using the same fish and seafood identified in Model 1, intakes of EPA+DHA and total LCn3PUFA were estimated if at least one, two or three serves of high LCn3PUFA fish were forced into the intake model (Model 2).

Model 3: Nutrient profile of a diet following the food group intake recommendation of the Australian Dietary Guidelines 2013 but maintaining the current fish consumption pattern (i.e. lower fish intake)

Dietary pattern

The dietary pattern was based on the Australian Dietary Guidelines Sample Daily Food Patterns for Adults 2013 (NHMRC 2013a) (Table 4.1) and the total number of serves of the various food groups over a 14-day period were calculated. Three serves (100 g serve) of non-oily fish per fortnight were included in the 'Lean meat/ poultry/ fish/ eggs/ tofu/ nuts & seeds/ legumes & beans' food group category, as data from the 1995 NNS suggested a mean intake of around 150 g per week.

Food available for selection in model

Foods included in the model were based on food intake data obtained from the 1995 NNS, as this is the most recent available national data on food consumption for Australian adults. Foods that were reported to have been consumed (intake amount greater than '0' gram) by at least 1% of the women were included in the model, except for those food items that either (i) provided no nutrient value, e.g. artificial sweeteners, water, or (ii) were considered as 'extras'[2] in the original Australian Guide to Healthy Eating. The aim was to have for selection from each food group a minimum of five food choices for variety. In cases where less than five items could be identified as frequently consumed food within one food group, the top five most frequently consumed foods were included in the model, even if they were consumed by less than 1% of the women.

[2] The terminology 'extra food' has been replaced by 'discretionary choices' in the new Australian Dietary Guidelines 2013

Food compositional data

The majority of the nutrient data for foods used in the modelling was obtained from FSANZ's NUTTAB 2010 database, the most recent composition database of Australian foods. Frequently consumed foods identified from the 1995 NNS were matched as closely as possible to the foods listed in the NUTTAB 2010 database. Since not every nutrient of interest for the foods was provided by the NUTTAB 2010 database, missing nutrient values were determined by one of the following methods:

- Imputation from FSANZ's AUSNUT 2007 database using food of similar description
- If total LCn3PUFA was listed as '0' for a particular food, then the amount of individual LCn3PUFA (EPA, DPA and DHA) was assumed to be '0' as well
- If the amount of total LCn3PUFA and two out of the three LCn3PUFA were known in a particular food, then the amount of the third LCn3PUFA was calculated by subtraction
- Some EPA, DPA and DHA values were estimated according to the EPA:DPA:DHA ratio obtained from similar foods listed in NUTTAB 2010
- A recipe method was used for several composite food items.

Recommended intakes

Nutrients obtained from the simulations were compared to recommended intake requirements. Estimated average requirement (EAR), recommended dietary intake (RDI), AI, and upper level of intake (UL) of nutrients were obtained from NRVs for Australia and New Zealand (NHMRC 2006) (Table 4.2). Suggested dietary targets and acceptable macronutrient distribution ranges for macronutrients (AMDR) for the reduction of risk of chronic diseases were also examined. These intake requirements were the same for women in the 19–30 years and 31–50 years age groups, except for magnesium. Women in the 31–50 years age group have a slightly higher magnesium intake requirement.

Simulation of diets

Risk Solver Premium V9.0.4.0 program (Frontline Systems, Inc.) was used to simulate dietary intakes. Foods were randomly chosen from the food groups according to the pre-set number of serves for each food group to represent consumption over a 14-day period, as previously mentioned. This random selection

of foods was repeated 1000 times and the average daily intakes of selected nutrients were generated by the computer. The proportion of these 1000 diets providing adequate nutrients to meet EAR, RDI, AI, SDT, AMDR or exceeding UL, where applicable, was also estimated.

Model 4: Nutrient profile of a diet following the food intake recommendation of the Australian Dietary Guidelines 2013 but including more fish and seafood (i.e. higher fish intake)

Model 4 followed the same procedure as Model 3 but included a higher fish component. The number of serves of oily and non-oily fish required to meet all LCn3PUFA recommendations as identified in Model 2 was included in this Model 4. The additional fish serves replaced other foods in the 'Lean meat/ poultry/ fish/ eggs/ tofu/ nuts & seeds/ legumes & beans' group. Intake of all other food groups remained the same.

Table 4.1 Australian Dietary Guidelines — Sample daily food patterns for women 19–50 years

Food groups	**Recommended average daily number of serves from each of the five food groups**		
	Non-pregnant / Non-lactating	Pregnant	Lactating
Vegetables and legumes / beans	5	5	7.5
Fruit	2	2	2
Grain (cereal) foods, mostly wholegrain and/or high fibre cereal varieties	6	8.5	9
Lean meat and poultry, fish, eggs, tofu, nuts and seeds, and legumes/ beans*	2.5	3.5	2.5
Milk, yoghurt, cheese and/or alternatives, mostly reduced fat	2.5	2.5	2.5
Approx. number of additional serves from the five food groups or unsaturated spreads and oils or discretionary choices	0–2.5	0–2.5	0–2.5

*Around 2 serves of fish per week is recommended.

Table 4.2 Nutrient reference values (NRVs) for Australia and New Zealand[a] on selected nutrients for non-pregnant, non-lactating women aged 19–50 years

Nutrients	RDI[b]	EAR[c]	AI[d]	UL[e]
Protein (g/day)	46	37	-	NP[f]
Linoleic acid (n-6) (g/day)	-	-	8	NP
α-linolenic acid (n-3) (g/day)	-	-	0.8	NP
LC n-3 (DHA/EPA/DPA) (mg/day)	-	-	90	3000
Carbohydrate (g/day)	-	-	NP	NP
Dietary fibre (g/day)	-	-	25	NP
Thiamin (mg/day)	1.1	0.9	-	NP
Riboflavin (mg/day)	1.1	0.9	-	NP
Niacin as niacin equivalents (mg/day)	14	11	-	35[g]
Folate as dietary folate equivalents (μg/day)	400	320	-	1000[h]
Vitamin A as retinol equivalents (μg/day)	700	500	-	3000
Vitamin C (mg/day)	45	30	-	NP
Vitamin E as α-tocopherol equivalents (mg/day)	-	-	7	300
Calcium (mg/day)	1000	840	-	2500
Iron (mg/day)	18	8	-	45
Iodine (μg/day)	150	100	-	1100
Magnesium (mg/day)	310[i], 320[j]	255[i], 265[j]	-	350
Phosphorus (mg/day)	1000	580	-	4000
Potassium (mg/day)	-	-	2800	NP
Sodium (mg/day)	-	-	460–920	2300
Zinc (mg/day)	8	6.5	-	40

a. NRVs according to NH&MRC (2006).

b. RDI – Recommended Dietary Intake is the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage and gender group.

c. EAR – Estimated Average Requirement is the daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.

d. AI – Adequate Intake is used when an RDI cannot be determined and is the average daily nutrient intake level based on observed or experimentally determined approximations of estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate.

e. UL – Upper Level of intake is the highest average daily nutrient level likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases.

f. NP – Not possible to set due to insufficient evidence or no clear level for adverse effects.

g. UL applied to supplemental nicotinic acid, UL for supplemental nicotinamide is 900 mg/day.

h. UL applied to intake from fortified foods and supplements.

i. Value is for non-pregnant, non-lactating women aged 19–30 years.

j. Value is for non-pregnant, non-lactating women aged 31–50 years.

4.3 Statistical analysis

PASW Statistics 17.0 was used to analyse data from the 1995 NNS for the identification of frequently consumed foods. Risk Solver Premium V9.0.4.0 program (Frontline Systems, Inc.) was used to simulate food intakes and to estimate the proportion of diets meeting recommendations.

4.4 Results and discussion

Fish and seafood included in Models 1 and 2

Based on data from women aged 19–49 years who participated in the 1995 NNS, 231 fish items were identified. Of the 231 individual fish items, those that were of mixed fish types (e.g. seafood marina) or those where no particular fish type could be identified were excluded from the modelling (e.g. Fish, Ns As to Type, Battered, Fried or Fish in Lemon Sauce, From Basic Ingredients). The others were grouped together according to the types of fish but regardless of how they were prepared (e.g. raw, battered, or crumbed) except for canned fish. Table 4.3 lists the types of fish and seafood consumed by the women aged 19–49 years in the 1995 NNS.

Table 4.3 Type of fish and seafood consumed by women aged 19–49 years in the 1995 National Nutrition Survey on the day of the survey using 24 hour recall (n=3506)

Fish type	No of women who reported to have consumed	Fish type	No of women who reported to have consumed
Tuna, canned	109	Salmon, Australian, canned	4
Prawn	98	Coral trout	3
Salmon, Pink, canned	37	Flathead	3
Calamari	36	Hake	3
Shark	28	Trevally	3
Scallop	21	Mussel	3
Sardine, canned	18	Lobster	3
Smoked salmon	15	Mackerel	2
Snapper	13	Blue grenadier	2
Perch, ocean	9	Flounder	2
Salmon, Red, canned	9	Garfish	2
Whiting	8	Herring	2
Cod	8	Atlantic	2
Baby octopus	8	Cod, Smoked	2
Bream	7	Oyster, canned	2
Dory	7	Prawn, canned*	2
Kingfish	6	Tailor	1
Mullet	6	Gemfish	1
Oyster	6	Trumpeter	1
Tuna	5	Scampi	1
Anchovy, canned	5	Yabby	1
Crab	5	Smoked trout*	1
Barramundi	4	Haddock*	1
Ling	4	Kipper canned*	1
Trout	4		

*Not included in the modelling due to lack of fatty acids data

The number of serves of fish required per week to achieve recommendations as per Model 1 is listed in Table 4.4. This demonstrates that if the types of fish and seafood consumed were similar to that of the 1995 NNS, eight serves of fish or seafood would need to be consumed each week in order to achieve the intake recommendations in 50% of the cases (SDT of 430 mg for LCn3PUFA and the FAO/WHO Expert Consultation recommendation of 250 mg per day of EPA+DHA combined). This is comparable to the concept of EAR, where the prevalence of inadequate intakes within a group could be estimated.

Table 4.4 Number of serves of fish and seafood required per week to achieve recommendations as per Model 1 (i.e. current fish and seafood consumption pattern)

Number of serves of fish or seafood per week (randomly selected but according to the current intake pattern)	Average daily EPA + DHA intake from fish or seafood (% of fish meals providing 250 mg per day or more)	Average daily LCn3PUFA intake from fish or seafood (% of fish meals providing 430 mg per day or more)	Average amount consumed (gram per week)
1 serve	56 mg (1%)	61 mg (0%)	94
2 serves	108 mg (8%)	118 mg (1%)	190
3 serves	163 mg (18%)	177 mg (4%)	284
4 serves	215 mg (30%)	234 mg (8%)	379
5 serves	270 mg (48%)	294 mg (17%)	475
6 serves	321 mg (63%)	349 mg (28%)	569
7 serves	373 mg (77%)	406 mg (40%)	663
8 serves	426 mg (85%)	463 mg (54%)	758
9 serves	478 mg (94%)	519 mg (66%)	853
10 serves	535 mg (98%)	581 mg (76%)	947
11 serves	591 mg (99%)	642 mg (84%)	1043
12 serves	643 mg (100%)	698 mg (90%)	1137

The number of serves of fish required per week to achieve recommendations as per Model 2 is listed in Table 4.5. This shows that the number of serves per week required for half of the population to achieve the intake recommendations can be reduced to three serves if at least two of the fish serves are of high LCn3PUFA content. If all three serves were of high LCn3 content, then everyone would achieve the recommendations.

Table 4.5 Number of serves of fish and seafood required per week to achieve recommendations as per Model 2 (i.e. high LCn3PUFA fish must be included)

Number of serves of fish or seafood per week (must include at least one high LCn3 fish)	Average daily EPA + DHA intake from fish or seafood (% of fish meals providing 500 mg per day or more)	Average daily LCn3PUFA intake from fish or seafood (% of fish meals providing 430 mg per day or more)	Average amount consumed (gram per week)
2 serves (1 high plus 1 med /low)	221 mg (36%)	240 mg (1%)	170
3 serves (1 high plus 2 med/low)	255 mg (48%)	277 mg (3%)	269
4 serves (1 high plus 3 med/low)	289 mg (64%)	313 mg (6%)	365
2 serves (2 high only)	382 mg (100%)	415 mg (31%)	143
3 serves (2 high plus 1 med/low)	404 mg (100%)	439 mg (58%)	241
4 serves (2 high plus 2 med/low)	441 mg (100%)	479mg (69%)	337
3 serves (3 high only)	570 mg (100%)	620mg (100%)	214
4 serves (3 high plus 1 med/low)	608 mg (100%)	660 mg (100%)	313

Foods included in Models 3 and 4

Of the 13 858 persons who took part in the 1995 NNS, there were 3506 women aged 19–49 years. A total of 3270 food and drink items were reported to have been consumed by women in this age group, of which 183 food items in total were included in the final modelling (Appendix 3). This drastic reduction in the number of food items included for selection in the modelling exercise occurred due to the fact that the remaining 3087 food items were either consumed by less than 1% of the women or the food items were considered as 'extras' in the original Australian Guide to Healthy Eating.

Proportion of diets meeting recommendations in Models 3 and 4

The number of serves of various food groups was pre-determined according to the recommendations of the Australian Dietary Guidelines 2013. One and a half serves of non-oily fish or seafood per week (or three serves per fortnight) were included in the lower fish diet as per estimated mean intake from the 1995 NNS. Three serves of oily fish of high LCn3PUFA content per week (or six serves per fortnight) were included in the higher fish diet as determined by the results of Model 2 (number of serves meeting recommendations at all times). The three additional fish serves over the 14-day period replaced one serve of poultry and two serves of red meat. Apart from the differing number of serves of poultry, red meat and fish, all other food groups remained the same for both diets. Table 4.6 lists the actual number of serves of various food groups allocated to a 14-day diet.

Table 4.6 Differences in food intakes between a lower fish diet (Model 3) and a higher fish diet (Model 4) assuming average height with sedentary to moderate physical activity levels

Food groups	Types within food groups	Serve size	Serves/fortnight (Lower fish diet)	Serves/fortnight (Higher fish diet)
Vegetables and legumes / beans	Starchy vegetables	75 g	10	10
	Green & brassica vegetables	75 g	14	14
	Orange vegetables	75 g	14	14
	Legumes	75 g	4	4
	Other vegetables	75 g	28	28
Fruit	Fresh fruit	150 g	28	28
	Dried fruit*	30 g		
	100% fruit juice*	125 ml		
Grain (cereal) foods, mostly wholegrain and/or high fibre cereal varieties	Wholegrain cereals / grains	Bread 40 g Breakfast cereals 30 g Oats, rice, pasta 120 g	56	56
	Refined cereals/grains	Bread 40 g Breakfast cereals 30 g Oats, rice, pasta 120 g	28	28
Lean meat and poultry, fish, eggs, tofu, nuts and seeds, and legumes/ beans	Poultry & other white meat	80 g	**8**	**7**
	Fish and seafood	100 g or small can	**3 (non-oily fish)**	**6 (oily fish)**
	Eggs	120 g	4	4
	Legumes	170 g	4	4
	Red meats	65 g	**9**	**7**
	Nuts and seeds	30 g	7	7
Milk, yoghurt, cheese and/or alternatives, mostly reduced fat	Milk (fresh, UHT long life or reconstituted dried	250 ml	5 High fat dairy 5 Medium fat dairy 25 Low fat dairy	5 High fat dairy 5 Medium fat dairy 25 Low fat dairy
	Yoghurt	200 g		
	Cheese (hard cheese)	40 g		
Approx. no of additional serves from the 5 food groups or unsaturated spreads/oils or discretionary choices	Margarine	10 g	28	28
	Oils	7 g		

*Only to be used occasionally as a substitute for other foods in the group, lower rate of selection was set in the Models.

The average daily nutrient intake of the two dietary patterns is listed in Table 4.7 (lower fish content) and Table 4.8 (higher fish content) respectively. The mean energy intake for both the lower and higher fish diet was around 7.6 MJ. This would satisfy the estimated energy requirement for women of 1.6 m tall and with a light physical activity level (PAL of around 1.4). Additional serves from the five food groups or discretionary choices would need to be added to meet higher energy requirements if women were taller or more active.

All of the simulated diets, whether it was the lower fish diet or the higher fish diet, met the EAR for protein, thiamin, riboflavin, niacin, folate, vitamin A, vitamin C, calcium, iron, iodine, magnesium, phosphorus and zinc, and the AI for linoleic acid, LCn3PUFA, dietary fibre, vitamin E, sodium and potassium. AIs for α-linolenic acid were achieved in 62% of the simulated diets following a lower fish intake pattern and 79% for those with a higher fish intake pattern.

The proportion of diets meeting RDI again were similar in both dietary patterns. Except for iron, 99–100% of all diets met the RDI for all nutrients reported. The proportion of diets meeting the RDI for iron was only marginally different between the two dietary patterns (16% in the lower fish diet *vs*. 19% in the higher fish diet). Iron requirements in for women 19–50 years are much higher than their male counterparts due to blood loss during menstruation (RDI of 18 mg/day for non-pregnant and non-lactating women *vs.* 8 mg/day for men) and therefore more difficult to meet requirements. The RDI for pregnancy women is even higher at 27 mg/day and for some women, iron supplements are required.

Table 4.7 Theoretical mean daily nutrient intake profile in women aged 19–50 years as estimated by the simulation of 1000 diets that followed the recommendations of the Australian Dietary Guidelines but with a lower fish content (around 1.5 serves of non-oily fish and seafood per week)

Nutrients	Mean (SD)	Minimum	Maximum
Energy, including dietary fibre (kJ)	7597 (119)	7252	7942
Protein (g)	98 (1)	94	103
Fat (g)	53 (2)	48	60
Total available carbohydrate (g)	215 (6)	196	234
Total sugars (g)	90 (4)	78	102
Starch (g)	124 (5)	111	145
Dietary fibre (g)	38 (2)	33	44
Ethanol (g)	0 (0)	0	0
Total saturated fatty acids (g)	16 (1)	13	18
Total monounsaturated fatty acids (g)	19 (1)	16	23
Total polyunsaturated fatty acids (g)	14 (1)	12	16
Linoleic acid (LA) (g)	13 (1)	11	15
α-linolenic acid (ALA) (g)	0.8 (0.1)	0.6	1.0
Total long chain n-3 polyunsaturated fatty acids (mg)	132 (18)	99	202
Vitamin A as retinol equivalents (μg)	1281 (135)	796	1661
Retinol (μg)	267 (30)	184	373
Thiamin, B1 (mg)	2.1 (0.1)	1.8	2.5
Riboflavin, B2 (mg)	2.9 (0.2)	2.4	3.5
Niacin equivalents (mg)	46 (2)	40	52
Total folates (μg)	685 (41)	553	851
Folate as dietary folate equivalents (μg)	884 (62)	699	1125
Vitamin C (mg)	156 (18)	107	219
Vitamin E (mg)	12 (1)	9	16
Calcium (mg)	1261 (48)	1132	1416
Iron (mg)	17 (1)	15	20
Iodine (μg)	167 (8)	140	191
Magnesium (mg)	432 (20)	373	499
Phosphorus (mg)	1828 (48)	1695	2006
Potassium (mg)	3556 (86)	3293	3863
Sodium (mg)	1814 (95)	1528	2173
Zinc (mg)	14.2 (0.5)	12.8	15.7
Cholesterol (mg)	291 (13)	251	333
Eicosapentaenoic acid (EPA) (mg)	33 (5)	22	52
Docosapentaenoic acid (DPA) (mg)	35 (3)	24	47
Docosahexaenoic acid (DHA) (mg)	64 (12)	39	109

Table 4.8 Theoretical mean daily nutrient intake profile in women aged 19–50 years as estimated by the simulation of 1000 diets that followed the recommendations of the Australian Dietary Guidelines but with a higher fish content (around three serves of oily fish and seafood per week)

Nutrients	Mean (SD)	Minimum	Maximum
Energy, including dietary fibre (kJ)	7583 (120)	7239	7975
Protein (g)	97 (1)	93	101
Fat (g)	54 (2)	48	62
Total available carbohydrate (g)	214 (6)	195	231
Total sugars (g)	90 (4)	79	102
Starch (g)	122 (5)	109	143
Dietary fibre (g)	38 (2)	33	44
Ethanol (g)	0 (0)	0	0
Total saturated fatty acids (g)	16 (1)	14	18
Total monounsaturated fatty acids (g)	19 (1)	17	24
Total polyunsaturated fatty acids (g)	14 (1)	12	16
Linoleic acid (LA) (g)	13 (1)	11	15
α-linolenic acid (ALA) (g)	0.8 (0.1)	0.6	1.1
Total long chain n-3 polyunsaturated fatty acids (mg)	568 (60)	400	728
Vitamin A as retinol equivalents (μg)	1269 (133)	774	1637
Retinol (μg)	254 (23)	180	329
Thiamin, B1 (mg)	2.1 (0.1)	1.8	2.5
Riboflavin, B2 (mg)	2.9 (0.2)	2.5	3.5
Niacin equivalents (mg)	46 (2)	41	52
Total folates (μg)	684 (41)	553	851
Folate as dietary folate equivalents (μg)	883 (62)	699	1125
Vitamin C (mg)	155 (18)	107	219
Vitamin E (mg)	12 (1)	9	15
Calcium (mg)	1329 (50)	1173	1495
Iron (mg)	17 (1)	15	20
Iodine (μg)	170 (8)	145	195
Magnesium (mg)	432 (20)	378	494
Phosphorus (mg)	1858 (48)	1727	2041
Potassium (mg)	3560 (88)	3269	3865
Sodium (mg)	1802 (98)	1538	2208
Zinc (mg)	13.8 (0.5)	12.3	15.3
Cholesterol (mg)	287 (10)	253	315
Eicosapentaenoic acid (EPA) (mg)	209 (23)	152	287
Docosapentaenoic acid (DPA) (mg)	75 (14)	48	128
Docosahexaenoic acid (DHA) (mg)	283 (30)	196	370

The main difference seen between the two dietary patterns was the proportion of diets achieving the SDT for LCn3PUFA and the FAO/WHO recommendation of 250 mg per day of EPA+DHA combined (Table 4.9). These recommendations were met in nearly all of the higher fish diets (at least 99% of cases) but not with the lower fish diet. This shows that it would be difficult to achieve SDT if a no or low fish diet is consumed unless LCn3PUFA fortified food or LCn3PUFA supplements are consumed. The proportion of diets meeting SDTs for vitamin A, vitamin C, vitamin E, potassium, sodium and fibre were very similar in both dietary patterns. Less than 10% of the diets (high or low fish) met SDT for vitamin C and vitamin E, which warrants further investigation. Very few (<0.02%) of the simulated diets met the SDT for sodium and provided more than 1,600mg of sodium per day. None of the diets met the SDTs for potassium and all provided less than 4,700 mg of potassium per day.

The mean intake of sodium was similar in both dietary patterns and was around 1800 mg per day ranging from 1528 mg to 2208 mg. Although almost all simulated diets failed to meet the SDT for sodium, none exceeded the upper level of sodium intake of 2300 mg per day. This has been made possible as no food from the ‘discretionary choices’ group was included in the modelling. Foods in the ‘discretionary choices’ group are generally higher in salt, sugar or saturated fat.

Table 4.9 Proportion of 1000 simulated diets meeting Suggested Dietary Targets (SDT) and FAO/WHO recommended daily intake of 250 mg of EPA+DHA

Nutrient (Recommended Intake)	Proportion of diets meeting requirement (%)	
	Lower fish diet	Higher fish diet
Vitamin A (1,220 µg*)	68	65
Vitamin C (190 mg*)	3	3
Vitamin E (14 mg*)	3	6
Folate as dietary folate equivalents (300–600 µg*)	100	100
Sodium (≤1,600 mg*)	0	0
Potassium (4,700 mg*)	0	0
Dietary fibre (28 g*)	100	100
LCn3PUFA (430 mg*)	0	99
EPA + DHA (250 mg†)	0	100

* SDT for Australian women, intake per day on average

† Daily intake recommendation by the FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition 2011

Both the mean percentage contributions to dietary energy and the proportion of diets that were within the AMDR were similar in both dietary patterns (Table 4.10).

Table 4.10 Mean percentage contribution to dietary energy of macronutrients and proportion of diets within acceptable macronutrient distribution ranges (AMDR)

Nutrient (AMDR)	Mean % contribution to dietary energy		Proportion of diets within AMDR (%)	
	Lower fish diet	Higher fish diet	Lower fish diet	Higher fish diet
Protein (15–25% of energy)	22	22	100	100
Fat (20–35% of energy)	26	26	100	100
Saturated fat (8–10% of energy)	8	8	100	100
Linoleic acid (n-6 fat) (4–10% of energy)	6	6	100	100
α-linolenic acid (n-3 fat) (0.4–1% of energy)	0.4	0.4	56	74
Carbohydrate (45–65% of energy)	45	45	65	60

In summary, both dietary patterns provided very similar nutrient profiles with the only difference relating to the LCn3PUFA. Including three serves of oily fish per week would easily meet the SDT of 430 mg of LCn3PUFA per day and the FAO/WHO recommended daily intake of 250 mg EPA+DHA in women. The extra serve of fish per week recommended here is not totally at odds with the current Australian Dietary Guidelines of two serves of fish per week. If consuming only two serves of fish per week, one would need to ensure the type of fish consumed is of higher LCn3PUFA contents in order to meet the targets. Whereas consuming three

serves of fish per week would allow a wider choices of fish type which is more practical in the long term.

Chapter 5 Compositional data of selected fish and fish products

5.1 Background

Food composition tables are available from around the world providing food composition data that are crucial in the fields of dietetics (clinical and research), nutritional epidemiology, health promotion and food legislation. Ideally each country should have their own food composition data, as food composition may differ between countries due to differing cultivars, soils, climates, agricultural or aquaculture practices (Greenfield & Southgate 2003). A list of food composition database websites (nationally and internationally) can be found at http://www.foodcomp.dk/v7/fcdb_links.asp.

In Australia, the two food composition databases, NUTTAB and AUSNUT, which are maintained by FSANZ, provide compositional data on Australian foods. NUTTAB 2010 is the latest version and contains nutrient data for 2668 foods and beverages and up to 245 nutrients per food including energy, proximates, minerals, vitamins, fatty acids, amino acids, caffeine and cholesterol. AUSNUT 2007 contains data for 3874 foods and beverages and provides information for 37 nutrients.

Fatty acid components are of particular interest for fish and seafood. LCn3PUFA data in AUSNUT 2007 are available as total LCn3PUFA only (i.e. combined value for EPA+DPA+DHA). In NUTTAB 2010, although not all listed foods have data for LCn3PUFA, some do have a listing of the individual fatty acids. The Australian RMIT fatty acids database, which is available on the dietary analysis software platforms FoodWorks and SERVE, provides fatty acid profiles for over 1000 common Australian and New Zealand food items. Two publications, 'Seafood the good food' (Nichols et al. 1998) and 'Seafood the good food II' (Mooney, Nichols & Elliott 2002), report on the content and composition of the oil from 268 Australian fish and seafood species. Nutrient information panels of packaged foods may be another source of information, however, since the listing of LCn3PUFA content is not mandatory, food companies generally only provide information about LCn3PUFA on the nutrient information panel when content claims are being made.

Mercury content in fish and seafood is also of interest as this type of food contains much higher levels of mercury than most other foods. Information on mercury content is less readily available. The FSANZ NUTTAB 2010 database provides data on around 70 food items with 24 of them being fish or seafood products. In a document published by the Heart Foundation (Colquhuon et al. 2008), a table was included that listed the mercury content of several Australian fish species.

Apart from the sources of information mentioned above, from time to time, researchers may publish food compositional data of selected foods relevant to their research.

Food composition tables sometimes provide information only on raw food products but food is often cooked prior to consumption, therefore there is the need to examine the effects of cooking on the nutrients, particularly fatty acids profile and contaminants in fish and seafood. And for this reason, the analysis presented in this thesis includes both raw and cooked samples.

5.2 Purpose of analysis

The purpose of the analysis was to (1) establish the compositional profile of fish and fish products used in the planned randomised controlled trial; (2) add to the existing database owned by the products' distributor and (3) observe the changes in composition between cooked and raw variants.

5.3 Process

5.3.1 Fish and fish products included for analysis

It was decided that the fish and fish products used in the randomised controlled trial would be those that are commonly consumed and readily available in the Australian market, and that a variety of fresh, canned and frozen products should be included. With this in mind, a total of 13 products was selected from the range of products marketed by the company Simplot Australia. Simplot Australia was one of the collaborators of this project and supplied fish products free of charge for the purpose

of analysis as well as subsequently for use in the trial. Data obtained from the analysis would in turn complement their existing compositional database and provide on-going compositional information of their products. Although the total number of products selected for analysis was limited to 13 due to budgetary constraints, it was believed that this range still provided adequate variety for an eight-week intervention trial. Products selected for analysis were:

(1) John West Atlantic Salmon (Skin Off) 300 g
(2) John West Yellowtail Kingfish 300 g
(3) John West Sardines in Tomato Sauce 110 g (undrained)
(4) John West Pink Salmon 210 g (drained)
(5) John West Red Salmon 105 g (drained)
(6) John West Salmon Tempters Onion & Tomato 95 g (undrained)
(7) John West Tuna Tempters Lemon & Cracked Pepper 95 g (drained)
(8) John West Tuna in Springwater 95 g (drained)
(9) Birds Eye Atlantic Salmon Lemon Pepper 270 g
(10) Birds Eye Lightly Seasoned Fish Fillets (Hoki) – Lemon & Cracked Pepper 400 g
(11) Birds Eye Fish Fingers 1 kg (Hoki/Hake)
(12) Birds Eye Oven Bake Fish Fillets (Hoki/Hake) Original Crumb 425 g
(13) Birds Eye Deep Sea Dory Fish Portions Original Crumb 425 g

5.3.2 Selection of suitable laboratory to conduct the analysis

Around the same time of the implementation of this project, another Seafood CRC project, the Australian seafood compositional profiles led by Mr David Padula (Project number 2008/905), was due to commence. The main aim of the Australian Seafood Compositional Profiles project was to support the industry by providing nutrient data on a range of Australian seafoods. The project employed the service of a consortium of four laboratories to undertake the analytical work following a tendering process with set evaluation criteria. As the nature of the analytical work between the two projects was very similar, it was deemed appropriate to also employ the services of these selected laboratories for this project. The benefits of this include (1) there would be uniformity in the sample handling and preparation procedures as well as methods of analysis; and (2) better pricing could be negotiated with the

overall increase in sample volume. The laboratories involved in the sample analysis for this project were AsureQuality Limited (AQ, Auckland New Zealand), Hill Laboratories (Hill, Hamilton New Zealand) and National Measurement Institute (NMI) in Victoria Australia. The service of the fourth laboratory involved with the Seafood Compositional Profiles project, National Institute of Water and Atmospheric Research (Wellington, New Zealand), was not required as this current project does not include DNA species identification.

5.3.3 Analytes included and method of analysis

In addition to the nutritional data required to meet labelling requirements (except sugars), several other key nutrients and chemicals relevant to fish and seafood were included in the analysis. Moreover, several analytes were also included as they did not incur extra cost when performed with the required tests.

The proposal for laboratory testing submitted by AsureQuality Limited provided the following brief description on the methods to be used for various analytes.

Protein

Nitrogen content was first determined by the Kjeldahl Block Digestion method which involved samples digestion with sulphuric acid, potassium sulphate and a copper/titanium catalyst, followed by steamed distillation of the liberated ammonia and then titration against standard acid. Protein content was then calculated by applying a factor (6.25) to convert from nitrogen to protein.

Protein (g/100 g) = Total nitrogen x 6.25

Moisture

Samples were dried to constant weight at 95–100 °C under pressure ≤100 mmHg. Loss in weight was reported as moisture.

Fat

Fat was first extracted from a hydrochloric acid digest of the sample with diethyl ether and petroleum ether. The solvents were then evaporated and the residue weighed.

Ash

Ash was determined by organic matter incineration at 525 °C.

Total Carbohydrate

Total carbohydrate was estimated by measurement of all the other components in the sample and calculated by difference from 100%.

Total Carbohydrate = 100 – Fat – Protein – Moisture – Ash

Energy

The amount of energy in a sample was calculated from its composition.

Energy (kJ per 100 g) = $\sum Wi\ Fi$ where Wi is the average weight of the food component (g/100 g food) and Fi is the energy factor assigned to that component.

Fatty Acids

Lipid material was first extracted from the sample by solvent extraction and the triglycerides were trans-esterified with methanolic potassium hydroxide. The fatty acid composition was then quantitatively determined by Gas Liquid Chromatography of the methyl esters.

Cholesterol

The sample was first saponified and then evaporated to dryness. The residue was re-dissolved in chloroform and the extract analysed directly by Gas Liquid Chromatography on a non-polar column using a Flame Ionisation Detector.

Minerals and heavy metals (except mercury and methyl mercury)

Samples were digested with nitric acid and a trace of hydrofluoric acid at 100 °C for one hour (for selenium and iodine analysis, samples were digested with tetramethyl ammonium hydroxide). The digest was then analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) or Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Mercury

Mercury was solubilised from the samples by digesting with a concentrated nitric acid/hydrochloric acid mixture. The solubilised elements were then measured by ICP-MS.

Methyl mercury

Methyl mercury was extracted with an alkaline (sodium hydroxide). The resulting extract was analysed using speciated isotope dilution mass spectrometry with solid phase microextraction (SPME).

Vitamin A and Vitamin E

The homogenised sample was saponified under reflux and extracted into organic solvent. Retinol (or vitamin E) was quantitated after evaporation by isocratic, reversed phase, high-performance liquid chromatography (HPLC), using fluorescent detection and external calibration.

Vitamin D

The sample was saponified and extracted with petroleum ether. The petroleum ether extract was then dried under nitrogen and added to heptane. The extract was then separated by normal phase Liquid Chromatography and any vitamin D &/or 25-hydroxyvitamin D identified by Ion Trap MS-MS and quantitated against individual calibration curves.

Table 5.1 to Table 5.4 list the proximates, fatty acids, minerals, and vitamins that have been selected for analysis respectively. The tables also list the reference methods of analysis and the laboratory responsible for the analysis of the individual analyte.

Table 5.1 Proximates selected for analysis and analytical method details

Lab*	Analyte	Limit of Reporting (LOR)	Limit of Detection (LOD)	Units	Reference Method
AQ	Energy	1	1	kJ/100 g	New Zealand (Australia New Zealand Food Standards Code) Food Standards 2002, Amendment No. 2
AQ	Moisture	0.1	0.1	g/100 g	AOAC 950.46
AQ	Protein	0.1	0.1	g/100 g	AOAC 988.05, 920.53, 955.04, 981.10, 920.87, 984.13, 920.103, 991.20, 930.33, 2001.11 as appropriate (modified)
AQ	Fat	0.1	0.1	g/100 g	Method by Folch et al. (1957)
AQ	Ash	0.1	0.1	g/100 g	AOAC 920.153 BS4401:Part1:1980/ISO 936-1978
AQ	Total carbohydrate	0.5	0.1	g/100 g	Calculation

*AQ = AsureQuality Limited

Table 5.2 Fatty acids selected for analysis and analytical method details

Lab*	Analyte	Limit of Reporting (LOR)	Limit of Detection (LOD)	Units	Reference Method
AQ	Monounsaturated fatty acids	0.1	0.1	g/100 g	In-house based on Bannon et al. (1985)
AQ	Polyunsaturated fatty acids	0.1	0.1	g/100 g	In-house based on Bannon et al. (1985)
AQ	Saturated Fat	0.1	0.1	g/100 g	In-house based on Bannon et al. (1985)
AQ	Omega-3 (total)	0.1	0.1	g/100 g	Calculated from fatty acid profile
AQ	Omega-6 (total)	0.1	0.1	g/100 g	Calculated from fatty acid profile
AQ	Omega-9 (total)	0.1	0.1	g/100 g	Calculated from fatty acid profile
AQ	EPA	10	10	mg/100 g	In-house based on Bannon et al. (1985)
AQ	DPA	10	10	mg/100 g	In-house based on Bannon et al. (1985)
AQ	DHA	10	10	mg/100 g	In-house based on Bannon et al. (1985)
AQ	Trans fatty acids	0.1	0.1	g/100 g	In-house based on Bannon et al. (1985)
AQ	Cholesterol	1	0.5	mg/100 g	Based on AOAC 933.08, 970.50, 970.51

*AQ = AsureQuality Limited

Table 5.3 Minerals and heavy metals selected for analysis and analytical method details

Lab*	Analyte	Limit of Reporting (LOR)	Limit of Detection (LOD)	Units	Reference Method
AQ	Antimony	0.01	0.005	mg/kg	Wet oxidation, ICP-MS
AQ	Boron	0.5	0.25	mg/kg	Wet oxidation, ICP-MS
AQ	Cadmium	0.002	0.001	mg/kg	Wet oxidation, ICP-MS
AQ	Calcium	2.8	1.1	mg/kg	Acid Digest, ICP-OES
AQ	Chromium	0.1	0.025	mg/kg	Wet oxidation, ICP-MS
AQ	Copper	0.10	0.05	mg/kg	Wet oxidation, ICP-OES
AQ	Iodine	0.05	0.01	mg/kg	TMAH Digestion, ICP-MS
AQ	Iron	0.62	0.1	mg/kg	Acid Digest, ICP-OES
AQ	Lead	0.01	0.005	mg/kg	Wet oxidation, ICP-MS
AQ	Magnesium	0.74	0.4	mg/kg	Wet oxidation, ICP-OES
AQ	Manganese	0.07	0.025	mg/kg	Acid Digest, ICP-OES
Hill	Mercury	0.010	0.002	mg/kg	Acid Digest, ICP-MS
Hill	Methyl mercury	0.005	0.002	mg/kg	SPME-GC-ICP-MS
AQ	Molybdenum	0.02	0.01	mg/kg	Wet oxidation, ICP-MS
AQ	Nickel	0.1	0.05	mg/kg	Wet oxidation, ICP-MS
AQ	Phosphorus	3.3	1.6	mg/kg	Acid Digest, ICP-OES
AQ	Potassium	5.7	2.9	mg/kg	Acid Digest, ICP-OES
AQ	Selenium	0.02	0.01	mg/kg	Wet oxidation, ICP-MS
AQ	Sodium	2.7	1.3	mg/kg	Acid Digest, ICP-OES
AQ	Sulphur	10	5	mg/kg	Wet oxidation, ICP-OES
AQ	Tin	0.03	0.01	mg/kg	Wet oxidation, ICP-MS
AQ	Zinc	1.5	0.75	mg/kg	Wet oxidation, ICP-OES

*AQ = AsureQuality Limited; Hill = Hill Laboratories

Table 5.4 Vitamins selected for analysis and analytical method details

Lab*	Analyte†	Limit of Reporting (LOR)	Limit of Detection (LOD)	Units	Reference Method
AQ	Vitamin A	10	10	IU/100 g	Method recommended by COST 91 (Brubacher, Müller-Mulot & Southgate 1986) (modified), EN Standard method 12823-1:2000, AOAC 992.04 and 002.06
NMI	Vitamin D	0.3	Not disclosed	µg/100 g	LC-MS-MS
AQ	Vitamin E	0.11	0.11	IU/100 g	Method recommended by COST 91 (Brubacher, Müller-Mulot & Southgate 1986)

* AQ = AsureQuality Limited; NMI = National Measurement Institute

† Analyses for these vitamins were conducted on those fish products that purportedly had a higher oily fish content and included John West Atlantic Salmon (Skin Off) 300g, John West Yellowtail Kingfish 300g, John West Sardines in Tomato Sauce 110g (undrained), John West Pink Salmon 210g (drained), John West Red Salmon 105g (drained), and Birds Eye Atlantic Salmon Lemon Pepper 270g

5.3.4 Preparation of samples

All samples were transported from Simplot Australia to the premises of South Australian Research and Development Institute (SARDI) under temperature-controlled conditions where necessary. Ideally, fish that are usually sold fresh should be processed while fresh. However, due to timing issues, fresh fish had to be stored frozen prior to their despatch for processing and analysis.

All samples submitted for analysis were composite samples from a minimum of three production dates and some up to six different production dates.

For canned fish, a method taken from Codex Standard for Canned Finfish (CODEX STAN 119 – 1981, REV. 1 – 1995) was followed for the draining of canned fish where indicated. In brief, each can was drained for two minutes in a stainless steel sieve, with aperture 2.8mm x 2.8mm and inclined at 18°. All cans were maintained at a temperature between 20 °C and 30 °C for a minimum of 12 hours prior to draining.

Figure 5.1 Illustration of the draining process for canned fish

Samples that required cooking prior to consumption were analysed both as raw and as cooked. Frozen convenient fish products were baked in an oven according to the instructions on the packs. Fresh fillets without skin (although now frozen) were wrapped in aluminium foil prior to being baked in the oven to prevent drying out. All visible ice was removed prior to cooking.

All samples, cooked and raw, were homogenised with stainless steel cutters, packed in plastic bags protected from light and frozen before being shipped to the selected laboratories.

Figure 5.2 Equipment used to homogenise samples

5.3.5 Quality assurance and quality control

All laboratories contracted to undertake the analyses were accredited and complied with the requirements for the competence of testing and calibration laboratories ISO/IEC 17025:2005.

All samples were analysed in duplicates. Reagent blank (same procedure but omitting the sample), spikes and standard reference materials (NIST 1849 infant formula and NIST 2383 baby food composite) were prepared according to the laboratory's standard operating procedure.

5.3.6 Calculated versus analysed results for cooked products

Since the products were cooked by oven baking with no added oil, the hypothesis was that this cooking process would only result in the loss of moisture while the other components of interest would remain unchanged.

Let *X* denotes the amount of moisture lost during the cooking process per 100g of raw product. *X* can therefore be calculated as follow:

$$\frac{Moisture_{raw}-X}{100-X} = \frac{Moisture_{cooked}}{100}$$

where $Moisture_{raw}$ was the moisture content per 100g of the raw product and $Moisture_{cooked}$ was the moisture content per 100g of the cooked product.

Once *X* was determined, then the content of other components in the cooked product could be calculated using the following formula, assuming only loss of moisture occurred during the cooking process:

$$Component\ A_{cooked}\ (\text{g/100g}) = \frac{Component\ A_{raw}}{100\ -X} * 100$$

where $Component\ A_{raw}$ was the amount of component A per 100g of the raw product.

The calculated and analysed results for the cooked products were then compared to determine if the original hypothesis could be supported.

5.4 Results and discussion

Thirteen products, some as both raw and cooked, totalling 20 samples were successfully analysed and their compositional profiles are listed in Table 5.5 to Table 5.14 at the end of this chapter. Reported are the average of the two results obtained from duplicate testings and their relative percentage difference (RPD). The following approaches were taken for the treatment of data.

- When calculating the average value, if one result was above the LOR and the other one below, the LOR would be used to calculate the average for the result below the LOR. If both results were below the LOR, the average would be reported as <LOR.
- RPD was calculated by dividing the absolute difference between the two results by their mean value and expressed as a percentage. For example, if the results for nutrient A were *X* mg/100 g and *Y* mg/100 g respectively, the RPD would be calculated as follows:

Relative percentage difference (RPD) = $\frac{Absolule\ value\ of\ (X-Y)}{(\frac{X+Y}{2})} * 100$

- No RPD was calculated if one or both results were below the LOR.
- All results were reported as per 100 g of food. If the results were provided as mg/kg, the results would be divided by 10 to convert to mg/100 g or multiplied by 100 to convert to µg/100 g.
- MeHg was reported as methylmercuric chloride (MeHgCl) in the report. In order to convert to MeHg, the results were divided by 251.076 (molecular weight of MeHgCl) and then multiplied by 215.623 (molecular weight of MeHg).

5.4.1 Proximates

Of the 20 analysed samples, the median energy provided was 707 kJ/100 g and ranged from 467 kJ (Tuna in Springwater) to 1150 kJ/100 g (Birds Eye Atlantic Salmon, cooked).

Energy generally correlates with the amount of total fats present, therefore, as expected, the product with the least amount of total fat was Tuna in Springwater (0.9 g/100 g) and the highest was cooked Birds Eye Atlantic Salmon (20.1 g/100 g).

Those products with almost 100% fish as the sole ingredient had an average protein value of 23 g/100 g. Protein value generally decreased as the fish content decreased. The product with the lowest protein value was Birds Eye Deep Sea Dory Fish Portions (9.7 g/100 g), which had a fish content of around 49%.

5.4.2 Long chain n-3 polyunsaturated fatty acids (LCn3PUFA)

Based on the classification suggested in the NHMRC commissioned document, 'A modelling system to inform the revision of the Australian Guide to Healthy Eating' (Dietitians Association of Australia 2011), fish and seafood can be classified into high LCn3PUFA (>1400 mg LCn3PUFA/100 g), medium LCn3PUFA (400–1399 mg LCn3PUFA/100 g) or low LCn3PUFA (<400 mg LCn3PUFA/100 g). Both Atlantic Salmon products (John West and Birds Eye), John West Yellowtail Kingfish (when cooked), John West canned sardine in tomato sauce, John West canned pink and red salmon in brine, could all be classified as high LCn3PUFA products with

levels greater than 1400 mg per 100 g. The product with the highest LCn3PUFA content was cooked Birds Eye Atlantic Salmon Lemon Pepper (3015 mg/100 g), followed by raw Birds Eye Atlantic Salmon Lemon Pepper (2175 mg/100 g) and then John West Sardines in Tomato Sauce 110 g (2056 mg/100 g). Except for raw John West Yellowtail Kingfish (1145 mg/100 g), the remaining analysed products had less than 400 mg per 100 g of LCn3PUFA and were considered low LCn3PUFA products. However, even at levels of <400 mg per 100 g with the lowest being Birds Eye Dory Portions Original Crumb at 157 mg/100 g, these low LCn3PUFA fish products still satisfy the claim for being 'good source of LCn3PUFA'.

5.4.3 Mercury and other metals

Mercury

All products analysed complied with FSANZ Standard 1.4.1, Contaminants and Natural Toxicants for mercury. According to Standard 1.4.1, the maximum level of mercury permitted to be present in fish and fish products is 0.5 mg/kg (or 50 µg/100 g) except for gemfish, billfish, southern bluefin tuna, barramundi, ling, orange roughy, rays and all species of shark, in which case the maximum level was set at 1 mg/kg (or 100 µg/100 g). The product that recorded the highest THg level was the John West Yellowtail Kingfish at 7.0 µg/100 g (both raw and cooked). The next highest was John West Tuna in Springwater at 6.0 µg/100 g, followed by Birds Eye Lightly Seasoned Fish Fillets (Hoki) Lemon & Cracked Pepper (raw) at 5.5 µg/100 g. The product with the lowest THg level was Birds Eye Fish Fingers (Hoki/Hake) (both raw and cooked) at 1.1 µg/100 g. These results therefore not only reflect the type of fish but also the amount present in the product. The fish content in Birds Eye Fish Fingers was listed at around 53%.

Cadmium, lead and tin

The other three metals for which maximum levels have been set in FSANZ Standard 1.4.1 and also analysed were cadmium, lead and tin. For cadmium, a maximum level of 2 mg/kg is set for molluscs but no maximum level is set for fish. Of the 20 fish samples, nine were below the LOR for cadmium (<0.002 mg/kg) and the highest level detected was from John West Sardines in Tomato Sauce at 0.019 mg/kg.

The maximum level set for lead in fish is 0.5 mg/kg. All samples analysed were below the LOR for lead (<0.01 mg/kg), except for John West Tuna Tempters with Lemon & Cracked Pepper, which registered a reading of 0.012 mg/kg.

For tin, a maximum level of 250 mg/kg is set for all canned foods. All 20 samples analysed, some of which were canned products, were below the LOR for tin (<0.03 mg/kg).

5.4.4 Vitamins A, D and E

The levels of vitamin A, D and E were determined in nine oily fish samples.

The product with the highest vitamin A content was John West Sardines in Tomato Sauce (1027 IU/100 g), followed by John West Red Salmon (drained, 137 IU/100 g), John West Pink Salmon (drained, 41 IU/100 g) and cooked Yellowtail Kingfish (17 IU/100 g). The remaining samples were below the LOR for vitamin A (<10 IU/100 g).

The levels of vitamin D2 and 25-hydroxy vitamin D2 were all below the LOR (<0.03 µg/100 g). John West Red Salmon had the highest vitamin D3 and 25-hydroxy vitamin D3 levels at 23 µg/100 g and 1.25 µg/100 g respectively.

The average vitamin E level amongst the 20 samples was 3.38 IU/100 g and ranged between 0.48 IU/100 g (John West Tuna in Springwater) and 8.80 IU/100 g (cooked Yellowtail Kingfish).

5.4.5 Raw and cooked products

Based on the results of the analysis, it appears that the hypothesis that the oven baking process (no oil added) would only result in the loss of moisture could generally be supported. The percentage differences between calculated and analysed results in cooked John West Atlantic Salmon (skin off) for energy, EPA, DHA, mercury and methyl mercury concentrations were 0.3%, 0.5%, 1.1%, 4.4% and 30.5% respectively (Table 5.5). The seemingly large percentage difference in methyl mercury content could be explained by the fact that the absolute value for methyl mercury was in the order of 2–3 µg/100 g and therefore even slight variation due to

measurement or random error could return a relatively large percentage difference. For John West Yellowtail Kingfish, the percentage differences between calculated and analysed results in the cooked products for energy EPA, DHA, mercury and methyl mercury concentrations were 2.9%, 18.1%, 21.0%, 6.1% and 5.4% respectively (Table 5.6).

These findings are in accordance with other studies that have examined the effects of cooking on nutrients and contaminants. In a study by Larsen et al. (2010), farmed New Zealand King Salmon (*Oncorhynchus tshawytscha*) were analysed after cooking with different methods: poaching, steaming, microwaving, pan-frying (no added oil), oven baking (no added oil) and deep-frying in sunflower oil. Moisture content was lower in all cooked samples with deep-fried samples having the lowest moisture content (50.45% *vs.* 63.86% when raw). There were no significant differences in the percentages of the LCn3PUFA across all cooked samples except for deep-fried salmon due to the absorption of fatty acids from the sunflower oil. Similarly, a study by Şengör et al. (2012) examining the effects of baking, steaming, grilling and microwaving on Atlantic Salmon (*Salmo salar*) showed no significant changes to fatty acids composition. Sioen et al. (2006) and Ansorena et al. (2010) showed that the level of changes in fat content and fatty acid profiles after frying in oil is inversely proportional to the initial fat content.

A review article published in 2011 concluded that fish lipid profiles indeed could change depending on the cooking processes used, fat content of the fish and the frying oil composition (Moradi et al. 2011) . Deep-frying tended to induce the largest change in fish lipids due to absorption of higher amounts of frying oil and the changes would depend on the type of frying oil used. When comparing the effect of frying between high fat content fish and low fat content fish, high fat content fish resulted in smaller changes as low fat content fish tended to absorb more fat.

In a review by Domingo (2010), the effect of cooking on the mercury of food was assessed. Studies have shown that commonly used cooking techniques such as frying and baking do not change the absolute content of mercury in fish. The increase in mercury level seen in the cooked food is due to a concentration effect with the loss of moisture or fat during cooking. For the various organic environmental pollutants, since they are associated with the fat portion of foods, Domingo suggests cooking

methods that remove fat from the product (and the fat discarded) should help reduce the amount of pollutants in the cooked food.

Overall, the determination of the nutrient profile in foods is a complex issue. As detailed in the publication by Greenfield & Southgate (2003), 'Food composition data: Production, management and use', the development of food nutrient profiles requires careful planning of procedures relating to sampling methods, analytical methods and data quality assurance. In situation where data are calculated or imputed from published or unpublished reports rather than analysed, the data must be scrutinized for their appropriateness for inclusion into the database.

Our national body, FSANZ, continuously updates existing nutrient data in their NUTTAB food composition database and generates data for foods that have limited data. This up-keeping of information is necessary with advances of new methods in food productions or processing, environmental changes potentially affecting the composition of foods, or new analytical technology which can produce more accurate results. Accurate nutritional evaluation of foods in turn allows the study of the relationship between diet and health. Although the NUTTAB 2010 database is quite comprehensive and provides nutrient data for around 2,000 foods, there are many food items with nutrient data still lacking. The compositional data obtained from our analyses thereby allowed the intervention diet to be formulated for the randomised controlled trial.

Table 5.5 Compositional profile for John West Atlantic Salmon (Skin Off)

Component (Unit, per 100 g of food)	Raw		Cooked		Calculated (Based on moisture loss in cooked sample)	Difference between calculated and analysed (%)
	Average of 2 results	% RPD	Average of 2 results	% RPD		
Energy (kJ/100 g)	704	1.1	744	1.3	742	0.3
Moisture (g/100 g)	68.1	0.1	66.4	0.3	66.4	0.0
Protein (g/100 g)	21.3	0.0	23.4	0.4	22.4	4.3
Fat (g/100 g)	9.1	3.3	9.8	3.1	9.6	2.2
Ash (g/100 g)	1.2	16.7	1.3	0.0	1.3	2.9
Total carbohydrate (g/100 g)	0.5	–	<0.5	–	0.5	–
Monounsaturated fatty acids (g/100 g)	3.8	5.3	4.1	4.9	4.0	2.4
Polyunsaturated fatty acids (g/100 g)	3.1	3.3	3.3	3.1	3.3	1.1
Saturated Fat (g/100 g)	2.2	4.7	2.4	4.3	2.3	3.6
Omega-3 (Total) (g/100 g)	1.9	5.4	2.0	5.1	2.0	0.1
Omega-6 (Total) (g/100 g)	0.8	0.0	0.9	0.0	0.8	6.8
Omega-9 (Total) (g/100 g)	2.8	3.6	3.0	3.4	2.9	1.7
EPA (mg/100 g)	630	3.2	660	3.0	664	0.5
DPA (mg/100 g)	285	3.5	305	3.3	300	1.6
DHA (mg/100 g)	690	2.9	735	4.1	727	1.1
Trans fatty acids (g/100 g)	<0.1	–	<0.1	–	–	–
Cholesterol (mg/100 g)	55	1.8	58	0.0	58	0.1
Antimony (µg/100 g)	<1	–	<1	–	–	–
Boron (mg/100 g)	<0.05	–	<0.05	–	–	–

Table 5.5 Compositional profile for John West Atlantic Salmon (Skin Off) (continued)

Component (Unit, per 100 g of food)	Raw		Cooked		Calculated (Based on moisture loss in cooked sample)	Difference between calculated and analysed (%)
	Average of 2 results	% RPD	Average of 2 results	% RPD		
Cadmium (µg/100 g)	<0.2	–	<0.2	–	–	–
Calcium (mg/100 g)	6.8	1.5	6.5	0.0	7.2	9.2
Chromium (µg/100 g)	<10	–	<10	–	–	–
Copper (mg/100 g)	0.04	0.0	0.05	8.7	0.04	18.7
Iodine (µg/100 g)	<5	–	<5	–	–	–
Iron (mg/100 g)	0.33	3.1	0.35	0.0	0.35	0.7
Lead (µg/100 g)	<1	–	<1	–	–	–
Magnesium (mg/100 g)	30	0.0	33	0.0	32	4.4
Manganese (mg/100 g)	0.008	6.1	<0.007	–	0.008	–
Mercury (µg/100 g)	3.0	13.3	3.3	12.1	3.2	4.4
Methyl mercury (µg/100 g)	2.4	3.6	3.3	5.1	2.5	30.5
Molybdenum (µg/100 g)	<2	–	<2	–	–	–
Nickel (µg/100 g)	<10	–	<10	–	–	–
Phosphorus (mg/100 g)	240	0.0	250	0.0	253	1.1
Potassium (mg/100 g)	375	2.7	390	0.0	395	1.3
Selenium (µg/100 g)	24	4.3	26	3.9	25	2.9
Sodium (mg/100 g)	40	2.5	39	2.6	42	7.4
Sulphur (mg/100 g)	230	0.0	250	0.0	242	3.2
Tin (µg/100 g)	<3	–	<3	–	–	–
Zinc (mg/100 g)	0.33	0.0	0.37	2.7	0.35	6.4

Table 5.5 Compositional profile for John West Atlantic Salmon (Skin Off) (continued)

Component (Unit, per 100 g of food)	Raw		Cooked		Calculated (Based on moisture loss in cooked sample)	Difference between calculated and analysed (%)
	Average of 2 results	% RPD	Average of 2 results	% RPD		
Vitamin A as Retinol (IU/100 g)	<10	–	<10	–	–	–
Ergocalciferol (Vitamin D2) (µg/100 g)	<0.3	–	<0.3	–	–	–
25-Hydroxy Vitamin D2 (µg/100 g)	<0.3	–	<0.3	–	–	–
Cholcalciferol (Vitamin D3) (µg/100 g)	5.0	10.1	4.2	9.5	5.3	20.3
25-Hydroxy Vitamin D3 (µg/100 g)	<0.3	–	<0.3	–	–	–
Vitamin E as Total Tocopherols (IU/100 g)	3.85	2.6	4.24	1.2	4.06	4.6

% RPD is the difference between the two results divided by the average result and expressed as a percentage. The lower the % RPD, the better the precision.
'–' Not calculated as at least one result was below the limit of reporting.

Table 5.6 Compositional profile for John West Yellowtail Kingfish

Component (Unit, per 100 g of food)	Raw		Cooked		Calculated (Based on moisture loss in cooked sample)	Difference between calculated and analysed (%)
	Average of 2 results	% RPD	Average of 2 results	% RPD		
Energy (kJ/100 g)	647	0.8	709	0.3	689	2.9
Moisture (g/100 g)	69.3	0.3	67.3	0.0	67.3	0.0
Protein (g/100 g)	22.6	0.0	23.7	0.0	24.1	1.5
Fat (g/100 g)	7.3	1.4	8.7	1.2	7.8	11.9
Ash (g/100 g)	1.2	0.0	1.2	0.0	1.3	6.1
Total carbohydrate (g/100 g)	<0.5	–	<0.5	–	–	–
Monounsaturated fatty acids (g/100 g)	2.9	3.5	3.4	3.0	3.1	10.1
Polyunsaturated fatty acids (g/100 g)	2.4	4.3	2.9	0.0	2.6	13.4
Saturated Fat (g/100 g)	2.0	0.0	2.4	4.3	2.1	12.7
Omega-3 (Total) (g/100 g)	1.4	7.4	1.7	0.0	1.5	14.0
Omega-6 (Total) (g/100 g)	0.8	0.0	1.0	10.5	0.9	17.4
Omega-9 (Total) (g/100 g)	2.0	0.0	2.4	4.3	2.1	12.7
EPA (mg/100 g)	505	2.0	635	1.6	538	18.1
DPA (mg/100 g)	155	6.5	190	0.0	165	15.1
DHA (mg/10 0g)	485	2.1	625	1.6	517	21.0
Trans fatty acids (g/100 g)	<0.1	–	<0.1	–	–	–
Cholesterol (mg/100 g)	57	1.8	64	1.6	61	5.4
Antimony (µg/100 g)	<1	–	<1	–	–	–
Boron (mg/100 g)	<0.05	–	<0.05	–	–	–

Table 5.6 Compositional profile for John West Yellowtail Kingfish (continued)

Component (Unit, per 100 g of food)	Raw		Cooked		Calculated (Based on moisture loss in cooked sample)	Difference between calculated and analysed (%)
	Average of 2 results	% RPD	Average of 2 results	% RPD		
Cadmium (µg/100 g)	<0.2	–	<0.2	–	–	–
Calcium (mg/100 g)	6.1	0.0	6.0	1.7	6.5	7.7
Chromium (µg/100 g)	<10	–	<10	–	–	–
Copper (mg/100 g)	0.05	12.2	0.05	2.1	0.05	6.1
Iodine (µg/100 g)	5.6	3.6	5.8	8.7	5.96	2.8
Iron (mg/100 g)	0.41	4.9	0.42	0.0	0.44	3.8
Lead (µg/100 g)	<1	–	<1	–	–	–
Magnesium (mg/100 g)	33	0.0	32	0.0	35	9.0
Manganese (mg/100 g)	<0.007	–	<0.007	–	–	–
Mercury (µg/100 g)	7.0	27.3	7.0	11.4	7.5	6.1
Methyl mercury (µg/100 g)	6.5	7.9	7.3	11.8	6.9	5.4
Molybdenum (µg/100 g)	<2	–	<2	–	–	–
Nickel (µg/100 g)	<10	–	<10	–	–	–
Phosphorus (mg/100 g)	220	0.0	220	0.0	234	6.1
Potassium (mg/100 g)	380	0.0	370	0.0	405	8.6
Selenium (µg/100 g)	43	2.4	39	0.0	46	14.8
Sodium (mg/100 g)	50	0.0%	47	0.0	53	11.7
Sulphur (mg/100 g)	235	4.3%	250	0.0	250	0.1
Tin (µg/100 g)	<3	–	<3	–	–	–
Zinc (mg/100 g)	0.46	2.2%	0.48	2.1	0.49	2.0

Table 5.6 Compositional profile for John West Yellowtail Kingfish (continued)

Component (Unit, per 100 g of food)	Raw		Cooked		Calculated (Based on moisture loss in cooked sample)	Difference between calculated and analysed (%)
	Average of 2 results	% RPD	Average of 2 results	% RPD		
Vitamin A as Retinol (IU/100 g)	<10	–	17.3	1.2	–	–
Ergocalciferol (Vitamin D2) (µg/100 g)	<0.3	–	<0.3	–	–	–
25-Hydroxy Vitamin D2 (µg/100 g)	<0.3	–	<0.3	–	–	–
Cholcalciferol (Vitamin D3) (µg/100 g)	1.2	8.7%	1.2	26.1	1.3	6.1
25-Hydroxy Vitamin D3 (µg/100 g)	<0.3	–	<0.3	–	–	–
Vitamin E as Total Tocopherols (IU/100 g)	4.07	6.4%	8.80	2.3	4.34	103.0

% RPD is the difference between the two results divided by the average result and expressed as a percentage. The lower the % RPD, the better the precision.
'–' Not calculated as at least one result was below the limit of reporting.

Table 5.7 Compositional profile for John West Sardines in Tomato Sauce and Pink Salmon

Component (Unit, per 100 g of food)	Sardines		Pink Salmon	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100 g)	618	1.1	613	0.3
Moisture (g/100 g)	72.8	0.4	69.0	0.0
Protein (g/100 g)	14.4	0.7	23.6	0.4
Fat (g/100 g)	9.7	0.0	6.2	1.6
Ash (g/100 g)	2.4	4.3	2.3	4.4
Total carbohydrate (g/100 g)	0.9	58.8	<0.5	–
Monounsaturated fatty acids (g/100 g)	3.6	0.0	1.8	0.0
Polyunsaturated fatty acids (g/100 g)	3.2	0.0	2.9	3.5
Saturated Fat (g/100 g)	2.8	0.0	1.5	0.0
Omega-3 (Total) (g/100 g)	2.6	0.0	2.4	0.0
Omega-6 (Total) (g/100 g)	0.4	0.0	0.2	0.0
Omega-9 (Total) (g/100 g)	2.6	0.0	1.0	0.0
EPA (mg/100 g)	750	0.0	635	1.6
DPA (mg/100 g)	66	0.0	180	0.0
DHA (mg/100 g)	1240	0.0	865	1.2
Trans fatty acids (g/100 g)	0.1	0.0	0.1	0.0
Cholesterol (mg/100 g)	100	1.0	69	0.0
Antimony (µg/100 g)	<1	–	2.1	4.9
Boron (mg/100 g)	0.068	1.5	<0.05	–
Cadmium (µg/100 g)	1.9	0.0	0.25	4.1
Calcium (mg/100 g)	220.0	9.1	265.0	3.8
Chromium (µg/100 g)	13	8.0	<10	–
Copper (mg/100 g)	0.09	2.3	0.07	8.6
Iodine (µg/100 g)	20.5	4.9	23.5	4.3
Iron (mg/100 g)	1.60	0.0	0.83	1.2
Lead (µg/100 g)	<1	–	<1	–
Magnesium (mg/100 g)	30	3.4	35	5.7
Manganese (mg/100 g)	0.165	6.1	0.033	3.1
Mercury (µg/100 g)	1.6	6.5	2.1	14.6
Methyl mercury (µg/100 g)	1.2	20.7	1.8	0.0
Molybdenum (µg/100 g)	2.9	3.5	<2	–
Nickel (µg/100 g)	<10	–	<10	–
Phosphorus (mg/100 g)	245	4.1	360	5.6
Potassium (mg/100 g)	320	0.0	320	0.0

Table 5.7 Compositional profile for John West Sardines in Tomato Sauce and Pink Salmon (continued)

Component (Unit, per 100 g of food)	Sardines		Pink Salmon	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Selenium (µg/100 g)	26	7.7	39	2.6
Sodium (mg/100 g)	400	0.0	355	2.8
Sulphur (mg/100 g)	165	6.1	260	0.0
Tin (µg/100 g)	<3	–	<3	–
Zinc (mg/100 g)	2.40	8.3	0.87	4.6
Vitamin A as Retinol (IU/100 g)	1027	7.4	41	7.4
Ergocalciferol (Vitamin D2) (µg/100 g)	<0.3	–	<0.3	–
25-Hydroxy Vitamin D2 (µg/100 g)	<0.3	–	<0.3	–
Cholcalciferol (Vitamin D3) (µg/100 g)	12	8.7	11	0.0
25-Hydroxy Vitamin D3 (µg/100 g)	<0.3	–	<0.3	–
Vitamin E as Total Tocopherols (IU/100 g)	3.64	5.8	0.85	3.6

% RPD is the difference between the two results divided by the average result and expressed as a percentage. The lower the % RPD, the better the precision.
'–' Not calculated as at least one result was below the limit of reporting.

Table 5.8 Compositional profile for John West Red Salmon and Salmon Tempters

Component (Unit, per 100 g of food)	Red Salmon		Salmon Tempters	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100 g)	705	1.0	589	0.2
Moisture (g/100 g)	66.5	0.2	72.5	0.3
Protein (g/100 g)	23.3	0.9	14.0	0.7
Fat (g/100 g)	8.8	1.1	7.2	1.4
Ash (g/100 g)	2.4	8.3	1.3	0.0
Total carbohydrate (g/100 g)	<0.5	–	5.1	3.9
Monounsaturated fatty acids (g/100 g)	3.4	3.0	2.1	0.0
Polyunsaturated fatty acids (g/100 g)	3.4	0.0	4.1	2.5
Saturated Fat (g/100 g)	1.9	5.4	1.0	0.0
Omega-3 (Total) (g/100 g)	2.9	0.0	0.6	0.0
Omega-6 (Total) (g/100 g)	0.2	0.0	3.4	3.0
Omega-9 (Total) (g/100 g)	1.7	0.0	1.9	0.0
EPA (mg/100 g)	625	1.6	120	0.0
DPA (mg/100 g)	160	0.0	33	0.0
DHA (mg/100 g)	965	1.0	245	4.1
Trans fatty acids (g/100 g)	0.1	0.0	<0.1	–
Cholesterol (mg/100 g)	72	5.6	28	7.1
Antimony (µg/100 g)	1.6	0.0	<1	–
Boron (mg/100 g)	<0.05	–	<0.05	–
Cadmium (µg/100 g)	0.60	6.7	0.46	0.0
Calcium (mg/100 g)	205.0	4.9	9.7	5.2
Chromium (µg/100 g)	<10	–	<10	–
Copper (mg/100 g)	0.07	5.6	0.04	7.6
Iodine (µg/100 g)	24.0	8.3	6.6	4.6
Iron (mg/100 g)	0.69	7.3	0.76	5.3
Lead (µg/100 g)	<1	–	<1	–
Magnesium (mg/100 g)	28	7.1	21	4.9
Manganese (mg/100 g)	0.014	0.0	0.052	9.7
Mercury (µg/100 g)	4.7	4.3	1.4	28.6
Methyl mercury (µg/100 g)	4.0	17.0	1.2	6.9
Molybdenum (µg/100 g)	<2	–	<2	–
Nickel (µg/100 g)	<10	–	<10	–
Phosphorus (mg/100 g)	290	6.9	130	0.0
Potassium (mg/100 g)	280	0.0	210	0.0
Selenium (µg/100 g)	40	0.0	21	9.5

Table 5.8 Compositional profile for John West Red Salmon and Salmon Tempters (continued)

Component (Unit, per 100 g of food)	Red Salmon		Salmon Tempters	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Sodium (mg/100 g)	410	0.0	285	3.5
Sulphur (mg/100 g)	255	3.9	160	0.0
Tin (µg/100 g)	<3	–	<3	–
Zinc (mg/100 g)	0.75	2.7	0.31	3.3
Vitamin A as Retinol (IU/100 g)	137	3.7	NA	NA
Ergocalciferol (Vitamin D2) (µg/100 g)	<0.3	–	NA	NA
25-Hydroxy Vitamin D2 (µg/100 g)	<0.3	–	NA	NA
Cholcalciferol (Vitamin D3) (µg/100 g)	23	8.7	NA	NA
25-Hydroxy Vitamin D3 (µg/100 g)	1.3	8.0	NA	NA
Vitamin E as Total Tocopherols (IU/100 g)	1.41	2.1	NA	NA

% RPD is the difference between the two results divided by the average result and expressed as a percentage. The lower the % RPD, the better the precision.
'–'Not calculated as at least one result was below the limit of reporting.
NA – Not analysed.

Table 5.9 Compositional profile for John West Tuna Tempters and Tuna in Springwater

Component (Unit, per 100 g of food)	Tuna Tempters		Tuna in Springwater	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100 g)	618	0.5	467	1.5
Moisture (g/100 g)	70.7	0.1	72.3	0.4
Protein (g/100 g)	19.9	0.5	26.2	2.7
Fat (g/100 g)	7.2	2.8	0.9	11.8
Ash (g/100 g)	1.5	0.0	1.3	0.0
Total carbohydrate (g/100 g)	0.8	50.0	<0.5	–
Monounsaturated fatty acids (g/100 g)	2.6	3.9	0.2	0.0
Polyunsaturated fatty acids (g/100 g)	3.8	2.7	0.3	0.0
Saturated Fat (g/100 g)	0.9	0.0	0.4	28.6
Omega-3 (Total) (g/100 g)	0.2	0.0	0.2	0.0
Omega-6 (Total) (g/100 g)	3.5	0.0	0.1	0.0
Omega-9 (Total) (g/100 g)	2.5	0.0	0.1	0.0
EPA (mg/100 g)	19	0.0	27	11.3
DPA (mg/100 g)	<10	–	<10	–
DHA (mg/100 g)	170	0.0	200	10.0
Trans fatty acids (g/100 g)	<0.1	–	<0.1	–
Cholesterol (mg/100 g)	38	2.7	53	1.9
Antimony (µg/100 g)	<1	–	1	–
Boron (mg/100 g)	<0.05	–	<0.05	–
Cadmium (µg/100 g)	1.2	0.0	1.8	0.0
Calcium (mg/100 g)	7.1	1.4	5.6	7.1
Chromium (µg/100 g)	<10	–	<10	–
Copper (mg/100 g)	0.07	4.0	0.07	5.4
Iodine (µg/100 g)	8.6	1.2	9.4	1.1
Iron (mg/100 g)	0.83	2.4	2.25	4.4
Lead (µg/100 g)	1.2	8.7	1	–
Magnesium (mg/100 g)	28	3.6	28	3.6
Manganese (mg/100 g)	0.072	2.8	0.009	3.4
Mercury (µg/100 g)	2.5	36.7	6.0	5.0
Methyl mercury (µg/100 g)	2.4	0.0	5.3	0.0
Molybdenum (µg/100 g)	<2	–	<2	–
Nickel (µg/100 g)	<10	–	<10	–
Phosphorus (mg/100 g)	155	6.5	220	0.0
Potassium (mg/100 g)	210	0.0	210	0.0

Table 5.9 Compositional profile for John West Tuna Tempters and Tuna in Springwater (continued)

Component (Unit, per 100 g of food)	Tuna Tempters		Tuna in Springwater	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Selenium (µg/100 g)	73	2.7	84	1.2
Sodium (mg/100 g)	375	2.7	285	3.5
Sulphur (mg/100 g)	215	4.7	265	3.8
Tin (µg/100 g)	<3	–	<3	–
Zinc (mg/100 g)	0.53	1.9	0.74	1.4

% RPD is the difference between the two results divided by the average result and expressed as a percentage. The lower the % RPD, the better the precision.
'–'Not calculated as at least one result was below the limit of reporting.

Table 5.10 Compositional profile for Birds Eye Atlantic Salmon Lemon Pepper

Component (Unit, per 100 g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100 g)	894	0.0	1150	0.0
Moisture (g/100 g)	63.0	0.2	54.5	0.2
Protein (g/100 g)	19.4	0.0	23.5	1.3
Fat (g/100 g)	14.6	0.7	20.1	0.0
Ash (g/100 g)	1.6	0.0	1.8	0.0
Total carbohydrate (g/100 g)	1.5	13.3	<0.5	–
Monounsaturated fatty acids (g/100 g)	5.8	0.0	7.9	0.0
Polyunsaturated fatty acids (g/100 g)	5.7	1.8	7.8	0.0
Saturated Fat (g/100 g)	3.0	0.0	4.2	0.0
Omega-3 (Total) (g/100 g)	3.8	0.0	5.2	0.0
Omega-6 (Total) (g/100 g)	1.5	0.0	2.0	0.0
Omega-9 (Total) (g/100 g)	4.6	0.0	6.3	0.0
EPA (mg/100 g)	750	0.0	1040	0.0
DPA (mg/100 g)	360	0.0	505	2.0
DHA (mg/100 g)	1065	0.9	1470	0.0
Trans fatty acids (g/100 g)	0.1	0.0	0.1	0.0
Cholesterol (mg/100 g)	58	3.4	70	1.4
Antimony (µg/100 g)	<1	–	<1	–
Boron (mg/100 g)	<0.05	–	<0.05	–
Cadmium (µg/100 g)	<0.2	–	<0.2	–
Calcium (mg/100 g)	64.5	41.9	79.5	8.8
Chromium (µg/100 g)	<10	–	<10	–
Copper (mg/100 g)	0.06	3.4	0.06	5.4
Iodine (µg/100 g)	7.8	10.3	7.9	3.8
Iron (mg/100 g)	0.31	3.3	0.33	0.0
Lead (µg/100 g)	<1	–	<1	–
Magnesium (mg/100 g)	29	0.0	33	6.1
Manganese (mg/100 g)	0.065	4.7	0.077	2.6
Mercury (µg/100 g)	2.5	32.0	2.1	19.0
Methyl mercury (µg/100 g)	2.4	3.6	2.6	0.0
Molybdenum (µg/100 g)	<2	–	<2	–
Nickel (µg/100 g)	<10	–	<10	–
Phosphorus (mg/100 g)	245	4.1	280	7.1
Potassium (mg/100 g)	350	0.0	400	0.0
Selenium (µg/100 g)	20	5.1	26	3.9

Table 5.10 Compositional profile for Birds Eye Atlantic Salmon Lemon Pepper (continued)

Component (Unit, per 100 g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Sodium (mg/100 g)	210	0.0	215	4.7
Sulphur (mg/100 g)	205	4.9	255	3.9
Tin (µg/100 g)	<3	–	<3	–
Zinc (mg/100 g)	0.44	6.9	0.61	3.3
Vitamin A as Retinol (IU/100 g)	<10	–	<10	–
Ergocalciferol (Vitamin D2) (µg/100 g)	<0.3	–	<0.3	–
25-Hydroxy Vitamin D2 (µg/100 g)	<0.3	–	<0.3	–
Cholcalciferol (Vitamin D3) (µg/100 g)	8.5	2.4	9.8	24.5
25-Hydroxy Vitamin D3 (µg/100 g)	<0.3	–	0.4	0.0
Vitamin E as Total Tocopherols (IU/100 g)	3.18	0.6	3.84	6.0

% RPD is the difference between the two results divided by the average result and expressed as a percentage. The lower the % RPD, the better the precision.
'–'Not calculated as at least one result was below the limit of reporting.

Table 5.11 Compositional profile for Birds Eye Lightly Seasoned Fish Fillets (Hoki) – Lemon & Cracked Pepper

Component (Unit, per 100 g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100 g)	670	1.5	663	0.5
Moisture (g/100 g)	68.2	0.9	67.9	0.0
Protein (g/100 g)	14.6	4.1	16.0	0.6
Fat (g/100 g)	7.4	0.0	6.9	2.9
Ash (g/100 g)	1.1	0.0	1.3	8.0
Total carbohydrate (g/100 g)	8.7	0.0	8.0	5.0
Monounsaturated fatty acids (g/100 g)	4.2	0.0	3.9	2.6
Polyunsaturated fatty acids (g/100 g)	2.2	0.0	2.1	0.0
Saturated Fat (g/100 g)	1.0	0.0	0.9	0.0
Omega-3 (Total) (g/100 g)	0.9	0.0	0.9	0.0
Omega-6 (Total) (g/100 g)	1.2	8.7	1.1	9.5
Omega-9 (Total) (g/100 g)	3.8	0.0	3.5	2.9
EPA (mg/100 g)	100	0.0	99	2.0
DPA (mg/100 g)	29	0.0	28	3.6
DHA (mg/100 g)	250	0.0	255	3.9
Trans fatty acids (g/100 g)	<0.1	–	<0.1	–
Cholesterol (mg/100 g)	29	3.5	29	3.5
Antimony (µg/100 g)	<1	–	<1	–
Boron (mg/100 g)	0.084	7.1	<0.05	–
Cadmium (µg/100 g)	<0.2	–	<0.2	–
Calcium (mg/100 g)	12.0	0.0	13.5	7.4
Chromium (µg/100 g)	<10	–	<10	–
Copper (mg/100 g)	0.04	6.9	0.05	4.4
Iodine (µg/100 g)	<5	–	<5	–
Iron (mg/100 g)	0.25	0.0	0.30	3.4
Lead (µg/100 g)	<1	–	<1	–
Magnesium (mg/100 g)	32	3.2	34	3.0
Manganese (mg/100 g)	0.120	0.0	0.130	0.0
Mercury (µg/100 g)	5.5	29.1	5.4	13.1
Methyl mercury (µg/100 g)	5.0	6.9	4.2	20.4
Molybdenum (µg/100 g)	2.2	0.0	2.2	4.7
Nickel (µg/100 g)	<10	–	<10	–
Phosphorus (mg/100 g)	160	0.0	170	0.0
Potassium (mg/100 g)	300	0.0	325	3.1

Table 5.11 Compositional profile for Birds Eye Lightly Seasoned Fish Fillets (Hoki) – Lemon & Cracked Pepper (continued)

Component (Unit, per 100 g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Selenium (µg/100 g)	44	0.0	49	2.1
Sodium (mg/100 g)	130	0.0	140	0.0
Sulphur (mg/100 g)	155	6.5	165	6.1
Tin (µg/100 g)	<3	–	<3	–
Zinc (mg/100 g)	0.28	0.0	0.30	10.2

% RPD is the difference between the two results divided by the average result and expressed as a percentage. The lower the % RPD, the better the precision.
'–'Not calculated as at least one result was below the limit of reporting.

Table 5.12 Compositional profile for Birds Eye Fish Fingers 1 kg (Hoki/Hake)

Component (Unit, per 100 g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100 g)	838	1.9	854	1.2
Moisture (g/100 g)	59.3	1.3	58.8	1.0
Protein (g/100 g)	9.9	2.0	11.0	2.7
Fat (g/100 g)	8.3	1.2	8.7	0.0
Ash (g/100 g)	1.1	0.0	1.2	0.0
Total carbohydrate (g/100 g)	21.5	2.3	20.4	4.4
Monounsaturated fatty acids (g/100 g)	4.8	0.0	5.1	0.0
Polyunsaturated fatty acids (g/100 g)	2.6	3.9	2.7	0.0
Saturated Fat (g/100 g)	0.9	0.0	0.9	0.0
Omega-3 (Total) (g/100 g)	0.9	0.0	0.9	0.0
Omega-6 (Total) (g/100 g)	1.6	0.0	1.7	0.0
Omega-9 (Total) (g/100 g)	4.5	0.0	4.7	0.0
EPA (mg/100 g)	57	1.8	64	1.6
DPA (mg/100 g)	<10	–	<10	–
DHA (mg/100 g)	150	0.0	175	5.7
Trans fatty acids (g/100 g)	<0.1	–	<0.1	–
Cholesterol (mg/100 g)	28	0.0	31	3.3
Antimony (µg/100 g)	<1	–	<1	–
Boron (mg/100 g)	<0.05	–	<0.05	–
Cadmium (µg/100 g)	0.4	0.0	0.4	6.9
Calcium (mg/100 g)	12.3	2.4	15.5	6.5
Chromium (µg/100 g)	12	8.7	12	8.7
Copper (mg/100 g)	0.06	0.0	0.06	3.2
Iodine (µg/100 g)	17.5	5.7	<5	–
Iron (mg/100 g)	0.43	4.7	0.46	6.6
Lead (µg/100 g)	<1	–	<1	–
Magnesium (mg/100 g)	21	4.9	21	0.0
Manganese (mg/100 g)	0.190	0.0	0.200	0.0
Mercury (µg/100 g)	1.1	9.5	1.1	9.5
Methyl mercury (µg/100 g)	1.0	0.0	1.1	30.8
Molybdenum (µg/100 g)	4.7	0.0	4.8	0.0
Nickel (µg/100 g)	13	0.0	<10	–
Phosphorus (mg/100 g)	170	0.0	170	0.0
Potassium (mg/100 g)	130	0.0	130	0.0
Selenium (µg/100 g)	30	3.4	31	6.5

Table 5.12 Compositional profile for Birds Eye Fish Fingers 1 kg (Hoki/Hake) (continued)

Component (Unit, per 100 g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Sodium (mg/100 g)	270	0.0	275	3.6
Sulphur (mg/100 g)	105	9.5	120	0.0
Tin (µg/100 g)	<3	–	<3	–
Zinc (mg/100 g)	0.33	0.0	0.39	5.1

% RPD is the difference between the two results divided by the average result and expressed as a percentage. The lower the % RPD, the better the precision.
'–'Not calculated as at least one result was below the limit of reporting.

Table 5.13 Compositional profile for Birds Eye Oven Bake Fish Fillets (Hoki/Hake) Original Crumb 425 g

Component (Unit, per 100 g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100 g)	899	0.0	914	0.2
Moisture (g/100 g)	58.2	0.2	57.4	0.2
Protein (g/100 g)	10.6	2.8	10.9	0.0
Fat (g/100 g)	10.2	0.0	10.3	1.9
Ash (g/100 g)	1.0	10.5	1.0	0.0
Total carbohydrate (g/100 g)	20.2	1.5	20.5	1.5
Monounsaturated fatty acids (g/100 g)	6.1	1.7	6.1	1.7
Polyunsaturated fatty acids (g/100 g)	3.0	0.0	3.2	3.2
Saturated Fat (g/100 g)	1.1	0.0	1.1	0.0
Omega-3 (Total) (g/100 g)	1.0	0.0	1.1	0.0
Omega-6 (Total) (g/100 g)	1.9	5.4	1.9	0.0
Omega-9 (Total) (g/100 g)	5.6	0.0	5.6	1.8
EPA (mg/100 g)	79	0.0	83	2.4
DPA (mg/100 g)	12	0.0	<10	–
DHA (mg/100 g)	160	0.0	190	0.0
Trans fatty acids (g/100 g)	<0.1	–	<0.1	–
Cholesterol (mg/100 g)	20	5.1	22	4.7
Antimony (µg/100 g)	<1	–	<1	–
Boron (mg/100 g)	<0.05	–	<0.05	–
Cadmium (µg/100 g)	<0.2	–	0.2	0.0
Calcium (mg/100 g)	9.4	0.0	9.8	2.0
Chromium (µg/100 g)	13	8.0	12	8.7
Copper (mg/100 g)	0.06	5.0	0.06	4.7
Iodine (µg/100 g)	<5	–	5.7	7.0
Iron (mg/100 g)	0.47	0.0	0.44	2.3
Lead (µg/100 g)	<1	–	<1	–
Magnesium (mg/100 g)	24	8.3	24	0.0
Manganese (mg/100 g)	0.260	7.7	0.265	3.8
Mercury (µg/100 g)	1.8	28.6	1.3	40.0
Methyl mercury (µg/100 g)	1.4	30.3	1.0	0.0
Molybdenum (µg/100 g)	4.5	6.7	4.9	4.1
Nickel (µg/100 g)	<10	–	<10	–
Phosphorus (mg/100 g)	115	8.7	120	0.0
Potassium (mg/100 g)	185	5.4	180	0.0

Table 5.13 Compositional profile for Birds Eye Oven Bake Fish Fillets (Hoki/Hake) Original Crumb 425 g (continued)

Component (Unit, per 100 g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Selenium (µg/100 g)	32	9.5	34	0.0
Sodium (mg/100 g)	175	5.7	180	0.0
Sulphur (mg/100 g)	115	8.7	120	0.0
Tin (µg/100 g)	<3	–	<3	–
Zinc (mg/100 g)	0.34	5.9	0.38	5.3

% RPD is the difference between the two results divided by the average result and expressed as a percentage. The lower the % RPD, the better the precision.
'–'Not calculated as at least one result was below the limit of reporting.

Table 5.14 Compositional profile for Birds Eye Deep Sea Dory Fish Portions Original Crumb 425 g

Component (Unit, per 100 g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100 g)	931	0.1	926	0.8
Moisture (g/100 g)	59.2	0.8	59.3	0.8
Protein (g/100 g)	10.4	2.9	9.7	1.0
Fat (g/100 g)	12.9	2.3	12.8	0.8
Ash (g/100 g)	1.3	8.0	1.3	0.0
Total carbohydrate (g/100 g)	16.4	6.1	17.1	4.1
Monounsaturated fatty acids (g/100 g)	7.9	1.3	7.8	1.3
Polyunsaturated fatty acids (g/100 g)	3.8	2.7	3.8	2.7
Saturated Fat (g/100 g)	1.2	0.0	1.2	0.0
Omega-3 (Total) (g/100 g)	1.2	8.7	1.2	0.0
Omega-6 (Total) (g/100 g)	2.5	4.1	2.4	0.0
Omega-9 (Total) (g/100 g)	7.3	1.4	7.3	1.4
EPA (mg/100 g)	50	6.1	47	0.0
DPA (mg/100 g)	10	0.0	10	0.0
DHA (mg/100 g)	105	9.5	100	0.0
Trans fatty acids (g/100 g)	<0.1	–	<0.1	–
Cholesterol (mg/100 g)	18	5.7	18	0.0
Antimony (µg/100 g)	<1	–	<1	–
Boron (mg/100 g)	<0.05	–	0.090	4.4
Cadmium (µg/100 g)	0.32	3.2	0.30	0.0
Calcium (mg/100 g)	11.0	0.0	11.5	8.7
Chromium (µg/100 g)	15	0.0	13	8.0
Copper (mg/100 g)	0.06	1.6	0.04	2.3
Iodine (µg/100 g)	<5	–	<5	–
Iron (mg/100 g)	0.48	4.2	0.50	6.1
Lead (µg/100 g)	<1	–	<1	–
Magnesium (mg/100 g)	22	4.7	23	4.4
Manganese (mg/100 g)	0.285	3.5	0.285	3.5
Mercury (µg/100 g)	3.0	16.9	2.7	26.4
Methyl mercury (µg/100 g)	3.2	13.3	2.4	18.2
Molybdenum (µg/100 g)	4.8	2.1	5.1	0.0
Nickel (µg/100 g)	10	0.0	12	0.0
Phosphorus (mg/100 g)	170	0.0	170	0.0
Potassium (mg/100 g)	160	0.0	160	0.0

Table 5.14 Compositional profile for Birds Eye Deep Sea Dory Fish Portions Original Crumb 425 g (continued)

Component (Unit, per 100 g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Selenium (µg/100 g)	32	6.3	33	0.0
Sodium (mg/100 g)	285	3.5	295	3.4
Sulphur (mg/100 g)	100	1.0	110	0.0
Tin (µg/100 g)	<3	–	<3	–
Zinc (mg/100 g)	0.32	6.2	0.36	5.6

% RPD is the difference between the two results divided by the average result and expressed as a percentage. The lower the % RPD, the better the precision.
'–'Not calculated as at least one result was below the limit of reporting.

Chapter 6 Acceptability and effects of a higher fish diet — a randomised controlled trial

Introduction

Studies have shown benefits with adequate fish and LCn3PUFA in terms of heart health in the general population, and for women of child-bearing age, optimal infant neurodevelopmental is also implicated. Not only is nutrition during pregnancy and lactation critical, nutrition pre-conception has also been shown to be important (Gardiner et al. 2008). In Australia, it is estimated that 40–50% of pregnancies are unplanned (Marie Stopes International 2008) and as such, women who are pregnant may not be aware of the pregnancy themselves when foetal development begins. It would therefore be logical for women to incorporate a healthy balanced diet with adequate fish and LCn3PUFA throughout child-bearing age. However, there is concern with the contaminants present in some fish, particularly MeHg, and whether increasing fish intake would lead to unwanted side effects of mercury. The results from the analysis of 13 fish products presented in Chapter 5 demonstrates that the amount of MeHg in the tested products would not pose any risk of MeHg toxicity unless the intake is unrealistically high. The aim of this study is to implement a diet of a higher fish content to provide sufficient LCn3PUFA and examine (i) the acceptability of such a diet, and (ii) the effects on various biological parameters including changes in mercury levels. Since polymorphisms in certain genes have been associated with differences in the metabolism of fatty acids and mercury, determination of several single nucleotide polymorphisms (SNPs) previously shown to be linked with the metabolism of fatty acids and mercury was included in the study to allow for any potential confounding effects related to genetic determinants of responses.

6.1 Methods

6.1.1 Study design

This study was a single-blinded, parallel randomised controlled trial of eight weeks' duration, preceded by a two-week run-in period to access the acceptability of a diet higher in fish and related changes in several biological parameters, if any. The study was approved by the Southern Adelaide Health Services/Flinders University Human Research Ethics Committee and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000572066).

6.1.2 Study population

Healthy premenopausal women aged between 18 and 50 years with a BMI of ≥18.5 to ≤30 kg/m2 and relatively stable weight were recruited via advertisements in newspapers as well as posters placed in Flinders Medical Centre and Flinders University. Exclusions were pregnant or lactating women; daily consumers of fish oil or other supplements which could interfere with lipid metabolism; women with bleeding disorders, Type 1 or Type 2 diabetes, cardiovascular disease (e.g. unstable angina, heart failure, hypertension), dyslipidaemia, chronic inflammatory disease (e.g. rheumatoid arthritis, Crohn's Disease, ulcerative colitis) or seafood allergies; regular users of non-steroidal anti-inflammatory drugs; vegetarian or vegan or usual dietary intake consisting of more than one oily fish per week on average. Women working in occupations (such as dentists) with regular exposure to mercury were also excluded.

Women who met the criteria at the initial telephone screening were scheduled to attend a screening session at Flinders Medical Centre. At the screening visit, fasting blood samples were collected for the testing of cholesterol, triglycerides and glucose levels. Blood pressure, height and weight were also measured. Women who had cholesterol levels of >5.5 mmol/L, triglyceride >2 mmol/L, glucose ≥7 mmol/L, systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg or BMI <18.5 or >30 kg/m2 were excluded. Women who met all the criteria were then invited to take part in the study.

Written informed consent was obtained from all participants.

6.1.3 Study intervention

Participants were randomised to follow either a higher fish diet (intervention) or a diet that was lower in fish and higher in meat (control) after a two-week run-in period while stabilised on the control diet. Overall, participants attended seven sessions within a two-month period. Whilst sessions one, three, five and seven had to be conducted at Flinders Medical Centre, sessions two, four and six were conducted at other locations if the participant so wished (e.g. at home or work place). Baseline (week 0), mid-trial (week 4) and end of study (week 8) assessments were conducted at sessions three, five and seven respectively. A graphical representation of the study protocol is shown in Figure 6.1.

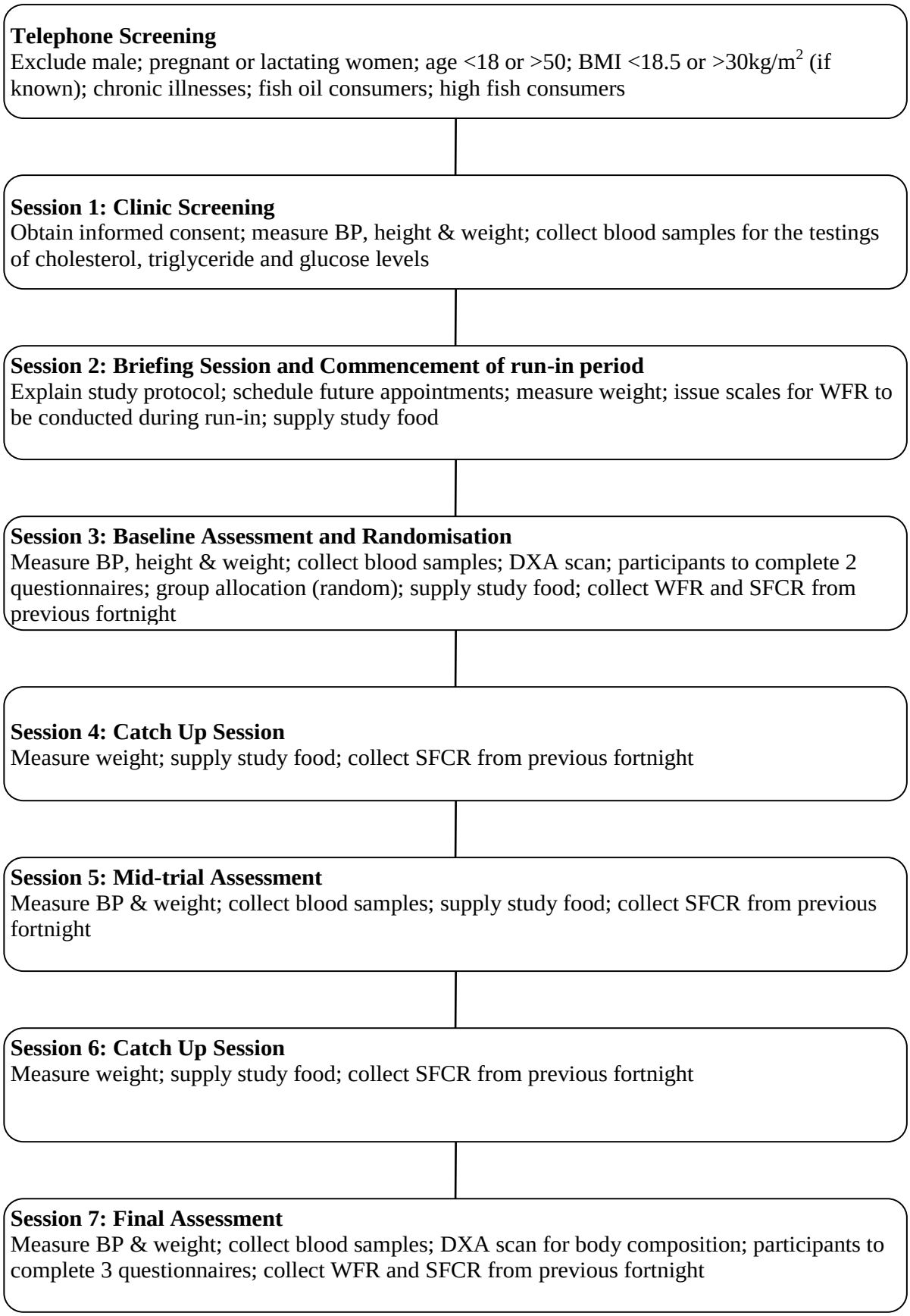


Figure 6.1 A graphical representation of study protocol for the randomised controlled trial examining the acceptability and effects of a higher fish diet

Intervention diet – a higher fish diet

The study foods for the higher fish diet provided on average 473 kJ of energy, 12.7 g of protein, 5.7 g of total fat, 138 mg of sodium, 243 mg of EPA, 338 mg of DHA and 0.46 mg of iron per day.

Fish products for the intervention (higher fish) group were supplied by Simplot Australia and included the following over a four-week period:

John West Atlantic Salmon (Skin Off)*, 2 x 150 g

John West Yellowtail Kingfish*#, 2 x 150 g

John West Pink Salmon, 105 g

John West Red Salmon, 105 g

John West Sardines in Tomato Sauce, 110 g

John West Salmon Tempters Onion & Tomato, 95 g

John West Tuna Tempters Lemon & Cracked Pepper, 2 x 95 g

John West Tuna in Springwater, 2 x 95 g

Birds Eye Atlantic Salmon Lemon Pepper, 135 g

Birds Eye Lightly Seasoned Fish Fillets (Hoki) – Lemon & Cracked Pepper, 200 g

Birds Eye Oven Bake Fish Fillets (Hoki/Hake) Original Crumb, 142 g

Birds Eye Deep Sea Dory Fish Portions Original Crumb, 142 g

These 16 fish meals were repeated for another four weeks to complete the 8-week study. Figure 6.2 depicts the fish products supplied over the entire 8-week study period.

* John West Atlantic Salmon and John West Yellowtail Kingfish were purchased from Coles Supermarkets (various stores in metropolitan Adelaide) and supplied to participants as fresh fish fillets.

Towards the end of October 2010, John West discontinued the sale of retail packs of Yellowtail Kingfish from Coles Supermarkets. Yellowtail Kingfish was therefore sourced from Cleanseas in Port Lincoln via Saltys, a meat, fish and seafood processor in Adelaide. Cleanseas was the same supplier for John West previously.

Figure 6.2:Intervention group study food

Control diet – a diet lower in fish and higher in meat

The study foods for the higher meat lower fish diet provided on average 465 kJ of energy, 12.9 g of protein, 5.1 g of total fat, 144 mg of sodium and 0.83 mg of iron per day. Participants in the control group were restricted to consume no more than one serving of low fat fish meal per week as per usual habit.

Meat products for the control (meat) group were purchased from Foodland Supermarkets at North Adelaide, Woolworths Supermarkets (various stores) and Lenard's (various stores) and included the following:

Chicken thigh fillet, 2 x 120 g

Beef scotch fillet, 2 x 150 g

Beef extra lean mince, 4 x 100 g

Primo Roast beef slices, 2 x 50 g

Select turkey slices, 2 x 40 g

Steggles Mini Roast with spinach and cheese, 175 g

Lenard's chicken schnitzel, 200 g

Lenard's chicken kiev, 200 g

Lenard's chicken cutlet cacciatore, 200 g

These 16 meat meals were repeated for another four weeks to complete the eight-week study. Figure 6.3 depicts the meat products supplied over the entire eight-week study period.

Figure 6.3: Control group study food

Background diet

Other than the substitution of four meat meals per week with the study food provided and to avoid or limit intake of omega-3 rich food, all participants were instructed to maintain their usual dietary intakes throughout the study period. A list of commonly consumed omega-3 rich food and drinks (e.g. canola oil, flaxseed or linseed oil, walnuts, food or drinks fortified with omega-3 fatty acids) was provided to study participants and they were advised to avoid these foods (Appendix 4). Olive oil and olive oil spreads were supplied to all participants for cooking and to use as spreads during the study period.

Blood samples collection and analysis

Trained phlebotomists collected blood samples in the morning before 10 am after the study participants had fasted between 12 and 14 hours. Participants were also instructed to avoid alcohol in the preceding 24 hours.

Blood samples were drawn into BD Vacutainer® tubes in the following order:

(a) 8.5 ml Gold top tube containing spray-coated silica and gel separator (REF 367958, SST™II Advance)

Blood was allowed to clot under room temperature for 30–60 minutes and centrifuged (Sigma® Laboratory Centrifuges 6K 15) at 1300 x g for 10 minutes. Serum was separated and stored in aliquots at -70 °C.

(b) 6.0 ml Royal Blue top tube containing dipotassium ethylene diamine tetraacetic acid (K2EDTA) 10.8 mg (REF 368381)

Two tubes of blood were drawn from each participant per assessment time point using this tube. Blood from one of the two tubes was stored as whole blood aliquots at -70 °C. The other tube was centrifuged (Beckman GS-6R Centrifuge) at 4 °C at 1300 x g for 10 minutes. Plasma was then separated and stored in aliquots at -70 °C.

(c) 6.0ml Pink top tube containing K2EDTA 10.8 mg (REF 367974)

Blood was centrifuged at 4 °C at 1300 x g for 10 minutes. Plasma was then separated and stored in aliquots at -70 °C. The red blood cells mass was re-suspended in normal saline at 4 °C and the samples despatched to the Fatty Acids Laboratory, University of Adelaide at Waite Campus within 72 hours.

(d) 2.0 ml Lavender top tube containing tripotassium ethylene diamine tetraacetic acid (K3EDTA) 3.6 mg (REF 367836)

Two tubes of blood were drawn from each participant at baseline assessment but only one tube at the final assessment using this tube. Fresh blood samples were despatched to an accredited commercial laboratory for haemoglobin analysis usually within three hours of collection while stored at room temperature. The extra tube collected at baseline was stored at 4 °C until deoxyribonucleic acid (DNA) extraction was performed.

Nunc™ CryoTube™ 1.8 ml vials (REF 377267) and 4.5 ml vials (REF 379146) were used as storage vials. All blood samples collected, except for the analysis of fatty acids and haemoglobin, were analysed after the trial had completed.

Randomisation

Participants were randomly assigned using random number generated by the StatsDirect Statistical Software system. Participants were block randomised using random block sizes to either the intervention or control group. Allocation only occurred after a two-week run-in period and after baseline assessment was completed. Sealed envelopes indicating group allocation were prepared by a person unrelated to the study according to the sequence generated. All researchers conducting assessments were blinded to treatment allocation.

6.2 Study outcomes – methods of assessment

6.2.1 Single nucleotide polymorphisms (SNPs) analysis

Three SNPs (rs953413, rs2277324, rs174537) shown to be associated with fatty acids synthesis (Tanaka et al. 2009) and three SNPs (rs1695, rs17883901, rs1138272) shown to be associated with mercury elimination were examined in this study (Custodio et al. 2004).

The following steps were involved in the SNPs detection:

(i) Extraction and purification of DNA from whole blood samples using QIAamp DNA Blood Midi Kit (QIAGEN, Germany). DNA was quantitated on a spectrometer.

(ii) Amplification of DNA via the polymerase chain reaction (PCR) with the addition of specific primers obtained from GeneWorks, Australia (Table 6.1) and a DNA polymerase (HotStarTaq® Plus, QIAGEN, Germany).

(iii) SNPs detection using ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, United States). After SNAPshot reactions were performed, samples were submitted to SouthPath and Flinders Sequencing Facility for electrophoresis and analysis with GeneScan® Analysis Software on the ABI PRISM® 3130X Genetic Analyzer platform.

(iv) Repeat analysis by sequencing was conducted in several randomly selected samples to confirm results by the SNaPshot protocol. Those samples that failed the SNaPshot protocol were also re-analysed by sequencing.

Table 6.1 Forward primers, reverse primers and probes used in the polymerase chain reaction

SNPs	Primer/Probe	Sequence
rs953413	Forward (Left)	TCT CAC AAA TGT ACT CAC CAT CA
	Reverse (Right)	TCT GTG ACG ATA AGG ACC ACA
	Probe	GAC TAA CGC TAA AGG TCA CAA AGC CC
rs174537	Forward	CAG GGG AGA GAG GTG GAG TA
	Reverse	CTG GCT GTC TCC CTC ACA G
	Probe	GAC TGA CTG ACT GCT CTC CCT CTG TCT TGG AC
rs2277324	Forward	AGG AAC CAG CCT CTC TCT CC
	Reverse	CCT GAA TCC CGC TCA TTC T
	Probe	CTC GGG TCT GGC GAG AGC
rs17883901	Forward	GGC CCT GTC CAA CTA AAA CA
	Reverse	AGC ATG CCC AGT CTT TGC
	Probe	TGC AAG GGT GAT TGG GTC
rs1695	Forward	GCT GGG GCT CAC AGA CAG
	Reverse	GCA ATA AGG GTG CAG GTT GT
	Probe	TAG TTG GTG TAG ATG AGG GAG A
rs1138272	Forward	GCA GCT GAA GTG GAC AGG AT
	Reverse	GTG GCG AGG AAG GAA CAG
	Probe	GAC TCT TCA CAT AGT CAT CCT TGC CC

6.2.2 Dietary assessment

6.2.2.1 3-day weighed-food record

Participants were instructed to record all food and drinks consumed over three days (including two weekdays and one weekend day) during the run-in period and also the last fortnight of the study. Digital scales with maximum of 3 kg capacity and 1 g graduations (Kenwood, DS607005) were provided to participants for weighing of foods. Weighed-food records were analysed by the candidate using FoodWorks 2009, Professional edition, Version 6 (Xyris Software, Australia). This software uses the Australia AUSNUT 2007 database to calculate nutrient intakes based on the food records.

6.2.2.2 Study food consumption record

Participants were instructed to record when (e.g. at breakfast, lunch or dinner) and how much (if not all consumed, e.g. ½, ¾) of the study foods provided were consumed on the day of consumption if possible.

6.2.2.3 Diet acceptability

Diet acceptability was assessed by a diet acceptability questionnaire (see Appendix 5) implemented on the day of the final assessment. This questionnaire was adapted from a study by Barnard et al. (2000) on the acceptability of a therapeutic low fat, vegan diet in premenopausal women. Only five out of the eight questions in the original questionnaire were included in the questionnaire used in this study.

6.2.3 Fatty acids analysis

Red blood cells were washed three times with normal saline. Erythrocyte lipids were then extracted with chloroform:propanol and separated by thin-layer chromatography (Broekhuyse 1974). The samples were methylated in 1% sulphuric acid in methanol for three hours at 70 °C. The resulting fatty acid methylesters were extracted with heptane and then quantified by gas chromatography (Smithers et al. 2008). Fatty acids analyses were conducted by the candidate under supervision at the Fatty Acid Lab at the Waite Campus of the University of Adelaide. The Omega-3 Index was calculated by adding the levels of EPA and DHA, expressed as a percent of total

erythrocyte fatty acids, and categorised as ‘undesirable’ (<4%), ‘intermediate’ (between 4% and <8%), and ‘desirable’ (≥8%) (Harris 2007).

Fatty acids were extracted from plasma phospholipids and processed in a similar manner as in red blood cells but using chloroform and methanol as extraction medium instead (Bligh & Dyer 1959).

6.2.4 Lipids study

Total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides were analysed on the Siemens Advia 2400 Chemistry platform. Low-density lipoprotein cholesterol (LDL-C) was calculated based on the concentrations of total cholesterol, HDL-C and triglycerides. Analyses were conducted by Healthscope Pathology (formerly Gribbles Pathology), at Wayville, South Australia. Healthscope is a National Association of Testing Authorities (NATA) accredited laboratory.

6.2.4.1 Total cholesterol

A standard enzymatic method was used to determine the concentration of total cholesterol. Cholesterol esters were hydrolysed by cholesterol esterase to cholesterol and free fatty acids. Cholesterol was then converted to cholest-4-en-3-one by cholesterol oxidase in the presence of oxygen to form hydrogen peroxide. The absorbance of the coloured complex formed from hydrogen peroxide, 4-aminoantipyrine and phenol under the catalytic influence of peroxidase, was measured at 505/694 nm (Trinder reaction).

6.2.4.2 HDL-C

A standard two-step enzymatic method was used to determine the concentration of HDL-C. Step 1 involved elimination of chylomicrons, very-low-density lipoproteins cholesterol (VLDL-C) and LDL-C by reaction with cholesterol esterase and cholesterol oxidase. Peroxide produced by the oxidase was removed by catalase. Catalase from Step 1 was inhibited by the addition of sodium azide. Step 2 involved the release of cholesterol in HDL particles by a surfactant. The HDL-C was then measured by a Trinder reaction. The intensity of the quinoneimine dye produced in

the Trinder reaction was directly proportional to the cholesterol concentration when measured at 596 nm.

6.2.4.3 Triglycerides

A standard enzymatic method was used to determine the concentration of total triglycerides, including the mono and diglycerides and the free glycerol fractions. Triglycerides were converted to glycerol and free fatty acids by lipase. Glycerol was then converted to glycerol-3-phosphate by glycerol kinase which in turned was converted to hydrogen peroxide by glycerol-3-phosphate-oxidase. The absorbance of the coloured complex formed from hydrogen peroxide, 4-aminophenazone and 4-cholorphenol under the catalytic influence of peroxidase was measured at 505/694 nm.

6.2.4.4 LDL-C

LDL-C was calculated using the Friedewald Equation. LDL-C calculations are invalid when triglyceride concentration is greater than 4.5 mmol/L and therefore not reported.

Friedewald Equation:

LDL-C = Total cholesterol – HDL-C – (Triglycerides /2.2)
(all values in mmol/L)

6.2.5 Iron study and haemoglobin

Iron and transferrin were analysed on the Siemens Advia 2400 Chemistry platform. Transferrin saturation was calculated based on the concentrations of serum iron and transferrin. Ferritin was analysed on the Siemens Advia Centaur Immunoassay platform. Analysis was conducted by Healthscope Pathology at Wayville, South Australia.

6.2.5.1 Serum iron

Ferric iron was dissociated from its carrier protein, transferrin, in an acid medium and simultaneously reduced to the ferrous form with ascorbic acid. The ferrous iron was then complexed with ferrozine to produce a coloured chromophore which was measured at 571/658 nm.

6.2.5.2 Transferrin

The concentration of transferrin was determined by a polyethylene glycol enhanced immunoturbidimetric assay. The method involves reacting human transferrin with specific antiserum to form a precipitate that can be measured turbidimetrically at 596/694 nm.

6.2.5.3 Transferrin saturation

The percentage of transferrin saturation was calculated according to the following formula:

% Transferrin Saturation = 3.982 x Iron (μmol/L) / Transferrin (g/L)

6.2.5.4 Serum ferritin

Ferritin concentration was determined by a two-site sandwich immunoassay using direct chemiluminometric technology. This assay uses constant amounts of two anti-ferritin antibodies. The first antibody is a polyclonal goat anti-ferritin antibody labelled with acridinium ester. The second antibody is a monoclonal mouse anti-ferritin antibody covalently coupled to paramagnetic particles. The amount of relative light units detected by the system is directly proportional to the amount of ferritin present in the blood sample.

6.2.5.5 Haemoglobin analysis

Haemoglobin analysis was performed on a Sysmex XE 2100 analyser using the manufacturer reagents. The Sysmex analysers used the SLE-Haemoglobin method. Sodium lauryl sulphate (SLS) was added to the red blood cells to convert haemoglobin into a stable SLS-haemoglobin complex. Concentration of SLS-haemoglobin was photometrically measured at an absorption maximum of 555 nm. Analysis was conducted by Healthscope Pathology at Wayville, South Australia.

6.2.6 Mercury analysis

Total mercury was analysed according to the Appendix to the EPA method 1631 (Jan 2001): Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and Bromine monochloride (BrCl) Oxidation. Blood samples were acid digested with heat and further oxidised with BrCl. Digestates were analysed by stannous chloride ($SnCl_2$) reduction, followed by gold amalgamation, thermal desorption and cold

vapour atomic fluorescence spectroscopy (CVFAS) using a Brooks Rand Labs Model III Analyzer. Methyl mercury was analysed according to the method of EPA 1630, Modified. Blood samples were digested in a potassium hydroxide/methanol solution. The digestates were then distilled in Teflon distillation vials. Samples were then analysed by ethylation Tenaz trap pre-concentration, gas chromatography separation, pyrolytic combustion and atomic fluorescence spectroscopy (CV-GC-AFS) using a BRL MERX-M Analyzer. Both THg and MeHg analyses were conducted by Brooks Rand Labs in Seattle, USA.

6.2.7 Selenium analysis

Total selenium was analysed by inductive coupled plasma mass spectrometry (ICP-MS) using a Perkin-Elmer ELAN DRC II. Analysis was conducted by Brooks Rand Labs in Seattle, USA, using both whole blood and plasma samples.

6.2.8 Inflammatory marker analysis (C-reactive protein)

Serum high-sensitivity C-reactive protein (CRP) was analysed on a Hitachi Cobas-Bio modular analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) by using a Tina-quant C-reactive protein (latex) high-sensitive assay kit (Roche Diagnostics, Mannheim, Germany). Analysis was conducted by Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Adelaide. CRP readings of >10 mg/L generally reflect the presence of acute infection or inflammation and were excluded from statistical analysis.

6.2.9 Anthropometric and other assessments

6.2.9.1 Height, weight, BMI and body composition

Height was measured using the Seca 284 (Germany) height and weight measuring station. Height was recorded without shoes to the nearest 0.1cm.

Weight was measured without shoes and in light clothing using a digital scale (Tanita, BF-679W, Japan) and recorded to the nearest 0.1 kg.

BMI was calculated by dividing the weight (in kilogram) by the square of the height (in metres):

$$\text{BMI (kg/m}^2\text{)} = \text{Weight (kg)} / [\text{Height (m)} \times \text{Height (m)}]$$

Dual energy X-ray absorptiometry technology (Lunar Prodigy, enCORE 2006 version 10.51.006, GE, Madison, USA) was used to measure body composition. Standard protocol was followed regarding the positioning of participants and removal of all metallic items (e.g. on clothes or accessories) during the scan. All analyses were performed by the same operator who was blinded to the allocation of the study participants.

6.2.9.2 Blood pressure

Blood pressure was measured using an automatic oscillometric device (Criticare 5070, Criticare Systems Inc., USA). Blood pressure was measured after five minutes of sitting and a second reading taken after one minute. If the systolic blood pressure differed by more than 10 mmHg or diastolic blood pressure by more than 6 mmHg, a third reading was taken. Blood pressure was reported as the average of all measurements taken.

6.2.9.3 The Center for Epidemiologic Studies Depression (CES-D) Scale

The CES-D Scale questionnaire (see Appendix 6) is a 20-item self-reported scale to measure symptoms associated with depression and can be used as a tool for epidemiologic studies of depression in the general population (Radloff 1977). This scale, however, is not a diagnostic tool for individuals.

6.3 Sample size and statistical analyses

The sample size was calculated based on data from a study conducted in pregnant women in Western Australia (Dunstan et al. 2004). In order to detect a 20% difference in DHA level in the erythrocyte membrane between groups, with a power (β) of 90% and a probability (α) of 0.05, 16 women needed to be included in each group. Allowing for a 'drop-out' rate of 20%, the recruitment target was set at 40 women (20 per group).

For the SNPs analyses, the PLINK (1.07) programme was used to test for association between allele/genotype and phenotype using linear regression (Purcell et al. 2007). All other statistical analyses were performed using IBM SPSS Statistics Version 20. Median and mode were shown for the result of the diet acceptability questionnaire; all other descriptive statistics are presented as means and standard deviations. The Mann-Whitney-U test was used to compare the responses of the diet acceptability questionnaire between the intervention and control group. Pearson's correlation was used to assess associations between fatty acids in red blood cells and plasma phospholipids. Mixed-design ANOVA was used to examine the main effect of time and diet as well as the interaction between time and diet. Data were transformed as needed using log or square root to follow a normal distribution prior to analysis. A p-value of <.05 was regarded as statistically significant.

6.4 Results

A total of 52 women were assessed for eligibility between August 2010 and April 2011. Of the 42 women who were eligible and initially enrolled into the study, 39 were randomised (Figure 6.4). In order to avoid the Christmas and New Year holiday period, participants commenced the 10-week trial either before mid-October 2011 or after February 2011. All 39 women who were randomised (intervention group, n=19; control group, n=20) completed the study. One participant was later ruled ineligible as her triglyceride level at baseline was found to be higher than the set inclusion criteria. Results from 38 women (19 from each group) were therefore included in the final analysis. Analysis was conducted according to the original assigned groups (i.e. intention-to-treat analysis).

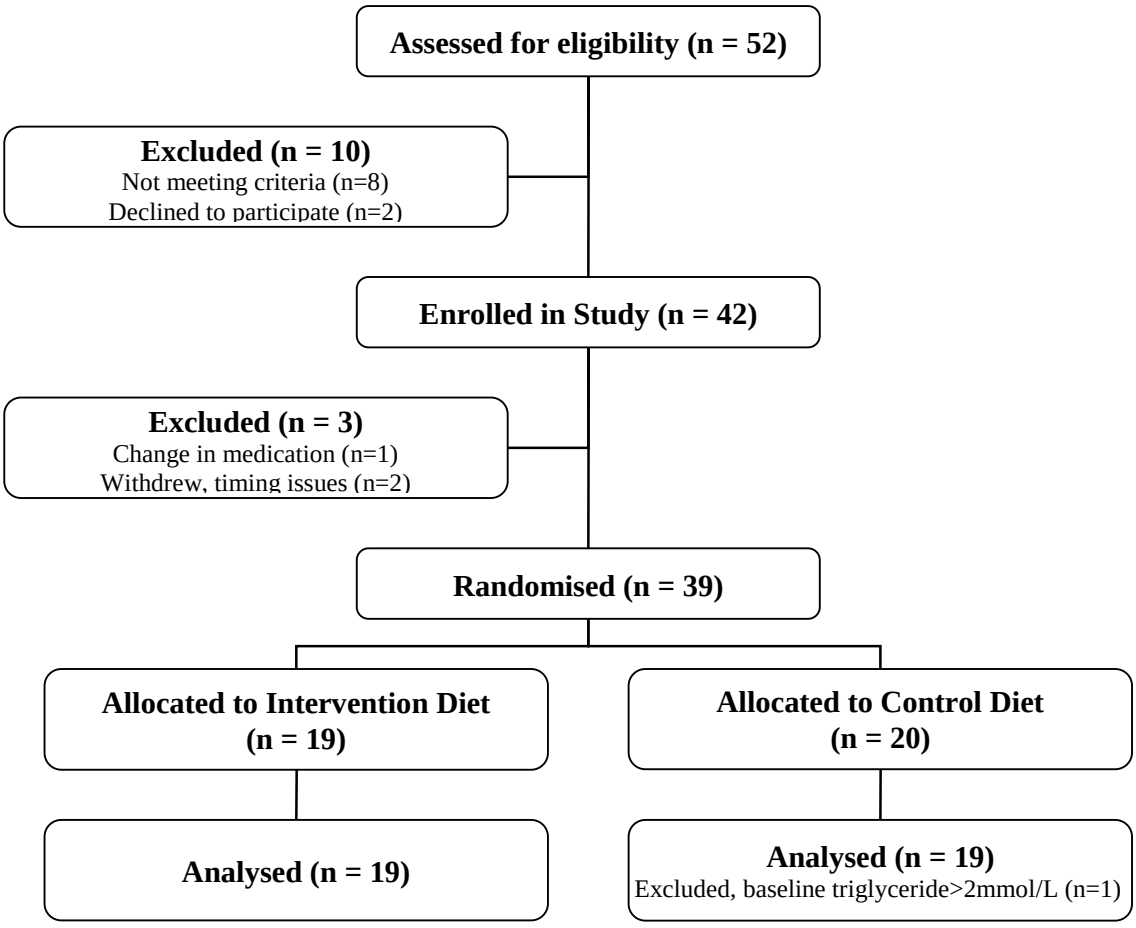


Figure 6.4 Flow diagram of the progress through phases of the randomised control trial aimed at examining the acceptability and effects of a higher fish diet

The characteristics of study participants at baseline are shown in Table 6.2 below. There was no statistically significant difference between the two groups in any of the parameters reported.

Table 6.2 Characteristics of study participants at baseline

Baseline characteristics	Intervention (n = 19)	Control (n = 19)	p-value
Age (years)	34.3 ± 9.5	33.5 ± 7.4	.78
BMI (kg/m^2)	23.3 ± 3.1	23.5 ± 2.3	.78
Caucasian (%)	84.2%	94.7%	.60
Number of amalgam fillings	1.7 ± 2.1	2.0 ± 2.3	.77
Erythrocyte EPA (% of total fatty acids)	0.89 ± 0.34	0.78 ± 0.20	.22
Erythrocyte DPA (% of total fatty acids)	2.70 ± 0.36	2.60 ± 0.34	.42
Erythrocyte DHA (% of total fatty acids)	4.80 ± 1.00	4.96 ± 0.57	.55

Values are mean ± standard deviation or percentages (%)

6.4.1 Single Nucleotide Polymorphisms (SNPs)

All 38 participants were successfully genotyped for the six selected SNPs. All SNPs investigated were in Hardy-Weinberg equilibrium and the frequencies for the alleles are listed in Table 6.3.

Table 6.4 and Table 6.5 show the mean long chain polyunsaturated fatty acids and mercury levels respectively at baseline according to the genotype. No statistically significant differences in the means were found except for AA with SNP rs174537. Since no substantial differences were observed between genotypes, no adjustment for genotypes was included in subsequent analysis.

Table 6.3 Minor allele frequencies and testing for Hardy-Weinberg equilibrium

Chromosome	SNP identifier	Location	Minor Allele	Major Allele	Minor Allele Frequency	Non-missing allele count	Genotype count	Observed hetero-zygosity	Expected hetero-zygosity	p-value
Fatty acids										
6	rs953413	11012609	A	G	0.41	76	7/17/14	0.45	0.48	.74
11	rs174537	61552430	A	C	0.32	76	3/18/17	0.47	0.43	.72
12	rs2277324	58012925	T	C	0.38	76	4/21/13	0.55	0.47	.49
Mercury										
6	rs17883901	53409787	A	G	0.07	76	0/5/33	0.13	0.12	1.00
11	rs1695	67352439	C	T	0.29	76	2/18/18	0.47	0.41	.46
11	rs1138272	67353329	A	G	0.08	76	1/4/33	0.11	0.15	.19

Table 6.4 Mean (standard deviation) fatty acids levels by genotypes (count, %) at baseline

Fatty acids (% of total fatty acids) Mean (standard deviation)	SNP (rs953413)			
	A/A (7, 18%)	A/G (17, 45%)	G/G (14, 37%)	p-value
EPA	0.69 (0.45)	0.89 (0.27)	0.85 (0.16)	.33
DPA	2.38 (0.36)	2.69 (0.32)	2.73 (0.34)	.05
DHA	4.78 (0.48)	4.94 (0.97)	4.85 (0.76)	.94
AA	13.49 (1.08)	13.70 (0.95)	13.86 (0.55)	.36

Fatty acids (% of total fatty acids) Mean (standard deviation)	SNP (rs174537)			
	A/A (3, 8%)	A/C (18, 47%)	C/C (17, 45%)	p-value
EPA	1.04 (0.48)	0.82 (0.33)	0.82 (0.16)	.38
DPA	2.81 (0.18)	2.57 (0.46)	2.70 (0.20)	.77
DHA	5.29 (1.96)	4.73 (0.73)	4.96 (0.63)	.97
AA	12.25 (0.71)	13.67 (0.79)	14.04 (0.62)	.001

Fatty acids (% of total fatty acids) Mean (standard deviation)	SNP (rs2277324)			
	T/T (4, 11%)	T/C (21, 55%)	C/C (13, 34%)	p-value
EPA	1.04 (0.24)	0.75 (0.20)	0.91 (0.36)	.85
DPA	2.70 (0.21)	2.56 (0.38)	2.78 (0.30)	.27
DHA	5.03 (0.39)	4.84 (0.78)	4.89 (0.97)	.88
AA	13.20 (0.45)	13.96 (0.58)	13.49 (1.14)	.79

Table 6.5 Mean (standard deviation) total mercury and methyl mercury levels by genotypes (count, %) at baseline

Mean (standard deviation)	SNP (rs17883901)			
	A/A (0, 0%)	A/G (5, 13%)	G/G (33, 87%)	p-value
Total mercury (ng/g)	-	1.65 (1.55)	1.71 (1.64)	.83
Methyl mercury (ng/g)	-	1.34 (1.33)	1.37 (1.28)	.80

Mean (standard deviation)	SNP (rs1695)			
	C/C (2, 5%)	C/T (18, 47%)	T/T (18, 47%)	p-value
Total mercury (ng/g)	0.99 (0.13)	1.83 (1.42)	1.64 (1.87)	.48
Methyl mercury (ng/g)	0.77 (0.01)	1.47 (1.24)	1.33 (1.38)	.81

Mean (standard deviation)	SNP (rs1138272)			
	A/A (1, 3%)	A/G (4, 11%)	G/G (33, 87%)	p-value
Total mercury (ng/g)	1.08 (0.00)	1.68 (0.91)	1.72 (1.70)	.80
Methyl mercury (ng/g)	0.76 (0.00)	1.39 (0.84)	1.38 (1.34)	.86

6.4.2 Dietary assessment

6.4.2.1 3-day weighed-food record

Of the 38 sets of 3-day weighed-food records returned by the participants, three sets of records from the intervention group were not included in the analysis. Data from one participant was incomplete as there was only one set of data from the run-in period (i.e. the two weeks prior to randomisation) and none during the trial period (i.e. Week 7 and Week 8 of the period study). Data from another two participants were also excluded as both 3-day food records were conducted during the trial period and none from the run-in period. In nearly all of the cases, daily nutrient intakes were estimated by averaging the intakes from three days of food intake records. However, one participant provided six days of intake record during the run-in period whilst another participant provided only two days of intake record for both periods. Analyses were therefore conducted based on available information.

The estimated intakes of several key nutrients of interest prior to (run-in period) and during the study period (trial periods) are listed in Table 6.6. Significant time x diet interactions were observed for vitamin D and LCn3PUFA intakes.

Table 6.6 Estimated key nutrients intake (per day) prior to (Run-in^) and during the study period (Trial^) as obtained from the averages of 3-day weighed-food record

Nutrient	Intervention (n=16)	Control (n=19)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
Energy including energy from dietary fibre (kJ)					
Run-in	7548 (1629)	7221 (2082)	$F(1,33)=.088$ p=.768	$F(1,33)=.560$ p=.460	$F(1,33)=2.956$ p=.095
Trial	7065 (1474)	6537 (2385)			
Protein (g)					
Run-in	83.4 (16.7)	83.5 (22.2)	$F(1,33)=3.141$ p=.086	$F(1,33)=.862$ p=.360	$F(1,33)=2.210$ p=.147
Trial	84.4 (23.7)	71.7 (26.2)			
Fat (g)					
Run-in	68.0 (21.7)	58.6 (22.4)	$F(1,33)=.165$ p=.688	$F(1,33)=1.491$ p=.231	$F(1,33)=3.311$ p=.078
Trial	58.3 (19.8)	52.5 (25.0)			
Saturated Fat (g)					
Run-in	26.0 (9.3)	22.1 (10.3)	$F(1,33)=.314$ p=.579	$F(1,33)=1.051$ p=.313	$F(1,33)=1.777$ p=.192
Trial	22.2 (9.0)	20.6 (10.5)			
Carbohydrate (g)					
Run-in	182.9 (43.0)	188.0 (52.3)	$F(1,33)=.263$ p=.611	$F(1,33)=.000$ p=.984	$F(1,33)=.314$ p=.579
Trial	182.4 (35.2)	177.9 (65.0)			
Iron (mg)					
Run-in	11.05 (2.98)	10.41 (2.82)	$F(1,33)=.001$ p=.981	$F(1,33)=.407$ p=.528	$F(1,33)=1.421$ p=.242
Trial	10.54 (2.77)	9.92 (3.88)			
Sodium (mg)					
Run-in	2133 (655)	2135 (602)	$F(1,33)=.011$ p=.917	$F(1,33)=.006$ p=.940	$F(1,33)=.111$ p=.741
Trial	2205 (973)	2172 (796)			
Vitamin D (µg)					
Run-in	2.51 (1.20)	2.01 (1.25)	$F(1,33)=20.718$ p<.001	$F(1,33)=30.432$ p<.001	$F(1,33)=17.325$ p<.001
Trial	5.47 (2.20)	1.88 (1.12)			
Long chain n-3 polyunsaturated fatty acids					
Run-in	128 (74)	124 (223)	$F(1,33)=36.674$ p<.001	$F(1,33)=43.664$ p<.001	$F(1,33)=32.769$ p<.001
Trial	1130 (684)	86 (71)			

^ Run-in period = the two weeks prior to randomisation; Trial = Week 7 and Week 8 of the study period
Descriptive statistics are presented as mean (standard deviation)
Time x Diet interactions, main effect of diet and main effect of time tested using mixed-design ANOVA
Data for long chain n-3 polyunsaturated fatty acids were log transformed prior to analysis

Table 6.7 and Table 6.8 show the estimated daily intakes of other vitamins and minerals, respectively, as obtained from the 3-day weighed-food records. There was no statistically significant main effect of diet, main effect of time or time x diet interaction for any other vitamins or minerals listed except for magnesium and zinc. There was a statistically significant time x diet interaction for magnesium and the mean magnesium intake in the intervention group increased from 318 mg to 340 mg per day whereas a reduction from 319 mg to 277 mg per day was observed in the control group-values. For zinc, there was no significant time x diet interaction but a main effect of time was observed. The mean zinc intake for both intervention and control groups combined decreased from 10.81 mg during run-in to 9.33 mg during the trial period.

Table 6.7 Estimated intakes (per day) of selected vitamins prior to (Run-in^) and during the study period (Trial^) as obtained from the averages of 3-day weighed-food record

Nutrient	Intervention (n=16)	Control (n=19)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
Vitamin A as retinol equivalents (μg)					
Run-in	759 (379)	705 (385)	$F(1,33)=.714$ p=.404	$F(1,33)=2.195$ p=.148	$F(1,33)=.380$ p=.542
Trial	780 (432)	574 (284)			
Thiamin, B1 (mg)					
Run-in	1.48 (0.65)	1.51 (0.79)	$F(1,33)=.406$ p=.529	$F(1,33)=.348$ p=.559	$F(1,33)=.259$ p=.614
Trial	2.24 (3.17)	1.51 (0.74)			
Riboflavin, B2 (mg)					
Run-in	2.28 (1.19)	2.12 (0.87)	$F(1,33)=.000$ p=.995	$F(1,33)=.270$ p=.607	$F(1,33)=.463$ p=.501
Trial	2.17 (0.93)	2.01 (1.06)			
Niacin equivalents (mg)					
Run-in	43.23 (9.57)	45.44 (13.37)	$F(1,33)=2.189$ p=.149	$F(1,33)=.086$ p=.771	$F(1,33)=.667$ p=.420
Trial	44.72 (12.51)	40.25 (15.65)			
Folate as dietary folate equivalents (μg)					
Run-in	353 (252)	408 (238)	$F(1,33)=.484$ p=.491	$F(1,33)=.424$ p=.520	$F(1,33)=.034$ p=.855
Trial	351 (193)	383 (192)			
Vitamin C (mg)					
Run-in	94 (102)	66 (43)	$F(1,33)=.209$ p=.651	$F(1,33)=.113$ p=.739	$F(1,33)=.000$ p=.995
Trial	67 (35)	72 (48)			
Vitamin E (mg)					
Run-in	7.07 (2.69)	7.33 (3.44)	$F(1,33)=3.324$ p=.077	$F(1,33)=.861$ p=.360	$F(1,33)=.674$ p=.418
Trial	8.77 (3.81)	6.69 (3.73)			

^ Run-in period = the two weeks prior to randomisation; Trial = Week 7 and Week 8 of the study period
Descriptive statistics are presented as mean (standard deviation)
Time x Diet interactions, main effect of diet and main effect of time tested using mixed-design ANOVA
Data for thiamin, folate as dietary folate equivalents, and vitamin C were log transformed prior to analysis

Table 6.8 Estimated intakes (per day) of selected minerals prior to (Run-in^) and during the study period (Trial^) as obtained from the averages of 3-day weighed-food record

Nutrient	Intervention (n=16)	Control (n=19)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
Calcium (mg)					
Run-in	733 (159)	786 (301)	$F(1,33)=2.259$ p=.142	$F(1,33)=.194$ p=.663	$F(1,33)=.439$ p=.512
Trial	866 (300)	735 (422)			
Iodine (μg)					
Run-in	101.2 (20.6)	96.6 (42.5)	$F(1,33)=2.216$ p=.146	$F(1,33)=2.143$ p=.153	$F(1,33)=.048$ p=.827
Trial	113.1 (45.7)	87.7 (32.0)			
Magnesium (mg)					
Run-in	318 (92)	319 (111)	$F(1,33)=6.010$ p=.020	$F(1,33)=.769$ p=.387	$F(1,33)=.656$ p=.424
Trial	340 (99)	277 (129)			
Phosphorus (mg)					
Run-in	1392 (263)	1407 (369)	$F(1,33)=3.437$ p=.073	$F(1,33)=.656$ p=.424	$F(1,33)=1.567$ p=.220
Trial	1428 (366)	1220 (510)			
Potassium (mg)					
Run-in	2758 (693)	2654 (830)	$F(1,33)=2.044$ p=.162	$F(1,33)=.895$ p=.351	$F(1,33)=1.227$ p=.276
Trial	2790 (659)	2399 (1025)			
Zinc (mg)					
Run-in	11.23 (3.68)	10.46 (4.85)	$F(1,33)=.166$ p=.687	$F(1,33)=.404$ p=.529	$F(1,33)=6.606$ p=.015
Trial	9.45 (3.77)	9.23 (4.11)			

^ Run-in period = the two weeks prior to randomisation; Trial = Week 7 and Week 8 of the study period
Descriptive statistics are presented as mean (standard deviation)
Time x Diet interactions, main effect of diet and main effect of time tested using mixed-design ANOVA
Data for zinc were log transformed prior to analysis

Since there appears little difference in energy and nutrient intakes between the intervention and control groups (except for vitamin D and LCn3PUFA), data and graphs presented on page 180 are based on the weighed-food records for both groups combined. The mean daily energy intake during the run-in period and the trial period for all participants was 7370 kJ (range: 3790–10 934 kJ) and 6779 kJ (range: 2767–11 585 kJ) respectively. These intakes corresponded to an average of 91% and 84% of the estimated energy requirement calculated using the Schofield equations (Schofield 1985) and assuming a PAL of 1.4 (very sedentary) during the two periods. The contribution of various macronutrients towards total energy intake and the ratio

of monounsaturated, polyunsaturated and saturated fats are shown in Figure 6.5 and Figure 6.6 respectively.

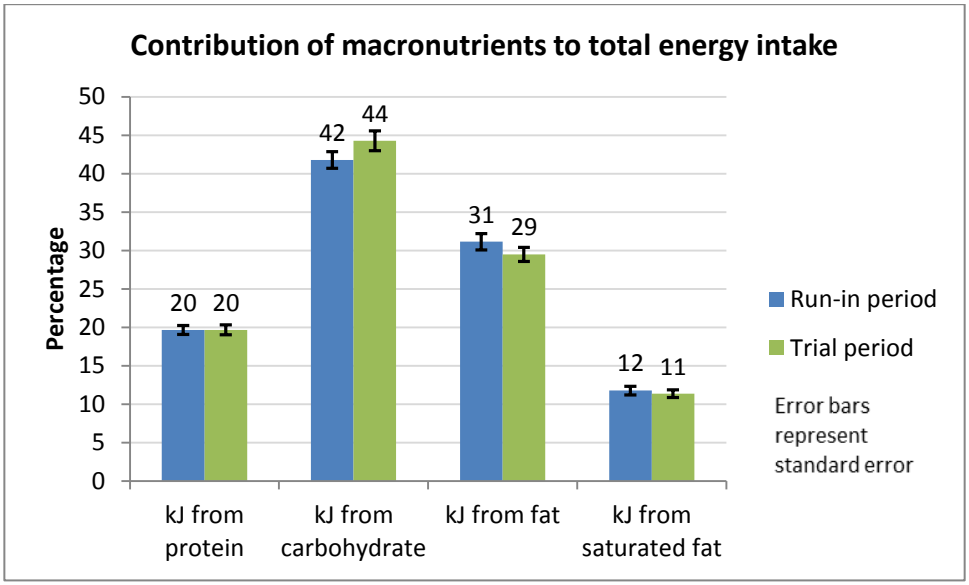


Figure 6.5 Contribution of various macronutrients towards total energy intake during the run-in period (i.e. two weeks prior to randomisation) and trial period (i.e. Week 7 and Week 8 of the study period)

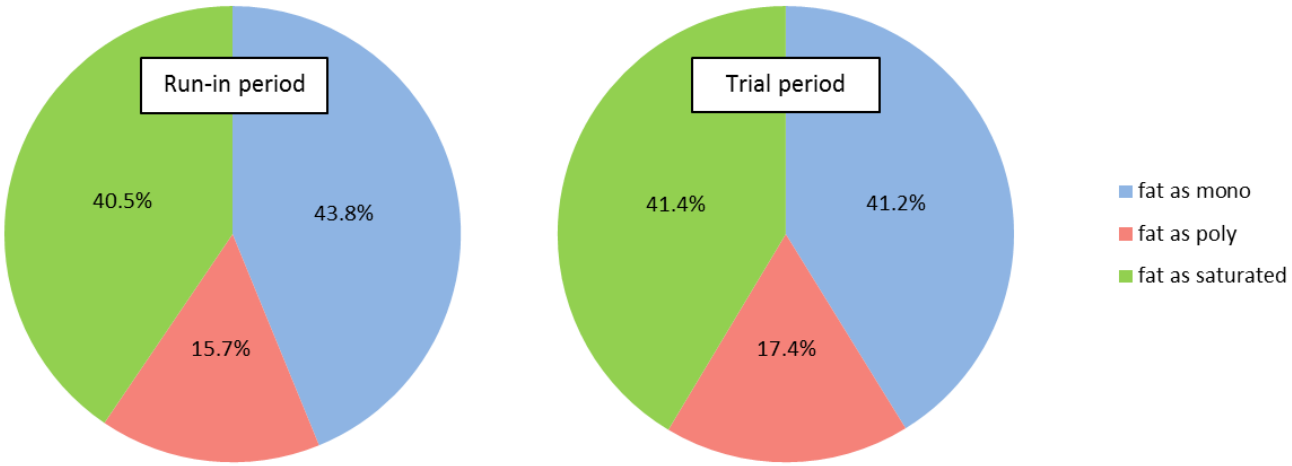


Figure 6.6 Ratio of monounsaturated, polyunsaturated and saturated fats observed during the run-in period (i.e. two weeks prior to randomisation) and trial period (i.e. Week 7 and Week 8 of the study period)

6.4.2.2 Compliance to allocated diets

Thirteen of the 19 participants in the control group consumed more than 90% of the study food provided. On average, 91% of the study food provided was consumed. Fish consumption in the control group was maintained at the pre-study level of no more than once fish meal each week.

In the intervention group, 18 of the 19 participants consumed more than 90% of the study foods provided. On average, 97% of the food provided was consumed. The number of additional fish meals (own supply) consumed by each participant during the eight-week study period ranged from zero to five serves.

6.4.2.3 Diet acceptability

There was no difference in the median scores for diet acceptability between the two groups.

Table 6.9 Acceptability of a diet higher in fish (intervention) versus a low fish diet (control) rated on a 1 to 7 scale

Questions	Intervention (n=19)		Control (n=19)		p-value
	Median	Mode	Median	Mode	
How well do you like the food? (1 = 'extremely unappealing', 7 = 'extremely good')	6	6	5	5	.055
How easy to prepare the food? (1 = 'extremely difficult', 7 = 'extremely easy')	6	7	6	6	.699
How much effort is needed to stay on diet? (1 = 'more than is possible', 7 = 'no effort at all')	6	6	6	6	.940
How easy to purchase, prepare and eat the foods in future? (1 = 'extremely difficult', 7 = 'extremely easy')	6	7	6	6	.844
How would you rate the acceptability of the diet? (1 = 'completely unacceptable', 7 = extremely acceptable')	6	7	6	6	.984

Responses for questionnaire items were tested using Mann-Whitney U Test.

6.4.3 Fatty acids

There were significant association between fatty acids levels in red blood cells and in plasma phospholipids as shown in Figure 6.7 to Figure 6.10 with correlation coefficients (*r*) ranging from 0.442 to .900, p-values all < .05.

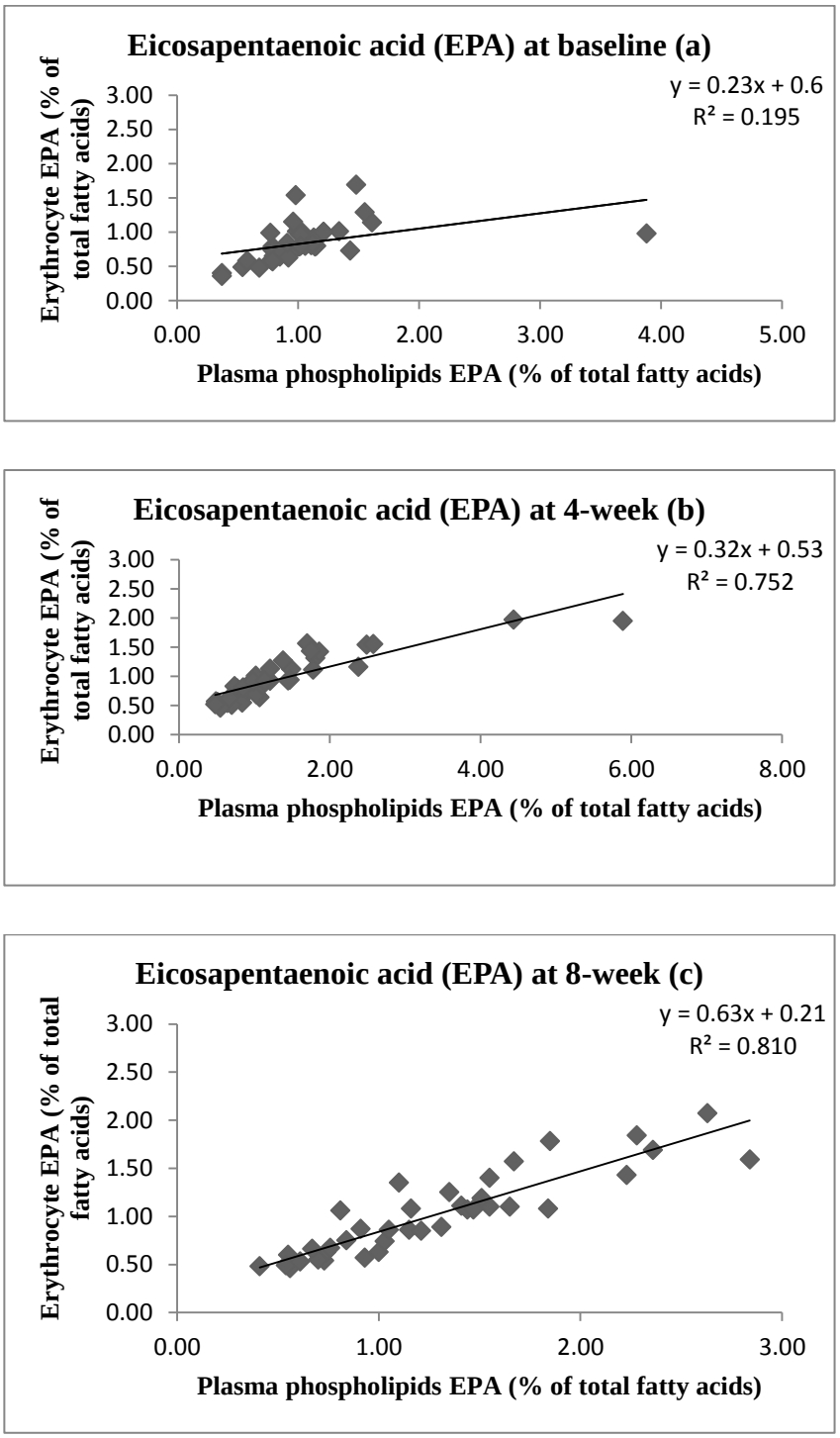


Figure 6.7 Correlations between erythrocyte EPA and plasma phospholipids EPA at baseline (a), 4-week (b) and 8-week (c).

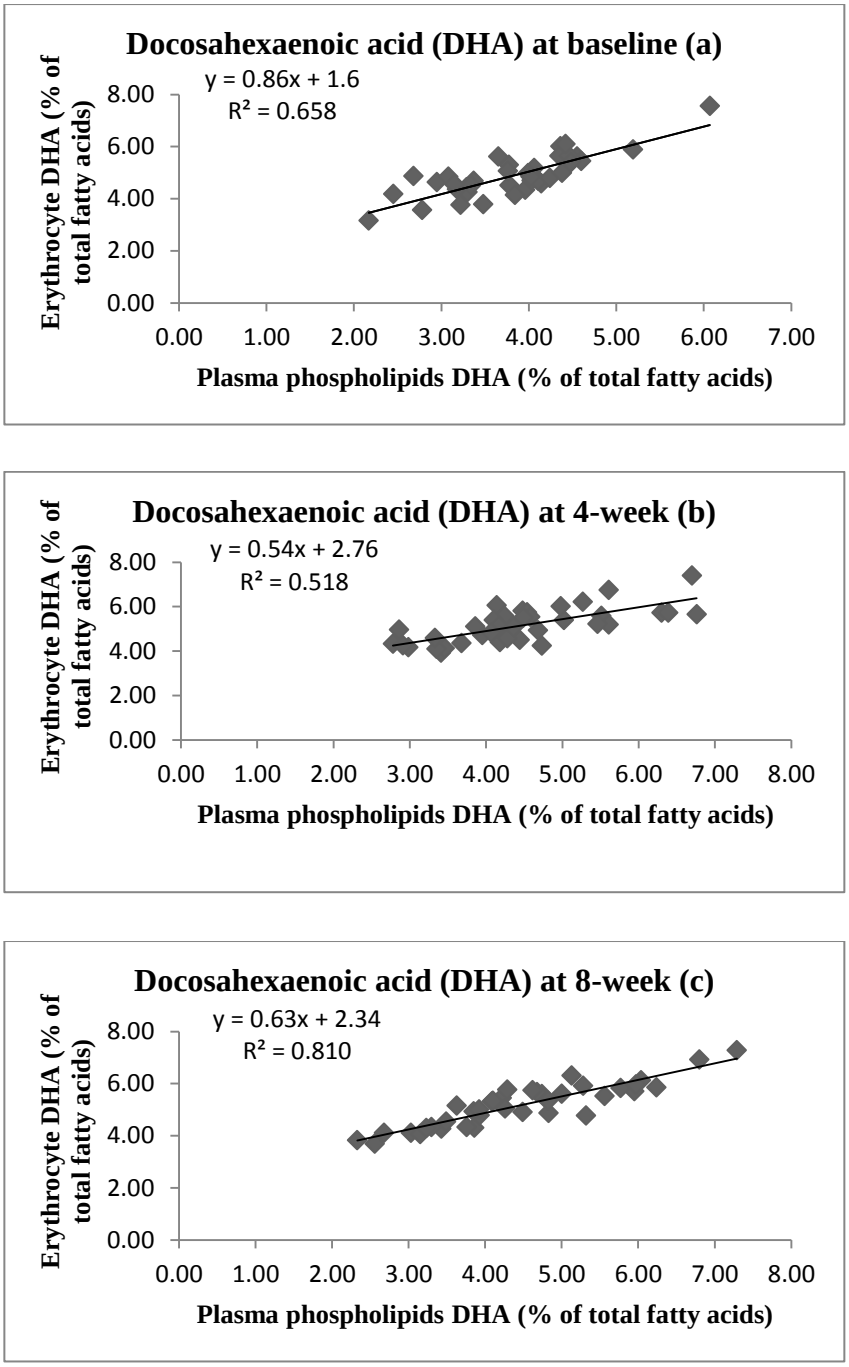
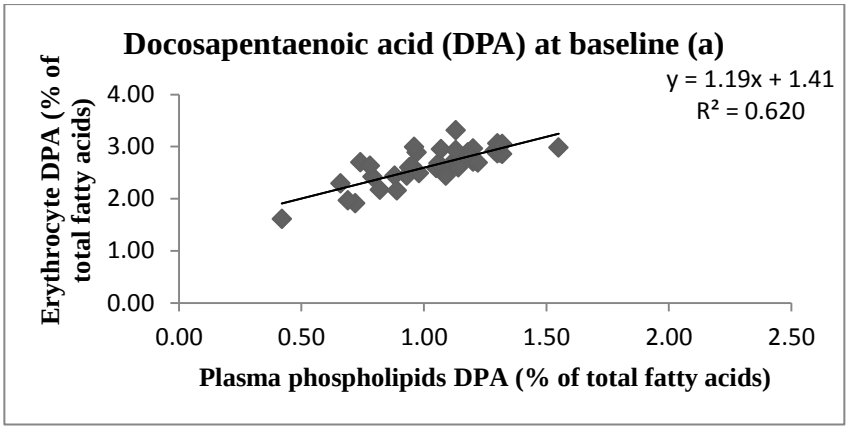
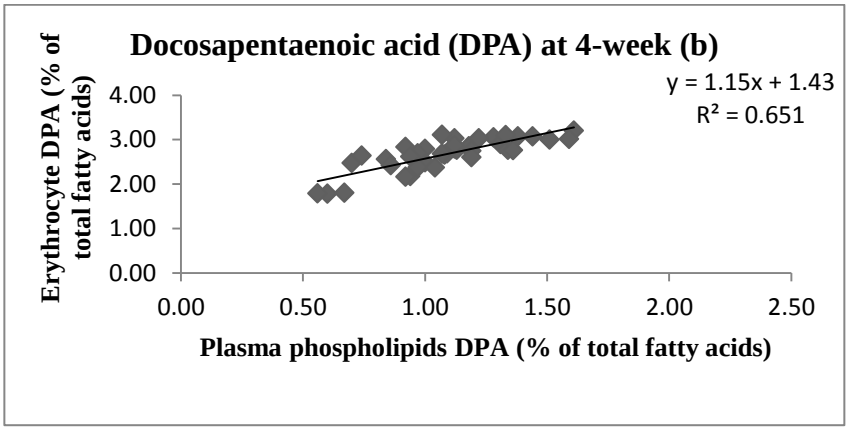


Figure 6.8 Correlations between erythrocyte DHA and plasma phospholipids DHA at baseline (a), 4-week (b) and 8-week (c).





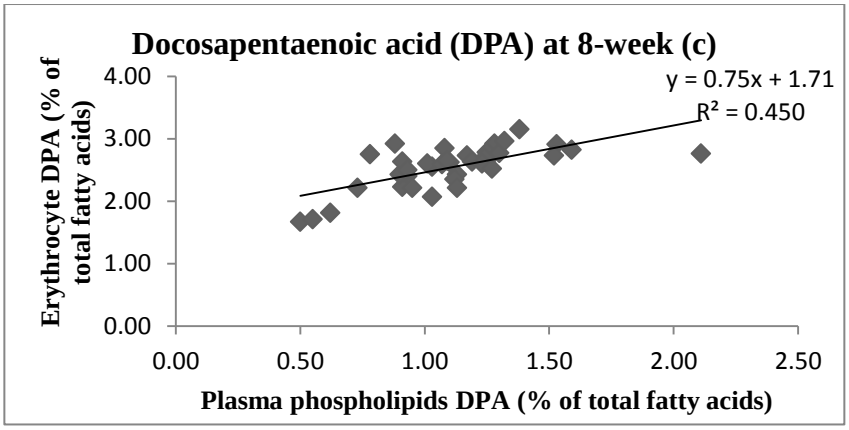


Figure 6.9 Correlations between erythrocyte DPA and plasma phospholipids DPA at baseline (a), 4-week (b) and 8-week (c).

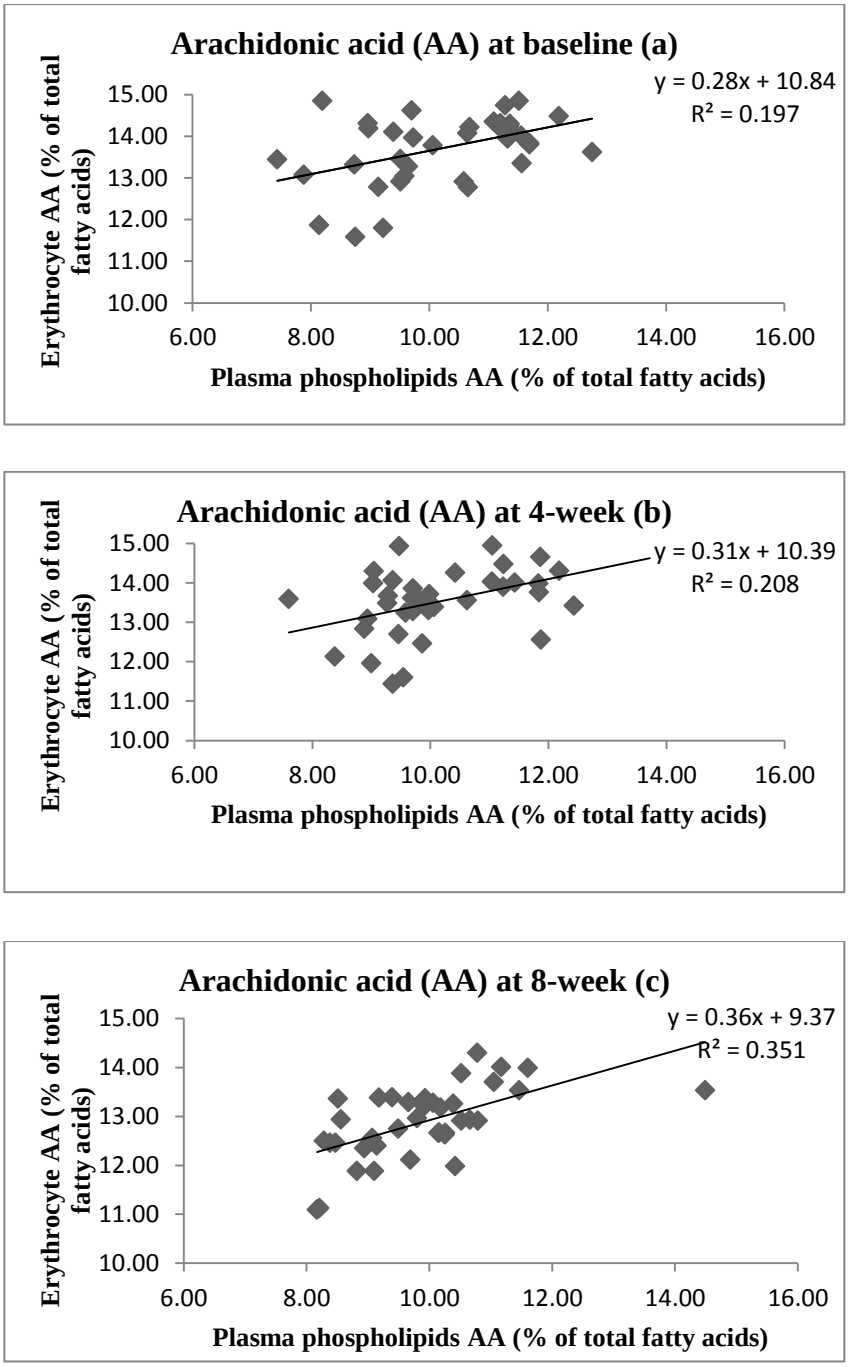


Figure 6.10 Correlations between erythrocyte AA and plasma phospholipids AA at baseline (a), 4-week (b) and 8-week (c).

Fatty acid profile of erythrocytes determined from blood collected at Week 0, Week 4, and Week 8 are shown in Table 6.10. The levels of the two LCn3PUFA commonly associated with positive health outcomes, EPA and DHA, were significantly higher in the erythrocytes of women in the intervention group at Week 4 and Week 8 when compared to the control group. For EPA, there was a significant interaction between time and the diet, $F(1.70, 61.26) = 42.96$, $p < .001$, partial eta squared = 0.54. There

was no statistically significant difference in EPA concentration between the groups at baseline, but there was a statistically significant difference in EPA concentration between intervention and control at mid-trial, $F(1,36) = 30.22$, $p<.001$, partial eta squared=.456 and at end of the study, $F(1,36) = 38.89$, $p<.001$, partial eta squared = .519. For the simple main effect of time, there was no statistically significant difference in EPA after Bonferroni adjustment in the control group ($p>.05$). For the intervention group, there was a statistically significant effect of time on EPA concentration, $F(2,36) = 42.38$, $p<.001$, partial eta squared=.702. EPA concentration significantly increased at mid-trial ($p<.001$) and post-intervention ($p<.001$) when compared to baseline, however, there was no statistically significant difference between EPA concentration at mid-trial and post-intervention ($p=.211$).

For DHA, the interaction between time and diet was significant, $F(1.40,50.45) = 42.61$, $p<.001$, partial eta squared = .542. There was no statistically significant difference in DHA concentration between groups at baseline, but there was a statistically significant difference in DHA concentration between intervention and control at mid-trial, $F(1,36) = 7.08$, $p=.012$, partial eta squared=.164 and at the end of the study, $F(1,36) = 19.68$, $p<.001$, partial eta squared = .353. For the simple main effect of time, there were significant differences in DHA for both the control and intervention group. DHA decreased significantly post-intervention when compared to baseline ($p=.005$) and mid-trial ($p=.025$) in the control group. For the intervention group, DHA concentration significantly increased from baseline to mid-trial ($p<.001$) and continued to rise from mid-trial to the end of the study ($p=.013$).

For docosapentaenoic acid (DPA), there was a significant interaction between time and diet, $F(2,72)=4.46$, $p=.015$, partial eta squared =.110. There was no statistically significant difference in DPA at baseline between the control and intervention, however DPA was lower in the control group at mid-trial ($p=.028$) and at the end of the study ($p=.020$). DPA concentration within the control group decreased significantly post-intervention when compared to baseline ($p<.001$) and mid-trial ($p<.001$). In the intervention group, there was no significant difference in DPA concentration between baseline and post-intervention however a transient increase at mid-trial was observed. There was no significant influence on AA concentration from time and diet interaction, $F(2,72)=2.04$, $p=.138$ or main effect of diet $F(1,36)=3.12$, $p=.086$, however both groups experienced significant decreases post-

intervention when compared to baseline (control group, p=.002; intervention group, p<.001) and mid-trial (control group, p=.010; intervention group, p<.001).

The Omega-3 Index of all 19 participants in the control group were in the ‘intermediate’ category and remained in the same category after the trial. The majority of the participants (16 out of 19) in the intervention group were also in the same ‘intermediate’ category both pre- and post-trial. Two participants saw a shift in their category, one from ‘undesirable’ to ‘intermediate’, and another one from ‘intermediate’ to ‘desirable’. One participant remained in the ‘desirable’ category throughout.

Table 6.10 Effects of a diet higher in fish (intervention) versus a low fish diet (control) on erythrocyte EPA, DPA, DHA and AA as a proportion of total fatty acids

Long chain polyunsaturated fatty acids (n-3 / n-6)	Intervention (n=19)	Control (n=19)	Time x Diet Interaction
Eicosapentaenoic acid (EPA) (% of total fatty acids)			
Week 0	0.89 (0.34)[a]	0.78 (0.20)[a]	*F*(1.702,61.261)
Week 4	1.23 (0.39)*,[b]	0.72 (0.17)[a]	= 42.958
Week 8	1.31 (0.38)*,[b]	0.71 (0.20)[a]	p<.001
Docosapentaenoic acid (DPA) (% of total fatty acids)			
Week 0	2.70 (0.36)[a,b]	2.60 (0.34)[a]	*F*(2,72) = 4.463
Week 4	2.79 (0.35)*,[a]	2.56 (0.35)[a]	p=.015
Week 8	2.66 (0.30)*,[b]	2.42 (0.34)[b]	
Docosahexaenoic acid (DHA) (% of total fatty acids)			
Week 0	4.80 (1.00)[a]	4.96 (0.57)[a]	*F*(1.401,50.454)
Week 4	5.46 (0.81)*,[b]	4.84 (0.62)[a]	=42.612
Week 8	5.66 (0.75)*,[c]	4.68 (0.60)[b]	p<.001
Arachidonic acid (AA) (% of total fatty acids)			
Week 0	13.59 (0.89)[a]	13.85 (0.79)[a]	*F*(2,72) = 2.039
Week 4	13.42 (0.86)[a]	13.67 (0.89)[a]	p=.138
Week 8	12.51 (0.55)*,[b]	13.25 (0.71)[b]	

Descriptive statistics are presented as mean (standard deviation)

Time x Diet interactions tested using mixed-design ANOVA

* Indicates significant between-group difference, p<.05 (One-way ANOVA at all three separate time points)

[a,b,c] Different superscripts indicate significant within-group difference, p<.05 at *post-hoc* analysis, with Bonferroni adjustment for multiple comparisons (Repeated measures ANOVA for both groups)

Data for EPA and DHA were log transformed prior to analysis

Data for DPA and AA were reflected and log transformed prior to analysis

Similar results in terms of EPA and DHA concentrations were observed in plasma phospholipids as shown in Table 6.11. There were significant increases of EPA and DHA when tested at mid-trial when compared to baseline, however no further statistically significant increases were observed when tested at the end of the study period at 8 weeks.

For DPA in plasma phospholipids, although there was a significant time x diet interaction, no statistically significant difference in DPA concentrations was observed between baseline and at 8 weeks in either the intervention group or the control group.

There was no significant influence on plasma phospholipids AA concentration from time and diet interaction, $F(2,72)=2.254$, p=.112. There was also no significant main effect of diet $F(1,36)=4.062$, p=.051, nor main effect of time, $F(2,72)=2.496$, p=.090.

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Table 6.11 Effects of a diet higher in fish (intervention) versus a low fish diet (control) on plasma phospholipids EPA, DPA, DHA and AA as a proportion of total fatty acids

Long chain polyunsaturated fatty acids (n-3 / n-6)	Intervention (n=19)	Control (n=19)	Time x Diet Interaction
Eicosapentaenoic acid (EPA) (% of total fatty acids)			
Week 0	1.04 (0.33)[a]	1.07 (0.72)[a]	$F(2,72) = 13.065$
Week 4	1.93 (1.28)*[b]	0.87 (0.28)[a]	p<.001
Week 8	1.61 (0.64)*[b]	0.93 (0.35)[a]	
Docosapentaenoic acid (DPA) (% of total fatty acids)			
Week 0	1.06 (0.25)[a]	1.02 (0.22)[a]	$F(1.420,51.102) = 4.220$
Week 4	1.19 (0.27)*[b]	0.99 (0.21)[a]	p=.032
Week 8	1.19 (0.34)[a,b]	1.01 (0.23)[a]	
Docosahexaenoic acid (DHA) (% of total fatty acids)			
Week 0	3.72 (0.88)[a]	3.90 (0.63)[a]	$F(2,72) = 38.211$
Week 4	5.16 (0.91)*[b]	3.77 (0.62)[a]	p<.001
Week 8	5.23 (1.03)*[b]	3.70 (0.76)[a]	
Arachidonic acid (AA) (% of total fatty acids)			
Week 0	10.10 (1.24)	10.40 (1.42)	F(2,72) = 2.254 p=.112 (Main effect of time and main effect of group also not significant)
Week 4	9.70 (1.01)	10.70 (1.36)	
Week 8	9.46 (0.82)	10.31 (1.41)	

Descriptive statistics are presented as mean (standard deviation)

Time x Diet interactions tested using mixed-design ANOVA

* Indicates significant between-group difference, p<.05 (One-way ANOVA at all three separate time points)

[a,b,c] Different superscripts indicate significant within-group difference, p<.05 at *post-hoc* analysis, with Bonferroni adjustment for multiple comparisons (Repeated measures ANOVA for both groups)

Data for EPA and AA were log transformed prior to analysis

6.4.4 Lipids study

There were no significant differences between groups in terms of lipids profile at baseline or at the end of the trial as shown in Table 6.12 below. There were also no significant changes within subjects pre- and post-trial.

Table 6.12 Effects of a diet higher in fish (intervention) versus a low fish diet (control) on serum lipid and lipoproteins

Lipids	Intervention (n=19)	Control (n=19)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
Total cholesterol (mmol/L)					
Week 0	4.2 (0.6)	4.3 (0.5)	$F(1,36) = .760$ p=.389	$F(1,36)=.038$ p=.846	$F(1,36)=.220$ p=.642
Week 8	4.3 (0.6)	4.3 (0.5)			
HDL cholesterol (mmol/L)					
Week 0	1.6 (0.2)	1.7 (0.4)	$F(1,36) =.2756$ p=.106	$F(1,36)=.115$ p=.737	$F(1,36)=.034$ p=.855
Week 8	1.7 (0.3)	1.7 (0.3)			
LDL cholesterol (mmol/L)					
Week 0	2.2 (0.5)	2.2 (0.3)	$F(1,36) = .371$ p=.546	$F(1,36)=.198$ p=.659	$F(1,36)=.032$ p=.859
Week 8	2.3 (0.4)	2.2 (0.5)			
Triglyceride (mmol/L)					
Week 0	0.8 (0.3)	0.9 (0.4)	$F(1,36) = .576$ p=.453	$F(1,36)=1.250$ p=.271	$F(1,36)=.753$ p=.391
Week 8	0.8 (0.3)	1.0 (0.4)			

Descriptive statistics are presented as mean (standard deviation)

Time x Diet interactions, main effect of diet and main effect of time tested using mixed-design ANOVA

6.4.5 Iron status and haemoglobin level

There were no significant differences between groups in terms of iron status at baseline or at the end of the trial as shown in Table 6.13 below. There were also no significant changes within subjects pre- and post-trial.

Table 6.13 Effects of a diet higher in fish (intervention) versus a low fish diet (control) on serum iron and haemoglobin levels

Iron study	Intervention (n=19)	Control (n=19)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
Serum iron (μmol/L)					
Week 0	18 (6)	18 (7)	$F(1,36)=.506$ p=.482	$F(1,36)=.652$ p=.425	$F(1,36)=.604$ p=.442
Week 8	20 (10)	18 (9)			
Transferrin (g/L)					
Week 0	2.73 (0.29)	2.93 (0.53)	$F(1,36)=.549$ p=.463	$F(1,36)=1.309$ p=.260	$F(1,36)=1.155$ p=.290
Week 8	2.72 (0.32)	2.85 (0.55)			
Transferrin saturation (%)					
Week 0	27 (9)	26 (13)	$F(1,36)=.382$ p=.540	$F(1,36)=.894$ p=.351	$F(1,36)=.951$ p=.336
Week 8	31 (15)	26 (15)			
Ferritin (μg/L)					
Week 0	48 (35)	47 (30)	$F(1,36)=.370$ p=.547	$F(1,36)=.009$ p=.924	$F(1,36)=.004$ p=.948
Week 8	46 (31)	48 (31)			
Haemoglobin (g/L)					
Week 0	136 (9)	131 (20)	$F(1,36)=2.140$ p=.152	$F(1,36)=.035$ p=.853	$F(1,36)=.077$ p=.783
Week 8	135 (9)	136 (10)			

Descriptive statistics are presented as mean (standard deviation)

Time x Diet interactions, main effect of diet and main effect of time tested using mixed-design ANOVA

Data for iron, transferrin, transferrin saturation and ferritin were transformed to their square roots prior to analysis

Data for haemoglobin were reflected and then transformed to their square roots prior to analysis

6.4.6 Mercury

For THg, there was significant interaction between time and diet $F(1.52,54.55)=10.98$, $p<.001$. There was no statistically significant difference in THg concentration between groups at baseline (p=.913) or at mid-trial (p=.124), but there was a statistically significant difference in THg concentration between intervention and control post-intervention, $F(1,36) = 7.96$, p=.008, partial eta squared = .181. For the control group, there was no statistically significant difference across times, $F(2,26) = 1.14$, p=.332, partial eta squared = .059. For the intervention group, there was a statistically significant simple main effect of time on THg concentration, $F(1.31,23.50) = 12.79$, p=.001, partial eta squared = .415. There were statistically significant differences in THg between baseline and mid-trial (p=.003) and baseline and post-intervention (p=.004). However, there was no significant difference between mid-trial and post-intervention THg concentration (p=.271).

Similarly, for MeHg, there was significant interaction between time and diet $F(1.28,45.99)=14.97$, $p<.001$, partial eta squared = .294. There was no statistically significant difference in MeHg concentration between groups at baseline (p=.805), but there was a statistically significant difference in MeHg concentration between groups at mid-trial, $F(1,36) = 4.57$, p=.040, partial eta squared = .113 and post-intervention, $F(1,36) = 10.95$, p=.002, partial eta squared = .233. For the control group, there was a statistically significant simple main effect of time on MeHg concentration, $F(2, 36) = 4.67$, p=.016, partial eta squared = .206. MeHg concentration was significantly lowered in the control group post-intervention when compared to baseline (p=.041) and mid-trial (p=.029). For the intervention group, MeHg concentration increased at mid-trial (p=.005) and post-intervention (p=.008) when compared to baseline. However, there was no significant difference between MeHg at mid-trial and post-intervention (p=.741).

Only one person in the intervention group exceeded the EPA reference dose of 5.8 µg/L, the level of mercury in foetal cord blood below which is assumed to cause no appreciable harm (Schober et al. 2003). This person had >5.8 µg/L at all assessment time points (7.1 µg/L, 7.9 µg/L and 7.0 µg/L of THg at baseline, mid-trial and post-trial respectively).

Since foetal cord blood mercury has been found to be higher than maternal blood level due to bioconcentration across the placenta, there is suggestion that maternal levels of as low as 3.5 µg/L may be of concern (Mahaffey, Clickner & Jeffries 2009). Four persons in this study had levels >3.5 µg/L at post-trial (two from intervention group and two from control group). However, these results cannot be attributed to the intervention as the two persons in the intervention group had similar levels even before the study started.

Table 6.14 Effects of a diet higher in fish (intervention) versus a low fish diet (control) on total mercury and methyl mercury levels

Total and methyl mercury	Intervention (n=19)	Control (n=19)	Time x Diet Interaction
Total mercury (µg/L)			
Week 0	1.91 (2.04)[a]	1.48 (1.03)	*F*(1.515,54.549)
Week 4	2.27 (1.90)[b]	1.69 (1.51)	= 10.977
Week 8	2.32 (1.54)*,[b]	1.48 (1.30)	p<.001
Methyl mercury (µg/L)			
Week 0	1.51 (1.55)[a]	1.22 (0.94)[a]	*F*(1.278,45.990)
Week 4	1.90 (1.41)*,[b]	1.30 (1.14)[a]	= 14.965
Week 8	1.92 (1.21)*,[b]	1.13 (1.11)[b]	p<.001

Descriptive statistics are presented as mean (standard deviation)

Time x Diet interactions tested using mixed-design ANOVA

* Indicates significant between-group difference, p<.05 (one-way ANOVA at all three separate time points)

[a,b] Different superscripts indicate significant within-group difference, p<.05 at *post-hoc* analysis, with Bonferroni adjustment for multiple comparisons (repeated measures ANOVA for both groups)

Data for THg and MeHg were log transformed prior to analysis

6.4.7 Selenium

There were moderate to strong correlations between plasma selenium and whole blood selenium levels throughout the three assessment time points (baseline, $r(36)=.439$, p=.006; mid-trial, $r(36)=.495$, p=.002; post-intervention, $r(36)=.572$, p<.001). Table 6.15 below shows the mean (standard deviation) of both plasma and whole blood for both intervention and control at various time points. No statistically significant time and diet interaction, main effect of diet or main effect of time was observed.

Table 6.15 Effects of a diet higher in fish (intervention) versus a low fish diet (control) on plasma and whole blood selenium levels

Selenium	Intervention (n=19)	Control (n=19)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
Plasma selenium (µg/L)					
Week 0	95 (18)	97 (16)	$F(1.494,53.800)$ = 2.959 p=.075	$F(1,36)$ =.316 p=.578	$F(1.494,53.800)$ = 1.756 p=.189
Week 4	98 (18)	95 (18)			
Week 8	104 (23)	96 (17)			
Whole blood selenium (µg/L)					
Week 0	155 (31)	152 (23)	$F(1.700,61.195)$ = 1.674 p=.199	$F(1,36)$ =.924 p=.343	$F(1.700,61.195)$ = 2.008 p=.150
Week 4	163 (32)	155 (25)			
Week 8	166 (38)	152 (24)			

Descriptive statistics are presented as mean (standard deviation)

Time x Diet interactions tested using mixed-design ANOVA

6.4.8 Inflammatory marker (C-Reactive Protein)

Two participants (one each from the intervention and control groups) had at least one CRP reading of >10 mg/L and were excluded from the analysis. CRP of >10 mg/L generally reflects the presence of an acute infection or inflammation. The mean (standard deviation) of the levels of CRP at the three assessment time points is listed in Table 6.16 below. No statistically significant time and diet interaction, main effect of diet or main effect of time was observed.

Table 6.16 Effects of a diet higher in fish (intervention) versus a low fish diet (control) on serum C-reactive protein (CRP) levels

Serum CRP (mg/L)	Intervention (n=18)	Control (n=18)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
Week 0	1.16 (0.94)	1.73 (2.13)	$F(2,68)$ = 1.185 p=.312	$F(1,34)$ = .888 p=.353	$F(2,68)$ = .028 p=.972
Week 4	1.12 (1.12)	2.16 (2.67)			
Week 8	1.19 (1.16)	1.82 (2.17)			

Descriptive statistics are presented as mean (standard deviation)

Time x Diet interactions tested using mixed-design ANOVA

Data were log transformed prior to analysis

6.4.9 Anthropometric and other assessments

6.4.9.1 Weight, BMI and body composition

There were no significant differences between groups in terms of weight, BMI and body composition at baseline or at the end of the trial as shown in Table 6.17 below. There were also no significant changes within subjects pre- and post-trial.

Table 6.17 Effects of a diet higher in fish (intervention) versus a low fish diet (control) on weight, BMI and body composition

Anthropometric measurement	Intervention (n=19)	Control (n=19)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
Weight (kg)					
Week 0	62.4 (7.7)	65.2 (9.0)	$F(1,36)=.248$ p=.621	$F(1,36)=1.083$ p=.305	$F(1,36)=.442$ p=.511
Week 8	62.3 (7.9)	65.2 (9.5)			
BMI (kg/m^2)					
Week 0	23.3 (3.1)	23.5 (2.3)	$F(1,36)=.151$ p=.700	$F(1,36)=.086$ p=.770	$F(1,36)=.393$ p=.535
Week 8	23.2 (3.3)	23.5 (2.4)			
Body fat (% of total mass)					
Week 0	32 (6)	33 (6)	$F(1,36)=.231$ p=.634	$F(1,36)=.083$ p=.775	$F(1,36)=.016$ p=.899
Week 8	32 (6)	33 (5)			

Descriptive statistics are presented as mean (standard deviation)
Time x Diet interactions, main effect of diet and main effect of time tested using mixed-design ANOVA

6.4.9.2 Blood pressure

For diastolic blood pressure, there was a significant main effect of time, $F(2,72) = 3.47$, $p = .036$, partial eta squared = .088 as shown in Table 6.18. There was a statistically significant decrease in diastolic blood pressure in the intervention group from baseline to post-intervention ($p = .002$) whereas there was no significant difference across time in the control group ($p = .912$). There were no significant interactions between time and diet, main effect of time, or main effect of diet detected for systolic blood pressure.

Table 6.18 Effects of a diet higher in fish (intervention) versus a low fish diet (control) on blood pressure

Blood pressure	Intervention (n=19)	Control (n=19)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
Systolic blood pressure (mmHg)					
Week 0	108 (8)	110 (8)	$F(1.709,61.513)$ = .269 p=.730	$F(1,36)$ =1.423 p=.241	$F(1.709,61.513)$ =2.092 p=.139
Week 4	105 (8)	108 (9)			
Week 8	105 (8)	108 (11)			
Diastolic blood pressure (mmHg)					
Week 0	64 (7)[a]	62 (7)	$F(2,72)$ = 2.233 p=.115	$F(1,36)$ =.026 p=.873	$F(2,72)$ =3.471 p=.036
Week 4	61 (7)[a,b]	62 (8)			
Week 8	60 (8)[b]	61 (6)			

Descriptive statistics are presented as mean (standard deviation)

Time x Diet interactions tested using mixed-design ANOVA

[a,b] Different superscripts indicate significant within-group difference, p<.05 at *post-hoc* analysis, with Bonferroni adjustment for multiple comparisons (repeated measures ANOVA for both groups)

Data for systolic and diastolic blood pressure were log transformed prior to analysis

6.4.9.3 Depression scale

There were no significant differences between groups in terms of depression scale at baseline or at the end of the trial as shown in Table 6.19 below. There were also no significant changes within subjects pre- and post-trial.

Table 6.19 Effects of a diet higher in fish (intervention) versus a low fish diet (control) on depression mood indicator

CES-Depression Scale	Intervention (n=19)	Control (n=19)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
Week 0	6 (4)	8 (6)	$F(1,36)=.184$ p=.671	$F(1,36)=.431$ p=.516	$F(1,36)=.337$ p=.565
Week 8	7 (9)	7 (7)			

Descriptive statistics are presented as mean (standard deviation)

Time x Diet interactions, main effect of diet and main effect of time tested using mixed-design ANOVA

6.5 Discussion

Participants of this study were recruited from newspaper advertisements and ranged from 18 to 50 years representing women of child-bearing age who fulfilled our inclusion and exclusion criteria. The participants within the intervention arm of this trial (higher fish intake) consumed an average amount of 425 g of fish per week from a variety of fish and fish products, which provided a daily average of 243 mg of EPA and 338 mg of DHA (581 mg EPA+DHA). Results from the 3-day weighed-food record showed that the main difference between the intervention and control diet was in the provision of LCn3PUFA from the diets. Vitamin D intake also increased in the intervention group with the increase in consumption of oily fish. The intake of the other macro- or micronutrients examined mostly remained similar between diets as well as before and during the study periods.

Currently, several recommendations exist for intake of fish and LCn3PUFA. FSANZ recommends that everyone, especially pregnant women, include fish regularly in their diet and that most fish can be safely eaten two to three times a week (serving size for adult = 150 g) (FSANZ 2011a). The level of LCn3PUFA intake considered to be adequate according to the NRVs is 160 mg per day for adult men and 90 mg per day for adult women. The SDT however is set at 610 mg for men and 430 mg for women for reduction of chronic disease risk (NHMRC 2006). To reduce the risk of coronary heart disease, the Heart Foundation recommends all adult Australians consume 500 mg per day of combined EPA and DHA through a combination of two to three serves of oily fish per week, fish oil capsules/liquid and foods/drinks enriched with omega-3 fatty acids (Colquhuon et al. 2008). The International Perinatal Lipid Intake Working Group recommends that pregnant and lactating women should aim to achieve a DHA intake of at least 200 mg per day by consuming one to two portions of oily fish per week (Koletzko et al. 2007). This study diet aligns satisfactorily with these recommendations with high acceptance of the higher fish intake (diet acceptability score 6 out of 7). There was also no difference in how well the participants liked the food and the ease of preparation of meals containing fish or fish products compared to the control diet.

The changes in the LCn3PUFA, EPA and DHA achieved in this study were comparable to other studies that have employed similar amounts of LCn3PUFA in

their interventions. Harris et al. (2007) examined the effects of fish and fish oil capsules on LCn3PUFA in blood cells and plasma phospholipids in a group of women aged between 21 and 49 years over a 16-week period (n=23). The fish intervention group received on average 95 mg of EPA and 390 mg of DHA per day (485 mg EPA+DHA) and the mean erythrocytes EPA and DHA increased from 0.80% of total fatty acids to 1.39% and from 3.22% to 4.52% respectively after eight weeks of intervention. This is comparable to our study where the mean EPA rose from 0.89% of total fatty acids to 1.31%, and mean DHA from 4.80% to 5.66%, a net increase of 0.42% and 0.86% respectively. Although EPA and DHA increased in the intervention group, the Omega-3 Index of the participants hardly shifted. Since Omega-3 Index is the summation of EPA and DHA levels in erythrocytes, it is likely that our eight-week intervention is not long enough in duration to demonstrate significant changes. A study by Arteburn et al. (2006) showed that while plasma phospholipid DHA reached equilibrium within one month of the DHA supplementation, it took four to six months of supplementation to reach steady state in red blood cells which is consistent with their turnover rate of 120 days (Allison 1960). It is therefore not possible to confirm what level of fish intake is required to achieve 'desirable' status from this study.

There was some fluctuation of DPA levels in the intervention group and a significant reduction in the control group after eight weeks according to readings from erythrocyte analysis but not plasma, although no change was expected. The reason for this observation is unclear. Since meat is a major source of DPA for most Australians (Howe et al. 2006), these changes in DPA levels might reflect the participants' meat intakes during the assessment period. The role of DPA is not as widely studied as the other LCn3PUFA (EPA and DHA) and limited information is available relating to its significance. Although both the intervention group and the control group experienced a reduction in AA in erythrocyte, a long chain n-6 polyunsaturated fatty acid (LCn6PUFA), with AA concentration in the intervention group significantly lower than the control group after eight weeks, no such reduction was observed in the plasma phospholipids analysis. The differences in fatty acids changes observed in erythrocyte and plasma phospholipids could be explained in part by the difference in their kinetics of their incorporation (Katan et al. 1997). Changes in consumption of foods containing LCn6PUFA (e.g. from vegetable oil to olive oil

in some participants) might partly explain the reduction in erythrocyte AA observed in both the intervention and control groups.

As expected, there were significant increases in THg and MeHg levels in blood in the intervention group, i.e. those who consumed more fish. It is well established that fish consumption is the major contributor of organic mercury in the diet and that those who consume more fish have higher mercury levels. In the US 1999–2000 National Health and Nutrition Examination Survey (NHANES), women aged 16–49 years who ate three or more servings of fish within the previous 30 days of the survey had blood mercury level almost four times higher than those women who did not eat any fish in that period (geometric mean mercury of 1.94 µg/L *vs* 0.51 µg/L; $p<.001$) (Schober et al. 2003). The mean blood mercury in participants within the higher fish intervention group in the current study rose from 1.91 µg/L to 2.32 µg/L after eight weeks and it is hypothesised that these levels would continue to rise until a steady-state was reached if this pattern of fish consumption were to be maintained long term. However, even though the mean THg and mean MeHg at Week 8 were higher than those at Week 4 in the intervention group, the difference did not reach statistical significance. This indicates further increases in THg and MeHg levels are likely to be small. The observed rise in mercury associated with increased fish intake, but not exceeding the safe consumption limit as suggested by FSANZ, is therefore unlikely to pose additional risk; particularly if a variety of fish is included or high mercury-containing fish are avoided. The current provisional tolerable weekly intake for MeHg as recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA 2004) is 1.6 µg per kg body weight per week in women of child-bearing age. For a woman of average build, say 60 kg, the amount of MeHg that could be safely consumed would be 96 µg per week. Assuming the contribution of MeHg from non-fish food is small and around 1 µg per week, the amount of MeHg that can safely be consumed from fish would be 95 µg per week. The quantity of fish that can be safely consumed therefore depends on the level of MeHg present in the fish. If fish consumption is limited to 450 g (three 150 g serves) per week, then fish with levels of up to around 21 µg of MeHg per 100 g would pose no problem. Most Australian fish and seafood have mercury levels below 21 µg/100g. For example, canned tuna, one of the more popular fish products consumed, contains 17.7 µg of mercury per 100 g (FSANZ NUTTAB 2010). All fish products used in

this study were analysed and found to have mercury levels below 10 µg/100 g as shown in Chapter 5.

There were no significant differences between groups or within subjects observed in terms of lipids profile, iron status, plasma or whole blood selenium levels, the inflammatory marker CRP, body weight and composition following the interventions. It was not unexpected that the lipids profile remained unchanged because studies that have shown reduction in triglycerides, especially in hypertriglyceridemia patients, used higher doses of LCn3PUFA (Harris 1997). The study participants in this current trial generally had lipid levels within the desirable range. This study also suggested that iron status was not compromised by the substitution of meat meals with fish meals over an eight-week period. Although meat, in particular red meat, is considered a good source of iron, fish also contains iron. The iron content of the fish products provided in this study contained approximately 50% less iron than the meat provided. The authors did not expect body weight to change because the study foods were isocaloric and participants were advised to adhere to their daily routine.

A significant reduction in diastolic blood pressure was observed in the intervention group post-trial but this drop did not reach statistical significance when compared with the control group. Participants in the trial were normotensive at baseline and reduction in blood pressure was not expected. Nevertheless, this trend towards a reduction in diastolic blood pressure warrants further investigation. A review article by (2006) concluded that the cardioprotective role of LCn3PUFA is partly related to its blood pressure-lowering effects.

Six SNPs were chosen for genotyping in the study as they have been shown to be associated with blood fatty acids or mercury levels in previous studies (Custodio et al. 2004; Tanaka et al. 2009). It has been increasingly common to include genotyping in research as study outcomes may be confounded by genotypes. However, due to the small number of participants in this study, none of the SNPs tested was significantly associated with blood levels. Therefore no adjustment for genotypes was included in all subsequent analysis.

This study was carried out on non-pregnant and non-lactating women and therefore it may be difficult to generalise the changes in fatty acids profile to pregnant or

breastfeeding women. However, a recent randomised controlled trial conducted by the University of Southampton, UK (Miles et al. 2011) indicated that these changes can also occur in pregnant women. In this UK study, pregnant women who reported low habitual consumption of oily fish were instructed to incorporate two portions of salmon per week (providing around 500 mg EPA + DHA per day) into their diet from 20 weeks of gestation until they gave birth. It was demonstrated that the EPA and DHA status of these women was increased and the expected pregnancy-associated decline in these fatty acids was prevented. The status of EPA and DHA in their offspring was also increased.

The strength of this study includes the complete follow-up of all participants. This was possible due to the rather short study duration and small sample size and as such, all protocols could be strictly followed. We also considered the potential differences in response to dietary changes due to differences in genetic make-up of participants and included the analysis of several key SNPs responsible for the metabolism of fatty acids and mercury in this study.

One limitation of the study relates to the supply of study foods throughout the trial period. The acceptability of the diet that was higher in fish was therefore tested in terms of taste and ease of preparation only and the cost factor eliminated. As cost is potentially a limiting factor to the consumption of fish and seafood, we could not predict if participants would include more fish in their diet post study. Another potential limitation of the study is the volunteering nature of participation. One would assume those women who dislike fish would be unlikely to volunteer to take part in the study. However, there were participants in this study who did indicate their preference was to be in the control group (low fish diet).

6.6 Conclusions

In summary, it has been demonstrated that consumption of a variety of fish and fish products four times a week can assist women of child-bearing age meet national dietary intake recommendations. This higher fish intake improves EPA and DHA status without compromising iron status over an eight-week period. Although MeHg levels increased in the higher fish intervention group, it was overall within safety limits.

Chapter 7 Cost-effective analysis of a higher fish diet

7.1 Introduction

Fish and seafood are excellent sources of macro- and micronutrients. In particular, they provide high quality protein, are rich in selenium, iodine and the long chain n-3 polyunsaturated fatty acids (LCn3PUFA), EPA and docasahexaenoic acid (DHA) and are low in saturated fat. For example, oily fish such as Atlantic Salmon (fillet, raw) on average provides, for each 100 g, 20.7 g protein, 22 µg selenium, 505 mg EPA, 812 mg DHA (FSANZ, NUTTAB 2010) and 6.9 µg iodine (FSANZ, AUSNUT 2007). However, fish consumption in Australia is much lower than other animal protein sources such as poultry and red meat. One of the barriers of fish consumption in Australia relates to the fact that fish is generally perceived as being expensive (Birch, Lawley & Hamblin 2012).

Chapter 6 describes a randomised controlled trial where women of child-bearing age were randomised to consume a higher fish diet or typical Australian diet lower in fish but higher in meat. The outcomes of interest were changes in red blood cells, fatty acids levels, mercury in whole blood, lipids, iron status, body composition and acceptability of the diet. This chapter will explore the cost effectiveness of this dietary pattern in relation to DHA intake and status.

7.2 Methods

Healthy women were recruited into the trial by newspaper advertisement and posters placed at the Flinders Medical Centre and Flinders University. Following a two-week run-in period, women were randomly allocated to the higher fish group (n=19) or the lower fish group (n=19) for a total of eight weeks. Assessment was conducted at baseline (prior to randomisation), at four weeks and at eight weeks. Details of the inclusion/exclusion criteria and study protocol have been previously described in Chapter 6.1.

Cost of resources used

Cost was calculated based on retail values of foods provided in the study and costs of staff time in providing counselling and supplying foods. Foods provided during the

intervention period are listed in Table 7.1. Normal retail price from two major supermarket chains (Woolworths Unley and Coles Unley) as on 22/10/2011 were used in the calculation unless the product was only available from Woolworths or from Coles (e.g. home brand product). If a particular item was not found on the supermarket shelf, then the price was sourced from its online shopping list or from a third supermarket chain (Foodland North Adelaide). For Lenards chicken products, prices were obtained from two to three outlets (Aberfoyle Park, Unley, Marion and Salisbury). Apart from the fish or meat study food, participants were also provided with olive oil spread and olive oil for cooking. Dietetics input included 30 minutes of initial consultation followed up by 3 x 10 minute appointments. Wages were based on South Australian Government wages parity (salaried) enterprise agreement 2010 at base rate, Allied Health Professional (AHP) stream (Table 7.2).

Table 7.1 Food provided to participants during the 8-week intervention period

Intervention Diet	Quantity
John West Atlantic Salmon	4 x 150 g (2 x 300 g packets)
John West Yellowtail Kingfish	4 x 150 g (2 x 300 g packets)
John West Sardine in Tomato Sauce	2 x 110 g can
John West Pink Salmon	2 x 105 g can
John West Red Salmon	2 x 105 g can
John West Salmon Tempter Onion & Tomato	2 x 95 g can
John West Tuna in Springwater	4 x 95 g can
John West Tuna Tempter Lemon & Pepper	4 x 95 g can
Birds Eye Atlantic Salmon Lemon & Pepper	2 x 135 g (1 x 270 g box)
Birds Eye Fillets Lightly Seasoned Lemon & Pepper	2 x 200 g (1 x 400 g box)
Birds Eye Deep Sea Dory	2 x 142 g (2/3 x 425 g box)
Birds Eye Oven Baked Fillets Original Crumbed	2 x 142 g (2/3 x 425 g box)
Control Diet	**Quantity**
Extra lean minced beef	8 x 100 g
Chicken thigh fillet	4 x 120 g
Scotch fillet	4 x 150 g
Steggles Chicken Mini Roast Spinach & Cheese	2 x 175 g (1 x 350 g box)
Select Turkey slices	4 x 40 g (2 x 80 g packets)
Primo Roast beef slices	4 x 50 g (2 x 100 g packets)
Lenards Chicken cacciatore	2 x 200 g
Lenards Chicken schnitzel	2 x 200 g
Lenards Chicken kiev	2 x 200 g

Table 7.2 Unit costs of resources used

Resources	Unit	Cost per unit	Details*
Cost of food (per person)		**$**	
Food supplied – intervention group	eight-week study period	125.90	Average normal retail prices from two different supermarket chain stores in the majority of cases
Food supplied – control group	eight-week study period	83.00	Average normal retail prices from two different supermarket chain stores or two to three different outlets of the same franchise in the majority of cases
Dietetic Input (per person)			
Initial consultation (1 unit)	30 minutes	13.90	South Australian Government wages parity (salaried) enterprise agreement 2010, Allied Health Professionals (AHP) stream, AHP-1, 4 year degree
Follow-up consultations (3 units)	10 minutes	4.65	South Australian Government wages parity (salaried) enterprise agreement 2010, Allied Health Professionals (AHP) stream, AHP-1, 4 year degree

* Pricing as of October 2011 except for the two discontinued products where the purchased price at the time of the study was used (no more than one year ago)

Effectiveness

Effectiveness was measured as (1) DHA provided in the foods and (2) changes in DHA levels in red blood cells. The incremental cost effectiveness ratio (ICER) is calculated as the difference in costs between the diets divided by the difference in changes in erythrocyte DHA achieved by the two diets.

(1) DHA provided in foods:

For intervention foods, DHA contents were analysed based on composite samples from a minimum of three production dates.

For control foods, DHA contents were estimated based on the NUTTAB 2010 food composition database.

(2) Changes in DHA levels in red blood cells:

Venous blood was drawn at baseline (prior to randomisation) and at eight weeks after the intervention. Erythrocyte phospholipids were extracted with chloroform:propanol and then separated by thin-layer chromatography. The

samples were methylated in 1% sulphuric acid in methanol for three hours at 70 °C. The resulting fatty acid methylesters were extracted with heptane and then quantified by gas chromatography.

7.3 Results

Table 7.3 below shows the comparative costs of foods providing 200 mg of DHA.

Table 7.3 Comparative costs of the study foods providing 200mg of DHA

Item	Intervention Diet	Control Diet
Cost of study foods provided within an eight-week period (per person)	$125.90	$83.00
Amount of DHA provided by study foods provided during an eight-week period	18944 mg	197 mg
Cost per 200 mg of DHA	$1.33	$84.26

Table 7.4 shows the differences between the intervention and control diets in terms of costs (study foods only) and changes in DHA levels in red blood cells.

Table 7.4 Cost effectiveness of a higher fish diet to achieve higher DHA intake and DHA level in red blood cells

Variable	Mean (SD) Intervention (Higher fish, lower meat diet)	Mean (SD) Control (Lower fish, higher meat diet)	Difference (95% Cl) in means
Mean cost* (A$)	153.70 (n=19)	110.80 (n=19)	42.90
Change in erythrocyte DHA level (% of total fatty acids)	+0.87 (0.55) (n=19)	-0.28 (0.32) (n=19)	1.15 (0.84–1.45)
Incremental cost effectiveness ratio (ICER) DHA	-	-	37.3 (29.6–51.1)

*Cost includes the cost of food and dietetic input

7.4 Discussion

Although cost is often quoted as the primary barrier to regular fish and seafood consumption (McManus et al. 2007), this study has demonstrated that consuming fish is a very cost-effective way of increasing DHA intake and DHA levels in the blood. The amount of fish consumed averaged to around 425 g per week, which is within the safe consumption recommendation from FSANZ. In this study, a variety of fish and fish products with varying DHA contents was chosen to mimic normal consumption pattern. The levels of DHA in the fish study foods ranged from 105 mg per 100 g to 750 mg per 100 g. Considering the costs of food alone, to obtain equal amount of DHA, it would have cost sixty times more if consuming the meat study food compared to the fish study food. Theoretically, DHA intake could be made even more cost-effective if we only chose those products that are cheaper in price but still high in DHA content, e.g. Sardine in Tomato Sauce averaged $2.40 per 110 g can and provided 750 mg of DHA per 100 g. One could argue that consuming fish oil would be much more economical in terms of DHA supplementation. However, fish oil does not provide the other nutrients in fish that form part of a healthy diet.

Fayet et al. (2010) studied the comparative costs of foods that provided 100 mg of LCn3PUFA. In that study, it was found that the cheapest option was fish oil capsules (8 cents), followed by salmon (12 cents), tuna (17 cents), enriched eggs (50 cents), seafood ($1.00), enriched bread ($1.30), enriched yoghurt ($1.50), enriched milk ($2.00), and lean red meat (approx. $2). Again, this demonstrates that fish is a more cost-effective means of obtaining LCn3PUFA.

The ICER is calculated as the difference in costs between the diets divided by the difference in changes in erythrocyte DHA achieved by the two diets. An ICER of 37.3 for the base case analysis indicates that an additional expenditure of $37.30 over an eight-week period (or $0.70/day) is associated with an increase in the erythrocyte DHA level by 1% of total fatty acids. Although participants in our study were non-pregnant and non-lactating women, other studies have demonstrated that DHA supplementation during gestation led to higher plasma or erythrocyte DHA levels (Van Houwelingen et al. 1995; Dunstan et al. 2004). This higher maternal DHA status in turn was associated with higher DHA status in the newborn (Van Houwelingen et al. 1995; Dunstan et al. 2004) and better developmental outcomes

(Judge, Harel & Lammi-Keefe 2007b; Dunstan et al. 2008). Although dietary DHA and erythrocytes/plasma DHA are well correlated and blood measures of DHA in turn are predictive of internal organ DHA status (Kuratko & Salem Jr 2009), there is currently no globally recognised DHA status that is conducive to good health. The Omega-3 Index, calculated from summing erythrocytes EPA and DHA (as a percentage of total fatty acids) has been proposed as a biomarker for assessing the risk of coronary heart disease and an Omega-3 Index of 8% is classified as desirable (Harris 2007).

The sustainability of fish stock warrants a mention here although detailed discussion is out of the scope of this study. Total world fish supply (i.e. including capture fisheries and aquaculture) in 2009 was estimated at 145 million tonnes and accounted for 16.6% of the world population's intake of animal protein and 6.5% of all protein consumed (FAO 2012). Although the percentage of over exploited fish stocks rose from 10% in 1974 to 26% in 1989 and continued to rise to 29.9% in 2009, good progress is being made in reducing overexploited stocks and rebuilding plans put in place in some areas (FAO 2012). For example, in the United States, 67% of all stocks are sustainably harvested and only 17% are still overexploited in 2009 (FAO 2012). In Australia, the figures released in 2014 showed that out of the 238 different assessments of stock undertaken, 129 fish stocks are being fished sustainably (87,5% of catch by volume), 7 stocks in recovery (0.9%), 19 stocks in decline (2.1%), 11 stocks (4.9%) as overfished, and 68 undefined stocks (4.6%) with insufficient information to determine their stock status (Flood et al. (eds) 2014). Ensuring effective management of marine capture fisheries and successful development of global aquaculture (which is projected to dominate future global fish supply) will assist in filling the global fish supply-demand gap (World Bank 2013).

In summary, adequate DHA is essential for optimal maternal and infant outcome and the findings from this study indicate that fish consumption is a cost-effective means of increasing DHA intake.

Chapter 8 Conclusion, implications for future practice and research

Fish is a nutritious food and is a good source of many macro- and micronutrients. In particular, fish provides high quality protein, is low in saturated fat, and is an excellent source of LCn3PUFA, EPA and DHA. Studies have consistently shown that maternal fish consumption reduces the risk of sub-optimal neurodevelopment in offspring (FAO & WHO 2011). The abundance of LCn3PUFA in fish is primarily responsible for this effect as DHA is the major component of the brain cells and visual neurons (Neuringer & Connor 1986). In women who were pregnant and had given birth, levels of LCn3PUFA were often reduced (Al et al. 1995; Otto et al. 1997). Although the body has its own mechanism to provide the developing foetus with adequate amounts of LCn3PUFA, it would seem logical to ensure there is adequate reserve even before falling pregnant and to avoid deficiency. Fish, being an excellent source of LCn3PUFA, therefore should form part of a healthy diet. LCn3PUFA also demonstrates an effect on prolongation of gestation which would reduce the incidence of pre-term babies (Makrides, Duley & Olsen 2006). Pre-term is known to be associated with many health problems (Moster, Lie & Markestad 2008; Boyle & Boyle 2013). Studies also show that fish consumption lowers the risk of mortality from coronary heart disease (Mozaffarian & Rimm 2006).

On the other hand, there is convincing evidence of adverse neurological/neurodevelopmental outcomes in infants and young children associated with methyl mercury exposure through maternal fish and seafood consumption during pregnancy (FAO & WHO 2011). The crux of the matter is therefore to balance the benefits and the risks associated with fish consumption. As the amounts of LCn3PUFA and methyl mercury present in fish differ according to species and the environment from which the fish are caught, it would make sense for consumers to choose those fish that are high in LCn3PUFA but low in methyl mercury. Monitoring of fish stock quality (both local and imported) and public awareness campaign by government and/or industry would be crucial to maximise the benefits of fish consumption.

The Australian Dietary Guidelines recommend that two 100 g serves of fish be included in the diet each week and the Heart Foundation recommends even a higher amount of two to three 150 g serves of oily fish each week. From our own modelling, it would appear that the amount needed to be consumed in order to achieve an average daily intake of 460 mg LCn3PUFA (the SDT) would be 241 g when including a combination of high and low fish and seafood. Obviously, a much lower amount is needed if only the high LCn3PUFA fish are selected, but in reality, one would probably prefer more fish choices than to adhere to oily fish only.

However, the consumption of fish in the overall Australian population, including women of child-bearing age, is often less than recommended. It is estimated that less than half of the Australian women of child-bearing age consume fish at least twice a week or 200 g or more each week, although there is an upward trend with time.

Reasons for the inadequate consumption of fish may include personal preferences, the cost and availability of good quality fish as well as concern about the presence of contaminants, such as mercury and other pollutants, in fish. In addition, some women have reported that they are not familiar with how fish or seafood should be cooked. From our analysis of the ALSWH data, women who were pregnant or had given birth less than 12 months ago consumed even less fish when compared to other women. One possible explanation for this observation could be that these pregnant or post-partum women were deliberately avoiding or reducing the amount of fish consumed for fear of contaminants that might be present in the fish. They may therefore miss out on the benefits derived from fish consumption. The FAO report assessing the risk and benefit of fish consumption concluded that, in the majority of cases, maternal fish consumption reduces the risk of sub-optimal neurodevelopment in infants and should form part of a healthy diet.

In our randomised controlled trial, it was demonstrated that the acceptability of the intervention higher fish diet was no different to the control usual diet that included more meat and limited amounts of fish. Women reported no problem with incorporating the variety of fish and fish products into their diets when some of the products were convenience products that required minimal cooking skills. Although the level of mercury in the blood did increase in the intervention group, it was still at

a level accepted as safe. Our analysis of the fish used in the trial showed that the level of mercury was relatively low and could be safely consumed.

The intervention diet significantly increased the level of LCn3PUFA in the blood. Although the cost of fish is generally perceived as expensive, consuming fish is actually quite a cost-effective way of obtaining LCn3PUFA when compared to a diet with only meat and no fish. One could argue that consuming fish oil would be much more economical in terms of DHA supplementation. However, fish oil does not provide the other nutrients in fish that form part of a healthy diet. Moreover, fish oil is not without its complications. Studies have shown that high doses of fish oil are associated with bleeding risk, taste problems and stomach upsets (Villani et al. 2013).

Future research could explore how fish consumption can be increased given that fish with a relatively low mercury content are readily available in Australia and the benefits are known. Perhaps more high-level evidence is required to convince people of the health benefits associated with fish consumption. Currently, most evidence was obtained from non-experimental studies, very few of which involved studying the effects of fish consumption in an experimental setting. Randomised controlled trials have largely been focused on the benefits of LCn3PUFA rather than fish per se. In addition, most RCTs have been conducted in women who were already pregnant and supplementation most commonly commenced in the second or third trimesters. What would happen if the EPA and DHA status was already at an optimal level well before conception? What is the evidence of increased LCn3PUFA or fish intake pre-conception and birth outcomes? More research should be directed at identifying the benefits, if any, of habitual fish consumption. And if benefits are found, how should this be communicated to women to effect a behavioural change?

Also more studies should be conducted to examine the amount that needs to be consumed for health benefits. Is there a particular blood level that should be achieved? Currently there is no universally accepted blood level of LCn3PUFA for good health, although the Omega-3 Index has been put forward to assess the risk of coronary heart disease. Our study, despite having double the amount of fish, still did not successfully push blood levels to an optimal level of LCn3PUFA according to the Omega-3 Index over the eight-week study period. Our modelling also supports that

the amount would seem adequate. There appears to be a disconnect between the amount consumed and blood levels achieved or was it simply because our intervention period was too short? Is this level of intake adequate? For pregnant or women contemplating pregnancy, what should be the optimal level? It may be that certain women need to consume more to achieve the optimal blood level and others might need less. Does genetic composition play a role here? In our analysis, blood levels of DHA and mercury at baseline and the change in the intervention group could not be explained by the difference in SNPs. However, bearing in mind that our sample size was very small (n=39) and only three SNPs each were selected for analyses, it was no surprise that a null effect was observed. Nevertheless, it is worth further investigation if a certain genotype influences responses and if so, allowances should be made in future studies. There is also increasing evidence to show that epigenetic changes can influence diets and there is a trend of more personalised prescriptive dietary advice based on the genetic make-up of the individual.

Since there is growing evidence to suggest regular consumption of fish may lower risks of cardiovascular disease, stroke, macular degeneration, and dementia in older adults (NHMRC 2013b) in addition to infant neurodevelopmental benefit (FAO & WHO 2011), encouraging the general population to increase fish consumption could potentially improve the health status of the nation. In addition, there will also be economical benefits with increases in trades and work opportunities. One point worth considering though is the impact on the sustainability of fish stock if the population intake was to increase. There are several schools of thought relating to the sustainability of fish, ranging from those who believe fisheries are adequately managed to those claiming that the world will run out of fish by 2048. Aquaculture has developed over the years and the ratio of aquaculture over wild-caught fish consumption has largely increased. This clearly could relieve the pressure on fish stocks. However, caution must be taken to ensure that the nutritional quality of aquaculture fish is maintained. Government and industry should monitor closely the quality of the fish available on the market to give consumers the confidence that fish consumption is safe.

Although the developmental gain from maternal dietary manipulation could be small when compared to genetic and other environmental influences, particularly in situations where the availability of food is not an issue, adequate fish consumption

could still be beneficial in providing all newborns with the best possible beginning. Moreover, the public, particularly, women of child-bearing and young children should be encouraged to consume those fish that are high in LCn3PUFA but low in methyl mercury so as to maximise the benefits while reducing potential risks.

References

ABS 2001, *Technical paper, National Nutrition Survey, Confidentialised Unit Record File,* ABS, Canberra.

Ailhaud, G & Guesnet, P 2004, 'Fatty acid composition of fats is an early determinant of childhood obesity: a short review and an opinion', *Obesity Reviews,* vol. 5**,** no**.** 1, pp. 21–6.

Al, MDM, Van Houwelingen, AC, Kester, ADM, Hasaart, THM, De Jong, AEP & Hornstra, G 1995, 'Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status', *British Journal of Nutrition,* vol. 74**,** no**.** 01, pp. 55–68.

Allison, AC 1960, 'Turnovers of erythrocytes and plasma proteins in mammals', *Nature,* vol. 188**,** no**.** 4744, pp. 37–40.

Ansorena, D, Guembe, A, Mendizabal, T & Astiasaran, I 2010, 'Effect of fish and oil nature on frying process and nutritional product quality', *Journal of Food Science,* vol. 75**,** no**.** 2, pp. H62–H7.

Arterburn, LM, Hall, EB & Oken, H 2006, 'Distribution, interconversion, and dose response of n-3 fatty acids in humans', *American Journal of Clinical Nutrition,* vol. 83**,** no**.** 6, pp. S1467–76.

Asserhoj, M, Nehammer, S, Matthiessen, J, Michaelsen, KF & Lauritzen, L 2009, 'Maternal fish oil supplementation during lactation may adversely affect long-term blood pressure, energy intake, and physical activity of 7-year-old boys', *Journal of Nutrition,* vol. 139**,** no**.** 2, pp. 298–304.

Bannon, CD, Craske, JD & Hilliker, AE 1985, 'Analysis of fatty acid methyl esters with high accuracy and reliability. IV. Fats with fatty acids containing four or more carbon atoms', *Journal of the American Oil Chemists' Society,* vol. 62**,** no**.** 10, pp. 1501–7.

Barden, AE, Dunstan, JA, Beilin, LJ, Prescott, SL & Mori, TA 2006, 'n-3 fatty acid supplementation during pregnancy in women with allergic disease: effects on blood pressure, and maternal and fetal lipids', *Clinical Science,* vol. 111**,** no**.** 4, pp. 289–94.

Barnard, N, Scialli, AR, Bertron, P, Hurlock, D & Edmonds, K 2000, 'Acceptability of a therapeutic low-fat, vegan diet in premenopausal women', *Journal of Nutrition Education,* vol. 32**,** no**.** 6, pp. 314–9.

Bergmann, RL, Bergmann, KE, Haschke-Becher, E, Richter, R, Dudenhausen, JW, Barclay, D, et al. 2007, 'Does maternal docosahexaenoic acid supplementation during pregnancy and lactation lower BMI in late infancy?', *Journal of Perinatal Medicine,* vol. 35**,** no**.** 4, pp. 295–300.

Bergmann, RL, Haschke-Becher, E, Klassen-Wigger, P, Bergmann, KE, Richter, R, Dudenhausen, JW, et al. 2008, 'Supplementation with 200 mg/day docosahexaenoic acid from mid-pregnancy through lactation improves the docosahexaenoic acid status of mothers with a habitually low fish intake and of their infants', *Annals of Nutrition & Metabolism,* vol. 52**,** no**.** 2, pp. 157–66.

Birch, D, Lawley, M & Hamblin, D 2012, 'Drivers and barriers to seafood consumption in Australia', *Journal of Consumer Marketing*, vol. 29**,** no**.** 1, pp. 64–73.

Bligh, EG & Dyer, WJ 1959, 'A rapid method of total lipid extraction and purification', *Canadian Journal of Biochemistry and Physiology*, vol. 37**,** no**.** 8, pp. 911–7.

Bloomingdale, A, Guthrie, LB, Price, S, Wright, RO, Platek, D, Haines, J, et al. 2010, 'A qualitative study of fish consumption during pregnancy', *American Journal of Clinical Nutrition*, vol. 92**,** no**.** 5, pp. 1234–40.

Boyle, JD & Boyle, EM 2013, 'Born just a few weeks early: does it matter?', *Archives of Disease in Childhood - Fetal and Neonatal Edition*, vol. 98**,** no**.** 1, pp. F85–8.

Broekaert, I, Campoy, C, Iznaola, C, Hoffmann, B, Müller-Felber, W & Koletzko, B 2005, 'Visual evoked potentials in infants after dietary supply of docosahexaenoic acid and 5-methyltetrahydrofolate during pregnancy', *Advances in Experimental Medicine & Biology*, vol. 569**,** no**.**, pp. 201–2.

Broekhuyse, RM 1974, 'Improved lipid extraction of erythrocytes', *Clinica Chimica Acta*, vol. 51**,** no**.** 3, pp. 341–3.

Browne, JC, Scott, KM & Silvers, KM 2006, 'Fish consumption in pregnancy and omega-3 status after birth are not associated with postnatal depression', *Journal of Affective Disorders*, vol. 90**,** no**.** 2–3, pp. 131–9.

Brubacher, G, Müller-Mulot, W & Southgate, DAT 1986, *Methods for the determination of vitamins in food – recommended by COST 91*, Elsevier Science Publishers, London.

Bulstra-Ramakers, MTEW, Huisjes, HJ & Visser, GHA 1995, 'The effects of 3g eicosapentaenoic acid daily on recurrence of intrauterine growth retardation and pregnancy induced hypertension', *BJOG: An International Journal of Obstetrics and Gynaecology*, vol. 102**,** no**.** 2, pp. 123–6.

Calder, PC 2008, 'The relationship between the fatty acid composition of immune cells and their function', *Prostaglandins, Leukotrienes and Essential Fatty Acids*, vol. 79**,** no**.** 3–5, pp. 101–8.

Calder, PC 2009, 'Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale', *Biochimie*, vol. 91**,** no**.** 6, pp. 791–5.

Campoy, C, Escolano-Margarit, MV, Anjos, T, Szajewska, H & Uauy, R 2012, 'Omega-3 fatty acids on child growth, visual acuity and neurodevelopment', *British Journal of Nutrition*, vol. 107**,** no**.** Suppl S2, pp. S85–S106.

Campoy, C, Escolano-Margarit, MV, Ramos, R, Parrilla-Roure, M, Csabi, G, Beyer, J, et al. 2011, 'Effects of prenatal fish-oil and 5-methyltetrahydrofolate supplementation on cognitive development of children at 6.5 y of age', *American Journal of Clinical Nutrition*, vol. 94**,** no**.** 6 Suppl, pp. 1880S–8S.

Cancer Council Victoria 2012, *Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2) User Information Guide*, Cancer Council Victoria, viewed 28 Feb 2013, <http://www.cancervic.org.au/downloads/cec/FFQs/DQES-Guide.pdf>

Cetin, I & Koletzko, B 2008, 'Long-chain omega-3 fatty acid supply in pregnancy and lactation', *Current Opinion in Clinical Nutrition and Metabolic Care,* vol. 11**,** no**.** 3, pp. 297–302.

Cheatham, CL, Nerhammer, AS, Asserhoj, M, Michaelsen, KF & Lauritzen, L 2011, 'Fish oil supplementation during lactation: effects on cognition and behavior at 7 years of age', *Lipids,* vol. 46**,** no**.** 7, pp. 637–45.

Cobiac, L, Clifton, P, Abbey, M, Belling, G & Nestel, P 1991, 'Lipid, lipoprotein, and hemostatic effects of fish *vs* fish-oil n-3 fatty acids in mildly hyperlipidemic males', *American Journal of Clinical Nutrition,* vol. 53**,** no**.** 5, pp. 1210–6.

Cohen, JT, Bellinger, DC, Connor, WE & Shaywitz, BA 2005, 'A quantitative analysis of prenatal intake of n-3 polyunsaturated fatty acids and cognitive development', *American Journal of Preventive Medicine,* vol. 29**,** no**.** 4, pp. 366–74.

Colombo, J, Kannass, KN, Shaddy, DJ, Kundurthi, S, Maikranz, JM, Anderson, CJ, et al. 2004, 'Maternal DHA and the development of attention in infancy and toddlerhood', *Child Development,* vol. 75**,** no**.** 4, pp. 1254–67.

Colquhuon, D, Ferreira-Jardim, A, Udell, T, B, E & the Nutrition and Metabolism Committee of the Heart Foundation 2008, *Review of evidence Fish, fish oils, n-3 polyunsaturated fatty acids and cardiovascular health,* National Heart Foundation of Australia, viewed 11 Dec 2011, <http://www.heartfoundation.org.au/SiteCollectionDocuments/Fish-FishOils-revie-of-evidence.pdf>

Commonwealth Department of Health 1986, *National Dietary Survey of Adults: 1983. No. 1. Foods consumed,* AGPS, Canberra.

Custodio, HM, Broberg, K, Wennberg, M, Jansson, JH, Vessby, B, Hallmans, G, et al. 2004, 'Polymorphisms in glutathione-related genes affect methylmercury retention', *Archives of Environmental Health,* vol. 59**,** no**.** 11, pp. 588–95.

D'Almeida, A, Carter, JP, Anatol, A & Prost, C 1992, 'Effects of a combination of evening primrose oil (gamma linolenic acid) and fish oil (eicosapentaenoic + docahexaenoic acid) versus magnesium, and versus placebo in preventing pre-eclampsia', *Women & Health,* vol. 19**,** no**.** 2, pp. 117–31.

Daniels, JL, Longnecker, MP, Rowland, AS, Golding, J & Alspac Study Team – University of Bristol Institute of Child Health 2004, 'Fish intake during pregnancy and early cognitive development of offspring', *Epidemiology,* vol. 15**,** no**.** 4, pp. 394–402.

Davidson, PW, Cory-Slechta, DA, Thurston, SW, Huang, L-S, Shamlaye, CF, Gunzler, D, et al. 2011, 'Fish consumption and prenatal methylmercury exposure: Cognitive and behavioral outcomes in the main cohort at 17 years from the Seychelles child development study', *Neurotoxicology,* vol. 32**,** no**.** 6, pp. 711–7.

Davidson, PW, Myers, GJ, Cox, C, Axtell, C & et al. 1998, 'Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: Outcomes at 66 months of age in the Seychelles Child Development Study', *Journal of the American Medical Association,* vol. 280**,** no**.** 8, pp. 701–7.

Davidson, PW, Myers, GJ, Cox, C, Shamlaye, CF, Marsh, DO, Tanner, MA, et al. 1995, 'Longitudinal neurodevelopmental study of Seychellois children following *in utero* exposure

to methylmercury from maternal fish ingestin: Outcomes at 19 and 29 months', *NeuroToxicity,* vol. 16**,** no**.** 4, pp. 677–88.

De-Regil, LM, Fernández-Gaxiola, AC, Dowswell, T & Peña-Rosas, JP 2010, 'Effects and safety of periconceptional folate supplementation for preventing birth defects ', *Cochrane Database of Systematic Reviews*, Issue 10, Art. No. CD007950, DOI: 10.1002/14651858.CD007950.pub2.

Debes, F, Budtz-Jørgensen, E, Weihe, P, White, RF & Grandjean, P 2006, 'Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years', *Neurotoxicology & Teratology,* vol. 28**,** no**.** 3, pp. 363–75.

Decsi, T, Campoy, C & Koletzko, B 2005, 'Effect of n-3 polyunsaturated fatty acid supplementation in pregnancy: the Nuheal trial', *Advances in Experimental Medicine & Biology,* vol. 569**,** no**.**, pp. 109–13.

Delgado-Noguera, MF, Calvache, JA & Bonfill Cosp, X 2010, 'Supplementation with long chain polyunsaturated fatty acids (LCPUFA) to breastfeeding mothers for improving child growth and development', *Cochrane Database of Systematic Reviews*, Issue 12, Art. No. CD007901, DOI: 10.1002/14651858.CD007901.pub2.

Department of Health 2012, *The Victorian Health Monitor Food and Nutrition report,* State Government of Victoria, Melbourne.

Dietitians Association of Australia 2011, *A modelling system to inform the revision of the Australian Guide to Healthy Eating*, Reference code N55c, NHMRC, viewed 29 Jun 2012, <http://www.nhmrc.gov.au/_files_nhmrc/file/publications/n55c_australian_dietary_guidelines_food_modelling.pdf>

Domingo, JL 2010, 'Influence of cooking processes on the concentrations of toxic metals and various organic environmental pollutants in food: A review of the published literature', *Critical Reviews in Food Science & Nutrition,* vol. 51**,** no**.** 1, pp. 29–37.

Doornbos, B, van Goor, SA, Dijck-Brouwer, DA, Schaafsma, A, Korf, J & Muskiet, FA 2009, 'Supplementation of a low dose of DHA or DHA+AA does not prevent peripartum depressive symptoms in a small population based sample', *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* vol. 33**,** no**.** 1, pp. 49–52.

Dunstan, JA, Mori, TA, Barden, A, Beilin, LJ, Holt, PG, Calder, PC, et al. 2004, 'Effects of n-3 polyunsaturated fatty acid supplementation in pregnancy on maternal and fetal erythrocyte fatty acid composition', *European Journal of Clinical Nutrition,* vol. 58**,** no**.** 3, pp. 429–37.

Dunstan, JA, Mori, TA, Barden, A, Beilin, LJ, Taylor, AL, Holt, PG, et al. 2003, 'Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial', *Journal of Allergy and Clinical Immunology,* vol. 112**,** no**.** 6, pp. 1178–84.

Dunstan, JA, Simmer, K, Dixon, G & Prescott, SL 2008, 'Cognitive assessment of children at age 2½ years after maternal fish oil supplementation in pregnancy: a randomised controlled trial', *Archives of Disease in Childhood Fetal & Neonatal Edition,* vol. vol. 93**,** no**.** 1, pp. F45–50.

Dziechciarz, P, Horvath, A & Szajewska, H 2010, 'Effects of n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on neurodevelopment and

visual function in children: a systematic review of randomized controlled trials', *Journal of the American College of Nutrition,* vol. 29**,** no**.** 5, pp. 443–54.

Eilander, A, Hundscheid, DC, Osendarp, SJ, Transler, C & Zock, PL 2007, 'Effects of n-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout childhood: a review of human studies', *Prostaglandins, Leukotrienes and Essential Fatty Acids,* vol. 76**,** no**.** 4, pp. 189–203.

Escolano-Margarit, MV, Ramos, R, Beyer, J, Csabi, G, Parrilla-Roure, M, Cruz, F, et al. 2011, 'Prenatal DHA status and neurological outcome in children at age 5.5 years are positively associated', *Journal of Nutrition,* vol. 141**,** no**.** 6, pp. 1216–23.

FAO 2012, *FAO Fisheries and Aquaculture Department: The state of world fisheries and aquaculture 2012*, viewed 5 Apr 2015, <http://www.fao.org/docrep/016/i2727e/i2727e.pdf>

FAO & WHO 2011, *Report of the Joint FAO/WHO Expert Consultation on the risks and benefits of fish consumption*, Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization.

FAOSTAT 2012, *Food Supply Livestock and Fish Primary Equivalent*, FAO, viewed 3 Jun 2013, <http://faostat.fao.org/site/610/DesktopDefault.aspx?PageID=610#ancor>

Fayet, F, Meyer, B & Baghurst, K 2010, 'Alternate ways to achieve dietary recommendations for long-chain omega-3 PUFA', poster presented to the 2010 Annual Scientific Meeting of the Nutrition Society of Australia, Perth, Australia, 30 Nov–3 Dec.

Flood, M, Stobutzki, I, Andrews, J, Ashby, C, Begg, G, Fletcher, R, et al. (eds) 2014, *Status of key Australian fish stocks reports 2014*, viewed 5 Apr 2015, <http://fish.gov.au/Pages/SAFS_Report.aspx>

Folch, J, Lees, M & Sloane Stanley, GH 1957, 'A simple method for the isolation and purification of total lipides from animal tissues', *Journal of Biological Chemistry,* vol. 226**,** no**.** 1, pp. 497–509.

Freeman, MP, Davis, M, Sinha, P, Wisner, KL, Hibbeln, JR & Gelenberg, AJ 2008, 'Omega-3 fatty acids and supportive psychotherapy for perinatal depression: a randomized placebo-controlled study', *Journal of Affective Disorders,* vol. 110**,** no**.** 1–2, pp. 142–8.

Freeman, MP & Davis, MF 2010, 'Supportive psychotherapy for perinatal depression: preliminary data for adherence and response', *Depression and Anxiety,* vol. 27**,** no**.** 1, pp. 39–45.

Freeman, MP, Hibbeln, JR, Wisner, KL, Brumbach, BH, Watchman, M & Gelenberg, AJ 2006, 'Randomized dose-ranging pilot trial of omega-3 fatty acids for postpartum depression', *Acta Psychiatrica Scandinavica,* vol. 113**,** no**.** 1, pp. 31–5.

FSANZ 2008, *AUSNUT 2007*, FSANZ, Canberra, Australia, viewed 17 Nov 2011, <http://www.foodstandards.gov.au/consumerinformation/ausnut2007/ausnut2007microsofte4060.cfm>

FSANZ 2011a, *Mercury in Fish: Advice on fish consumption*, FSANZ, Canberra, Australia, viewed 17 Feb 2014, <http://www.foodstandards.gov.au/consumer/chemicals/mercury/documents/mercury_in_fish_brochure_lowres.pdf>

FSANZ 2011b, *NUTTAB 2010 Online Searchable Database*, FSANZ, Canberra, Australia, viewed 17 Nov 2011, <http://www.foodstandards.gov.au/consumerinformation/nuttab2010/nuttab2010onlinesearchabledatabase/onlineversion.cfm>

Furuhjelm, C, Warstedt, K, Larsson, J, Fredriksson, M, Bottcher, MF, Falth-Magnusson, K, et al. 2009, 'Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy', *Acta Paediatrica,* vol. 98**,** no**.** 9, pp. 1461–7.

Gardiner, PM, Nelson, L, Shellhaas, CS, Dunlop, AL, Long, R, Andrist, S, et al. 2008, 'The clinical content of preconception care: Nutrition and dietary supplements', *American Journal of Obstetrics and Gynecology,* vol. 199**,** no**.** 6 Suppl B, pp. S345–56. English.

Gibson, RA, Neumann, MA & Makrides, M 1997, 'Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants', *European Journal of Clinical Nutrition,* vol. 51**,** no**.** 9, pp. 578–84.

Golding, J, Steer, C, Emmett, P, Davis, JM & Hibbeln, JR 2009, 'High levels of depressive symptoms in pregnancy with low omega-3 fatty acid intake from fish', *Epidemiology,* vol. 20**,** no**.** 4, pp. 598–603.

Gould, JF, Smithers, LG & Makrides, M 2013, 'The effect of maternal omega-3 (n−3) LCPUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials', *American Journal of Clinical Nutrition,* vol. 97**,** no**.** 3, pp. 531–44.

Grandjean, P, Weihe, P, Jørgensen, PJ, Clarkson, T, Cernichiari, E & Viderø, T 1992, 'Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead', *Archives of Environmental Health,* vol. 47**,** no**.** 3, pp. 185–95.

Grandjean, P, Weihe, P, White, RF, Debes, F, Araki, S, Yokoyama, K, et al. 1997, 'Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury', *Neurotoxicology and Teratology,* vol. 19**,** no**.** 6, pp. 417–28.

Greenfield, H & Southgate, DAT 2003, *Food composition data Production, Management and Use*, 2nd edn, viewed 26 Jul 2013, <ftp://ftp.fao.org/docrep/fao/008/y4705e/y4705e.pdf>

Haider, BA & Bhutta, ZA 2006, 'Multiple-micronutrient supplementation for women during pregnancy', *Cochrane Database of Systematic Reviews*, Issue 4, Art. No. CD004905, DOI: 10.1002/14651858.CD004905.pub2.

Halldorsson, TI, Meltzer, HM, Thorsdottir, I, Knudsen, V & Olsen, SF 2007, 'Is high consumption of fatty fish during pregnancy a risk factor for fetal growth retardation? A study of 44,824 Danish pregnant women', *American Journal of Epidemiology,* vol. 166**,** no**.** 6, pp. 687–96.

Harper, M, Thom, E, Klebanoff, MA, Thorp, J, Jr., Sorokin, Y, Varner, MW, et al. 2010, 'Omega-3 fatty acid supplementation to prevent recurrent preterm birth: a randomized controlled trial', *Obstetrics & Gynecology,* vol. 115**,** no**.** 2 Pt 1, pp. 234–42.

Harris, W 1997, 'n-3 fatty acids and serum lipoproteins: human studies', *American Journal of Clinical Nutrition,* vol. 65**,** no**.** 5, pp. 1645S–54S.

Harris, WS 2007, 'Omega-3 fatty acids and cardiovascular disease: A case for omega-3 index as a new risk factor', *Pharmacological Research,* vol. 55**,** no**.** 3, pp. 217–23.

Harris, WS, Pottala, JV, Sands, SA & Jones, PG 2007, 'Comparison of the effects of fish and fish-oil capsules on the n–3 fatty acid content of blood cells and plasma phospholipids', *American Journal of Clinical Nutrition,* vol. 86**,** no**.** 6, pp. 1621–5.

Hauner, H, Much, D, Vollhardt, C, Brunner, S, Schmid, D, Sedlmeier, E-M, et al. 2012, 'Effect of reducing the n-6:n-3 long-chain PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial', *American Journal of Clinical Nutrition,* vol. 95**,** no**.** 2, pp. 383–94.

Helland, IB, Saugstad, OD, Smith, L, Saarem, K, Solvoll, K, Ganes, T, et al. 2001, 'Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women', *Pediatrics,* vol. 108, no. 5, e82, viewed 19 Jan 2010, <http://pediatrics.aappublications.org/content/108/5/e82.full.html>

Helland, IB, Smith, L, Blomen, B, Saarem, K, Saugstad, OD & Drevon, CA 2008, 'Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age', *Pediatrics,* vol. 122, no. 2, e472–e479, viewed 19 Jan 2010, <http://www.pediatrics.org/cgi/content/full/122/2/e472>

Helland, IB, Smith, L, Saarem, K, Saugstad, OD & Drevon, CA 2003, 'Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age', *Pediatrics,* vol. 111, no. 1, e39–e44, viewed 19 Jan 2010, <http://www.pediatrics.org/cgi/content/full/111/1/e39>

Heppe, DHM, Steegers, EAP, Timmermans, S, Breeijen, Hd, Tiemeier, H, Hofman, A, et al. 2011, 'Maternal fish consumption, fetal growth and the risks of neonatal complications: the Generation R Study', *British Journal of Nutrition,* vol. 105**,** no**.** 06, pp. 938–49.

Hibbeln, JR 2002, 'Seafood consumption, the DHA content of mothers' milk and prevalence rates of postpartum depression: a cross-national, ecological analysis', *Journal of Affective Disorders,* vol. 69**,** no**.** 1-3, pp. 15-29.

Hibbeln, JR, Davis, JM, Steer, C, Emmett, P, Rogers, I, Williams, C, et al. 2007, 'Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study', *Lancet,* vol. 369**,** no**.** 9561, pp. 578–85.

Higgins, JPT & Green S. (eds.) 2011, *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]*. viewed 16 Jul 2014, <www.cochrane-handbook.org>

Horvath, A, Koletzko, B & Szajewska, H 2007, 'Effect of supplementation of women in high-risk pregnancies with long-chain polyunsaturated fatty acids on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials', *British Journal of Nutrition,* vol. 98**,** no**.** 2, pp. 253–9.

Howe, P, Meyer, B, Record, S & Baghurst, K 2006, 'Dietary intake of long-chain n-3 polyunsaturated fatty acids: contribution of meat sources', *Nutrition,* vol. 22**,** no**.** 1, pp. 47–53.

Hure, A, Young, A, Smith, R & Collins, C 2009, 'Diet and pregnancy status in Australian women', *Public Health Nutrition,* vol. 12**,** no**.** 6, pp. 853-61.

Imhoff-Kunsch, B, Briggs, V, Goldenberg, T & Ramakrishnan, U 2012, 'Effect of n-3 long-chain polyunsaturated fatty acid intake during pregnancy on maternal, infant, and child health outcomes: a systematic review', *Paediatric and Perinatal Epidemiology,* vol. 26 no**.** Suppl 1, pp. 91–107.

Innis, SM & Friesen, RW 2008, 'Essential n-3 fatty acids in pregnant women and early visual acuity maturation in term infants', *American Journal of Clinical Nutrition,* vol. 87**,** no**.** 3, pp. 548–57.

Jaddoe, VW, van Duijn, CM, van der Heijden, AJ, Mackenbach, JP, Moll, HA, Steegers, EA, et al. 2010, 'The Generation R Study: design and cohort update 2010', *European Journal of Epidemiology,* vol. 25**,** no**.** 11, pp. 823–41.

Jans, LAW, Giltay, EJ & Van der Does, AJW 2010, 'The efficacy of n-3 fatty acids DHA and EPA (fish oil) for perinatal depression', *British Journal of Nutrition,* vol. 104**,** no**.** 11, pp. 1577–85.

JECFA 2004, *Safety evaluation of certain food additives and contaminants / prepared by the sixty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA),* WHO food additives series:52, WHO, Geneva.

Jensen, C, Prager, T, Zou, Y, Kennard Fraley, J, Maude, M, Anderson, R, et al. 1999, 'Effects of maternal docosahexaenoic acid supplementation on visual function and growth of breast-fed term infants', *Lipids,* vol. 34**,** no**.** Suppl, pp. S225.

Jensen, CL, Voigt, RG, Llorente, AM, Peters, SU, Prager, TC, Zou, YL, et al. 2010, 'Effects of early maternal docosahexaenoic acid intake on neuropsychological status and visual acuity at five years of age of breast-fed term infants', *Journal of Pediatrics,* vol. 157**,** no**.** 6, pp. 900–5.

Jensen, CL, Voigt, RG, Prager, TC, Zou, YL, Fraley, JK, Rozelle, JC, et al. 2005, 'Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants', *American Journal of Clinical Nutrition,* vol. 82**,** no**.** 1, pp. 125–32.

Judge, MP, Cong, X, Harel, O, Courville, AB & Lammi-Keefe, CJ 2012, 'Maternal consumption of a DHA-containing functional food benefits infant sleep patterning: An early neurodevelopmental measure', *Early Human Development,* vol. 88**,** no**.** 7, pp. 531–7.

Judge, MP, Harel, O & Lammi-Keefe, CJ 2007a, 'Maternal consumption of a docosahexaenoic acid-containing functional food during pregnancy: benefit for infant performance on problem-solving but not on recognition memory tasks at age 9 mo', *American Journal of Clinical Nutrition,* vol. 85**,** no**.** 6, pp. 1572–7.

Judge, MP, Harel, O & Lammi-Keefe, CJ 2007b, 'A docosahexaenoic acid-functional food during pregnancy benefits infant visual acuity at four but not six months of age', *Lipids,* vol. 42**,** no**.** 2, pp. 117–22.

Karlsson, T, Birberg-Thornberg, U, Duchen, K & Gustafsson, PA 2010, 'LC-PUFA supplemented to mothers during pregnancy and breast-feeding improves cognitive performance in their children four years later - an RCT study', abstract presented to the International Society for the Study of Fatty Acids and Lipids Maastricht, Netherlands, 29 May–2 June.

Katan, MB, Deslypere, JP, van Birgelen, AP, Penders, M & Zegwaard, M 1997, 'Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes,

and adipose tissue: an 18-month controlled study', *Journal of Lipid Research,* vol. 38**,** no**.** 10, pp. 2012–22.

Kjellström, T, Kennedy, P, Wallis, S & Mantell, C 1986, *Physical and mental development of children with prenatal exposure to mercury from fish Stage 1: Preliminary tests at age 4,* National Swedish Environmental Protection Board, Solna.

Kjellström, T, Kennedy, P, Wallis, S, Stewart, A, Friberg, L, Lind, B, et al. 1989, *Physical and mental development of children with prenatal exposure to mercury from fish Stage 2: Interviews and psychological tests at age 6,* National Swedish Environmental Protection Board, Solna.

Klemens, CM, Berman, DR & Mozurkewich, EL 2011, 'The effect of perinatal omega-3 fatty acid supplementation on inflammatory markers and allergic diseases: a systematic review', *BJOG: An International Journal of Obstetrics and Gynaecology,* vol. 118**,** no**.** 8, pp. 916–25.

Knudsen, VK, Hansen, HS, Osterdal, ML, Mikkelsen, TB, Mu, H & Olsen, SF 2006, 'Fish oil in various doses or flax oil in pregnancy and timing of spontaneous delivery: a randomised controlled trial', *BJOG: An International Journal of Obstetrics and Gynaecology,* vol. 113**,** no**.** 5, pp. 536–43.

Koletzko, B, Cetin, I, Brenna, JT, Perinatal Lipid Intake Working, G, Child Health, F, Diabetic Pregnancy Study, G, et al. 2007, 'Dietary fat intakes for pregnant and lactating women', *British Journal of Nutrition,* vol. 98**,** no**.** 5, pp. 873–7.

Kramer, MS & Kakuma, R 2003, 'Energy and protein intake in pregnancy', *Cochrane Database of Systematic Reviews,* Issue 4, Art. No. CD000032, DOI: 10.1002/14651858.CD000032.

Krauss-Etschmann, S, Hartl, D, Rzehak, P, Heinrich, J, Shadid, R, Del Carmen Ramirez-Tortosa, M, et al. 2008, 'Decreased cord blood IL-4, IL-13, and CCR4 and increased TGF-beta levels after fish oil supplementation of pregnant women', *Journal of Allergy and Clinical Immunology,* vol. 121**,** no**.** 2, pp. 464–70.

Krauss-Etschmann, S, Shadid, R, Campoy, C, Hoster, E, Demmelmair, H, Jiménez, M, et al. 2007, 'Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial', *American Journal of Clinical Nutrition,* vol. 85**,** no**.** 5, pp. 1392–400.

Kremmyda, L-S, Vlachava, M, Noakes, PS, Diaper, ND, Miles, EA & Calder, PC 2011, 'Atopy risk in infants and children in relation to early exposure to fish, oily fish, or long-chain omega-3 fatty acids: a systematic review', *Clinical Reviews in Allergy & Immunology,* vol. 41**,** no**.** 1, pp. 36–66.

Kromann, N & Green, A 1980, 'Epidemiological studies in the Upernavik District, Greenland. Incidence of some chronic diseases 1950–1974', *Acta Medica Scandinavica,* vol. 208**,** no**.** 5, pp. 401–6.

Kuratko, CN & Salem Jr, N 2009, 'Biomarkers of DHA status', *Prostaglandins, Leukotrienes and Essential Fatty Acids,* vol. 81**,** no**.** 2-3, pp. 111–8.

Laivuori, H, Hovatta, O, Viinikka, L & Ylikorkala, O 1993, 'Dietary supplementation with primrose oil or fish oil does not change urinary excretion of prostacyclin and thromboxane

metabolites in pre-eclamptic women', *Prostaglandins, Leukotrienes and Essential Fatty Acids,* vol. 49**,** no**.** 3, pp. 691–4.

Lando, AM, Fein, SB & Choinière, CJ 2012, 'Awareness of methylmercury in fish and fish consumption among pregnant and postpartum women and women of childbearing age in the United States', *Environmental Research,* vol. 116, July**,** no**.**, pp. 85–92.

Larnkjaer, A, Christensen, JH, Michaelsen, KF & Lauritzen, L 2006, 'Maternal fish oil supplementation during lactation does not affect blood pressure, pulse wave velocity, or heart rate variability in 2.5-y-old children', *Journal of Nutrition,* vol. 136**,** no**.** 6, pp. 1539–44.

Larqué, E, Gil-Sánchez, A, Prieto-Sánchez, MT & Koletzko, B 2012, 'Omega 3 fatty acids, gestation and pregnancy outcomes', *British Journal of Nutrition,* vol. 107**,** no**.** Suppl S2, pp. S77–S84.

Larsen, D, Quek, SY & Eyres, L 2010, 'Effect of cooking method on the fatty acid profile of New Zealand King Salmon (Oncorhynchus tshawytscha)', *Food Chemistry,* vol. 119**,** no**.** 2, pp. 785–90.

Lauritzen, L, Hoppe, C, Straarup, EM & Michaelsen, KF 2005a, 'Maternal fish oil supplementation in lactation and growth during the first 2.5 years of life', *Pediatric Research,* vol. 58**,** no**.** 2, pp. 235–42.

Lauritzen, L, Jorgensen, MH, Mikkelsen, TB, Skovgaard, M, Straarup, EM, Olsen, SF, et al. 2004, 'Maternal fish oil supplementation in lactation: effect on visual acuity and n-3 fatty acid content of infant erythrocytes', *Lipids,* vol. 39**,** no**.** 3, pp. 195–206.

Lauritzen, L, Jorgensen, MH, Olsen, SF, Straarup, EM & Michaelsen, KF 2005b, 'Maternal fish oil supplementation in lactation: effect on developmental outcome in breast-fed infants', *Reproduction, Nutrition, Development,* vol. 45**,** no**.** 5, pp. 535–47.

Lauritzen, L, Kjaer, TM, Fruekilde, MB, Michaelsen, KF & Frokiaer, H 2005c, 'Fish oil supplementation of lactating mothers affects cytokine production in 2 ½-year-old children', *Lipids,* vol. 40**,** no**.** 7, pp. 669–76.

Leermakers, ETM, Sonnenschein-van der Voort, AMM, Heppe, DHM, de Jongste, JC, Moll, HA, Franco, OH, et al. 2013, 'Maternal fish consumption during pregnancy and risks of wheezing and eczema in childhood: The Generation R Study', *European Journal of Clinical Nutrition,* vol. 67**,** no**.** 4, pp. 353–9.

Lewin, GA, Schachter, HM, Yuen, D, Merchant, P, Mamaladze, V, Tsertsvadze, A, et al. 2005, 'Effects of omega-3 fatty acids on child and maternal health. Evidence report/Technology assessment No. 118', viewed 21 Jan 2010, <http://archive.ahrq.gov/downloads/pub/evidence/pdf/o3maternalchild/o3mch.pdf>

Liu, L, Johnson, HL, Cousens, S, Perin, J, Scott, S, Lawn, JE, et al. 2012, 'Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000', *The Lancet,* vol. 379**,** no**.** 9832, pp. 2151–61.

Llorente, AM, Jensen, CL, Voigt, RG, Fraley, JK, Berretta, MC & Heird, WC 2003, 'Effect of maternal docosahexaenoic acid supplementation on postpartum depression and information processing', *American Journal of Obstetrics and Gynecology,* vol. 188**,** no**.** 5, pp. 1348–53.

Lo, A, Sienna, J, Mamak, E, Djokanovic, N, Westall, C & Koren, G 2012, 'The effects of maternal supplementation of polyunsaturated fatty acids on visual, neurobehavioural, and developmental outcomes of the child: A systematic review of the randomized trials', *Obstetrics and Gynecology International*, vol. 2012, Article ID 591531, viewed 29 Apr 2013, <http://dx.doi.org/10.1155/2012/591531>

Mahaffey, KR, Clickner, RP & Jeffries, RA 2009, 'Adult women's blood mercury concentrations vary regionally in the United States: Association with patterns of fish consumption (NHANES 1999-2004)', *Environmental Health Perspectives*, vol. 117**,** no**.** 1, pp. 47–53.

Makrides, M, Duley, L & Olsen, SF 2006, 'Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction', *Cochrane Database of Systematic Reviews*, Issue 3, Art. No. CD003402, DOI: 10.1002/14651858.CD003402.pub2.

Makrides, M, Gibson, RA, McPhee, AJ, Yelland, L, Quinlivan, J, Ryan, P, et al. 2010, 'Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial', *Journal of the American Medical Association,* vol. 304**,** no**.** 15, pp. 1675–83.

Malcolm, CA, Hamilton, R, McCulloch, DL, Montgomery, C & Weaver, LT 2003a, 'Scotopic electroretinogram in term infants born of mothers supplemented with docosahexaenoic acid during pregnancy', *Investigative Ophthalmology & Visual Science*, vol. 44**,** no**.** 8, pp. 3685–91.

Malcolm, CA, McCulloch, DL, Montgomery, C, Shepherd, A & Weaver, LT 2003b, 'Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomised trial', *Archives of Disease in Childhood Fetal & Neonatal Edition,* vol. 88**,** no**.** 5, pp. F383–90.

Marangell, LB, Martinez, JM, Zboyan, HA, Chong, H & Puryear, LJ 2004, 'Omega-3 fatty acids for the prevention of postpartum depression: negative data from a preliminary, open-label pilot study', *Depression and Anxiety,* vol. 19**,** no**.** 1, pp. 20–3.

Marie Stopes International 2008, *Real Choices: Women, contraception and unplanned pregnancy*, viewed 07 Jun 2013, <http://www.mariestopes.org.au/images/stories/libraryfiles/Real-Choices-Key-Findings.pdf>

Marsh, DO, Clarkson, TW, Myers, GJ, Davidson, PW, Cox, C, Cernichiari, E, et al. 1995, 'The Seychelles Study of fetal methylmercury exposure and child development: Introduction', *NeuroToxicology,* vol. 16**,** no**.** 4, pp. 583–96.

Maslova, E, Strøm, M, Oken, E, Campos, H, Lange, C, Gold, D, et al. 2013, 'Fish intake during pregnancy and the risk of child asthma and allergic rhinitis – longitudinal evidence from the Danish National Birth Cohort', *British Journal of Nutrition,* vol. 110**,** no**.** 07, pp. 1313–25.

Mattes, E, McCarthy, S, Gong, G, van Eekelen, JAM, Dunstan, J, Foster, J, et al. 2009, 'Maternal mood scores in mid-pregnancy are related to aspects of neonatal immune function', *Brain, Behavior, and Immunity,* vol. 23**,** no**.** 3, pp. 380–8.

McLennan, W & Podger, A 1998, *National Nutrition Survey users' guide 1995,* no. 4801.0, ABS, Commonwealth of Australia, Canberra.

McLennan, W & Podger, A 1999, *National Nutrition Survey foods eaten Australia 1995*, no. 4808.0, ABS, Commonwealth of Australia, Canberra.

McManus, A, Burns, SK, Howat, PA, Cooper, L & Fielder, L 2007, 'Factors influencing the consumption of seafood among young children in Perth: a qualitative study', *BMC Public Health*, vol. 7, no.119, viewed 31 Oct 2009, <http://www.biomedcentral.com/content/pdf/1471-2458-7-119.pdf>

Meis, PJ, Klebanoff, M, Thom, E, Dombrowski, MP, Sibai, B, Moawad, AH, et al. 2003, 'Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate', *New England Journal of Medicine*, vol. 348**,** no**.** 24, pp. 2379–85.

Meltzer, HM, Brantsæter, AL, Ydersbond, TA, Alexander, J, Haugen, M & The MoBa Dietary Support, G 2008, 'Methodological challenges when monitoring the diet of pregnant women in a large study: experiences from the Norwegian Mother and Child Cohort Study (MoBa)', *Maternal and Child Nutrition*, vol. 4**,** no**.** 1, pp. 14–27.

Miles, EA, Noakes, PS, Kremmyda, L-S, Vlachava, M, Diaper, ND, Rosenlund, G, et al. 2011, 'The Salmon in Pregnancy Study: study design, subject characteristics, maternal fish and marine n–3 fatty acid intake, and marine n–3 fatty acid status in maternal and umbilical cord blood', *American Journal of Clinical Nutrition*, vol. 94**,** no**.** 6 Suppl, pp. 1986S–92S.

Mooney, BD, Nichols, PD & Elliott, NG 2002, *Seafood The Good Food II: Oil profiles for further Australian seafoods, and influencing factors*, CSIRO Marine Research and Fisheries Research & Development Corporation (FRDC), Hobart, Tasmania.

Moradi, Y, Bakar, J, Motalebi, AA, Muhamad, SHS & Man, YC 2011, 'A review on fish lipid: Composition and changes during cooking methods', *Journal of Aquatic Food Product Technology*, vol. 20**,** no**.** 4, pp. 379–90.

Mori, TA 2006, 'Omega-3 fatty acids and hypertension in humans', *Clinical and Experimental Pharmacology and Physiology*, vol. 33**,** no**.** 9, pp. 842–6.

Moster, D, Lie, RT & Markestad, T 2008, 'Long-term medical and social consequences of preterm birth', *New England Journal of Medicine*, vol. 359**,** no**.** 3, pp. 262–73.

Mozaffarian, D & Rimm, EB 2006, 'Fish intake, contaminants, and human health: evaluating the risks and the benefits', *Journal of the American Medical Association*, vol. 296**,** no**.** 15, pp. 1885–99. [Erratum appears in 2007 *Journal of the American Medical Association*, vol. 297, no. 6, p. 590].

Muhlhausler, BS, Gibson, RA & Makrides, M 2010, 'Effect of long-chain polyunsaturated fatty acid supplementation during pregnancy or lactation on infant and child body composition: a systematic review', *American Journal of Clinical Nutrition*, vol. 92**,** no**.** 4, pp. 857–63.

Myers, GJ, Davidson, PW, Cox, C, Shamlaye, CF, Palumbo, D, Cernichiari, E, et al. 2003, 'Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study', *Lancet*, vol. 361**,** no**.** 9370, pp. 1686–92.

Myers, GJ, Davidson, PW, Shamlaye, CF, Axtell, CD, Cernichiari, E, Choisy, O, et al. 1997, 'Effects of prenatal methylmercury exposure from a high fish diet on developmental milestones in the Seychelles Child Development Study', *NeuroToxicity*, vol. 18**,** no**.** 3, pp. 819–30.

Myers, GJ, Marsh, DO, Davidson, PW, Cox, C, Shamlaye, CF, Tanner, M, et al. 1995, 'Main neurodevelopment study of Seychellois children following *in utero* exposure to methylmercury from a maternal fish diet: Outcome at six months', *NeuroToxicology,* vol. 16**,** no**.** 4, pp. 653–64.

Neale, EP, Nolan-Clark, D, Probst, YC, Batterham, MJ & Tapsell, LC 2012, 'Comparing attitudes to fish consumption between clinical trial participants and non-trial individuals', *Nutrition & Dietetics,* vol. 69**,** no**.** 2, pp. 124–9.

Nesheim, M & Yaktine, A (eds) 2006, *Seafood choices: Balancing benefits and risks,* The National Academies Press, Washington, D. C.

Neuringer, M & Connor, WE 1986, 'n-3 fatty acids in the brain and retina: evidence for their essentiality', *Nutrition Reviews,* vol. 44**,** no**.** 9, pp. 285–94.

NH&MRC 1992, 'Dietary Guidelines for Australians', viewed 30 Mar 2015, <https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/n4.pdf>

NHMRC 2006, *Nutrient Reference Values for Australia and New Zealand,* Commonwealth of Australia, Canberra.

NHMRC 2012, *Review: Nutritional requirements and dietary advice targeted for pregnant and breastfeeding women*, NHMRC, Canberra.

NHMRC 2013a, *Australian Dietary Guidelines Summary,* Publication reference N55a, NHMRC, Canberra.

NHMRC 2013b, *Australian Dietary Guidelines,* Publication reference N55, NHMRC, Canberra.

Nichols, PD, Virtue, P, Mooney, BD, Elliott, NG & Yearsley, GK 1998, *Seafood The Good Food*, CSIRO Marine Research, Hobart, Tasmania.

Noakes, PS, Vlachava, M, Kremmyda, L-S, Diaper, ND, Miles, EA, Erlewyn-Lajeunesse, M, et al. 2012, 'Increased intake of oily fish in pregnancy: effects on neonatal immune responses and on clinical outcomes in infants at 6 mo', *American Journal of Clinical Nutrition,* vol. 95**,** no**.** 2, pp. 395–404.

Oken, E, Kleinman, KP, Olsen, SF, Rich-Edwards, JW & Gillman, MW 2004, 'Associations of seafood and elongated n-3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort', *American Journal of Epidemiology,* vol. 160**,** no**.** 8, pp. 774–83.

Oken, E, Ning, Y, Rifas-Shiman, SL, Rich-Edwards, JW, Olsen, SF & Gillman, MW 2007, 'Diet during pregnancy and risk of preeclampsia or gestational hypertension', *Annals of Epidemiology,* vol. 17**,** no**.** 9, pp. 663–8.

Oken, E, Osterdal, ML, Gillman, MW, Knudsen, VK, Halldorsson, TI, Strom, M, et al. 2008a, 'Associations of maternal fish intake during pregnancy and breastfeeding duration with attainment of developmental milestones in early childhood: a study from the Danish National Birth Cohort', *American Journal of Clinical Nutrition,* vol. 88**,** no**.** 3, pp. 789–96.

Oken, E, Radesky, JS, Wright, RO, Bellinger, DC, Amarasiriwardena, CJ, Kleinman, KP, et al. 2008b, 'Maternal fish intake during pregnancy, blood mercury levels, and child cognition

at age 3 years in a US cohort', *American Journal of Epidemiology,* vol. 167**,** no**.** 10, pp. 1171–81.

Oken, E, Wright, RO, Kleinman, KP, Bellinger, D, Amarasiriwardena, CJ, Hu, H, et al. 2005, 'Maternal fish consumption, hair mercury, and infant cognition in a U.S. Cohort', *Environmental Health Perspectives,* vol. 113**,** no**.** 10, pp. 1376–80.

Olsen, J, Melbye, M, Olsen, SF, Sørensen, TIA, Aaby, P, Nybo Andersen, A, et al. 2001, 'The Danish National Birth Cohort – its background, structure and aim', *Scandinavian Journal of Public Health,* vol. 29**,** no**.** 4, pp. 300–7.

Olsen, S, Sorensen, TI, Secher, N, Hansen, H, Jensen, B, Sommer, S, et al. 1986, 'Intake of marine fat, rich in (n-3)-polyunsaturated fatty acids, may increase birthweight by prolonging gestation', *The Lancet,* vol. 328**,** no**.** 8503, pp. 367–9.

Olsen, SF, Dalby Sorensen, J, Secher, NJ, Hedegaard, M, Brink Henriksen, T, Hansen, HS, et al. 1992, 'Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration', *Lancet,* vol. 339**,** no**.** 8800, pp. 1003–7.

Olsen, SF, Osterdal, ML, Salvig, JD, Mortensen, LM, Rytter, D, Secher, NJ, et al. 2008, 'Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial', *American Journal of Clinical Nutrition,* vol. 88**,** no**.** 1, pp. 167–75.

Olsen, SF, Secher, NJ, Tabor, A, Weber, T, Walker, JJ & Gluud, C 2000, 'Randomised clinical trials of fish oil supplementation in high risk pregnancies', *BJOG: An International Journal of Obstetrics and Gynaecology,* vol. 107**,** no**.** 3, pp. 382–95.

Onwude, JL, Lilford, RJ, Hjartardottir, H, Staines, A & Tuffnell, D 1995, 'A randomised double blind placebo controlled trial of fish oil in high risk pregnancy', *BJOG: An International Journal of Obstetrics and Gynaecology,* vol. 102**,** no**.** 2, pp. 95–100.

Otto, SJ, vanHouwelingen, AC, Antal, M, Manninen, A, Godfrey, K, LopezJaramillo, P, et al. 1997, 'Maternal and neonatal essential fatty acid status in phospholipids: An international comparative study', *European Journal of Clinical Nutrition,* vol. 51**,** no**.** 4, pp. 232–42.

Palmer, DJ, Sullivan, T, Gold, MS, Prescott, SL, Heddle, R, Gibson, RA, et al. 2012, 'Effect of n-3 long chain polyunsaturated fatty acid supplementation in pregnancy on infants' allergies in first year of life: randomised controlled trial', *BMJ,* vol. 344, e184, viewed 4 Jun 2012, <http://www.bmj.com/highwire/filestream/563331/field_highwire_article_pdf/0.pdf>

Purcell, S, Neale, B, Todd-Brown, K, Thomas, L, Ferreira, MAR, Bender, D, et al. 2007, 'PLINK: A tool set for whole-genome association and population-based linkage analyses', *American Journal of Human Genetics,* vol. 81**,** no**.** 3, pp. 559–75.

Radloff, L 1977, 'The CES-D Scale: A self-report depression scale for research in the general population', *Applied Psychological Measurement,* vol. 1**,** no**.** 3, pp. 385–401.

Ramakrishnan, U, Grant, F, Goldenberg, T, Zongrone, A & Martorell, R 2012, 'Effect of women's nutrition before and during early pregnancy on maternal and infant outcomes: A systematic review', *Paediatric and Perinatal Epidemiology,* vol. 26**,** no**.** Suppl 1, pp. 285–301.

Ramakrishnan, U, Martorell, R, Stein, AD, Wang, M, DiGirolamo, A, Schnaas, L, et al. 2010b, 'Effect of prenatal supplementation with docosahexanoic acid on child size and

development at 18 mo: randomized placebo-controlled trial in Mexico.', abstract presented to the International Society for the Study of Fatty Acids and Lipids, Maastricht, Netherlands, 29 May–2 June.

Ramakrishnan, U, Stein, AD, Parra-Cabrera, S, Wang, M, Imhoff-Kunsch, B, Juarez-Marquez, S, et al. 2010a, 'Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico', *Food & Nutrition Bulletin,* vol. 31**,** no**.** 2 (Suppl), pp. S108–16.

Rees, AM, Austin, MP & Parker, GB 2008, 'Omega-3 fatty acids as a treatment for perinatal depression: randomized double-blind placebo-controlled trial', *Australian and New Zealand Journal of Psychiatry,* vol. 42**,** no**.** 3, pp. 199–205. [Erratum appears in 2008 *Australian and New Zealand Journal of Psychiatry*, vol. 42, no. 5, p. 438].

Rodríguez, G, Iglesia, I, Bel-Serrat, S & Moreno, LA 2012, 'Effect of n-3 long chain polyunsaturated fatty acids during the perinatal period on later body composition', *British Journal of Nutrition,* vol. 107**,** no**.** Suppl S2, pp. S117–28.

Salvig, JD & Lamont, RF 2011, 'Evidence regarding an effect of marine n-3 fatty acids on preterm birth: a systematic review and meta-analysis', *Acta Obstetricia et Gynecologica Scandinavica,* vol. 90**,** no**.** 8, pp. 825–38.

Salvig, JD, Olsen, SF & Secher, NJ 1996, 'Effects of fish oil supplementation in late pregnancy on blood pressure: a randomised controlled trial', *BJOG: An International Journal of Obstetrics and Gynaecology,* vol. 103**,** no**.** 6, pp. 529–33.

Sanjurjo, P, Ruiz-Sanz, JI, Jimeno, P, Aldamiz-Echevarria, L, Aquino, L, Matorras, R, et al. 2004, 'Supplementation with docosahexaenoic acid in the last trimester of pregnancy: maternal-fetal biochemical findings', *Journal of Perinatal Medicine,* vol. 32**,** no**.** 2, pp. 132–6.

Schober, SE, Sinks, TH, Jones, RL, Bolger, PM, McDowell, M, Osterloh, J, et al. 2003, 'Blood mercury levels in US children and women of childbearing age, 1999-2000', *Journal of the American Medical Association,* vol. 289**,** no**.** 13, pp. 1667–74.

Schofield, WN 1985, 'Predicting basal metabolic rate, new standards and review of previous work', *Human Nutrition-Clinical Nutrition,* vol. 39**,** no**.** Suppl 1, pp. 5–41.

Şengör, GFÜ, Alakavuk, DÜ & Tosun, ŞY 2012, 'Effect of cooking methods on proximate composition, fatty acid composition, and cholesterol content of Atlantic Salmon (Salmo salar)', *Journal of Aquatic Food Product Technology,* vol. 22**,** no**.** 2, pp. 160–7.

Simopoulos, AP 1991, 'Omega-3 fatty acids in health and disease and in growth and development', *The American Journal of Clinical Nutrition,* vol. 54**,** no**.** 3, pp. 438–63.

Sioen, I, Haak, L, Raes, K, Hermans, C, De Henauw, S, De Smet, S, et al. 2006, 'Effects of pan-frying in margarine and olive oil on the fatty acid composition of cod and salmon', *Food Chemistry,* vol. 98**,** no**.** 4, pp. 609–17.

Smithers, LG, Gibson, RA & Makrides, M 2011, 'Maternal supplementation with docosahexaenoic acid during pregnancy does not affect early visual development in the infant: a randomized controlled trial', *American Journal of Clinical Nutrition,* vol. 93**,** no**.** 6, pp. 1293–9.

Smithers, LG, Gibson, RA, McPhee, A & Makrides, M 2008, 'Effect of two doses of docosahexaenoic acid (DHA) in the diet of preterm infants on infant fatty acid status: results from the DINO trial', *Prostaglandins Leukotrienes & Essential Fatty Acids*, vol. 79**,** no**.** 3–5, pp. 141–6.

Smuts, CM, Borod, E, Peeples, JM & Carlson, SE 2003a, 'High-DHA eggs: feasibility as a means to enhance circulating DHA in mother and infant', *Lipids*, vol. 38**,** no**.** 4, pp. 407–14.

Smuts, CM, Huang, M, Mundy, D, Plasse, T, Major, S & Carlson, SE 2003b, 'A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy', *Obstetrics & Gynecology*, vol. 101**,** no**.** 3, pp. 469–79.

Stein, AD, Wang, M, Martorell, R, Neufeld, LM, Flores-Ayala, R, Rivera, JA, et al. 2011, 'Growth to age 18 months following prenatal supplementation with docosahexaenoic acid differs by maternal gravidity in Mexico', *Journal of Nutrition*, vol. 141**,** no**.** 2, pp. 316–20. [Erratum appears in 2011 *Journal of Nutrition*, vol. 141, no. 9, p. 1762].

Stein, AD, Wang, M, Rivera, JA, Martorell, R & Ramakrishnan, U 2012, 'Auditory- and visual-evoked potentials in Mexican infants are not affected by maternal supplementation with 400 mg/d docosahexaenoic acid in the second half of pregnancy', *Journal of Nutrition*, vol. 142**,** no**.** 8, pp. 1577–81.

Strom, M, Mortensen, EL, Halldorsson, TI, Thorsdottir, I & Olsen, SF 2009, 'Fish and long-chain n-3 polyunsaturated fatty acid intakes during pregnancy and risk of postpartum depression: a prospective study based on a large national birth cohort', *American Journal of Clinical Nutrition*, vol. 90**,** no**.** 1, pp. 149–55.

Su, KP, Huang, SY, Chiu, TH, Huang, KC, Huang, CL, Chang, HC, et al. 2008, 'Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial', *Journal of Clinical Psychiatry*, vol. 69**,** no**.** 4, pp. 644–51.

Szajewska, H, Horvath, A & Koletzko, B 2006, 'Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials', *American Journal of Clinical Nutrition*, vol. 83**,** no**.** 6, pp. 1337–44.

Tanaka, T, Shen, J, Abecasis, GR, Kisialiou, A, Ordovas, JM, Guralnik, JM, et al. 2009, 'Genome-wide association study of plasma polyunsaturated fatty acids in the InCHIANTI study', *PLoS Genetics*, vol. 5, no.1, e1000338, viewed 26 Feb 2010, <http://dx.doi.org/10.1371%2Fjournal.pgen.1000338>

Tofail, F, Kabir, I, Hamadani, JD, Chowdhury, F, Yesmin, S, Mehreen, F, et al. 2006, 'Supplementation of fish-oil and soy-oil during pregnancy and psychomotor development of infants', *Journal of Health, Population and Nutrition*, vol. 24**,** no**.** 1, pp. 48–56.

van Goor, SA, Dijck-Brouwer, DAJ, Erwich, JJHM, Schaafsma, A & Hadders-Algra, M 2011, 'The influence of supplemental docosahexaenoic and arachidonic acids during pregnancy and lactation on neurodevelopment at eighteen months', *Prostaglandins, Leukotrienes and Essential Fatty Acids*, vol. 84**,** no**.** 5–6, pp. 139–46.

van Goor, SA, Janneke Dijck-Brouwer, DA, Doornbos, B, Erwich, JJHM, Schaafsma, A, Muskiet, FAJ, et al. 2010, 'Supplementation of DHA but not DHA with arachidonic acid during pregnancy and lactation influences general movement quality in 12-week-old term infants', *British Journal of Nutrition*, vol. 103**,** no**.** 02, pp. 235–42.

Van Houwelingen, AC, Søsrensen, JD, Hornstra, G, Simonis, MMG, Boris, J, Olsen, SF, et al. 1995, 'Essential fatty acid status in neonates after fish-oil supplementation during late pregnancy', *British Journal of Nutrition,* vol. 74**,** no**.** 5, pp. 723–31.

Vaz, JdS, Kac, G, Emmett, P, Davis, JM, Golding, J & Hibbeln, JR 2013, 'Dietary patterns, n-3 fatty acids intake from seafood and high levels of anxiety symptoms during pregnancy: Findings from the Avon Longitudinal Study of Parents and Children', *Plos One,* vol. 8, no. 7, e67671, viewed 11 Dec 2013, <http://dx.doi.org/10.1371%2Fjournal.pone.0067671>

Villani, AM, Crotty, M, Cleland, LG, James, MJ, Fraser, RJ, Cobiac, L, et al. 2013, 'Fish oil administration in older adults: is there potential for adverse events? A systematic review of the literature', *BMC Geriatrics,* vol. 13, no. 41, viewed 8 Jan 2014, <http://www.biomedcentral.com/1471-2318/13/41>

Walsh, SW 1985, 'Preeclampsia: an imbalance in placental prostacyclin and thromboxane production', *American Journal of Obstetrics and Gynecology,* vol. 152**,** no**.** 3, pp. 335–40.

Wojcicki, JM & Heyman, MB 2011, 'Maternal omega-3 fatty acid supplementation and risk for perinatal maternal depression', *Journal of Maternal-Fetal & Neonatal Medicine,* vol. 24**,** no**.** 5, pp. 680–6.

World Bank 2013, *Fish to 2030: Prospects for fisheries and aquaculture. Agriculture and environmental services discussion paper: no.3,* viewed 5 Apr 2015, <http://www-wds.worldbank.org/external/default/WDSContentServer/WDSP/IB/2014/01/31/000461832_20140131135525/Rendered/PDF/831770WP0P11260ES003000Fish0to02030.pdf>

Zhou, SJ, Yelland, L, McPhee, AJ, Quinlivan, J, Gibson, RA & Makrides, M 2012, 'Fish-oil supplementation in pregnancy does not reduce the risk of gestational diabetes or preeclampsia', *American Journal of Clinical Nutrition,* vol. 95**,** no**.** 6, pp. 1378–84.

Appendices

Appendix 1 Search Strategy for the literature review

No.	Searches	Results
1	*Eicosapentaenoic Acid/	2116
2	*Eicosapentaenoic Acid/ or *Fatty Acids, Omega-3/ or eicosapent?enoic acid$.mp	10489
3	*Docosahexaenoic Acids/ or *Eicosapentaenoic Acid/ or Docosapentaenoic acid$.mp	4735
4	docosapent?enoic acid$.mp	600
5	Docosahexaenoic Acid$.mp. or *Docosahexaenoic Acids/ or *Fatty Acids, Essential/	10371
6	docosahex?enoic acid$.mp. or *Docosahexaenoic Acids/	8089
7	*Fatty Acids, Omega-3/ or *Eicosapentaenoic Acid/ or omega-3.mp. or *Docosahexaenoic Acids/	14082
8	Docosahexaenoic Acids/ or Fatty Acids, Omega-3/ or DHA.mp.	15087
9	Eicosapentaenoic Acid/ or EPA.mp.	9732
10	DPA.mp.	1700
11	("n-3" adj4 "FA$").mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]	7208
12	("n-3" adj4 "PUFA$").mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]	2733
13	("n3" adj4 "fatty$").mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]	116
14	("n3" adj4 "PUFA$").mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]	50
15	*Fish Products/ or seafood.mp. or *Shellfish/ or *Seafood/ or *Fishes/	36028
16	*Fishes/	28913
17	pregnant.mp. or *Pregnancy/ or *Pregnant Women/ or *Placenta/	174651

18	pregnancy.mp. or *Pregnancy/ or *Pregnancy Outcome/	710827
19	maternal.mp.	197267
20	mother$.mp. or *Mothers/	150551
21	*Lactation/ or lactation.mp.	41810
22	*Milk, Human/ or *Pregnancy/ or *Lactation/ or lactating.mp.	80945
23	*Infant, Newborn/ or *Pregnancy/ or *Breast Feeding/ or *Mothers/ or breastfeed.mp. or *Infant/	139993
24	*Pregnancy/ or *Infant/ or Infant, Premature/ or *Milk, Human/ or *Mothers/ or *Infant, Newborn/ or *Breast Feeding/ or breast-feed.mp.	181595
25	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16	66323
26	17 or 18 or 19 or 20 or 21 or 22 or 23 or 24	953466
27	25 and 26	3221
28	Random Allocation/	76943
29	Double-Blind Method/	119238
30	Single-Blind Method/	17338
31	randomised controlled trial.mp.	8275
32	randomized controlled trial.mp. or Randomized Controlled Trial/	351276
33	controlled clinical trial.mp. or Controlled Clinical Trial/	92455
34	meta-analysis.mp. or Meta-Analysis/	59291
35	Randomized Controlled Trials as Topic/ or systematic review.mp.	109617
36	28 or 29 or 30 or 31 or 32 or 33 or 34 or 35	639725
37	27 and 36	407
38	limit 37 to (english language and humans and yr="2003-Current")	213

Appendix 2 Description of studies included in the literature review

Trial ID	Year*	Country	Subject characteristics	Duration of supplementation	Intervention – Intake per day (n=number at randomisation)	Control – Intake per day (n=number at randomisation)
1	1992	Angola	Women receiving prenatal care at a hospital	From within first four months of pregnancy to delivery	8 evening primrose + fish oil capsules providing in total 0.30 g of GLA, 0.14 g of EPA & 0.08 g of DHA (n=50) **OR** 2 x 500 mg Magnesium tablets (n=50)	8 olive oil capsules (n=50)
2	1992	Denmark	Women presenting for routine assessment	From 30 week gestation to delivery	4 fish oil capsules providing in total 2.7 g of LCn3PUFA (1.28 g of EPA & 0.92 g of DHA) (n=266)	4 x 1g olive oil capsules (n=136) **OR** No supplement given (n=131)
3	1993	Finland	Women diagnosed with pre-eclampsia	From 26–36 week gestation to delivery	10 fish oil capsules providing in total 1.80 g of EPA & 1.20 g of DHA (n=5) **OR** 10 primrose oil capsules providing in total 3.75 g of LA & 0.45 g of GLA (n=7)	10 capsules providing in total 5 g of maize oil & 5 g of corn oil (n=6)
4	1994	Netherlands	Women with a history of IUGR +/- PIH in previous pregnancy	From 12–14 week gestation to delivery	12 capsules providing in total 3 g of EPA, DHA also present but dose NR (n=34)	12 capsules containing coconut oil (n=34)

Trial ID	Year*	Country	Subject characteristics	Duration of supplementation	Intervention – Intake per day (n=number at randomisation)	Control – Intake per day (n=number at randomisation)
5	1995	United Kingdom	Women with a history of IUGR, PIH or unexplained stillbirth or first time pregnant women who developed abnormal uterine blood flow	Majority from 19–26 week to 38 week gestation	9 MaxEPA fish oil capsules providing in total 1.62 g of EPA & 1.0 8g of DHA (n=113)	9 air-filled capsules (n=119)
6	1997	Australia	Women from middle class families who intended to breastfeed and their healthy term babies	For 12 weeks after delivery	Maternal daily intake of a DHA-rich algal oil in varying doses: 0.2 g of DHA (n=10) 0.4 g of DHA (n=12) 0.9 g of DHA (n=10) 1.3 g of DHA (n=8)	Placebo containing 0 g of DHA (n=12)
7	1999	United States	Women who intended to breastfeed and their babies, characteristics not further specified	For 4 months after delivery	Maternal daily intake of 0.20–0.25 g of DHA as either algal DHA (n=42) **OR** Refined high-DHA fish oil (n=42)	Placebo (n=42)
8	2000	9 European countries	Women with history of pre-term delivery	From ~20 week gestation to delivery	4 fish oil capsules providing in total 2.7 g of LCn3PUFA (1.28 g of EPA & 0.92 g of DHA) (n=110)	4 x 1 g olive oil capsules (n=122)

Trial ID	Year*	Country	Subject characteristics	Duration of supplementation	Intervention – Intake per day (n=number at randomisation)	Control – Intake per day (n=number at randomisation)
9	2000	9 European countries	Women with history of IUGR	From ~20 week gestation to delivery	4 fish oil capsules providing in total 2.7 g of LCn3PUFA (1.28 g of EPA & 0.92 g of DHA) (n=141)	4 x 1 g olive oil capsules (n=139)
10	2000	9 European countries	Women with history of pregnancy-induced hypertension	From ~20 week gestation to delivery	4 fish oil capsules providing in total 2.7 g of LCn3PUFA (1.28 g of EPA & 0.92 g of DHA) (n=184)	4 x 1 g olive oil capsules (n=202)
11	2000	9 European countries	Women pregnant with twins	From ~20 week gestation to delivery	4 fish oil capsules providing in total 2.7 g of LCn3PUFA (1.28 g of EPA & 0.92 g of DHA) (n=289)	4 x 1 g olive oil capsules (n=290)
12	2000	9 European countries	Women with signs of pre-eclampsia in current pregnancy	From ~33 week gestation to delivery	9 fish oil capsules providing in total 6.1 g of LCn3PUFA (2.88 g of EPA & 2.07 g of DHA) (n=44)	9 x 1 g olive oil capsules (n=35)
13	2000	9 European countries	Women with signs of IUGR in current pregnancy	From ~33 week gestation to delivery	9 fish oil capsules providing in total 6.1 g of LCn3PUFA (2.88 g of EPA & 2.07 g of DHA) (n=36)	9 x 1 g olive oil capsules (n=27)
14	2001	Norway	Healthy women who intended to breastfeed &	From 17–19 week gestation to 3 months after delivery	10 ml of cod liver oil providing 2.63 g of LCn3PUFA (0.80 g of EPA, 1.18 g of	10 ml of corn oil providing 4.75 g of n-6 fatty acids (no EPA, DHA or AA)

Trial ID	Year*	Country	Subject characteristics	Duration of supplementation	Intervention – Intake per day (n=number at randomisation)	Control – Intake per day (n=number at randomisation)
			their healthy term babies		DHA & 0.03 g of AA) (n=301)	(n=289)
15	2003	United States	Healthy women	From 24–28 week gestation to delivery	Eggs high in DHA (mean intake of 184 mg DHA per day from eggs) (n=27)	Regular egg (mean intake of 35 mg DHA per day from eggs) (n=25) OR Low egg intake group (no egg provided, mean intake of 11 mg DHA per day from eggs, n=21)
16	2003	United States	Healthy women	From 24–28 week gestation to delivery	Eggs high in DHA (mean intake of 146 mg DHA per day from eggs) (n=176)	Regular eggs (mean intake of 32 mg DHA per day from eggs) (n=174)
17	2003	United Kingdom	Healthy women and their healthy term babies	From 15 week gestation to delivery	2 fish oil capsules providing in total 200 mg DHA (n=50)	2 sunflower oil placebo capsules containing oleic acid (n=50)
18	2003	United States	Healthy women who intended to breastfeed and their healthy babies	For 4 months after delivery	1 high-DHA algal triacylglycerol capsule providing ~200 mg of DHA (n=114 mothers, 115 infants)	1 soy & corn oil capsule (n=113, mothers, 115 infants)
19	2003	Australia	Healthy women & their healthy term babies; All women have a history of allergic rhinitis or asthma	From 20 week gestation to delivery	4 fish oil capsules providing in total 1.11 g of EPA & 2.24 g of DHA (n=52)	4 olive oil capsules (n=46)

Trial ID	Year*	Country	Subject characteristics	Duration of supplementation	Intervention – Intake per day (n=number at randomisation)	Control – Intake per day (n=number at randomisation)
20	2004	Spain	Healthy women presenting for routine examination	From 26–27 week gestation to delivery	Dietary formula containing 2 g of fat providing 40 mg of EPA & 200 mg of DHA (n=10)	Placebo dietary formula (n=10)
21	2004	Denmark	Women with fish intake below the 50th percentile of the Danish National Birth Cohort population who intended to breastfeed and their healthy term babies	For 4 months after delivery	Fish oil muesli bar, cookies or capsules providing in total 1.5 g of LCn3PUFA (0.6 g of EPA & 0.8 g of DHA) (n=62)	Olive oil muesli bar, cookies or capsules (n=60)
22	2005	Germany, Hungary & Spain (NUHEAL)	Healthy women	From 22 week gestation to delivery	Milk-based supplement providing 150 mg of EPA & 500 mg of DHA (n=77) **OR** 400 µg of folic acid (n=77) OR 150 mg of EPA, 500 mg of DHA & 400 µg of folic acid (n=77)	Placebo milk-based supplement (n=80)
23	2006	Denmark	Women from the Danish National Birth Cohort who had low fish intake	From 17–27 week gestation to expected date of delivery	Fish oil providing varying doses of long chain fatty acids per day: 0.1 g EPA+DHA (n=374); 0.3 g EPA+DHA (n=370); 0.7 g EPA+DHA (n=367);	No treatment (n=748)

Trial ID	Year*	Country	Subject characteristics	Duration of supplementation	Intervention – Intake per day (n=number at randomisation)	Control – Intake per day (n=number at randomisation)
					1.4 g EPA+DHA (n=358); 2.8 g EPA+DHA (n=373) **OR** Flax oil providing in total 2.2 g of ALA per day (n=369)	
24	2006	Bangladesh	Women recruited from a house-to-house survey where illiteracy, poverty and poor living environment were common; and their babies	From 25 week gestation to delivery	4 fish oil capsules providing in total 1.8 g of EPA & 1.2 g of DHA (n=200)	4 soy oil capsules providing in total 2.25 g of LA & 0.27 g of ALA (n=200)
25	2007	Germany	Healthy women who intended to breastfeed and their healthy term babies	From 21 week gestation to 3 months after delivery	Supplement with vitamins & minerals, 4.5 g of fructo-oligosaccharide and 200 mg of DHA (n=48)	Supplement with vitamins & minerals only (n=49) **OR** Supplement with vitamins & minerals + 4.5 g of fructo-oligosaccharide (n=47)
26	2007	US	Healthy women and their healthy babies	From 24 week gestation to delivery	DHA-containing cereal-based bar providing on average 214 mg of DHA per day (n=27 at latest count)	Placebo cereal-based bar containing corn oil (n=21 at latest count)

Trial ID	Year*	Country	Subject characteristics	Duration of supplementation	Intervention – Intake per day (n=number at randomisation)	Control – Intake per day (n=number at randomisation)
27	2008	Taiwan	Women diagnosed with major depressive disorder between 16–32 week gestation	For eight weeks after randomisation	5 fish oil capsules providing in total 2.2 g of EPA & 1.2 g of DHA (n=18)	5 olive oil capsules (n=18)
28	2008	United States	Women diagnosed with perinatal major depressive disorder who were either pregnant (12–32 week gestation) or postpartum (within 6 months of childbirth)	For 8 weeks after randomisation	4 capsules providing in total 1.1 g of EPA & 0.8 g of DHA (n=28) Supportive psychotherapy was also provided	4 corn oil capsules with small amount of fish oil added (n=23) Supportive psychotherapy was also provided
29	2008	Australia	Women in their third trimester of pregnancy to 6 months postnatal and diagnosed with major depressive disorder	For 6 weeks after randomisation	6 g of fish oil capsules providing 0.4 g of EPA & 1.6 g of DHA (n=13)	Sunola oil as placebo (n=13)
30	2008	Canada	Healthy pregnant women	From 16 week gestation to delivery	2 capsules providing in total ~400 mg of algal DHA (n=68)	2 capsules of corn-soybean oil blend (n=67)

Trial ID	Year*	Country	Subject characteristics	Duration of supplementation	Intervention – Intake per day (n=number at randomisation)	Control – Intake per day (n=number at randomisation)
31	2009	Netherlands	Healthy pregnant women	From 14–20 week gestation to 3 months after delivery	220 mg of DHA (n=63) **OR** 220 mg each of DHA+AA (n=58)	Soy bean oil as placebo (n=62)
32	2009	Sweden	Women from families with allergy symptoms	From 25 week gestation to 3–4 months after delivery	Fish oil capsules providing 1.6 g EPA+1.1 g DHA (n=70)	Soy oil capsules (2.5 g LA, n-6) (n=75)
33	2010	Australia (DOMInO)	Women with singleton pregnancies at less than 21 week gestation who attended routine antenatal appointments	From study entry to delivery	DHA-rich fish oil concentrate, providing 100 mg of EPA and 800 mg of DHA (n=1197)	Vegetable oil capsules (blend of rapeseed, sunflower and palm oil) (n=1202)
34	2010	Mexico (POSGRAD)	Women recruited during routine prenatal care visits who planned to exclusively or predominantly breast feed for at least 3 months	From 18–22 week gestation to delivery	2 capsules providing in total 400 mg of algal DHA (n=547)	2 placebo capsules containing corn-soy oil blend (n=547)
35	2011	United Kingdom (SiPS)	Women who habitually had low fish intake and had family history of atopy, allergy or asthma	From 20 week gestation to delivery	Incorporate 2 x 150 g salmon portions into the diet per week, resulting in daily median intake (from total diet) of 134 mg of EPA & 269 mg of DHA (n=62)	Maintain usual diet consisting of <2 portions per month of oily fish, resulting in daily median intake (from total diet) of 12 mg of EPA & 20 mg of DHA (n=61)

Trial ID	Year*	Country	Subject characteristics	Duration of supplementation	Intervention – Intake per day (n=number at randomisation)	Control – Intake per day (n=number at randomisation)
36	2012	Germany (INFAT)	Healthy pregnant women	From 15 week gestation to 4 months after delivery	3 fish oil capsules providing in total 180 mg of EPA & 1020 mg of DHA while reducing intake of AA to ~90 mg per day (n=104)	Healthy balanced diet and to refrain from taking fish oil or DHA supplements (n=104)

* Year of first publication of study results included in this review

Appendix 3 Summary of risk of bias assessment for each included study

Legend: '+' = low risk; '-' = high risk; '?' = unclear risk; L = low risk (all '+'); H = high risk (at least one '-'); U = Unclear risk (at least one '?', rest either '?' or '+')

Trial ID	Publication	Random sequence (Selection bias)	Allocation concealment (Selection bias)	Blinding of participants (Performance bias)	Blinding of outcome assessment (Detection bias)	Incomplete outcome data (Attrition bias)	Selective reporting (Reporting bias)	Overall rating
1.	D'Almeida 1992	?	?	-	-	+	+	H
2.	Olsen 1992; Salvig 1996; Olsen 2008	+	+	-	+	+	+	H
3.	Laivuori 1993	+	+	+	?	-	+	H
4.	Bulstra-Ramakers 1994	+	+	+	+	+	+	L
5.	Onwude 1995	+	+	+	+	+	+	L
6.	Gibson 1997	+	?	?	?	+	+	U
7.	Jensen 1999	+	?	+	?	+	+	U
8.	Olsen 2000	+	+	+	+	+	+	L
9.	Olsen 2000	+	+	+	+	+	+	L
10.	Olsen 2000	+	+	+	+	+	+	L
11.	Olsen 2000	+	+	+	+	+	+	L
12.	Olsen 2000	+	+	+	+	+	+	L
13.	Olsen 2000	+	+	+	+	+	+	L

Trial ID	Publication	Random sequence (Selection bias)	Allocation concealment (Selection bias)	Blinding of participants (Performance bias)	Blinding of outcome assessment (Detection bias)	Incomplete outcome data (Attrition bias)	Selective reporting (Reporting bias)	Overall rating
14.	Helland 2001; Helland 2003; Helland 2008	+	+	+	+	-	?	H
15.	Smuts 2003a	+	?	-	?	+	+	H
16.	Smuts 2003b	+	+	-	+	+	+	H
17.	Malcolm 2003a; Malcolm 2004b	+	?	+	+	-	+	H
18.	Llorente 2003; Jensen 2005	+	+	+	+	-	+	H
19.	Dunstan 2003; Dunstan 2004; Barden 2006; Duncan 2008; Mattes 2009	+	+	+	+	+	+	L
20.	Sanjurjo 2004	+	?	+	?	+	+	U
21.	Lauritzen 2004; Lauritzen 2005a; Lauritzen 2005b; Lauritzen 2005c; Larnkjaer 2006; Cheatham 2011	+	+	+ (- long term)	+	+ (- long term)	+	L (H-long term)
22.	Broekaert 20005; Krauss-Etschmann 2007; Krauss-Etschmann2008; Escolano-Margarit 2001; Campoy 2011	+	+	+	?	+ (- long term)	+	U (H-long term)
23.	Knudsen 2006	+	?	-	-	-	+	H
24.	Tofail 2006	?	?	+	+	-	+	H
25.	Bergmann 2007; Bergmann 2008	+	+	+	+	-	+	H

Trial ID	Publication	Random sequence (Selection bias)	Allocation concealment (Selection bias)	Blinding of participants (Performance bias)	Blinding of outcome assessment (Detection bias)	Incomplete outcome data (Attrition bias)	Selective reporting (Reporting bias)	Overall rating
26.	Judge 2007a; Judge 2007b; Courville 2001	-	?	+	+	+	+	H
27.	Su 2008	?	?	+	+	-	+	H
28.	Freeman 2008; Freeman 2010	?	?	+	+	+	-	H
29.	Rees 2008	+	+	+	+	+	+	L
30.	Innis 2008	+	+	+	+	+	+	L
31.	Doornbos 2009; van Goor 2010; van Goor 2011;	?	?	+	+	-	+	H
32.	Furuhjelm 2009	+	+	+	+	+	+	L
33.	Makrides 2010; Smithers 2011; Zhou 2012; Palmer 2012	+	+	+	+	+	+	L
34.	Ramakrishnan 2010a; Ramakrishnan 2010b; Stein 2011, Stein 2012	+	+	+	+	+ (- long term)	+	L (H-long term)
35.	Miles 2011; Noakes 2012	+	?	-	+	+ (- long term)	+	H
36.	Hauner 2012	+	+	-	-	+	+	H

Appendix 4 Foods included in the dietary modelling

Starchy vegetables

Potato, Coliban, peeled, boiled
Potato, Desiree, peeled, baked
Potato, new, peeled, boiled
Potato, pale skin, peeled, mashed with milk & butter
Potato, Pontiac, peeled, baked
Potato, red skin, peeled, mashed with milk & butter
Potato, Sebago, unpeeled, baked
Potato, Sebago, unpeeled, boiled
Salad, potato, commercial
Sweetcorn, fresh on cob, boiled, with salt, drained
Sweetcorn, kernels, canned in brine, drained
Sweetcorn, kernels, purchased frozen, boiled in brine, drained
Sweetcorn, kernels, purchased frozen, boiled, drained

Green and brassica vegetables

Bean, green, fresh, boiled, drained
Bean, green, frozen, boiled, drained
Broccoli, fresh, boiled, drained
Brussels sprout, fresh, boiled, drained
Cabbage, bok choy, stir-fried without oil
Cabbage, savoy, boiled, drained
Cauliflower, boiled, drained
Lettuce, iceberg, raw
Lettuce, mignonette, raw
Pea, green, frozen, boiled, drained
Salad, coleslaw, commercial
Snowpea, boiled, drained

Orange vegetables

Carrot, baby, peeled, boiled, drained
Carrot, baby, peeled, raw
Carrot, mature, peeled, boiled, drained
Pumpkin, butternut, peeled, boiled
Pumpkin, peeled, baked

Legumes

Baked beans, canned in tomato sauce
Baked beans, canned in tomato sauce, salt reduced
Bean, red, kidney, canned, drained
Dhal (legume curry), Indian restaurant-style
Lentil, dried, boiled, drained

Nuts and seeds

Nut, almond, with skin
Nut, cashew, roasted, salted
Nut, peanut, without skin, roasted, with oil, unsalted
Peanut butter, smooth & crunchy, added sugar & salt
Peanut butter, smooth & crunchy, no added sugar or salt

Other vegetables

Avocado, raw
Beetroot, canned, drained
Capsicum, green, raw
Capsicum, green, stir-fried without oil
Capsicum, red, raw
Capsicum, red, stir-fried without oil
Celery, raw
Celery, stir-fried without oil
Cucumber, common, unpeeled, raw
Cucumber, Lebanese, unpeeled, raw
Mushroom, common, raw
Mushroom, common, stir-fried without oil
Mushroom, straw, Asian, canned in brine, drained
Onion, mature, brown skinned, peeled, stir-fried without oil
Onion, mature, white skinned, peeled, raw
Onion, mature, white skinned, peeled, stir-fried without oil
Sprout, alfalfa, raw
Sprout, bean, raw
Squash, button, boiled, drained
Tomato, cherry, raw
Tomato, common, raw
Tomato, hydroponic, raw
Tomato, whole, canned in tomato juice, boiled
Tomato, whole, canned in tomato juice, boiled, drained
Zucchini, green skin, boiled

Fruit

Apple, green skin, unpeeled, raw
Apple, red delicious, unpeeled, raw
Apple, red skin, unpeeled, raw
Apricot, dried
Banana, Cavendish, peeled, raw
Banana, lady finger or sugar, peeled, raw
Grape, black muscatel, raw
Grape, Thompson seedless or sultana, raw
Juice, apple, shelf stable, added vitamin C
Juice, apple, shelf stable, no added vitamin C
Juice, lemon
Juice, orange, added vitamin C
Juice, orange, home squeezed
Juice, orange, no added vitamin C
Kiwifruit, Hayward, peeled, raw
Mandarin (Imperial), peeled, raw
Mango, peeled, raw
Melon, rockmelon (cantaloupe), peeled, raw
Melon, watermelon, peeled, raw
Nectarine, unpeeled, raw
Orange, navel (all varieties), peeled, raw
Orange, navel (Washington), peeled, raw
Orange, Valencia, peeled, raw
Peach, unpeeled, raw

Pear, Packham's Triumph, unpeeled, raw
Pineapple (cayenne), peeled, raw
Plum, unpeeled, raw
Strawberry, raw
Sultana

Wholegrain cereals

Biscuit, savoury, wholemeal wheat flour, crispbread
Bread roll, from wholemeal flour
Bread roll, mixed grain
Bread, from wheat flour, added dried fruit, toasted
Bread, from wholemeal flour
Bread, from wholemeal flour, toasted
Bread, mixed grain
Bread, mixed grain, toasted
Breakfast cereal, mixed grain (wheat, corn, rice & oat), flakes, added dried fruit & nuts, added vitamins B1, B2, B3, C & folate, Ca & Fe
Breakfast cereal, mixed grain flakes (wheat, oats), added dried fruit, added vitamins B1, B2, B3 & folate & Fe
Breakfast cereal, wheat bran, flakes, sultanas, added vitamins B1, B2, B3, B6 & folate, Fe & Zn
Breakfast cereal, wheat bran, pellets, added vitamins B1, B2 & folate, Fe, Mg & Zn
Breakfast cereal, whole wheat, biscuit, added vitamins B1, B2, B3 & folate, Fe & Zn
Breakfast cereal, whole wheat, biscuit, organic, added vitamins B1, B2 & B3
Muesli, untoasted or natural style, unfortified
Oats, rolled, boiled, no added salt
Rice, brown, boiled, no added salt
Wheat bran, unprocessed

Refined cereals

Bread roll, from white flour
Bread, flat (pita or Lebanese), white
Bread, from white flour
Bread, from white flour, added fibre
Bread, from white flour, added fibre, toasted
Bread, from white flour, toasted
Breakfast cereal, flakes of corn, added vitamins B1, B2, B3, C & folate, Fe & Zn
Breakfast cereal, mixed grain (rice & wheat), flakes, sweetened, added vitamins B1, B2, B3, B6 & folate, Ca, Fe & Zn
Breakfast cereal, mixed grain (wheat, oat & corn), extruded shapes, added vitamins B1, B2, B3, B6 & C, Ca & Fe
Breakfast cereal, puffed or popped rice, added vitamins B1, B2, B3, C & folate, Fe & Zn
Crumpet, from white flour, toasted
Muffin, English style, from white flour, toasted
Pasta, vegetable filled, fresh, boiled, without added sauce
Pasta, white wheat flour, boiled from dry, no added salt
Pasta, white wheat flour, boiled from dry, with added salt
Rice, white, boiled, no added salt

Poultry

Chicken, barbecued, with skin
Chicken, breast, lean, baked
Chicken, breast, lean, grilled
Chicken, breast, lean, skin & fat, baked

Chicken, thigh, lean, baked

Oily fish

Salmon, Atlantic, grilled
Salmon, Atlantic, steamed or poached
Salmon, pink, canned in brine, drained
Salmon, red, canned in brine, drained
Sardine, canned in tomato sauce

Non-oily fish

Fish finger, crumbed, purchased frozen, grilled
Fish, crumbed, purchased frozen, baked
Prawn, king (large size), flesh only, purchased cooked
Tuna, canned in water, added salt, drained
Tuna, flavoured, canned in water, added salt, drained

Egg

Egg, chicken, scrambled, no added fat
Egg, chicken, whole, fried, peanut oil
Egg, chicken, whole, hard-boiled
Egg, chicken, whole, poached
Omelette, chicken egg, added butter

Red meat

Beef, casserole cuts, fully-trimmed, cooked
Beef, mince, low fat (lean/heart smart), dry fried (2008)
Beef, mince, regular, dried fried (2006)
Beef, sirloin steak, fully-trimmed, grilled
Lamb, leg roast, fully-trimmed, roasted

Low fat dairy

Milk, cow, fluid, reduced fat (1%)
Milk, cow, fluid, reduced fat (1.5%), added Ca, Mg, Zn & vitamin D
Milk, cow, fluid, reduced fat (1.5%), increased Ca, folate & vitamin D
Milk, cow, fluid, skim (~0.15% fat)
Milk, cow, fluid, skim (~0.15% fat), added milk solids
Yoghurt, low fat (<0.5%), apricot pieces or flavoured
Yoghurt, low fat (<0.5%), fruit pieces or flavoured, intense sweetened
Yoghurt, low fat (<0.5%), strawberry pieces or flavoured

High fat dairy

Cheese, cheddar, processed
Cheese, cheddar, reduced fat (~25%)
Cheese, cheddar, regular fat
Cheese, parmesan, shaved
Cheese, Swiss

Unsaturated fats and oils

Margarine spread, polyunsaturated (70% fat)
Margarine spread, polyunsaturated (70% fat), reduced salt (sodium = 380 mg/100 g)
Margarine spread, polyunsaturated, reduced fat (40% fat) & salt (sodium = 300 mg/100 g)
Margarine spread, polyunsaturated, reduced fat (50% fat) & salt (sodium = 380 mg/100 g)
Margarine spread, polyunsaturated, reduced fat (60% fat), reduced salt
Margarine, polyunsaturated

Margarine, polyunsaturated, reduced salt (sodium = 300 mg/100 g)
Oil, blend of polyunsaturated vegetable oils
Oil, grapeseed
Oil, maize
Oil, soybean
Oil, sunflower

Appendix 5 List of omega-3 rich food or drinks to avoid or limit to small amounts during the trial period

Fish or seafood*

[If you wish, you may choose from the following fish or seafood and consume no more than one serving of these low omega-3 fish or seafood (<400mg per 100 g) once a week: some tuna (check label), snapper, barramundi aquacultured, blue grenadier (or hoki), flathead, trevally, dory, ling, cod, flounder, whiting, basa, squid or calamari, prawn, lobster, crab]

Fats or oils

Canola oil or rapeseed oil, flaxseed or linseed oil, blended polyunsaturated vegetable oil, walnut oil, soybean oil, cod liver oil

Nuts and seeds

Walnut, linseed or flaxseed, LSA mixture (linseed, sunflower and almond mixture), lecithin soy granules, wheat germ

Breads and cereals

Breads with soy and/or linseed added

Foods fortified with omega-3 fatty acids

It is increasingly common to have foods fortified with omega-3, for example, breads, breakfast cereals, milk, eggs, juice, yoghurt etc. Please check product labels carefully.

Other

Lambs brain, lamb liver

* If you are in the higher fish group, other than the fish-study food provided, you may similarly have an additional one serve of low omega-3 fish or seafood once a week if you wish.

Appendix 6 Diet acceptability questionnaire

Study ID: _____ Date: ____/ ____/ _____

Please answer the following questions relating to the foods that you have been eating in the past 2 weeks:

1. How well do you like the foods that you have been eating in the past 2 weeks?

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

1 = "extremely good" 7 = "extremely unappealing"

2. How easy or difficult has it been for you to prepare the foods you have been eating during the past 2 weeks?

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

1 = " extremely easy" 7 = "extremely difficult"

3. How much effort does it take for you to stay on this diet?

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

1 = "more than is possible" 7 = "no effort at all"

4. If, in the future, you were to continue to eat the kinds of meals you have been having during the past 2 weeks, how easy or difficult would it be for you to purchase, prepare, and eat these foods?

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

1= "extremely easy" 7 = "extremely difficult"

5. How would you rate the acceptability of the diet?

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

1 = "completely unacceptable" 7 = "extremely acceptable"

Please describe any benefits or problems you experienced while on this diet.

_____

_____

Appendix 7 Center for Epidemiologic Studies Depression Scale (CES-D)

Study ID. _____ Date:_____ /_____/ _____

The Center for Epidemiologic Studies Depression Scale (CES-D) was developed as a tool for epidemiologic studies of depression in the general population. It was not designed as a diagnostic tool for depression for individuals. The 20 items below refer to how you have felt and behaved during the last week. Choose the appropriate answer.

1. I was bothered by things that don't usually bother me.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

2. I did not feel like eating; my appetite was poor.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

3. I felt that I could not shake off the blues even with the help of my family or friends.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

4. I felt that I was just as good as other people.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

5. I had trouble keeping my mind on what I was doing.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

6. I felt depressed.

- Rarely or none of the time (<1 day)
- Some or a little of the time (1–2 days)
- Occasionally or a moderate amount of the time (3–4 days)
- Most or all of the time (5–7 days)

7. I felt everything I did was an effort.

- Rarely or none of the time (<1 day)
- Some or a little of the time (1–2 days)
- Occasionally or a moderate amount of the time (3–4 days)
- Most or all of the time (5–7 days)

8. I felt hopeful about the future.

- Rarely or none of the time (<1 day)
- Some or a little of the time (1–2 days)
- Occasionally or a moderate amount of the time (3–4 days)
- Most or all of the time (5–7 days)

9. I thought my life had been a failure.

- Rarely or none of the time (<1 day)
- Some or a little of the time (1–2 days)
- Occasionally or a moderate amount of the time (3–4 days)
- Most or all of the time (5–7 days)

10. I felt fearful.

- Rarely or none of the time (<1 day)
- Some or a little of the time (1–2 days)
- Occasionally or a moderate amount of the time (3–4 days)
- Most or all of the time (5–7 days)

11. My sleep was restless.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

12. I was happy.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

13. I talked less than usual.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

14. I felt lonely.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

15. People were unfriendly.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

16. I enjoyed life.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

17. I had crying spells.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

18. I felt sad.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

19. I felt that people disliked me.

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

20. I could not get "going".

- ☐ Rarely or none of the time (<1 day)
- ☐ Some or a little of the time (1–2 days)
- ☐ Occasionally or a moderate amount of the time (3–4 days)
- ☐ Most or all of the time (5–7 days)

Thank you for completing this questionnaire.