The Australian Seafood Diet for Intergenerational Health: Development of a healthy high Australian seafood diet that will be acceptable to women of child-bearing age

Lynne Cobiac
Jocelyn Midgley
Michelle Miller
Campbell Thompson
Lily Chan

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Nutrition and Dietetics
GPO Box 2100
Adelaide SA 5001
AUSTRALIA

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Office Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042
Postal Box 26, Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042
Tollfree 1300 732 213 Phone 08 8201 7650 Facsimile 08 8201 7659
Website www.seafoodcrc.com ABN 51 126 074 048

Australian Government
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Table of Contents

Non-Technical Summary **6**

1. Introduction and Background **9**

1.1 Need 10

1.2 Objectives 11

2. Method Overview **12**

3. Phase 1: Literature and database reviews **13**

3.1 Long chain n-3 polyunsaturated fatty acids (LCn3PUFA) intakes and maternal and infant outcomes from randomised controlled trials (RCTs) 13

3.1.1 Length of gestation, pre-term delivery rate and post-term delivery rate 14

3.1.2 Incidence of pregnancy-induced hypertension, pre-eclampsia and eclampsia 28

3.1.3 Perinatal Depression 34

3.1.4 Foetal development (Anthropometry at birth) 40

3.1.5 Growth pattern 48

3.1.6 Neurological (cognitive) development 54

3.1.7 Visual function 63

3.1.8 Atopic disease 71

3.2 Fish intakes and maternal & infant outcomes 79

4. PHASE 2: Dietary Modelling – Comparing nutrient profiles of diets of differing fish and seafood contents 81

4.1 Background 81

4.2 Method 82

4.3 Statistical analysis 89

4.4 Results and Discussion 89

5. PHASE 3: Analytical testing of selected fish and fish products **101**

5.1 Background 101

5.2 Purpose of analysis 102

5.3 Process 103

5.3.1 Fish and fish products included for analyses 103

5.3.2 Selection of suitable laboratory to conduct the analyses 103

5.3.3 Analytes included and method of analyses 104
5.3.4 Preparation of samples 110
5.3.5 Quality assurance and quality control 112
5.4 Results and Discussions 112
5.4.1 Proximates 113
5.4.2 Long chain n-3 polyunsaturated fatty acids (LCn3PUFA) 114
5.4.3 Mercury and other metals 114
5.4.4 Vitamins A, D and E 115
6. PHASE 4: Acceptability and effects of a higher fish diet – a randomised controlled trial 136
6.1 Introduction 136
6.2 Study protocol 136
6.2.1 Subjects 136
6.2.2 Study design 137
6.3 Methods of assessment 142
6.3.1 Fatty acids analysis 142
6.3.2 Lipids study 142
6.3.3 Iron study and Haemoglobin 144
6.3.4 Mercury analysis 145
6.3.5 Dietary assessments 145
6.3.6 Anthropometric and other assessments 146
6.4 Statistical analyses 148
6.5 Results 148
6.5.1 Fatty acids contents 151
6.5.2 Lipids study 154
6.5.3 Iron status and haemoglobin level 155
6.5.4 Mercury 156
6.5.5 Dietary assessment 158
6.5.6 Anthropometric and other assessments 160
6.6 Discussions 163
6.7 Conclusions 166
7. PHASE 4: Cost effective analysis of a higher fish diet 167
7.1 Introduction 167

7.2 Methods 167
7.3 Results 171
7.4 Discussion 172
8. Benefits and Adoption 175
9. Further Development 175
10. Planned Outcomes 176
10.1 Public Benefit Outcomes 176
10.2 Private Benefit Outcomes 176
10.3 Linkages with CRC Milestone Outcomes 176
11. Conclusion 177
12. References 178
13. Appendices 188
Appendix 1: Intellectual Property 188
Appendix 2: Staff 189
Appendix 3: Search Strategy for the literature review 190
Appendix 4: Description of studies included in the literature review 192
Appendix 5: Foods included in the dietary modelling 201
Appendix 6: Effects of cooking on nutrients and contaminants 207
Appendix 7: List of omega-3 rich food or drinks to avoid or limit to small amounts during the trial period 209
Appendix 8: Diet acceptability questionnaire 210
Appendix 9: Center for Epidemiologic Studies Depression Scale (CES-D) 211

Non-Technical Summary

PROJECT NUMBER: 2008/731
The Australian Seafood Diet for Intergenerational Health: Development of a healthy high Australian seafood diet that will be acceptable to women of child-bearing age

PRINCIPAL INVESTIGATOR: Lynne Cobiac

ADDRESS: Flinders University
Nutrition and Dietetics
GPO Box 2100
Adelaide SA 5001
AUSTRALIA

PROJECT OBJECTIVES:

1. To provide an up-to-date review of the benefits of fish and long chain n-3 polyunsaturated fatty acids (LCn3PUFA) intakes in relation to maternal and infant health.
2. To develop a healthy Australian seafood dietary pattern to achieve sufficient intakes of LCn3PUFA.
3. To add to the existing database of compositional profile of fish products.
4. To explore the effects of cooking methods on the contents of fish and seafood.
5. To assess the acceptability and effects of a diet that is higher in fish.

OUTCOMES ACHIEVED

1. A literature review was conducted to establish the benefits of fish and long chain n-3 polyunsaturated fatty acids (LCn3PUFA) intakes in relation to maternal and infant health.
2. Diet modelling was conducted and a high DHA diet using Australian fish was defined.
3. Fish products used in the human intervention trial and supplied by Simplot Australia were analysed by a consortium of laboratories led by AsureQuality Limited thus provided additional information to the existing fish compositional database.
4. A literature review was conducted to provide an overview of the effects of cooking methods on the contents of fish and seafood.
5. An 8-week randomised controlled trial (Women's Dietary Study) was conducted between September 2010 and May 2011. A cost-effectiveness analysis was undertaken post trial.

LIST OF OUTPUTS PRODUCED

1. Literature review on benefits of fish and LCn3PUFA intakes in relation to maternal and infant health.
2. Report which contains analytical test results of 13 fish and fish products for key nutrients (proximates, minerals, fatty acids, vitamins) and contaminants (mercury and methyl mercury).
3. Literature review on effects of cooking methods on contents of fish and seafood.
4. Results obtained from the human intervention trial. Some results were presented at the Thirty-Fifth Joint Annual Scientific Meeting of the Nutrition Society of New Zealand and the Nutrition Society of Australia held in Queenstown, New Zealand, 29 November – 2 December 2011. Conference abstract published in the Australasian Medical Journal (AMJ 2011, 4,12, 789-813).
5. Cost-effectiveness analysis of the intervention trial.

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1. Introduction and Background

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 methylmercury 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. 2008) 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 for 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 1g 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 consumption of fish to pregnant women to achieve higher LCn3PUFA intakes relates to the levels of methylmercury 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 other contaminants contained in fish. However, fish also provides 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 (eg cardiovascular) beyond that of the n-3 fatty acids (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 n-3 fatty acids.

In the US, the general guidelines for pregnancy are to consume 340g (or 2 serves) of fish per week. In Australia, the serving size of fish is 100g cooked (or 115g raw) fish fillet according to the Australian Dietary Guidelines released in 2013. The

recommended amount in the US of 340g/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.

The net outcome of this communiqué may result in fewer women of childbearing age, those who are pregnant or those who wish to become pregnant from consuming fish or consuming inadequate amounts. It is highly important that this key group of consumers receive 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 200mg 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 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.

1.1 Need

This proposal therefore targets a need in women of child bearing age

1. In the understanding of the benefits of fish consumption in the diet
2. To determine if a diet high in DHA (providing an average of at least 200mg/d) obtained from Australian seafood (2-4 times a week) is acceptable and
3. If such diet can improve health-related outcomes (erythrocyte n-3 concentrations, lipids, blood pressure, weight and body composition, mood) when compared to a low DHA diet (~30 mg/d) without providing adverse levels of methyl mercury

1.2 Objectives

1. To provide an up-to-date review of the benefits of fish and long chain n-3 polyunsaturated fatty acids (LCn3PUFA) intakes in relation to maternal and infant health.
2. To develop a healthy Australian seafood dietary pattern to achieve sufficient intakes of LCn3PUFA.
3. To add to the existing database of compositional profile of fish products.
4. To explore the effects of cooking methods on the contents of fish and seafood.
5. To assess the acceptability and effects of a diet that is higher in fish.

2. Method Overview

There were four phases to the design methodology.

PHASE 1.

Undertook a literature/data-base review 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.

PHASE 2.

Conducted computer modelling approach to determine what a theoretical “higher fish diet” would look like that achieved desirable levels of n-3 fatty acids.

PHASE 3

Analysed several commercially available and commonly consumed fish and fish products to ascertain their nutrient profiles as well as their mercury and methylmercury contents. These products were incorporated in the “higher fish diet” in the human intervention study.

PHASE 4

Conducted a human intervention study to investigate what levels of n-3 fatty acids are achieved and what levels of intakes of methylmercury are in women volunteers of child-bearing age who consume the “higher fish diet”.

3. Phase 1: Literature and database reviews

3.1 Long chain n-3 polyunsaturated fatty acids (LCn3PUFA) intakes and maternal and infant outcomes from randomised controlled trials (RCTs)

Introduction

It is well known that maternal nutrition impacts on the foetus and the subsequent growth and development of the child. Several Cochrane Systematic Reviews have highlighted the importance of macro- and micronutrients intake during pregnancy as well as the pre-conception period (Haider & Bhutta 2006; Kramer & Kakuma 2003; Lumley et al. 2001). The LCn3PUFA are essential fatty acids that have received a great deal of attention ever 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, it was decided that our search would commence from January 2003 assuming previous work would have been covered in this comprehensive Lewin et al. (2005) review. The electronic database Medline® was searched from January 2003 to March 2013 using search terms such as omega-3, n-3 fatty acids, eicosapentaenoic acid (EPA),

docosahexaenoic acid (DHA), maternal, pregnancy, breast-feeding. The detailed search strategy can be found in Appendix 3. This review will focus on randomised controlled trials (RCTs) and other systematic reviews identified from this search as data generated from these study designs provide more reliable estimates of effects.

Results

Thirty-six RCTs were identified that had 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. There were several RCTs that used LCn3PUFA containing functional foods (e.g. DHA enriched eggs or cereal bars) but only one RCT used oily fish as intervention. Intervention doses varied from as low as 100mg of EPA+DHA through to 4.95g of EPA+DHA combined. Brief description of these RCTs can be found in the tables below as well as in Appendix 4. Since the publication of the comprehensive systematic review by Lewin et al. in 2005, 2 Cochrane Systematic Reviews (Delgado-Noguera et al. 2010; Makrides et al. 2006) and 17 other systematic reviews (Campoy et al. 2012; Dziechciarz et al. 2010; Eilander et al. 2007; Gould et al. 2013; Horvath et al. 2007; Imhoff-Kunsch et al. 2012; Jans et al. 2010; Klemens et al. 2011; Kremmyda et al. 2011; Larqué et al. 2012; Lo et al. 2012; Muhlhausler et al. 2010; Rodríguez et al. 2012; Salvig & Lamont 2011; Szajewska et al. 2006; Wojcicki & Heyman 2011), some including meta-analysis, have been conducted in an attempt to consolidate the available evidence relating to LCn3PUFA supplementation. Findings from individual trials, systematic reviews or meta-analyses are summarised below.

3.1.1 Length of gestation, pre-term delivery rate and post-term delivery rate

Results from individual trials

Length of gestation

Twenty-five RCTs have reported the length of gestation as either a primary or secondary outcome. Eight of these trials demonstrated a significant increase in the duration of pregnancy in the supplemented group when compared with the control

group while the remaining 17 studies 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 1.3g of EPA and 0.9g 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 have reported on the incidence of pre-term birth (<37 weeks gestation) (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 (OR, 0.54; 95% CI, 0.30 to 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 were 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 BMI and the number of prior pregnancies (Smuts et al. 2003b). In another much larger trial (n=2399), the DOMInO study by Makrides et al. (2010), supplementation of 800mg of DHA & 100mg of EPA per day did not influence the occurrence of 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 to 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 to 0.94; p=.03). No statistically significant differences in the rate of pre-term births were seen the remaining 12 RCTs.

Post-term delivery rates

Few RCTs report 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 rate when the results of the 6 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 to 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 to 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 to 4.07 days). This was based on a meta-analysis of 3 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 to 3.80 days; 1393 women of low/moderate risk; WMD, 8.50 days; 95% CI, 2.05 to 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 significantly longer duration of pregnancy (WMD 1.57 days; 95% CI, 0.35 to 2.78 days). This meta-analysis included 6 RCTs (Helland et al. 2001; Malcolm et al. 2003b; Olsen et al. 1992; Sanjurjo et al. 2004; Smuts et al. 2003a; Smuts et al. 2003b) totalling 1278 women. The same authors further explored the effect LCn3PUFA supplementation in women with high-risk pregnancies (Horvath et al.

2007) and found no evidence that supplementation influenced the duration of pregnancy.

Salvig & Lamont published a systematic review in 2011 and has 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) suggests gestational age is 4.51 days (95% Cl, 2.26 to 6.76) longer with supplementation.

Conversely, Imhoff-Kunsch et al. (2012) found no such effect in their meta-analysis of 8 RCTs (Bergmann et al. 2008; Dunstan et al. 2004; Judge et al. 2007b; Olsen et al. 1992; Olsen et al. 2000; Ramakrishnan et al. 2010a; Sanjurjo et al. 2004; Smuts et al. 2003b) totalling 2802 women (WMD, 0.87 day; 95% CI, -0.11 to 1.84 days).

The systematic review by Larqué et al. (2012) examined 9 RCTs (Bergmann et al. 2008; Helland et al. 2001; Innis & Friesen 2008; Knudsen et al. 2006; Krauss-Etschmann et al. 2007; Makrides et al. 2010; Olsen et al. 1992; Ramakrishnan et al. 2010a; Smuts et al. 2003b) 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 preterm 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 8 RCTs involving 1574 women was performed comparing intake of EPA and DHA vs. control for the incidence of pre-term delivery. No significant difference was found (Odds ratio [OR] 0.88; 95% CI, 0.62 to 1.25) compared to the control groups. Meta-analysis of two other trials involving a total of 328 women comparing intake of DHA vs. control also demonstrated no significant difference between supplemented and non-supplemented groups (OR, 0.53; 95% CI, 0.13 to 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 to 1.07; 5 RCTs, 1916 women) and when women were classified into

low/moderate risk (RR, 0.95 with supplementation; 95% CI, 0.80 to 1.13; 3 RCTs, 1393 women) and high risk pregnancy groups (RR, 0.82 with supplementation; 95% CI, 0.60 to 1.12; 3 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 to 0.99, p=.044; 2 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 to 3.66; p=.19; 2 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 3 RCTs, totalling 861 women (RR, 0.67 with supplementation; 95% CI, 0.41 to 1.10). Similar to the results of Makrides et al. (2010) Horvarth et al. (2007) found no significant difference in pre-term delivery in the group of women with high-risk pregnancies from 3 RCTs (RR, 0.82 with supplementation; 95% CI, 0.6 to 1.12, 523 women) but did show a reduction in early pre-term delivery (RR, 0.39 with supplementation; 95% CI, 0.18 to 0.84; 2 RCTs, 291 women).

A meta-analysis of 3 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, 0.4 to 0.93) and early pre-term birth (RR, 0.32; 95% Cl, 0.09 to 0.95).

The result of the more recent systematic review by Imhoff-Kunsch et al. (2012) is also in agreeance with that of Makrides et al.(2010) and Horvarth et al. (2007). The meta-analysis from 9 RCTs involving 6505 women showed that the difference in the risk of pre-term birth was non-significant between LCn3PUFA supplemented and non-supplemented group (RR, 0.09; 95% CI, 0.82 to 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 5 RCTs involving 4343 women (RR, 0.74; 95% CI, 0.58 to 0.94). One of the trials (Harper et al. 2010) included in both of these 2 meta-analyses 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 2 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 to 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 to 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	1.28g of EPA & 0.92g 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 3 groups	↔ between the 3 groups
4	Bulstra-Ramakers et al. 1994/ Netherlands	3g 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	1.62g of EPA & 1.08g 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)	1.28g of EPA & 0.92g of DHA (n=108); from ~20 week gestation to delivery	Olive oil capsules (n=120)	↑*	↓*	↑* when all six trials (Trial ID 8 to 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)
9	Olsen et al. 2000/ 9 European countries (Earl-IUGR)	1.28g of EPA & 0.92g of DHA (n=131); from ~20 week gestation to delivery	Olive oil capsules (n=132)	↑*	↔	↑* when all six trials (Trial ID 8 to 13) were combined
10	Olsen et al. 2000/ 9 European countries (Earl-PIH)	1.28g of EPA & 0.92g of DHA (n=167); from ~20 week gestation to delivery	Olive oil capsules (n=183)	↔	↔	↑* when all six trials (Trial ID 8 to 13) were combined
11	Olsen et al. 2000/ 9 European countries (Twins)	1.28g of EPA & 0.92g of DHA (n=286); from ~20 week gestation to delivery	Olive oil capsules (n=283)	↔	↔	↑* when all six trials (Trial ID 8 to 13) were combined
12	Olsen 2000 et al./ 9 European countries (Threat-PE)	2.88g of EPA & 2.07g of DHA (n=42) ;from ~33 week gestation to delivery	Olive oil capsules (n=34)	↔	↔	↑* when all six trials (Trial ID 8 to 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)
13	Olsen 2000/ 9 European countries (Susp-IUGR)	2.88g of EPA & 2.07g of DHA (n=36); from ~33 week gestation to delivery	Olive oil capsules (n=27)	↑*	↔	↑* when all six trials (Trial ID 8 to 13) were combined
14	Helland et al. 2001/ Norway	0.80g of EPA, 1.18g of DHA & 0.03g 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 184mg DHA (n=18); from 24-28 week gestation to delivery	Regular eggs providing 35mg DHA (n=19) **OR** Low egg intake, 11mg DHA from egg (n=16)	↑ (p=NR)	↓ (p=NR)	NR
16	Smuts et al. 2003b/ United States	High-DHA eggs providing 146mg DHA (n=142); from 24-28 week gestation to delivery	Regular eggs providing 32mg DHA (n=149)	↑**	↔	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)
17	Malcolm et al. 2003a/ United Kingdom	200mg DHA (n=31); from 15 week gestation to delivery	Sunflower oil placebo capsules (n=29)	↔	NR	NR
19	Dunstan et al. 2004/ Australia	1.11g of EPA & 2.24g of DHA (n=40); from 20 week gestation to delivery	Olive oil capsules (n=43)	↔	NR	NR
20	Sanjurjo et al. 2004/ Spain	40mg EPA & 200mg 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)	150mg of EPA & 500mg of DHA (n=69) OR 400ug of folic acid (n=65) OR 150mg of EPA, 500mg of DHA & 400ug 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	0.1g EPA+DHA (n=374) OR 0.3g EPA+DHA (n=370) OR 0.7g EPA+DHA (n=367) OR 1.4g EPA+DHA (n=358) OR 2.8g EPA+DHA (n=373) OR 2.2g 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	1.8g of EPA & 1.2g of DHA (n=125); from 25 week gestation to delivery	Soy oil capsules (n=124)	↔	↔	NR
25	Bergmann et al. 2008/ Germany	Basic supplement + 4.5g of fructo-oligosaccharide + 200mg of DHA (n=43); from 21 week gestation to 3 months after delivery	Basis supplement (n=37) **OR** Basis supplement + 4.5g of fructo-oligosaccharide (n=36)	↔	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)
26	Judge et al. 2012/ United States	214mg of DHA (n=22); from 24 week gestation to delivery	Placebo cereal bars with corn oil (n=25)	↔	NR	NR
31	van Goor et al. 2010/ Netherlands	220mg of DHA (n=42) **OR** 220mg of DHA & 220mg 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	1.6g of EPA & 1.1g 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	100mg of EPA & 800mg of DHA (n=1197); from ~22 week gestation to delivery	Vegetable oil capsules (n=1202)	↑ (p=.05)	↓* (in the number of very preterm birth, i.e. <34 weeks' gestation)	↑*

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)
34	Ramakrishnan et al. 2010/ Mexico	400mg 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 150g salmon portions per week resulting in median daily intake (from total diet) of 134mg of EPA and 269mg 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 12mg of EPA & 20mg of DHA (n=54)	↔	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)
36	Hauner et al. 2012/ Germany (INFAT)	180mg of EPA & 1020mg 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<0.05; ** = p<0.01; *** = p<0.005; NR = not reported; ↑ = increase/higher; ↓ = decrease/lower; ↔ = no significant difference; cf. = compared with

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

Results from individual trials

Eleven RCTs were identified that had reported the effect of LCn3PUFA supplementation on pregnancy-induced hypertension (PIH), pre-eclampsia and/or eclampsia (Table 2.2). Of the 9 studies that have blood pressure as outcome, none demonstrated a statistically significant effect on the increase of blood pressure with pregnancy or the incidence of PIH with LCn3PUFA supplementation. In one of these studies, D'Almeida et al. (1992) showed that the group supplemented with 1g of magnesium had the lowest number of cases of pregnancy-induced hypertension (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 9 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 a control group using olive oil (p=.0005). No significant effect was demonstrated in the remaining 8 RCTs. Eclampsia, a life threatening condition characterized by the appearance of seizures usually in a patient who has developed pre-eclampsia, was reported in 2 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 5 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 were low, most studies were not powered to detect a meaningful clinical difference in these outcomes. However, even in the large supplementation trial such as Makrides et al. (2010), no beneficial effect was found with LCn3PUFA supplementation.

Results from systematic reviews and meta-analysis

Lewin et al (2005) reviewed 8 RCTs published between 1992 and 2003 (totalling 2335 pregnant women) on the effect of LCn3PUFA supplementation to the incidence of PIH, and/or preeclampsia and/or eclampsia (Bulstra-Ramakers et al. 1994; D'Almeida et al. 1992; Laivuori et al. 1993; Olsen et al. 2000; Onwude et al. 1995; Salvig et al. 1996; Smuts et al. 2003b) (Table 2.2). Meta-analysis by Lewin of two (Olsen et al. 2000; Onwude et al. 1995) 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 to 1.51).

Makrides et al. (2006) reported no significant difference in the incidence of PIH from 5 RCTs (Bulstra-Ramakers et al. 1994; D'Almeida et al. 1992; Olsen et al. 2000; Onwude et al. 1995; Salvig et al. 1996) (RR, 1.09 with supplementation; 95% CI, 0.90 to 1.33; 1831 women), preeclampsia from 4 RCTs (D'Almeida et al. 1992; Olsen et al. 2000; Onwude et al. 1995; Salvig et al. 1996) (RR, 0.86 with supplementation; 95% CI, 0.59 to 1.27; 1683 women) or eclampsia from 1 RCT (D'Almeida et al. 1992) (RR, 0.14 with supplementation; 95% CI, 0.01 to 2.70; 100 women) following marine fatty acids supplementation when compared to control. No significant difference was seen in the incidence of preeclampsia when women were classified into low/moderate (RR, 1.01 with supplementation; 95% CI, 0.52 to 1.98, 3 RCTs, 1130 women) or high risk pregnancy group (RR, 0.80 with supplementation; 95% CI, 0.50 to 1.29; 2 RCTs, 553 women).

Szajewska et al. (2006) found no significant difference in the rate of preeclampsia or eclampsia between supplemented and non-supplemented women with low pregnancy risk from 2 RCTs (Smuts et al. 2003a; Smuts et al. 2003b) (RR, 0.73; 95% CI, 0.22 to 2.37; 328 women). No significant difference was observed in the incidence of PIH from 3 RCTs (Bulstra-Ramakers et al. 1994; Olsen et al. 2000; Onwude et al. 1995) (RR, 1.06; 95%CI, 0.87 to 1.29; 645 women) or preeclampsia from 1 RCT (Olsen et al. 2000) (RR, 0.72; 95% CI, 0.35 to 1.49; p=.37; 321 women) between supplemented and non-supplemented women with high pregnancy risk either (Horvath et al. 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, preeclampsia, 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	0.30g of GLA, 0.14g of EPA & 0.08g of DHA (n=50) **OR** 1.0g 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	1.28g of EPA & 0.92g 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	1.80g of EPA & 1.20g of DHA (n=3) **OR** 3.75g of LA & 0.45g 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

Trial ID	Reference/ Location	Intervention dosage (n=number in analysis)	Control (n=number in analysis)	Outcomes		
				Pregnancy-Induced Hypertension (cases/total)	Pre-eclampsia	Eclampsia
4	Bulstra-Ramakers et al. 1994/ Netherlands	3g 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
5	Onwude et al. 1995/ United Kingdom	1.62g of EPA & 1.08g 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)	1.28g of EPA & 0.92g 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)	1.28g of EPA & 0.92g 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 184mg DHA (n=18); from 24-28 week gestation to delivery	Regular eggs providing 35mg DHA (n=19) **OR** Low egg intake, 11mg DHA from egg (n=16)	NR	High-DHA eggs (1/18) Regular eggs group (0/19) Low egg (0/16)	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
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 32mg 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) ↔
19	Barden et al. 2006/ Australia	1.11g of EPA & 2.24g 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	100mg of EPA + 800mg 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

* = $p<0.05$; ** = $p<0.01$; *** = $p<0.005$; NR = not reported; ↑ = increase/higher; ↓ = decrease/lower; ↔ = no significant difference; cf. = compared with

3.1.3 Perinatal Depression

Results from individual trials

Three RCTs were identified conducted in women with depressive disorder during the perinatal period (varying from 12 week gestation up to 6 months postpartum) and had used doses ranging from 2.0g to 3.4g per day of LCn3PUFA (EPA+DHA) with intervention lasting between 6 to 8 weeks (Freeman et al. 2008; Rees et al. 2008; Su et al. 2008) (Table 2.3). Whilst all 3 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 demonstrated significant difference between intervention and control group (Su et al. 2008).

Five other RCTs conducted in healthy pregnant women had also assessed mood status following LCn3PUFA supplementation (Doornbos et al. 2009; Krauss-Etschmann et al. 2007; Llorente et al. 2003; Makrides et al. 2010; Mattes et al. 2009) (Table 2.3) and involved a total of 2958 women. Dosage used varied from 200mg of DHA to 3.35g EPA and DHA combined. Three studies commenced supplementation from around 20-22 weeks gestation and ceased at delivery (Krauss-Etschmann et al. 2007; Makrides et al. 2010; Mattes et al. 2009). One study supplemented for 4 months after delivery (Llorente et al. 2003) while another study commenced at 14-20 week gestation and continued for 3 months after delivery (Doornbos et al. 2009). No significant difference was 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 7 studies (Doornbos et al. 2009; Freeman et al. 2008; Krauss-Etschmann et al. 2007; Llorente et al. 2003; Mattes et al. 2009; Rees et al. 2008; Su et al. 2008) 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 to 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 to 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 (3 prospective longitudinal cohorts, 5 RCTs, 2 pilot trials). Six of these studies found no association between LCn3PUFA and perinatal depression (Browne et al. 2006; Doornbos et al. 2009; Freeman et al. 2008; Llorente et al. 2003; Marangell et al. 2004; Rees et al. 2008), 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 2g per day of EPA+DHA in combination with n-6 fatty acids.

Larqué et al (2012) examined the evidence from seven studies (Doornbos et al. 2009; Freeman et al. 2008; Freeman & Davis 2010; Freeman et al. 2006; Makrides et al. 2010; Rees et al. 2008; Su et al. 2008) and one meta-analysis (Jans et al. 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	~200mg 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	1.11g of EPA & 2.24g 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	150mg of EPA + 500mg of DHA **OR** 400ug of folic acid **OR** 150mg of EPA + 500mg of DHA + 400ug 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	2.2g of EPA & 1.2g 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 HRS 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

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes (compared with control)
28	Freeman et al. 2008/ US	1.1.g of EPA & 0.8g 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	0.4g of EPA & 1.6g 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
31	Doornbos et al. 2009/ Netherlands	220mg DHA **OR** 220mg 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	100mg of EPA + 800mg 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

* = $p<0.05$; ** = $p<0.01$; *** = $p<0.005$; 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

3.1.4 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 of the 19 RCTs examined (Bergmann et al. 2007; Dunstan et al. 2004; Helland et al. 2001; Innis & Friesen 2008; Judge et al. 2007b; Krauss-Etschmann et al. 2007; Malcolm et al. 2003a; Olsen et al. 1992; Olsen et al. 2000; Onwude et al. 1995; Sanjurjo et al. 2004; Smuts et al. 2003b; Tofail et al. 2006). 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 demonstrated increases in birth weight (Makrides et al. 2010; Olsen et al. 2000; Ramakrishnan et al. 2010a; Smuts et al. 2003a) although the effect was only evident in 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, majority of the RCTs reported non-significant differences in birth length (10 out of 13 RCTs) and head circumference (9 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 (Ramakrishnan et al. 2010a; Smuts et al. 2003a). The study by Smuts et al. (2003a) did not report the p-value while the effect of increase head circumference with supplementation was observed in primigravid women in the study by Ramakrishnan et al. (2010).

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; Makrides et al. 2010; Smuts et al. 2003a)

Only six studies out of the 21 RCTs have reported the incidence of IUGR. The majority of studies (5 out of 6 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 weights as outcome variables. Two trials (Olsen et al. 2000; Smuts et al. 2003a) demonstrated a significant increase in birth weights in n-3 fatty acids 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 weights was observed in the remaining eight trials (Dunstan et al. 2004; Helland et al. 2001; Malcolm et al. 2003a; Olsen et al. 1992; Olsen et al. 2000; Onwude et al. 1995; Smuts et al. 2003b). Data from two of these eleven trials were used to conduct a meta-analysis and no significant different was observed in mean birth weight between supplemented and non-supplemented groups (WMD, -61.51g; 95% CI, -256.21 to 133.18g; p=.54). Recurrence of IUGR did not differ in the 3 trials that had investigated the effect of n-3 fatty acids in women with history of IUGR in previous pregnancies (Table 2.4). A meta-analysis of these three trials (Bulstra-Ramakers et al. 1994; Olsen et al. 2000; Onwude et al. 1995) demonstrated no significant different in the incidence of IUGR (OR, 1.14; 95% CI, 0.79 to 1.64; p=.48). Incidence of low birth weight did not differ in 5 trials (Bulstra-Ramakers et al. 1994; 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 4 trials (Dunstan et al. 2004; Helland et al. 2001; Malcolm et al. 2003a; Olsen et al. 1992), was higher in 2 trials (Smuts et

al. 2003a; Smuts et al. 2003b) although no p-value was report in one (Smuts et al. 2003a). An increase in head circumference was observed only in one trial with small number (n=37) with no p-value (Smuts et al. 2003a); no significant difference was observed in the other 4 trials that had reported head circumference at birth (Dunstan et al. 2004; Helland et al. 2001; Malcolm et al. 2003a; Smuts et al. 2003b).

Makrides et al. (2006) reported in a meta-analysis a significant increase in birth weight (WMD, 47.24g; 95% CI, 1.05 to 93.44; p=.045; 3 RCTs, 2440 infants) and birth length (WMD, 0.48cm; 95% CI, 0.13 to 0.83; p=.008; 2 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, 55.79g; 95% CI, 4.83 to 106.74g; p=.032; 3 RCTs, 1946 infants) but not in high-risk pregnancy (WMD, 12.11g; 95% CI, -97.34 to 121.56g; p=.83; 1 RCT, 494 women). There appears no difference, however in the incidence of small-for-gestational age (SGA) (RR, 1.13; 95% CI, 0.96 to 1.34; p=.15; 1 RCT, 1374 infants) or low birth weight (RR, 1.00; 95% CI, 0.88 to 1.12; p=.94; 5 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, 53.97g; 95% CI, -3.11 to 111.04g; p=.06; 6 RCTs, 1278 infants) or birth length (WMD, 0.23cm; 95% CI, -0.04 to 0.50cm; p=.09; 5 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, 0.26cm; 95% CI, 0.02 to 0.49cm; p=.03; 4 RCTs, 729 infants) (Table 2.4). In the meta-analysis of women with high-low pregnancies, Horvath et al. (2007) observed that n-3 fatty acids supplementation did not result in significant reduction in the incidence of IUGR (RR, 1.03; 95% CI, 0.73 to 1.47; p=.85; 2 RCTs, 295 infants) or low birth weight in the newborn (RR, 1.03; 95% CI, 0.71 to 1.51; p=.87; 2 RCTs, 494 infants).

A meta-analysis of 4 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 to 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 which demonstrated significant increase in birth weight with supplementation (WMD, 42.22g; 95% CI, 14.76 to 69.68) but no difference in birth length (WMD, 0.27cm; 95% CI, -0.13 to 0.67) nor head circumference (WMD, -0.21cm; 95% CI, -0.84 to 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 to 1.02) or risk of IUGR (RR, 1.06; 95% CI, 0.2 to 1.21).

Based on the evidence from 8 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 mean 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 Circumference	Incidence of LBW	Recurrence/Incidence of IUGR
1[a,b,f]	D'Almeida et al. 1992/ Angola	0.30g of GLA, 0.14g of EPA & 0.08g of DHA (n=50)	Olive oil capsules as placebo (n=50)	NR	NR	NR	↓ (p=NR) (Weight <2000g)	NR
2[a,b,c,e,f,g]	Olsen 1992/ Denmark	1.28g of EPA & 0.92g 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	3g 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	1.62g of EPA & 1.08g 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)	1.28g of EPA & 0.92g 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)	1.28g of EPA & 0.92g of DHA (n=131-135)	Olive oil capsules (n=132-133)	↓*	NR	NR	↔	↔
11[a,b,f]	Olsen 2000/ 9 European countries (Twins)	1.28g of EPA & 0.92g of DHA (n=554-556 infants)	Olive oil capsules (n=566-557 infants)	↔	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 Circumference	Incidence of LBW	Recurrence/Incidence of IUGR
13[a,f]	Olsen 2000/ 9 European countries (Susp-IUGR)	2.88g of EPA & 2.07g of DHA (n=34)	Olive oil capsules (n=26)	↔	NR	NR	NR	NR
14[a,c,g]	Helland 2001/ Norway	0.80g of EPA, 1.18g of DHA & 0.03g AA (n=175)	Corn oil (n=166)	↔	↔	↔	NR	NR
15[a,c]	Smuts 2003a/ US	High-DHA eggs providing 184mg DHA (n=18)	Regular eggs providing 35mg 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 146mg DHA (n=142)	Regular eggs providing 32mg DHA (n=149)	↔	↑*	↔	↔	NR
17[a,c]	Malcolm 2003a/ UK	200mg DHA (n=31)	Sunflower oil placebo capsules (n=29)	↔	↔	↔	NR	NR
19[a,f]	Dunstan 2004/ Australia	1.11g of EPA & 2.24g of DHA (n=40)	Olive oil capsules (n=43)	↔	↔	↔	NR	NR
20[c,f]	Sanjurjo 2004/ Spain	40mg EPA & 200mg DHA (n=8)	Placebo dietary formula (n=8)	↔	NR	NR	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 Circumference	Incidence of LBW	Recurrence/Incidence of IUGR
22[g]	Krauss-Etschmann 2007; Germany, Hungary & Spain (NUHEAL)	150mg of EPA & 500mg of DHA (n=69) OR 400ug of folic acid (n=65) OR 150mg of EPA, 500mg of DHA & 400ug of folic acid (n=64)	Placebo (n=72)	↔	↔	↔	NR	NR
24	Tofail 2006/ Bangladesh	1.8g of EPA & 1.2g of DHA (n=125)	Soy oil capsules (n=124)	↔	↔	↔	NR	NR
25[f,g]	Bergmann 2007/ Germany	Basic supplement + 4.5g of fructo-oligosaccharide + 200mg of DHA (n=43)	Basic supplement +/- 4.5g of fructo-oligosaccharide (n=74)	↔	↓*	↔	NR	NR
26[f]	Judge 2007b/ US	DHA-containing cereal-based bar providing on average 214mg of DHA per day (n=16)	placebo cereal-based bar containing corn oil (n=14)	↔	↔	↔	NR	NR
30[g]	Innis & Friesen 2008/ Canada	400mg of algal DHA	Corn oil/soy-bean oil	↔	↔	NR	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 Circumference	Incidence of LBW	Recurrence/Incidence of IUGR
33[f,g]	Makrides 2010/ Australia	Maternal intake of DHA-rich fish oil concentrate, providing 800mg/day of DHA and 100mg/day of EPA (n=1197)	Vegetable oil capsules (blend of rapeseed, sunflower and palm oil) (n=1202)	↑ ***	↔	↔	↓*	↔
34[f,g]	Ramakrishnan 2010/ Mexico	Maternal intake of 400mg 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 different, 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 Horvarth 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

3.1.5 Growth pattern

Results from individual trials

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

Supplementation during pregnancy:

No significant difference was observed in growth pattern (with respect to weight, height and head circumference) when assessed at 3 or 7 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 0.2g DHA to 1.8g EPA+1.2g DHA) and control group.

Maternal supplementation during lactation:

No significant difference was observed in weight or height of children between intervention (ranging from 0.2g DHA to 0.4g EPA+0.9g DHA) and 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 (0.68cm) in head circumference was observed by Lauritzen et al. (2005a) in the intervention group when assessed at 2.5 years but not at birth, 2, 4 or 9 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 (1.54cm±0.63; p=.017) and body mass index (BMI) (0.65±0.28; p=.022) in the intervention group at 2.5 years when compared to control. However, the difference in BMI was no longer apparent when re-assessed at 7 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

(3.8mmHg±1.7; p<.05) and mean arterial pressure (2.8mmHg±1.3; p<.05) was observed in the fish oil group when compared to 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 6 weeks, 3, 6, 9 or 12 months after birth between intervention and control groups. Hauner et al. (2012) also found no significant difference in these parameters between supplemented and non-supplemented group when assessed at 6 week, 4 months or 12 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 had 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, 0.24kg; 96% CI, -0.07 to 0.55; 712 participants), medium term (12 to 24 months; WMD, -0.56kg; 95% CI, -0.64 to 0.48; 117 participants) or long term (beyond 24 months; WMD, 0.22; 95% CI, -0.13 to 0.57; 834 participants) outcomes. For child's length, their analysis favours the control group at long term (WMD, -0.75cm; 95% CI, -1.38 to -0.12; 834 participants) and for head circumference, significantly larger circumference was reported in the supplemented group both at medium term (WMD, 0.70cm; 975% CI, 0.56 to 0.84; 117 participants) and long term (WMD, 0.69cm; 95% CI, 0.35 to 1.02; 244 participants).

A systematic review by Muhlhausler et al. (2010) which included 3 human trials showed contrasting results, thus no conclusion can be made. One study (Bergmann et al. 2007) 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 (Helland et al. 2008). The remaining trial 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 7 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, no conclusion can be made regarding physical growth pattern in children born to mothers supplemented with LCn3PUFA during pregnancy and/or lactation.

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 0.20-0.25g 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 0.80g of EPA, 1.18g of DHA & 0.03g 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 n3/n6 fatty acids and the children's BMI at 7 years of age (n=143)
17[e]	Malcolm 2003b/ UK	Maternal daily intake of 200mg 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 1.11g of EPA & 2.24g of DHA during pregnancy	Olive oil capsules	↔ in weight, length or head circumference when assessed at 2.5 years of age (n=64)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
21[b,c,d]	Lauritzen 2005a, Larnkjaer 2006, Asserhoj 2009/ Denmark	Maternal daily intake of 0.6g EPA & 0.8g 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 different 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)
24[e]	Tofail 2006/ Bangladesh	Maternal daily intake of 1.8g of EPA & 1.2g 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 + 4.5g of fructo-oligosaccharide + 200mg 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

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
34[e]	Stein 2011/ Mexico	Maternal intake of 400mg 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	180mg of EPA & 1020mg 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)	↔ 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 different, 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

3.1.6 Neurological (cognitive) development

Results from individual trials

Twelve trials have been found assessing the maternal n-3 fatty acids supplementation during pregnancy and/or lactation on neurological development of their offspring (Table 2.6). Six trials involved supplementation during pregnancy only (Dunstan et al. 2008; Escolano-Margarit et al. 2011; Judge et al. 2007a; Makrides et al. 2010; Ramakrishnan et al. 2010b; Tofail et al. 2006), three trials commenced supplementation after delivery and continued for 3 to 4 months (Gibson et al. 1997; Jensen et al. 2005; Lauritzen et al. 2005b) and three trials commenced supplementation at between 14 to 25 week gestation and continued until 3 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 (1.1g EPA and 2.2g DHA per day) during pregnancy. At 2.5 years of age, no significant difference was observed in developmental quotients (except higher score for eye and hand coordination in the fish oil groups), behaviour or linguistic development between intervention and control groups. Similarly, the NUHEAL study conducted across three European countries did not show any significant difference with maternal supplementation of 0.15g EPA and 0.5g 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 4 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-MDI), Psychomotor Development Index (PDI) or behaviour rating in children when assessed at 10 months of age after maternal supplementation of n-3 fatty acids (1.8g EPA & 1.2g DHA per day). Judge

et al. (2007a) observed no difference in cognitive functioning at 9 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 (214mg DHA per day) when the Infant Planning Test was applied. In the study by Makrides et al. (2010), maternal supplementation of 800mg per day of DHA during second half of the pregnancy did not result in improved cognitive and language development in their offspring when assessed at 18 month of age. Ramakrishnan et al. (2010b) also concluded from their study that prenatal DHA supplementation (400mg per day of DHA) did not improve global development scores.

Maternal supplementation during lactation:

Gibson et al. (1997) using increasing doses of DHA (0g to 1.3g) demonstrated a positive association between infant erythrocyte DHA status and BSID MDI at 1 year of age but this association was lost when re-assessed at 2 years old. No association was found between infant erythrocyte DHA status and BSID PDI at either 1 or 2 years of age (Gibson et al. 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 mother received 200mg DHA supplementation. These children also performed significantly better on a test of sustained attention when assessed at 5 years of age (2010). Lauritzen et al. (2005b) observed no significant difference in problem solving ability between intervention and control group (0.4g EPA+0.9g DHA vs. olive oil) when infants were assessed at 9 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 Infant Planning Test. A negative effect in vocabulary comprehension at 1 year and sentence complexity at 2 years of age was seen in the boys from the supplemented groups (0.4g EPA + 0.9g DHA) but not in girls. When these children were re-assessed at 7 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 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 n-3 fatty acids supplementation, Helland et al. (2001) did not observe significant difference in electroencephalogram (EEG) maturity scores measured at 1 day or 3 months after birth when compared to the control group. There was also no significant difference in cognitive functioning when assessed at 6 or 9 months of age. Mental Processing Composite score was significantly higher in the intervention group when children were assessed at 4 years of age (Helland et al. 2003) but was no longer significantly different when assessed at 7 years of age (Helland et al. 2008). van Goor et al. conducted a study comparing the effect of supplementation of DHA alone (220mg per day) *vs.* DHA + AA (220mg 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 2 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 3 months (van Goor et al. 2010) of age. In another study, pregnant women were supplemented with either 1.6g EPA and 1.1g DHA per day or placebo from 25 week gestation and during 3.5 months of lactation (Karlsson et al. 2010). Improved performance on visuospatial task and executive task 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 difference 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 et al. 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 (8 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 level.

Four independent RCTs (6 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 available evidence do not support supplementing all expecting mothers for improvement of infant neurodevelopment although it is important to maintain a healthy diet that provides sufficient LCn3PUFA.

Out 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 do not provide 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 findings of increased developmental standard score in the supplemented group when compared to the non-supplemented group in children 2 to 5 years of age (WMD, 3.92 points; 95% CI, 0.77 to 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

Overall, available evidence show inconsistency and therefore no conclusion can be made to refute or support maternal LCn3PUFA supplementation during pregnancy and/or lactation for improvement in neurodevelopment in their offspring. However, several studies did demonstrate positive relationship between maternal/infant DHA status and better 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: 0.2g of DHA; 0.4g of DHA; 0.9g of DHA; 1.3g of DHA	Placebo containing 0g 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 0.80g of EPA, 1.18g of DHA & 0.03g 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)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
19[c,d,e,f,g]	Dunstan 2008/ Australia	Maternal daily intake of 1.11g of EPA & 2.24g 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 1½-5 years (n=72) • Language Development Survey (n=49-51)
21[b,c,f,h]	Lauritzen 2005b, Cheatham 2011/ Denmark	Maternal daily intake of 0.4g EPA & 0.9g 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 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 150mg of EPA & 500mg of DHA OR 400ug of folic acid OR 150mg of EPA, 500mg of DHA & 400ug 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)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
24[b,c,d,e,f,g]	Tofail 2006/ Bangladesh	Maternal daily intake of 1.8g of EPA & 1.2g 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 214mg 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	220mg DHA OR 220mg each of DHA + AA; from 14-20 week gestation to 3 months after delivery	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) ↑* 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 1.6g EPA+1.1g DHA; from 25 week gestation to 3-4 months after delivery	Soy oil capsules (2.5g LA, n-6)	At 46 months (n=NR), ↓* in errors in the executive task and visuospatial block design task ↔ in memory and language

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
33[d,f,g]	Makrides 2010/ Australia	Maternal intake of DHA-rich fish oil concentrate, providing 800mg/day of DHA and 100mg/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 400mg 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 different, 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] = RCTs 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

3.1.7 Visual function

Results from individual trials

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

Supplementation during pregnancy:

Malcolm et al. did not observe any significant different in visual function in the offspring following maternal fish oil supplementation (200mg DHA per day) as assessed by sceptic electroretinogram at birth (Malcolm et al. 2003a) or flash and pattern-reversal visual evoked potential (VEP) latency at 3 and 7 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 (214mg DHA per day) and control group when the infants were assessed at 4 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 6 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 400mg per day of DHA or 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 500mg of DHA, 500mg of

DHA+400µg of folic acid, 400µg 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 differences in latencies, amplitudes and minimum angle of resolution between the four supplemented group. 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 LCn3PUFA supplemented group (100mg EPA + 800mg 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 4 months of age (Smithers et al. 2011). Similarly, in the study by Stein et al. (2012), no significant difference in VEP latencies or amplitude were observed between the offspring of DHA-supplemented women (400mg per day) and control women (corn/soy oil).

Maternal supplementation during lactation:

Gibson et al. (1997) using increasing doses of DHA (0g to 1.3g per day) observed no significant different in VEP acuity among dietary groups when infants were assessed at 3 and 4 months after birth. Similarly, no significant different in visual acuity was evident in the study by Lauritzen et al. (2004) after fish oil supplementation when infants were assessed at 4 and 8 months of age. Lauritzen et al. (2004) however, demonstrated that infants with higher red blood cell levels of LCn3PUFA had better visual acuity at 4 months of age thus suggesting that LCn3PUFA might influence visual maturation. Jensen et al. (1999) randomised breastfeeding women into 3 study groups where one group received ~200-250mg of DHA per day as algal DHA, another groups 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, 200mg 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 4 and 8 months of age. Transient VEP amplitude again was significantly lower in the DHA supplemented group at both 4 and 8 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 difference 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 to 0.14; 3 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. 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 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 the Lo et al. (2012) in their systematic review of effectiveness of LCn3PUFA supplementation in pregnant women on visual outcomes of their offspring. One study found 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 difference between supplemented and non-supplemented group 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 4 RCTs and 2 RCTs where women were supplemented with LCn3PUFA during pregnancy and during lactation respectively. The authors concluded that clear and consistent short or long-term benefit of LCn3PUFA supplementation could not be demonstrated from the available evidence. In addition, 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: 0.2g of DHA; 0.4g of DHA; 0.9g of DHA; 1.3g of DHA	Placebo containing 0g 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 0.20-0.25g 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 200mg 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)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
21[b,c,d,e]	Lauritzen 2004/ Denmark	Maternal daily intake of 0.4g EPA & 0.9g 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 150mg of EPA & 500mg of DHA OR 400ug of folic acid OR 150mg of EPA, 500mg of DHA & 400ug of folic acid from 22 week gestation to delivery (Infant formula provided for 6 months if needed)	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)
26[d,e,f]	Judge et al. 2007b/ US	Maternal daily intake of 214mg 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 ~400mg 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)

Trial ID	Reference / Location	Intervention dosage	Control	Outcomes
33[e,f]	Smithers et al. 2011/ Australia	Maternal intake of DHA-rich fish oil concentrate, providing 800mg/day of DHA and 100mg/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 400mg 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 different, 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

3.1.8 Atopic disease

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 amount of the n-3 fatty acids EPA and DHA into cell membranes has been shown to reduce production of pro-inflammatory eicosanoid mediators such as prostaglandin E2 (PGE2) from n-6 fatty acid arachidonic acid (AA) (Calder 2008). Metabolism of EPA and DHA have 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 n-3 fatty acids or food containing high n-3 fatty acids content such as fish may be beneficial to the prevention or treatment of atopic disease where inflammation plays a major role.

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

Results from individual trials

Supplementation during pregnancy:

There were five RCTs (Dunstan et al. 2003; Krauss-Etschmann et al. 2008; Noakes et al. 2012; Olsen et al. 2008; Palmer et al. 2012) involving LCn3PUFA supplementation during pregnancy that had clinical and/or immunological outcomes. Dunstan et al. (2003) conducted a RCT in a group of healthy pregnant women who had a history of allergic rhinitis or asthma. The authors reported that children were less likely to have a positive skin prick test to egg at 1 year of age (OR, 0.34; 95% CI, 0.11 to 1.02; p=.055) following maternal fish oil supplementation from 20-week 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 to 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 6-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 diagnoses in their children from birth to 16 years old (n=528). The hazard rate of asthma was reduced by 63% (95% CI, 8 to 85%; p=.03) and allergic asthma by 87% (95% CI, 40 to 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 pregnancy women were supplemented with either fish oil capsules (100mg EPA + 800mg 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 to 1.09, p=.12) or without sensitisation (Adjusted RR, 1.10; 95% CI, 0.79 to 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 significant after adjustment (Adjusted RR, 0.64; 95% CI, 0.40 to 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 to 0.93; p=.02). In the study by Noakes et al. (2012), pregnant women were randomly assigned to either consume two 150g 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 6 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 0.15g EPA+0.5g DHA or 0.15g EPA+0.5 DHA+400µg folic acid or 400µg folic acid alone or placebo from 22 week gestation to delivery. The study demonstrated 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 4 months after birth with either 1.5g of LCn3PUFA per day or olive oil (in muesli bar 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 reported to have a diagnosis of eczema, wheezing or food allergy at 2 ½ 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 a 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 1.6g EPA + 1.1g DHA per day or with placebo (soy oil) from 25 weeks gestation to three to four months of breastfeeding. At 12 months of age, there was a lower prevalence of IgE-associated eczema ($p<.05$) and food allergy (p=.01) in the fish group compared to the placebo group when the infants were assessed at 12 months of age (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 et al. 2011; Kremmyda et al. 2011; Larqué et al. 2012). Incidentally, the same five RCTs were considered in these reviews and therefore 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 pre-natal period during programming of the foetus and before disease is established.

Conclusion

Overall, there is promising evidence to suggest maternal LCn3PUFA supplementation, particularly when initiated during pregnancy influences allergic biomarkers in children and therefore have 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	1.28g of EPA & 0.92g 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 1.11g of EPA & 2.24g 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 bar, cookies or capsules providing in total 1.5g of n-3 fatty acids (0.4g of EPA & 0.9g of DHA) for 4 months after delivery	Olive oil muesli bar, cookies or capsules	No difference in the percentage of children reported to have a diagnosis of eczema, wheezing or food allergy between groups when follow-up at 2.5 years of age (n=91)
22 [a,b,c]	Krauss-Etschmann 2008/ Germany, Hungary & Spain (NUHEAL)	Milk-based supplement providing 150mg of EPA & 500mg of DHA OR 400ug of folic acid OR 150mg of EPA, 500mg of DHA & 400ug 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	1.6g EPA+1.1g 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 800mg/day of DHA and 100mg/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 150g salmon portions per week resulting in median daily intake (from total diet) of 134mg of EPA and 269mg 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 12mg of EPA & 20mg 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<0.05, ** = p<0.01, *** = p<0.005, NR = not reported, ↑ = increase/higher, ↓ = decrease/lower, ↔ = no significant different, 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

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 2 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 et al. 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 in neurological development with LCn3PUFA intake initially, the association was no longer evident when reassessed in later years. It is possible that the positive effect might have been diluted with other external factors since birth such as socioeconomic factors.

LCn3PUFA appear 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.

3.2 Fish intakes and maternal & infant outcomes

There have been many randomised trials that examine the effects of LC n-3 PUFA supplementation in the form of fish oil in pregnancy and infant outcomes as shown in earlier section of this chapter. However, evidence related to fish consumption and health outcomes for mothers and infants *per se* have largely emerged from observational studies with the exception of a recently conducted randomised controlled trial in the United Kingdom, the Salmon in Pregnancy Study (SiPS). SiPS was the first intervention trials 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 between the salmon group and control groups when assessed at 6 months of age (Noakes et al. 2012).

There have been several large scale prospective cohort observational studies that examined the effects of fish consumption in relation to maternal and infant health outcomes. Earlier study conducted in New Zealand (Kjellstrom et al. 1986; Kjellstrom et al. 1989) and Faroe Islands (Debes et al. 2006; Steuerwald et al. 2000) pointed to negative outcomes with higher fish/seafood intake whereas the studies conducted in the Seychelles Islands did not support this view (Davidson et al. 2011).

A study in the UK (The Avon Longitudinal Study of Parents and Children, ALSPAC study) which enrolled more than 14,000 pregnant women between 1991 and 1992 suggested that maternal seafood intake of less than 340g per week was associated with increased risk of suboptimum outcomes in their children for verbal intelligence quotient, prosocial behaviour, fine motor, communication and social development scores (Hibbeln et al. 2007). Similarly, higher maternal fish intake was shown to be associated with higher child developmental scores at 18 months in a Denmark study involving 25,446 mother-child pairs enrolled between 1997 and 2002 (Oken et al.

2008). 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 preeclampsia (Oken et al. 2007), higher infant cognition at 6 months of age (Oken et al. 2005) and better child cognitive test performance at 3 years of age (Oken et al. 2008). Frequency of fish consumption during pregnancy was shown not to be associated with length of gestation or risk of preterm birth in the study; in fact there was a trend toward 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).

A recently published report by the Food and Agricultural Organization and World Health Organization (FAO & WHO 2011) attempted to quantify the relationship between maternal DHA consumption and neurodevelopment based on the findings of two of the previously mentioned studies, the ALSPAC study and 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.

Overall, the general consensus is that maternal fish consumption lowers the risk of suboptimal neurodevelopment in their offspring particularly when fish with lower methylmercury contents are selected.

4. PHASE 2: Dietary Modelling – Comparing nutrient profiles of diets of differing fish and seafood contents

4.1 Background

Fish is a good source of protein and many other nutrients such as the 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 recommends 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 (140g-280g) of fish per week for adults (NH&MRC 2013a). According to the NRVs, the level of LCn3PUFA intake considered to be adequate is 160mg per day for adult men and 90mg per day for adult women. These references of adequate intakes (AI) were based on gender specific median population intakes in Australia who seemingly had no apparent essential fatty acid deficiency and therefore does not necessarily represent optimal intakes. The suggested dietary target (SDT), equivalent to the 90th centile of the Australian/New Zealand population, is set at 610mg for men and 430mg for women for the reduction of chronic disease risk (NH&MRC 2006). This SDT is closer to the Heart Foundation's recommendation of daily intake of 500mg 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 250mg per day of EPA and DHA combined. For adult pregnant and lactating females, the minimum intake recommended is 300mg per day of EPA and DHA combined, of which at least 200mg per day should be DHA. The Consensus statements developed by the Perinatal Lipid Intake Working Group in Europe also recommends a minimum intake of 200mg 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 22.6g per day (McLennan & Podger 1999) and intake of LCn3PUFA was 195mg per day (EPA: 60mg; DPA:52mg; DHA:83mg) (Howe et al. 2006) . 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 the types of fish and seafood commonly consumed (Model 1) and secondly including fish with higher LCn3PUFA content (Model 2). Using the Australian Dietary Guidelines Sample Daily Food Patterns for Adults as food group intake recommendations (NH&MRC 2013b), we compared the nutrient profile between a low fish/seafood diet (Model 3) and 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.

4.2 Method

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

Establishing fish consumption pattern 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 we are interested in women of child-bearing age, only data from women aged 19 to 49 years were used for this modelling exercise.

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

The EPA and DHA contents of fish and seafood identified in Step 1 were 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 EXCEL

spread sheet. 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 Australian Dietary Guidelines. The suggested serving size for fish is 100g cooked fish, or about 115g if raw. The serving size for canned fish is one small tin which typically equates to around 70g 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 EXCEL as 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 1,000 times (known as trials) and the average nutrient intake value of all 1,000 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 in 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 current fish consumption pattern (i.e. lower fish intake)

Dietary pattern

Dietary pattern was based on the Australian Dietary Guidelines Sample Daily Food Patterns for Adults 2013 (NH&MRC 2013b) (Table 4.1) and the total number of

serves of the various food groups over a 14-day period were calculated. Three serves (100g serve) of non-oily fish per fortnight were included in the 'Lean meat/ poultry/ fish/ eggs/ tofu/ nuts & seeds/ legumes & beans' category as data from the 1995 NNS suggested a mean intake of around 150g 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' in the original Australian Guide to Healthy Eating. In general, statistical analyses conducted using the 1995 NNS data should be weighted by an appropriate population weighting factor as instructed by the 1995 NNS Technical Paper. No population weighting factor in this instance was applied to obtain frequency of consumption as previous analysis demonstrated minimum differences between weighted and unweighted data analysis. Less than 1% of the food would be considered wrongly classified if data were weighted when it should not have been or vice versa. We aimed to have for selection from each food group a minimum of five food choices for variety. In cases where less than 5 items could be identified as frequently consumed food within one food group, the top 5 most frequently consumed food were included in the model, even if they were consumed by less than 1% of the women.

Food compositional data

The majority of the nutrient data for foods used in the modelling were 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 were 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), adequate intake (AI) and upper level of intake (UL) of nutrients were obtained from Nutrient Reference Values for Australia and New Zealand (NH&MRC 2006) (Table 4.2). Suggested dietary targets and acceptable macronutrient distribution ranges for macronutrients (AMDR) for the reduction of risks 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 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 1,000 times and the average daily intakes of selected nutrients were generated by the computer. The proportion of these 1,000 diets providing adequate nutrients to meet EAR, RDI, AI, SDT, AMDR or exceeding UL, where applicable, were 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 fish and its type required to meet all LCn3PUFA recommendations as identified in Model 2 were 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 ½
Fruit	2	2	2
Grain (cereal) foods, mostly wholegrain and/or high fibre cereal varieties	6	8 ½	9
Lean meat and poultry, fish, eggs, tofu, nuts and seeds, and legumes/ beans*	2 ½	3 ½	2 ½
Milk, yoghurt, cheese and/or alternatives, mostly reduced fat	2 ½	2 ½	2 ½
Approx. number of additional serves from the five food groups or unsaturated spreads and oils or discretionary choices	0 – 2 ½	0 – 2 ½	0 – 2 ½

*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 to 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	3,000
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	-	1,000[h]
Vitamin A as retinol equivalents (μg/day)	700	500	-	3,000
Vitamin C (mg/day)	45	30	-	NP
Vitamin E as α-tocopherol equivalents (mg/day)	-	-	7	300
Calcium (mg/day)	1,000	840	-	2,500
Iron (mg/day)	18	8	-	45
Iodine (μg/day)	150	100	-	1,100
Magnesium (mg/day)	310[i], 320[j]	255 [i], 265 [j]	-	350
Phosphorus (mg/day)	1,000	580	-	4,000
Potassium (mg/day)	-	-	2,800	NP
Sodium (mg/day)	-	-	460 – 920	2,300
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 per cent) 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 900mg/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 Model 1 and 2

Based on data from women aged 19 to 49 years who participated in the 1995 NNS, 231 fish items were identified. Out 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 to 49 years in the 1995 NNS.

Table 4.3: Type of fish and seafood consumed by women in the 1995 National Nutrition Survey on the day of the survey using 24 hour recall

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 are listed in Table 4.4. This demonstrates that if the types of fish and seafood consumed were similar to that of the 1995 NNS, 8 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 430mg for LCn3PUFA and the FAO/WHO Expert Consultation recommendation of 250mg 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 250mg per day or more)	Average daily LCn3PUFA intake from fish or seafood (% of fish meals providing 430mg 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 are 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 3 serves if at least 2 of the fish serves are of high LCn3PUFA content. If all three serves were of high LCn3 content, then everyone would have achieved 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 1 high LCn3 fish)	Average daily EPA + DHA intake from fish or seafood (% of fish meals providing 500mg per day or more)	Average daily LCn3PUFA intake from fish or seafood (% of fish meals providing 430mg 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 the Model 3 and 4

Out of the 13,858 persons who took part in the 1995 NNS, there were 3506 women aged 19 to 49 years. A total of 3270 food and drinks 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 5).

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

*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 intakes of the two dietary patterns are listed in Table 4.7 (lower fish content) and Table 4.8 (higher fish content) respectively. The mean energy intakes for both lower and higher fish diet was around 7.6MJ. This would satisfy the estimated energy requirement for women of 1.6m 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 if women were taller or more active to meet higher energy requirements.

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. Adequate intakes 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 marginal different between the two dietary patterns (16% in the lower fish diet vs. 19% in the higher fish diet).

Table 4.7: Theoretical mean daily nutrient intake profile in women aged 19-50 years as estimated by the simulation of 1,000 diets which 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)

	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 (ug)	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 (ug)	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 1,000 diets which followed the recommendations of the Australian Dietary Guidelines but with a higher fish content (around 3 serves of oily fish and seafood per week)

	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 (ug)	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 (ug)	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 250mg 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. 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. None of the diets met the SDTs for sodium or potassium.

The mean intake of sodium was similar in both dietary patterns and was around 1,800mg per day ranging from 1,528mg to 2,208mg. Although almost all simulated diets failed to meet the SDT for sodium, none has exceeded the upper level of sodium intake of 2,300mg per day. This has been made possible as no food from the 'extra' group was included in the modelling. Foods in the 'extra' group are generally higher in salt, sugar or saturated fat.

Upper levels of intakes have also been set for niacin and folate. However, these ULs were intended for intakes from fortified foods and supplements. Since no supplement was included in the modelling and breakfast cereal is likely to be the only fortified food included with added niacin and folate, we were not concerned that the mean intakes of these two nutrients seemed to be at the higher end.

Table 4.9: Proportion of 1,000 simulated diets meeting Suggested Dietary Targets (SDT) and FAO/WHO recommended daily intake of 250mg of EPA+DHA

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

recommended daily intake of 250mg EPA+DHA in women. It is therefore important to include fish, in particular oily fish in the diet in order to achieve these dietary targets for optimal health outcomes.

5. PHASE 3: Analytical testing 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 field of dietetics (clinical and research), nutritional epidemiology, health promotion as well as 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 2 food composition databases, NUTTAB and AUSNUT, maintained by FSANZ provide compositional data on Australian foods. NUTTAB 2010 is the latest version and contains nutrient data for 2,668 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 3,874 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 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 on over 1,000 common Australian and New Zealand food items. Two publications, 'Seafood the good food' and 'Seafood the good food II' report on the content and composition of the oil from 268 Australian fish and seafood species. Nutrient information panel of packaged food may be another source of information, however, since listing of LCn3PUFA content is not mandatory, food companies generally only provide information on LCn3PUFA on the nutrient information panel when content claims are being made.

Mercury content in fish and seafood is also of interest as they contain much higher levels of mercury than most other foods. Information of mercury content is less readily available. FSANZ NUTTAB 2010 database provide 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 cooking on the nutrients, particularly fatty acids profile in fish and seafood. And for this reason, our analysis included both raw and cooked samples. A review of the literature regarding effects of cooking can be found in Appendix 6.

5.2 Purpose of analysis

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

5.3 Process

5.3.1 Fish and fish products included for analyses

It was agreed that the fish and fish products used in the randomised controlled trial would be those that were commonly consumed, 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 thirteen (13) products were 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 the analyses as well as subsequently for use in the trial. Data obtained from the analyses 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 still provided adequate variety for an 8-week intervention trial.
Products selected for analyses were:

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

5.3.2 Selection of suitable laboratory to conduct the analyses

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 nutrients data on a range of Australian seafood. They employed the service of a consortium of 4 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 for 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 analyses for this project were AsureQuality Limited (AQ, Auckland New Zealand), Hill Laboratories (Hill, Hamilton New Zealand) and National Measurement Institute (NMI) in Victoria Australia.

5.3.3 Analytes included and method of analyses

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 analyses. 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 AsureQualitiy 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/100g) = 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 100g) = $\sum Wi\ Fi$ where *Wi* is the average weight of the food component (g/100g 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 methylmercury)

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

Mercury

Mercury was solubilised from the samples by digesting with a concentrated nitric acid/hydrochloric acid mixture. The solubilised elements were than measured by ICPMS

Methylmercury

Methylmercury was extracted with an alkaline (NaOH). 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 analyses respectively. The tables also list the reference methods of analyses 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/100g	New Zealand (Australia New Zealand Food Standards Code) Food Standards 2002, Amendment No.2
AQ	Moisture	0.1	0.1	g/100g	AOAC 950.46
AQ	Protein	0.1	0.1	g/100g	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/100g	Method by Folch et al, The Journal of Biological Chemistry, 226:497-509, 1957
AQ	Ash	0.1	0.1	g/100g	AOAC 920.153 BS4401:Part1:1980/ISO 936-1978
AQ	Total carbohydrate	0.5	0.1	g/100g	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/100g	In-house based on JAOCS, 62 (1985) p1501-1507
AQ	Polyunsaturated fatty acids	0.1	0.1	g/100g	In-house based on JAOCS, 62 (1985) p1501-1507
AQ	Saturated Fat	0.1	0.1	g/100g	In-house based on JAOCS, 62 (1985) p1501-1507
AQ	Omega 3 (total)	0.1	0.1	g/100g	Calculated from fatty acid profile
AQ	Omega 6 (total)	0.1	0.1	g/100g	Calculated from fatty acid profile
AQ	Omega 9 (total)	0.1	0.1	g/100g	Calculated from fatty acid profile
AQ	EPA	10	10	mg/100g	In-house based on JAOCS, 62 (1985) p1501-1507
AQ	DPA	10	10	mg/100g	In-house based on JAOCS, 62 (1985) p1501-1507
AQ	DHA	10	10	mg/100g	In-house based on JAOCS, 62 (1985) p1501-1507
AQ	Trans fatty acids	0.1	0.1	g/100g	In-house based on JAOCS, 62 (1985) p1501-1507
AQ	Cholesterol	1	0.5	mg/100g	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	Methylmercury	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/100g	COST 91 P23-32, G. Brubacher, W. Muller-Mulot (Modified), EN 12823-1:2000, AOAC 992.04 and 002.06
NMI	Vitamin D	0.3	Not disclosed	µg/100g	LC-MS-MS
AQ	Vitamin E	0.11	0.11	iu/100g	COST '91, 97-106 (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 products (1), (2), (3), (4), (5) and (9)

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 were 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 analyses.

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

For canned fish, 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 2 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 as per 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 as per the laboratory's standard operating procedure.

5.4 Results and Discussions

Twenty 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/100g and *Y* mg/100g respectively, the RPD would be calculated as follows:

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

- No RPD was calculated if one or both results were below the LOR.
- All results were reported as per 100g of food. If the results were provided as mg/kg, the results would be divided by 10 to convert to mg/100g or multiplied by 100 to convert to µg/100g.
- Methylmercury was reported as methylmercuric chloride (MeHgCl) in the report. In order to convert to methylmercury (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 around 700kJ/100g and ranged from 467kJ (Tuna in springwater) to 1150kJ/100g (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 (0.9g/100g) and the highest was cooked Birds Eye Atlantic Salmon (20.1g/100g).

Those products with almost 100% fish as the sole ingredient had an average protein value of 23g/100g. Protein value generally decreased as the fish content decreased. The product with the lowest protein value was Birds Eye Deep Sea Dory Fish Portions (9.7g/100g) and 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 NH&MRC document, 'A modelling system to inform the revision of the Australian Guide to Healthy Eating' (2011), fish and seafood can be classified into high LCn3PUFA (>1400mg LCn3PUFA/100g), medium LCn3PUFA (400-1399mg LCn3PUFA/100g) or low LCn3PUFA (<400mg LCn3PUFA/100g). 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 1400mg per 100g. The product with the highest LCn3PUFA content was cooked Birds Eye Atlantic Salmon Lemon Pepper (3015mg/100g), followed by raw Birds Eye Atlantic Salmon Lemon Pepper (2175mg/100g) and then John West Sardines in Tomato Sauce 110g (2056mg/100g). Except for raw John West yellowtail kingfish (1145mg/100g), the remaining analysed products had less than 400mg per 100g of LCn3PUFA and were considered low LCn3PUFA products. However, even at levels of <400mg per 100g with the lowest being Birds Eye Dory Portions Original Crumb at 157mg/100g, 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 0.5mg/kg (or 50µg per 100g) 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 1mg/kg (or 100µg per 100g). The product that recorded the highest total mercury level in our analyses was the John West yellowtail kingfish at 7.0µg per 100g (both raw and cooked). The next highest was John West Tuna in Springwater at 6.0µg per 100g followed by Birds Eye Lightly Seasoned Fish Fillets (Hoki) Lemon & Cracked Pepper (raw) at 5.5µg per 100g. The product with the lowest total mercury level was Birds Eye Fish Fingers (Hoki/Hake) (both raw and cooked) at 1.1µg per 100g. 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 2mg/kg is set for molluscs but no maximum level is set for fish. In our analyses of 20 fish samples, 9 were below the LOR for cadmium (<0.002mg/kg) and the highest level detected was from John West Sardines with tomato sauce at 0.019mg/kg.

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

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

5.4.4 Vitamins A, D and E

The levels of vitamin A, D and E were determined in 9 oily fish samples.

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

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

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

The compositional data obtained from these analyses thereby allowed the intervention diet to be formulated for randomised controlled trial.

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

Component (Unit, per 100g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100g)	704	1.1%	744	1.3%
Moisture (g/100g)	68.1	0.1%	66.4	0.3%
Protein (g/100g)	21.3	0.0%	23.4	0.4%
Fat (g/100g)	9.1	3.3%	9.8	3.1%
Ash (g/100g)	1.2	16.7%	1.3	0.0%
Total carbohydrate (g/100g)	0.5	–	<0.5	–
Monounsaturated fatty acids (g/100g)	3.8	5.3%	4.1	4.9%
Polyunsaturated fatty acids (g/100g)	3.1	3.3%	3.3	3.1%
Saturated Fat (g/100g)	2.2	4.7%	2.4	4.3%
Omega 3 (Total) (g/100g)	1.9	5.4%	2.0	5.1%
Omega 6 (Total) (g/100g)	0.8	0.0%	0.9	0.0%
Omega 9 (Total) (g/100g)	2.8	3.6%	3.0	3.4%
EPA (mg/100g)	630	3.2%	660	3.0%
DPA (mg/100g)	285	3.5%	305	3.3%
DHA (mg/100g)	690	2.9%	735	4.1%
Trans fatty acids (g/100g)	<0.1	–	<0.1	–
Cholesterol (mg/100g)	55	1.8%	58	0.0%
Antimony (µg/100g)	<1	–	<1	–
Boron (mg/100g)	<0.05	–	<0.05	–
Cadmium (µg/100g)	<0.2	–	<0.2	–
Calcium (mg/100g)	6.8	1.5	6.5	0.0%
Chromium (µg/100g)	<10	–	<10	–
Copper (mg/100g)	0.04	0.0%	0.05	8.7%
Iodine (µg/100g)	<5	–	<5	–
Iron (mg/100g)	0.33	3.1%	0.35	0.0%
Lead (µg/100g)	<1	–	<1	–
Magnesium (mg/100g)	30	0.0%	33	0.0%
Manganese (mg/100g)	0.008	6.1%	<0.007	–
Mercury (µg/100g)	3.0	13.3%	3.3	12.1%
Methylmercury (µg/100g)	2.4	3.6%	3.3	5.1%

Component (Unit, per 100g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Molybdenum (µg/100g)	<2	–	<2	–
Nickel (µg/100g)	<10	–	<10	–
Phosphorus (mg/100g)	240	0.0%	250	0.0%
Potassium (mg/100g)	375	2.7%	390	0.0%
Selenium (µg/100g)	24	4.3%	26	3.9%
Sodium (mg/100g)	40	2.5%	39	2.6%
Sulphur (mg/100g)	230	0.0%	250	0.0%
Tin (µg/100g)	<3	–	<3	–
Zinc (mg/100g)	0.33	0.0%	0.37	2.7%
Vitamin A as Retinol (iu/100g)	<10	–	<10	–
Ergocalciferol (Vitamin D2) (µg/100g)	<0.3	–	<0.3	–
25-Hydroxy Vitamin D2 (µg/100g)	<0.3	–	<0.3	–
Cholcalciferol (Vitamin D3) (µg/100g)	5.0	10.1%	4.2	9.5%
25-Hydroxy Vitamin D3 (µg/100g)	<0.3	–	<0.3	–
Vitamin E as Total Tocopherols (iu/100g)	3.85	2.6%	4.24	1.2%

% 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 5.6 Compositional profile for John West Yellowtail Kingfish

Component (Unit, per 100g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100g)	647	0.8%	709	0.3%
Moisture (g/100g)	69.3	0.3%	67.3	0.0%
Protein (g/100g)	22.6	0.0%	23.7	0.0%
Fat (g/100g)	7.3	1.4%	8.7	1.2%
Ash (g/100g)	1.2	0.0%	1.2	0.0%
Total carbohydrate (g/100g)	<0.5	–	<0.5	–
Monounsaturated fatty acids (g/100g)	2.9	3.5%	3.4	3.0%
Polyunsaturated fatty acids (g/100g)	2.4	4.3%	2.9	0.0%
Saturated Fat (g/100g)	2.0	0.0%	2.4	4.3%
Omega 3 (Total) (g/100g)	1.4	7.4%	1.7	0.0%
Omega 6 (Total) (g/100g)	0.8	0.0%	1.0	10.5%
Omega 9 (Total) (g/100g)	2.0	0.0%	2.4	4.3%
EPA (mg/100g)	505	2.0%	635	1.6%
DPA (mg/100g)	155	6.5%	190	0.0%
DHA (mg/100g)	485	2.1%	625	1.6%
Trans fatty acids (g/100g)	<0.1	–	<0.1	–
Cholesterol (mg/100g)	57	1.8%	64	1.6%
Antimony (µg/100g)	<1	–	<1	–
Boron (mg/100g)	<0.05	–	<0.05	–
Cadmium (µg/100g)	<0.2	–	<0.2	–
Calcium (mg/100g)	6.1	0.0%	6.0	1.7%
Chromium (µg/100g)	<10	–	<10	–
Copper (mg/100g)	0.05	12.2%	0.05	2.1%
Iodine (µg/100g)	5.6	3.6%	5.8	8.7%
Iron (mg/100g)	0.41	4.9%	0.42	0.0%
Lead (µg/100g)	<1	–	<1	–
Magnesium (mg/100g)	33	0.0%	32	0.0%
Manganese (mg/100g)	<0.007	–	<0.007	–
Mercury (µg/100g)	7.0	27.3%	7.0	11.4%
Methylmercury (µg/100g)	6.5	7.9%	7.3	11.8%
Molybdenum (µg/100g)	<2	–	<2	–
Nickel (µg/100g)	<10	–	<10	–
Phosphorus (mg/100g)	220	0.0%	220	0.0%
Potassium (mg/100g)	380	0.0%	370	0.0%
Selenium (µg/100g)	43	2.4%	39	0.0%

Component (Unit, per 100g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Sodium (mg/100g)	50	0.0%	47	0.0%
Sulphur (mg/100g)	235	4.3%	250	0.0%
Tin (µg/100g)	<3	–	<3	–
Zinc (mg/100g)	0.46	2.2%	0.48	2.1%
Vitamin A as Retinol (iu/100g)	<10	–	17.3	1.2%
Ergocalciferol (Vitamin D2) (µg/100g)	<0.3	–	<0.3	–
25-Hydroxy Vitamin D2 (µg/100g)	<0.3	–	<0.3	–
Cholcalciferol (Vitamin D3) (µg/100g)	1.2	8.7%	1.2	26.1%
25-Hydroxy Vitamin D3 (µg/100g)	<0.3	–	<0.3	–
Vitamin E as Total Tocopherols (iu/100g)	4.07	6.4%	8.80	2.3%

% 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 5.7 Compositional profile for John West Sardine in Tomato Sauce and Pink Salmon

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

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

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

Component (Unit, per 100g of food)	Red Salmon		Salmon Tempters	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Sodium (mg/100g)	410	0.0%	285	3.5%
Sulphur (mg/100g)	255	3.9%	160	0.0%
Tin (µg/100g)	<3	–	<3	–
Zinc (mg/100g)	0.75	2.7%	0.31	3.3%
Vitamin A as Retinol (iu/100g)	137	3.7%	NA	NA
Ergocalciferol (Vitamin D2) (µg/100g)	<0.3	–	NA	NA
25-Hydroxy Vitamin D2 (µg/100g)	<0.3	–	NA	NA
Cholcalciferol (Vitamin D3) (µg/100g)	23	8.7%	NA	NA
25-Hydroxy Vitamin D3 (µg/100g)	1.3	8.0%	NA	NA
Vitamin E as Total Tocopherols (iu/100g)	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 100g of food)	Tuna Tempters		Tuna Springwater	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100g)	618	0.5%	467	1.5%
Moisture (g/100g)	70.7	0.1%	72.3	0.4%
Protein (g/100g)	19.9	0.5%	26.2	2.7%
Fat (g/100g)	7.2	2.8%	0.9	11.8%
Ash (g/100g)	1.5	0.0%	1.3	0.0%
Total carbohydrate (g/100g)	0.8	50.0%	<0.5	–
Monounsaturated fatty acids (g/100g)	2.6	3.9%	0.2	0.0%
Polyunsaturated fatty acids (g/100g)	3.8	2.7%	0.3	0.0%
Saturated Fat (g/100g)	0.9	0.0%	0.4	28.6%
Omega 3 (Total) (g/100g)	0.2	0.0%	0.2	0.0%
Omega 6 (Total) (g/100g)	3.5	0.0%	0.1	0.0%
Omega 9 (Total) (g/100g)	2.5	0.0%	0.1	0.0%
EPA (mg/100g)	19	0.0%	27	11.3%
DPA (mg/100g)	<10	–	<10	–
DHA (mg/100g)	170	0.0%	200	10.0%
Trans fatty acids (g/100g)	<0.1	–	<0.1	–
Cholesterol (mg/100g)	38	2.7%	53	1.9%
Antimony (µg/100g)	<1	–	1	–
Boron (mg/100g)	<0.05	–	<0.05	–
Cadmium (µg/100g)	1.2	0.0%	1.8	0.0%
Calcium (mg/100g)	7.1	1.4%	5.6	7.1%
Chromium (µg/100g)	<10	–	<10	–
Copper (mg/100g)	0.07	4.0%	0.07	5.4%
Iodine (µg/100g)	8.6	1.2%	9.4	1.1%
Iron (mg/100g)	0.83	2.4%	2.25	4.4%
Lead (µg/100g)	1.2	8.7%	1	–
Magnesium (mg/100g)	28	3.6%	28	3.6%
Manganese (mg/100g)	0.072	2.8%	0.009	3.4%
Mercury (µg/100g)	2.5	36.7%	6.0	5.0%
Methylmercury (µg/100g)	2.4	0.0%	5.3	0.0%
Molybdenum (µg/100g)	<2	–	<2	–
Nickel (µg/100g)	<10	–	<10	–
Phosphorus (mg/100g)	155	6.5%	220	0.0%
Potassium (mg/100g)	210	0.0%	210	0.0%

Component (Unit, per 100g of food)	Tuna Tempters		Tuna Springwater	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Selenium (µg/100g)	73	2.7%	84	1.2%
Sodium (mg/100g)	375	2.7%	285	3.5%
Sulphur (mg/100g)	215	4.7%	265	3.8%
Tin (µg/100g)	<3	–	<3	–
Zinc (mg/100g)	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 100g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100g)	894	0.0%	1150	0.0%
Moisture (g/100g)	63.0	0.2%	54.5	0.2%
Protein (g/100g)	19.4	0.0%	23.5	1.3%
Fat (g/100g)	14.6	0.7%	20.1	0.0%
Ash (g/100g)	1.6	0.0%	1.8	0.0%
Total carbohydrate (g/100g)	1.5	13.3%	<0.5	–
Monounsaturated fatty acids (g/100g)	5.8	0.0%	7.9	0.0%
Polyunsaturated fatty acids (g/100g)	5.7	1.8%	7.8	0.0%
Saturated Fat (g/100g)	3.0	0.0%	4.2	0.0%
Omega 3 (Total) (g/100g)	3.8	0.0%	5.2	0.0%
Omega 6 (Total) (g/100g)	1.5	0.0%	2.0	0.0%
Omega 9 (Total) (g/100g)	4.6	0.0%	6.3	0.0%
EPA (mg/100g)	750	0.0%	1040	0.0%
DPA (mg/100g)	360	0.0%	505	2.0%
DHA (mg/100g)	1065	0.9%	1470	0.0%
Trans fatty acids (g/100g)	0.1	0.0%	0.1	0.0%
Cholesterol (mg/100g)	58	3.4%	70	1.4%
Antimony (µg/100g)	<1	–	<1	–
Boron (mg/100g)	<0.05	–	<0.05	–
Cadmium (µg/100g)	<0.2	–	<0.2	–
Calcium (mg/100g)	64.5	41.9%	79.5	8.8%
Chromium (µg/100g)	<10	–	<10	–
Copper (mg/100g)	0.06	3.4%	0.06	5.4%
Iodine (µg/100g)	7.8	10.3%	7.9	3.8%
Iron (mg/100g)	0.31	3.3%	0.33	0.0%
Lead (µg/100g)	<1	–	<1	–
Magnesium (mg/100g)	29	0.0%	33	6.1%
Manganese (mg/100g)	0.065	4.7%	0.077	2.6%
Mercury (µg/100g)	2.5	32.0%	2.1	19.0%
Methylmercury (µg/100g)	2.4	3.6%	2.6	0.0%
Molybdenum (µg/100g)	<2	–	<2	–
Nickel (µg/100g)	<10	–	<10	–
Phosphorus (mg/100g)	245	4.1%	280	7.1%
Potassium (mg/100g)	350	0.0%	400	0.0%
Selenium (µg/100g)	20	5.1%	26	3.9%

Component (Unit, per 100g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Sodium (mg/100g)	210	0.0%	215	4.7%
Sulphur (mg/100g)	205	4.9%	255	3.9%
Tin (µg/100g)	<3	–	<3	–
Zinc (mg/100g)	0.44	6.9%	0.61	3.3%
Vitamin A as Retinol (iu/100g)	<10	–	<10	–
Ergocalciferol (Vitamin D2) (µg/100g)	<0.3	–	<0.3	–
25-Hydroxy Vitamin D2 (µg/100g)	<0.3	–	<0.3	–
Cholcalciferol (Vitamin D3) (µg/100g)	8.5	2.4%	9.8	24.5%
25-Hydroxy Vitamin D3 (µg/100g)	<0.3	–	0.4	0.0%
Vitamin E as Total Tocopherols (iu/100g)	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 100g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Energy (kJ/100g)	670	1.5%	663	0.5%
Moisture (g/100g)	68.2	0.9%	67.9	0.0%
Protein (g/100g)	14.6	4.1%	16.0	0.6%
Fat (g/100g)	7.4	0.0%	6.9	2.9%
Ash (g/100g)	1.1	0.0%	1.3	8.0%
Total carbohydrate (g/100g)	8.7	0.0%	8.0	5.0%
Monounsaturated fatty acids (g/100g)	4.2	0.0%	3.9	2.6%
Polyunsaturated fatty acids (g/100g)	2.2	0.0%	2.1	0.0%
Saturated Fat (g/100g)	1.0	0.0%	0.9	0.0%
Omega 3 (Total) (g/100g)	0.9	0.0%	0.9	0.0%
Omega 6 (Total) (g/100g)	1.2	8.7%	1.1	9.5%
Omega 9 (Total) (g/100g)	3.8	0.0%	3.5	2.9%
EPA (mg/100g)	100	0.0%	99	2.0%
DPA (mg/100g)	29	0.0%	28	3.6%
DHA (mg/100g)	250	0.0%	255	3.9%
Trans fatty acids (g/100g)	<0.1	–	<0.1	–
Cholesterol (mg/100g)	29	3.5%	29	3.5%
Antimony (µg/100g)	<1	–	<1	–
Boron (mg/100g)	0.084	7.1%	<0.05	–
Cadmium (µg/100g)	<0.2	–	<0.2	–
Calcium (mg/100g)	12.0	0.0%	13.5	7.4%
Chromium (µg/100g)	<10	–	<10	–
Copper (mg/100g)	0.04	6.9%	0.05	4.4%
Iodine (µg/100g)	<5	–	<5	–
Iron (mg/100g)	0.25	0.0%	0.30	3.4%
Lead (µg/100g)	<1	–	<1	–
Magnesium (mg/100g)	32	3.2%	34	3.0%
Manganese (mg/100g)	0.120	0.0%	0.130	0.0%
Mercury (µg/100g)	5.5	29.1%	5.4	13.1%
Methylmercury (µg/100g)	5.0	6.9%	4.2	20.4%
Molybdenum (µg/100g)	2.2	0.0%	2.2	4.7%
Nickel (µg/100g)	<10	–	<10	–
Phosphorus (mg/100g)	160	0.0%	170	0.0%
Potassium (mg/100g)	300	0.0%	325	3.1%

Component (Unit, per 100g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Selenium (µg/100g)	44	0.0%	49	2.1%
Sodium (mg/100g)	130	0.0%	140	0.0%
Sulphur (mg/100g)	155	6.5%	165	6.1%
Tin (µg/100g)	<3	–	<3	–
Zinc (mg/100g)	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 1kg (Hoki/Hake)

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

Component (Unit, per 100g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Sodium (mg/100g)	270	0.0%	275	3.6%
Sulphur (mg/100g)	105	9.5%	120	0.0%
Tin (µg/100g)	<3	–	<3	–
Zinc (mg/100g)	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 (Hake/Hoki) Original Crumb 425g

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

Component (Unit, per 100g of food)	Raw		Cooked	
	Average of 2 results	% RPD	Average of 2 results	% RPD
Selenium (µg/100g)	32	9.5%	34	0.0%
Sodium (mg/100g)	175	5.7%	180	0.0%
Sulphur (mg/100g)	115	8.7%	120	0.0%
Tin (µg/100g)	<3	–	<3	–
Zinc (mg/100g)	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 425g

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

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

6. PHASE 4: Acceptability and effects of a higher fish diet – a randomised controlled trial

6.1 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 neurodevelopment is also implicated. Not only is nutrition during pregnancy and lactation in women critical, nutrition pre-conception has also been shown to be important. In Australia, it is estimated that around 50% of women of reproductive age have experienced an unplanned pregnancy (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 methyl mercury, and whether increasing fish intake would lead to unwanted side effects of mercury. 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.

6.2 Study protocol

6.2.1 Subjects

Healthy pre-menopausal women aged between 18 and 50 years with a body mass index (BMI) of ≥18.5 to ≤30 kg/m2 and relatively weight stable were recruited via advertisements in newspaper 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 which consisted 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 ≥7mmol/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.

Recruitment commenced in August 2010 and the trial completed in May 2011. In order to avoid the Christmas and New Year holiday period, participants commenced the 10-week trial either no later than mid-October 2011 or after February 2011.

Written informed consent was obtained from all participants. The study had been reviewed by the Southern Area Health Services/Flinders University Human Research Ethics Committee and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000572066) .

6.2.2 Study design

This study was a single-blinded, parallel randomised controlled trial of eight weeks duration preceded by a two-week run-in period. Participants were block randomised (varying block sizes) 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. A graphical representation of the study protocol is shown in Figure 6.1 below. Overall, participants attended seven sessions within a two-month period. Whilst sessions one, three, five and seven had to be conducted at FMC, sessions two, four and six were conducted at other locations if the participant so wished (e.g. at home or work place). Baseline, mid-trial and final assessments were conducted at session three, five and seven respectively.

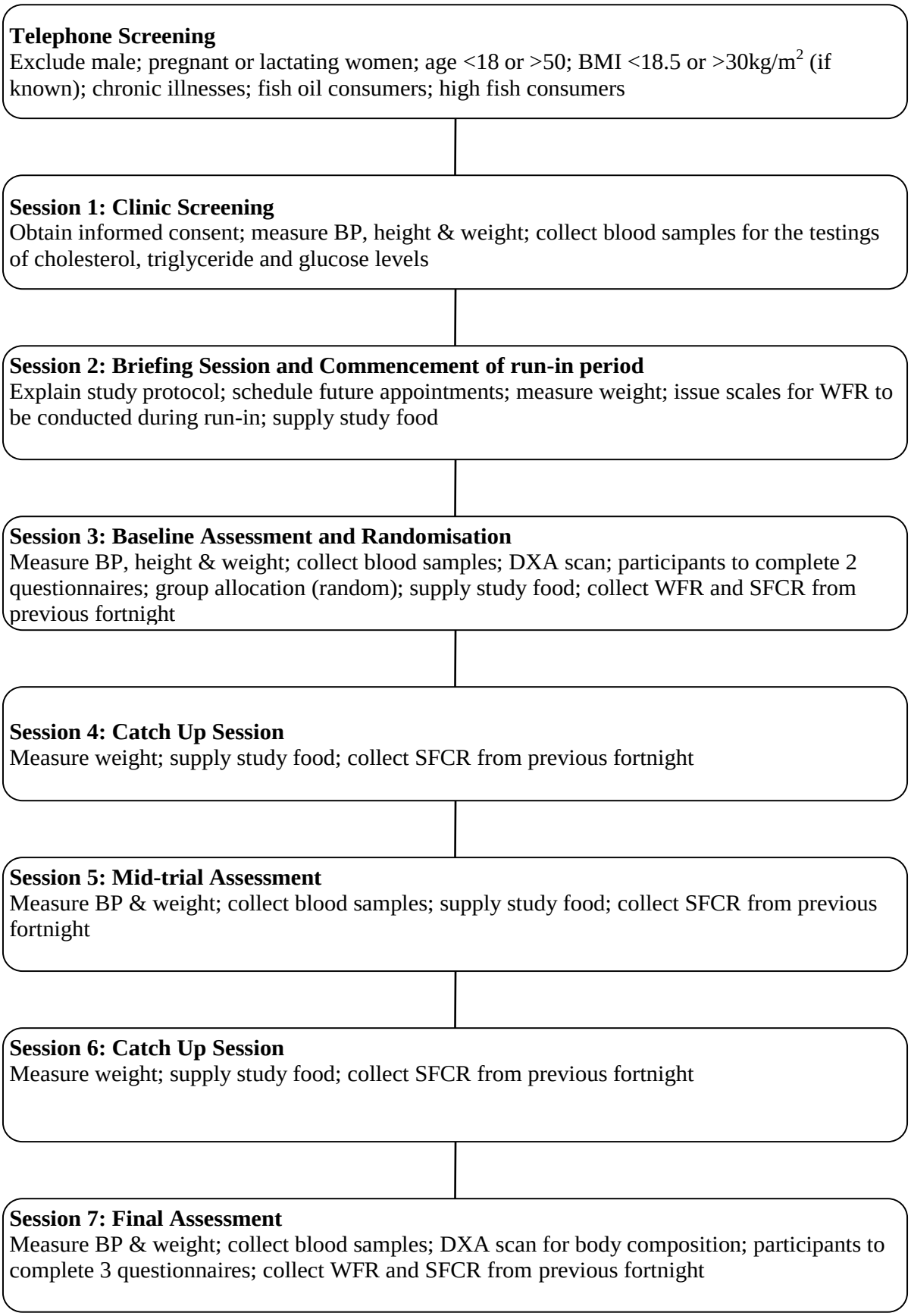

Figure 6.1 A graphical representation of study protocol for the randomised controlled trial examining the acceptability and effects of a higher fish diet

Intervention diet

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

Fish products for the intervention (higher fish) group were supplied by Simplot Australia and included the following over a 4-week period:

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

John West Yellowtail Kingfish*#, 2 x 150g

John West Pink Salmon, 105g

John West Red Salmon , 105g

John West Sardines in Tomato Sauce, 110g

John West Salmon Tempters Onion & Tomato, 95g

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

John West Tuna in Springwater, 2 x 95g

Birds Eye Atlantic Salmon Lemon Pepper, 135g

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

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

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

These 16 fish meals were repeated for another 4 weeks to complete the 8-week study

*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 pack 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

The study foods for the higher meat lower fish diet provided on average 465kJ of energy, 12.9g of protein, 5.1g of total fat, 144mg of sodium and 0.83mg 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 120g

Beef scotch fillet, 2 x 150g

Beef extra lean mince, 4 x 100g

Primo Roast beef slices, 2 x 50g

Select turkey slices, 2 x 40g

Steggles Mini Roast with spinach and cheese, 175g

Lenard's chicken schnitzel, 200g

Lenard's chicken kiev, 200g

Lenard's chicken cutlet cacciatore, 200g

These 16 meat meals were repeated for another 4 weeks to complete the 8-week study.

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 the participants was provided to study participants (Appendix 7). 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 analyses

Trained phlebotomists collected blood samples in the morning before 10am 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) 8.5ml 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 to 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) 6.0ml Royal Blue top tube containing dipotassium ethylene diamine tetraacetic acid (K2EDTA) 10.8mg (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 10.8mg (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) 2.0ml Lavender top tube containing tripotassium ethylene diamine tetraacetic acid (K3EDTA) 3.6mg (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 3 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™ 1.8ml vials (REF 377267) and 4.5ml 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.

6.3 Methods of assessment

6.3.1 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. The samples were methylated in 1% sulphuric acid in methanol for 3 hours at 70°. The resulting fatty acid methylesters was extracted with heptane and then quantified by gas chromatography. Fatty acids analyses were conducted by the candidate under supervision at the Fatty Acid Lab at the Waite Campus of the University of Adelaide.

6.3.2 Lipids study

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), 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.

6.3.2.1 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 505/694nm (Trinder reaction).

6.3.2.2 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 596nm.

6.3.2.3 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 505/694nm.

6.3.2.4 LDL-C

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

Friedewald Equation:

LDL-C = Total cholesterol – HDL-C – (Triglycerides /2.2)

(all values in mmol/L)

6.3.3 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. Analyses were conducted by Healthscope Pathology at Wayville, South Australia.

6.3.3.1 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 571/658nm.

6.3.3.2 Transferrin

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

6.3.3.3 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)

6.3.3.4 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.

6.3.3.5 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 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 555nm. Analysis was conducted by Healthscope Pathology at Wayville, South Australia.

6.3.4 Mercury analysis

Total mercury was analysed according to the method of EPA 1631, Appendix. Blood samples were acid digested and heat and further oxidized with bromine monochloride (BrCl). Digestates were analysed by stannous chloride (SnCl2) reduction, followed by gold amalgamation, thermal desorption and 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 total and methyl mercury analyses were conducted by Brooks Rand Labs in Seattle, USA.

6.3.5 Dietary assessments

6.3.5.1 3-day weighed food record

Participants were instructed to record all food and drinks consumed over 3 days (including 2 weekdays and 1 weekend day) during the run-in period and also the last

fortnight of the study. Digital scales with maximum of 3kg capacity and 1g 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 nutrients intakes based on the food records.

6.3.5.2 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.

6.3.5.3 Diet acceptability

Diet acceptability was assessed by a diet acceptability questionnaire (see Appendix 8) 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 our questionnaire.

6.3.6 Anthropometric and other assessments

6.3.6.1 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 0.1kg.

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

BMI (kg/m2) = Weight (kg) / [Height (m) x 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.

6.3.6.2 Blood pressure

Blood pressure was measured using an automatic oscillometric device (Criticare 5070, Criticare Systems Inc., USA). Blood pressure was measured after 5 minutes of seating and a second reading taken after one minute. If the systolic blood pressure differed more than 10mmHg or diastolic blood pressure more than 6mmHg, a third reading was taken. Blood pressure was reported as the average of all measurements taken.

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

The CES-D Scale questionnaire (see Appendix 9) 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.

6.4 Statistical analyses

Analyses were performed using IBM SPSS Statistics Version 19. Median and mode were shown for the result of the diet acceptability questionnaire; all other descriptive statistics were presented as means and standard deviations. The Mann Whitney-U test was used to compare the responses of the diet acceptability questionnaire and mixed-design ANOVA were used to compare the data at various time points both between and within groups. Data was transformed using log or square root to follow a normal distribution prior to analysis. A p-value of <.05 was regarded as statistically significant.

Sample size calculation:

This 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 have to be included in each group. Allowing for a 'drop-out' rate of 20%, the recruitment target was set at 40 women.

6.5 Results

Forty-two eligible women were enrolled into the study. Three women withdrew during the run-in phase (change of medication, n=1; timing issues, n=2). 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 were therefore included in the final analysis (see Figure 6.4 below).

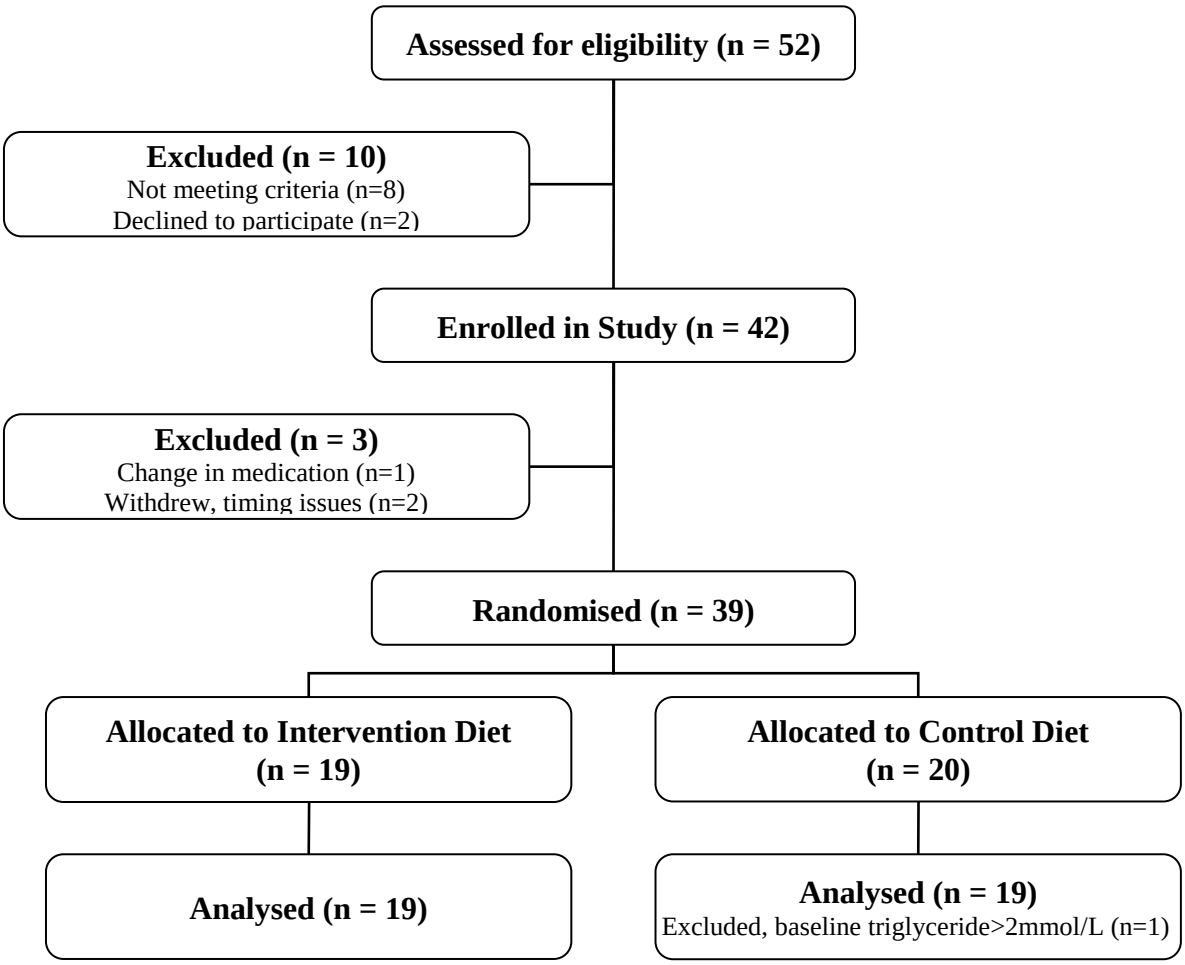

Figure 6.4: Flow diagram of the progress through phases of the randomised control trial aimed at examining the acceptability and effects of a higher fish diet

The characteristics of study participants at baseline are shown in Table 6.1 below. There was no statistically significant difference between the two groups in any of the parameters reported.

Table 6.1: Characteristics of study participants at baseline

	Intervention (n = 19)	**Control (n = 19)**	**p-value**
Age (years)	34.3 ± 9.5	33.5 ± 7.4	0.78
BMI (kg/m2)	23.3 ± 3.1	23.5 ± 2.3	0.78
Number of amalgam fillings	1.7 ± 2.1	2.0 ± 2.3	0.77
Erythrocyte EPA (% of total fatty acids)	0.89 ± 0.34	0.78 ± 0.20	0.22
Erythrocyte DPA (% of total fatty acids)	2.70 ± 0.36	2.60 ± 0.34	0.42
Erythrocyte DHA (% of total fatty acids)	4.80 ± 1.00	4.96 ± 0.57	0.55

All values are mean ± standard deviation

6.5.1 Fatty acids contents

Fatty acid profile of erythrocytes was determined from blood collected at Week 0 (baseline), Week 4 (mid-trial) and Week 8 (end of study) and are shown in Table 6.2. 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 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 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 arachidonic acid (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, $p<.001$).

Table 6.2: 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

	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)	$F(1.702,61.261)$
Week 4	1.23 (0.39)*,[b]	0.72 (0.17)	= 42.958
Week 8	1.31 (0.38)*,[b]	0.71 (0.20)	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)

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

6.5.2 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 6.3 below. There were also no significant changes within subjects pre- and post-trial.

Table 6.3: Effects of a diet higher in fish (intervention) versus a low fish diet (control) on serum lipid and lipoproteins

	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)	$F(1,36) = .760$	$F(1,36)=.038$ 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)	$F(1,36) =.2756$	$F(1,36)=.115$ 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)	$F(1,36) = .371$	$F(1,36)=.198$ 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)	$F(1,36) = .576$	$F(1,36)=1.250$ 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

6.5.3 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 6.4 below. There were also no significant changes within subjects pre- and post-trial.

Table 6.4: Effects of a diet higher in fish (intervention) versus a low fish diet (control) on serum iron and haemoglobin levels

	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

6.5.4 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 midtrial (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 midtrial (p=.003) and baseline and post-intervention (p=.004). However, there was no significant difference between midtrial 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<0.001, 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 midtrial, 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 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).

If using the EPA reference dose of 5.8µg/L, a level of mercury in foetal cord blood below which is assumed to cause no appreciable harm (Schober et al. 2003), then only one person in the intervention group exceeded this limit and had levels >5.8µg/L at all

assessment time points (7.1µg/L, 7.9µg/L and 7.0µg/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 level of as low as 3.5µg/L may be of concern (Mahaffey et al. 2009). If this reference of 3.5µg/L is used, then four persons had levels >3.5µg/L at post-trial (2 from intervention group and 2 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 6.5: Effects of a diet higher in fish (intervention) versus a low fish diet (control) on total mercury and methyl mercury levels

	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)

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

6.5.5 Dietary assessment

6.5.5.1 Compliance with allocated diets

Thirteen out 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 around one fish meal every one or two weeks.

In the intervention group, 18 out 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 8-week study period ranged from zero to five serves.

6.5.5.2 Diet acceptability

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

Table 6.6: Acceptability of a diet higher in fish (intervention) versus a low fish diet (control) rated on a 1 to 7 scale

	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

6.5.6 Anthropometric and other assessments

6.5.6.1 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 6.7 below. There were also no significant changes within subjects pre- and post-trial.

Table 6.7: Effects of a diet higher in fish (intervention) versus a low fish diet (control) on weight, BMI and body composition

	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)			
CES-Depression Scale					
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

6.5.6.2 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 6.8. 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 was no significant interactions between time and diet, main effect of time or main effect of diet detected for systolic blood pressure.

Table 6.8: Effects of a diet higher in fish (intervention) versus a low fish diet (control) on 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	108 (8)	110 (8)	$F(1.709,61.513)$	$F(1,36)=1.423$	$F(1.709,61.513)$
Week	105 (8)	108 (9)	= .269		=2.092
Week	105 (8)	108	p=.730	p=.241	p=.139
Diastolic blood pressure (mmHg)					
Week	64 (7)[a]	62 (7)	$F(2,72)$	$F(1,36)=.026$	$F(2,72)$
Week	61 (7)[a,b]	62 (8)	= 2.233		=3.471
Week	60 (8)[b]	61 (6)	p=.115	p=.873	p=.036

Descriptive statistics are presented as mean (standard deviation)

Time x Diet interactions tested using mixed-design ANOVA

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

6.5.6.3 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 6.9 below. There were also no significant changes within subjects pre- and post-trial.

Table 6.9: Effects of a diet higher in fish (intervention) versus a low fish diet (control) on depression mood indicator

	Intervention (n=19)	Control (n=19)	Time x Diet Interaction	Main effect of Diet	Main effect of Time
CES-Depression Scale					
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

6.6 Discussions

The participants within the intervention arm of this trial (higher fish intake) consumed an average amount of 425g of fish per week from a variety of fish and fish products which provided a daily average of 243mg of EPA and 338mg of DHA (581mg EPA+DHA).Currently, several recommendations exist for intake of fish and LCn3PUFA. Food Standards Australia New Zealand (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 = 150g) (FSANZ, Mercury in Fish). The level of LCn3PUFA intake considered to be adequate according to the Nutrient Reference Values is 160mg per day for adult men and 90mg per day for adult women. The suggested dietary target however is set at 610mg for men and 430mg for women for reduction of chronic disease risk (NH&MRC 2006). To reduce the risk of coronary heart disease, the Heart Foundation recommends all adult Australians consume 500mg 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, Ferreira-Jardim et al. 2008). The International Perinatal Lipid Intake Working Group recommends pregnant and lactating women should aim to achieve a DHA intake of at least 200mg per day by consuming one to two portions of oily fish per week (Koletzko et al. 2007). This 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.

The changes in the LCn3PUFA, EPA and DHA achieved in this study were comparable to other studies that have employed similar amounts of long-chain n-3 fatty acids in their interventions. Harris et al (Harris et al. 2007) examined the effects of fish and fish-oil capsules on n-3 fatty acids 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 95mg of EPA and 390mg of DHA per day (485mg 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 8 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. There was some fluctuation of DPA levels in the intervention group and a significant reduction in the control group after 8 weeks although no change was expected. The reason for this observation is unsure. 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. Both the intervention group and control group experienced a reduction in AA, a long chain n-6 polyunsaturated fatty acid (LCn6PUFA) with AA concentration in the intervention group significantly lower than the control group after 8 weeks. Changes in consumption of foods containing LCn6PUFA (e.g. from vegetable oil to olive oil in some participants) might partly explain the reduction in AA observed in the control group.

As expected, there were significant increases in THg and MeHg level in blood in the intervention group, ie 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 to 49 years who ate 3 or more servings of fish within past 30 days of 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 our study rose from1.91 µg/L to 2.32 µg/L after 8 weeks and it is hypothesised these levels would continue to rise until a steady-state is 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 slightly higher than those at week 4 in the intervention group, the difference was smaller than the increment seen between weeks 0 and 4 and 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 increase in 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 are 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 1.6µg 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 450g (three 150g serves) per week, then fish with levels of up to around 21 µg of MeHg per 100g 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 100g (FSANZ NUTTAB 2010). All fish products used in this study were analysed and found to have mercury levels below 10 µg/100g (unpublished data).

There were no significant differences between groups or within subjects observed in terms of lipids profile, iron status, 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 LC n-3 PUFA (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 8-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 Mori (2006) concluded that the cardioprotective role of n-3 fatty acids is partly related to its blood pressure lowering effects.

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 2 portions of salmon (providing around 500mg 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 were increased and the expected pregnancy-associated decline in these fatty acids were prevented. The status of EPA and DHA in their offspring was also increased.

6.7 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 THg and MeHg levels increased in the higher fish intervention group, it was overall within safety limits.

7. PHASE 4: Cost effective analysis of a higher fish diet

7.1 Introduction

Fish and seafood are excellent sources of macro- and micro-nutrients. In particular, they provide high quality protein, are rich in selenium, iodine and the long chain n-3 polyunsaturated fatty acids (LCn3PUFA), eicosapentaenoic acid (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 100g, 20.7g protein, 22µg selenium, 505mg EPA, 812mg DHA (FSANZ, NUTTAB 2010) and 6.9µg iodine (FSANZ, AUSNUT 2007). However, fish consumption in Australia are 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 (Australian Seafood CRC Fish Bite Series).

In the previous section, we reported on an 8-week clinical 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 outcome of interests were changes in red blood cells fatty acids levels, mercury in whole blood, lipids, iron status, body composition and acceptability of the diet. In this section, we will look at 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. Included were pre-menopausal women aged between 18 and 50 years with body mass index of ≥18.5 to ≤30 kg/m^2 and relatively weight stable. Excluded 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 consisted of more

than one oily fish per week on average. Women working in occupations with regular exposure to mercury were also excluded. Following a 2-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 8 weeks. Assessment was conducted at baseline (prior to randomisation), at 4 weeks and at 8 weeks.

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 2 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 minutes appointments. Wages 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 150g (2 x 300g packets)
John West Yellowtail kingfish	4 x 150g (2 x 300g packets)
John West Sardine in tomato sauce	2 x 110g can
John West Pink salmon	2 x 105g can
John West Red salmon	2 x 105g can
John West Salmon tempter onion & tomato	2 x 95g can
John West Tuna in springwater	4 x 95g can
John West Tuna tempter lemon & pepper	4 x 95g can
Birds Eye Atlantic salmon lemon & pepper	2 x 135g (1 x 270g box)
Birds Eye fillets lightly seasoned lemon & pepper	2 x 200g (1 x 400g box)
Birds Eye Deep sea dory	2 x 142g (2/3 x 425g box)
Birds Eye Oven baked fillets original crumbed	2 x 142g (2/3 x 425g box)

Control Diet	**Quantity**
Extra lean minced beef	8 x 100g
Chicken thigh fillet	4 x 120g
Scotch fillet	4 x 150g
Steggles Chicken Mini Roast Spinach & Cheese	2 x 175g (1 x 350g box)
Select Turkey slices	4 x 40g (2 x 80g packets)
Primo Roast beef slices	4 x 50g (2 x 100g packets)
Lenards Chicken cacciatore	2 x 200g
Lenards Chicken schnitzel	2 x 200g
Lenards Chicken kiev	2 x 200g

Table 7.2: Unit costs of resources used

Resources	Unit	Cost per unit	Details*
Cost of food (per person)			
Food supplied – intervention group	8-week study period	125.90	Average normal retail prices from two different supermarket chain stores in the majority of cases.
Food supplied – control group	8-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 2 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 (I) DHA provided in the foods and (2) changes in DHA levels in red blood cells.

(1) DHA provided in foods:

For intervention foods, DHA were analysed data based on composite samples from a minimum of 3 production dates.

For control foods, DHA contents were estimated based 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 8 weeks after the intervention. Erythrocyte phospholipids was extracted with chloroform:propanol and then separated by thin-layer chromatography. The samples were methylated in 1% sulphuric acid in methanol for 3 hours at 70°C. The resulting fatty acid methylesters was extracted with heptane and then quantified by gas chromatography.

7.3 Results

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

Table 7.3: Cost per 200mg of DHA

	Intervention Diet	Control Diet
Cost of study foods provided within an 8-week period (per person)	125.90	83.00
Amount of DHA provided by study foods	18944 mg	197 mg
Cost per 200mg of DHA	$1.33	$84.26

Table 7.4 shows the differences between the intervention and control diets in terms of costs 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 to 1.45)
Incremental cost effectiveness ratio (ICER) DHA	-	-	37.3 (29.6 to 51.1)

*Cost included 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 blood. The amount of fish consumed averaged to around 425g per week which is within the safe consumption recommendation from FSANZ. In this study we have chosen a variety of fish and fish products with varying DHA contents to mimic normal consumption pattern. The levels of DHA in our fish study foods ranged from 105mg per 100g to 750mg per 100g. Considering the costs of food alone, to achieve an intake 200mg DHA per day (as per recommendation), it would cost sixty times more if consuming only meat. 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 110g can and provided 750mg of DHA per 100g. 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 e.g. protein and other vitamins and minerals, which form part of a healthy diet.

Fayet et al. (2010) conducted a study and examined the comparative costs of foods that provided 100mg 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 read meat (approx. $2). Again this demonstrated that fish is a more cost-effective means of obtaining LCn3PUFA.

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. 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 in our study, participants were non-pregnant and non-lactating women, other studies have demonstrated DHA supplementation during gestation led to higher plasma or erythrocyte DHA levels (Dunstan et al. 2004; Van Houwelingen et al. 1995). This higher maternal DHA status in turn was associated with higher DHA status in the newborn (Dunstan et al. 2004; Van Houwelingen et al. 1995) and better developmental outcomes (Dunstan et al. 2008; Judge et al. 2007b). 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 % of total fatty acids) has been proposed as a biomarker for assessing risk of coronary heart disease and an Omega-3 Index of 8% is classified as desirable (Harris 2007).

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 mean of increasing DHA intakes.

8. Benefits and Adoption

It has been demonstrated a diet that included more fish (consumed four times a week) is acceptable and could improve LCn3PUFA status without compromising iron status or causing unsafe increase in mercury level. If more people would adopt this diet of higher fish content, then there would be increased demand in those seafood that are shown to be low in mercury but high in LCn3PUFA.

Simplot Australia intends to utilise the information in this project to educate consumers on the benefits of increased omega-3 in their diets. Specifically, this would involve summarising pertinent information for:

- Publication on websites such as John West http://johnwest.com.au/nutrition/omega3
- On-pack information/education eg. http://johnwest.com.au/nutrition/reading-a-john-west-pack
- Media or promotional advertorials.

More consumers are accessing the internet and social platforms for increased information on products, brands and services. In the past, Simplot developed an omega-3 website to help consumers understand how much omega-3 they are consuming (http://omega-3.com.au/). The cost effectiveness of the dietary patterns in relation to DHA intake, is suitable to be included on this website.

9. Further Development

It is anticipated that the result of this project be widely disseminated via journal publication and/or conference presentation.

It is important for consumers to have the confident to consume seafood knowing that it is safe and of high nutrient quality. Industries and/or government should consider regularly testing and updating compositional data of all fish and seafood available in the Australian market. Public should be educated on the types of commonly available fish and seafood that are low in contaminant (e.g. mercury) but high in LCn3PUFA.

The sustainability of fish stock has been a worldwide concern in recent years. With demand expected to increase further due to increase in population as well as awareness of its healthful properties, industries are advised to invest in efficient and sustainable aquaculture that can continue to provide quality seafood.

10. Planned Outcomes

10.1 Public Benefit Outcomes

The outcome of this project is a dietary pattern that is sufficiently high in omega-3 fatty acids for women of childbearing age but also one that is designed to mitigate risks from potential contamination. This dietary pattern – or The Australian Seafood Diet for Intergenerational Health – can be disseminated to all women and avoid the need to have specific and different recommendations for women either pregnant or wishing to become pregnant. This should minimise confusion and avoid any spill-over effect into the general population of specific information for at-risk sub-groups.

Compositional data obtained from the analysis of 13 fish and fish products could potentially be made available to the public to complement existing data.

10.2 Private Benefit Outcomes

This project will ultimately benefit all Seafood CRC participants as it will lay the framework for determining seafood diets with high omega-3 fatty acids and by raising the level of awareness, will encourage the consumption of seafood by all women (not just those intending to become pregnant) and as primary purchasers in the house-hold, will also increase overall family consumption.

10.3 Linkages with CRC Milestone Outcomes

This outcome is directly aligned with SellFish 2007-2014 Strategy 1 - Develop new approaches that CRC participants can use to communicate the health benefits and risks of seafood consumption. This project supports a PhD student who is due to graduate by the end of 2014.

11. Conclusion

Available literature demonstrated good evidence that long chain n-3 polyunsaturated fatty acids (LCn3PUFA) are beneficial for maternal and infant health. Fish is a good source of LCn3PUFA and other nutrients but its consumption within Australian women is overall less than optimal. A single-blinded randomised controlled 8-week trial conducted in healthy women 18 to 50 years who normally consumed ≤ one oily fish meal per week reported good acceptability and improvement of LCn3PUFA status while placed on a diet that included more fish. This higher fish diet which included a variety of fresh and convenient fish products, consumed four times a week, did not compromise iron status. Although blood mercury level did increase, it was still at a level accepted as safe as the levels of mercury present in the fish study food provided were relatively low (ranged from 1.1µg to 7.0µg per 100g). This showed that larger amount of fish can be consumed without compromising safety in term of mercury intake provided the fish consumed are low in mercury level.

A cost-effectiveness study conducted post-trial demonstrated that including fish in a diet is an economical means to obtain LCn3PUFA. Based on the food used in the randomised controlled trial, to achieve an intake 200mg DHA per day (as per DHA intake recommendation for pregnant and lactating women) it would cost sixty times more if consuming 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 which form part of a healthy diet.

Compositional data (including nutrients and contaminants such as mercury) of fish and seafood available in the Australian market are not always available. It is important for consumers to have the confident to consume seafood knowing that it is safe and of high nutrient quality. Industries and/or government should consider regularly testing and updating compositional data of all fish and seafood available in the Australian market. Public should be educated on the types of commonly available fish and seafood that are low in contaminant (e.g. mercury) but high in LCn3PUFA.

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13. Appendices

Appendix 1: Intellectual Property

Not applicable

Appendix 2: Staff

Lynne Cobiac
Nutrition and Dietetics
Flinders University, SA 5042
Contact telephone number: +61 8 8204 6406
Email address: lynne.cobiac@flinders.edu.au

Jocelyn Midgley
Simplot Australia Pty Ltd
2 Chifley Drive, Mentone VIC 3195
Contact telephone number: +61 3 9588 3562
Email address: Jocelyn.Midgley@simplot.com.au

Michelle Miller
Nutrition and Dietetics
Flinders University, SA 5042
Contact telephone number: +61 8 8204 5328
Email address: michelle.miller@flinders.edu.au

Campbell Thompson
Discipline of Medicine
University of Adelaide, SA 5005
Contact telephone number: +61 8 8222 4176
Email address: campbell.thompson@adelaide.edu.au

Lily Chan
Nutrition and Dietetics
Flinders University, SA 5042
Contact telephone number: +61 8 8204 7077
Email address: lily.chan@flinders.edu.au

Appendix 3: Search Strategy for the literature review

	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 4: 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 0.30g of GLA, 0.14g of EPA & 0.08g of DHA (n=50) **OR** 2 x 500mg 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 2.7g of n-3 fatty acids (1.28g of EPA & 0.92g of DHA) (n=266)	4 x 1g olive oil capsules (n=136) **OR** No supplement given (n=131)
3.	1993	Finland	Women diagnosed with preeclampsia	From 26-36 week gestation to delivery	10 fish oil capsules providing in total 1.80g of EPA & 1.20g of DHA (n=5) **OR** 10 primrose oil capsules providing in total 3.75g of LA & 0.45g of GLA (n=7)	10 capsules providing in total 5g of maize oil & 5g 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 3g 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 1.62g of EPA & 1.08g 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: 0.2g of DHA (n=10) 0.4g of DHA (n=12) 0.9g of DHA (n=10) 1.3g of DHA (n=8)	Placebo containing 0g 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 0.20-0.25g of DHA as either algal DHA (n=42) **OR** Refined high-DHA fish oil (n=42)	Placebo (n=42)

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)
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 2.7g of n-3 fatty acids (1.28g of EPA & 0.92g of DHA) (n=110)	4 x 1g olive oil capsules (n=122)
9.	2000	9 European countries	Women with history of IUGR	From ~20 week gestation to delivery	4 fish oil capsules providing in total 2.7g of n-3 fatty acids (1.28g of EPA & 0.92g of DHA) (n=141)	4 x 1g 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 2.7g of n-3 fatty acids (1.28g of EPA & 0.92g of DHA) (n=184)	4 x 1g 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 2.7g of n-3 fatty acids (1.28g of EPA & 0.92g of DHA) (n=289)	4 x 1g olive oil capsules (n=290)
12.	2000	9 European countries	Women with signs of preeclampsia in current pregnancy	From ~33 week gestation to delivery	9 fish oil capsules providing in total 6.1g of n-3 fatty acids (2.88g of EPA & 2.07g of DHA) (n=44)	9 x 1g 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 6.1g of n-3 fatty acids (2.88g of EPA & 2.07g of DHA) (n=36)	9 x 1g olive oil capsules (n=27)

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)
14.	2001	Norway	Healthy women who intended to breastfeed & their healthy term babies	From 17-19 week gestation to 3 months after delivery	10ml of cod liver oil providing 2.63g of n-3 fatty acids (0.80g of EPA, 1.18g of DHA & 0.03g AA) (n=301)	10ml of corn oil providing 4.75g of n-6 fatty acids (no EPA, DHA or AA) (n=289)
15.	2003	United States	Healthy women	From 24-28 week gestation to delivery	Eggs high in DHA (mean intake of 184mg DHA per day from eggs) (n=27)	Regular egg (mean intake of 35mg DHA per day from eggs) (n=25) OR Low egg intake group (no egg provided, mean intake of 11mg 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 146mg DHA per day from eggs) (n=176)	Regular eggs (mean intake of 32mg 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 200mg 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 ~200mg 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 1.11g of EPA & 2.24g 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 2g of fat providing 40mg of EPA & 200mg 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 1.5g of n-3 fatty acids (0.6g of EPA & 0.8g 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; All mothers were encouraged to breastfeed, those requiring formula feed were provided with one of two standard formulas for 6 months: DHA or DHA+folic acid group received formula with 0.5% of total fatty acid as DHA and 0.4% as AA, folic acid and placebo group received	Milk-based supplement providing 150mg of EPA & 500mg of DHA (n=77) **OR** 400ug of folic acid (n=77) OR 150mg of EPA, 500mg of DHA & 400ug of folic acid (n=77)	Placebo milk-based supplement (n=80)

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)
				formula free of DHA and AA		
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: 0.1g EPA+DHA (n=374); 0.3g EPA+DHA (n=370); 0.7g EPA+DHA (n=367); 1.4g EPA+DHA (n=358); 2.8g EPA+DHA (n=373) **OR** Flax oil providing in total 2.2g of ALA per day (n=369)	No treatment (n=748)
24.	2006	Bangladesh	Women recruited from a house-to-house survey where illiteracy, poverty and poor living environment was common; and their babies	From 25 week gestation to delivery	4 fish oil capsules providing in total 1.8g of EPA & 1.2g of DHA (n=200)	4 soy oil capsules providing in total 2.25g of LA & 0.27g 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, 4.5g of fructo-oligosaccharide and 200mg of DHA (n=48)	Supplement with vitamins & minerals only (n=49) **OR** Supplement with vitamins & minerals + 4.5g of fructo-oligosaccharide (n=47)

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)
26.	2007	US	Healthy women and their healthy babies	From 24 week gestation to delivery	DHA-containing cereal-based bar providing on average 214mg of DHA per day (n=27 at latest count)	Placebo cereal-based bar containing corn oil (n=21 at latest count)
27.	2008	Taiwan	Women diagnosed with major depressive disorder between 16-32 week gestation	For 8 weeks after randomisation	5 fish oil capsules providing in total 2.2g of EPA & 1.2g 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 & 0.8g 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	6g of fish oil capsules providing 0.4g of EPA & 1.6g of DHA (n=13)	Sunola oil as placebo (n=13)

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)
30.	2008	Canada	Healthy pregnant women	From 16 week gestation to delivery	2 capsules providing in total ~400mg of algal DHA (n=68)	2 capsules of corn-soybean oil blend (n=67)
31.	2009	Netherlands	Healthy pregnant women	From 14-20 week gestation to 3 months after delivery	220mg of DHA (n=63) **OR** 220mg 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 1.6g EPA+1.1g DHA (n=70)	Soy oil capsules (2.5g 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 100mg of EPA and 800mg 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 400mg of algal DHA (n=547)	2 placebo capsules containing corn-soy oil blend (n=547)
35.	2011	United Kingdom (SiPS)	Women habitually had low fish intake and had family history of atopy, allergy or asthma	From 20 week gestation to delivery	Incorporate 2 x 150g salmon portions into the diet per week, resulting in daily median intake (from total diet) of 134mg of EPA & 269mg 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 12mg of EPA & 20mg 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 180mg of EPA & 1020mg of DHA while reducing intake of AA to ~90mg 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 5: Foods included in the dietary modelling

Starchy vegetables

Sweetcorn, fresh on cob, boiled, with salt, drained
Sweetcorn, kernels, purchased frozen, boiled in brine, drained
Sweetcorn, kernels, purchased frozen, boiled, drained
Sweetcorn, kernels, canned in brine, drained
Potato, new, peeled, boiled
Potato, sebago, unpeeled, boiled
Potato, sebago, unpeeled, baked
Potato, pontiac, peeled, baked
Potato, desiree, peeled, baked
Potato, red skin, peeled, mashed with milk & butter
Potato, pale skin, peeled, mashed with milk & butter
Potato, coliban, peeled, boiled
Salad, potato, commercial

Green and brassica vegetables

Bean, green, fresh, boiled, drained
Bean, green, frozen, boiled, drained
Lettuce, iceberg, raw
Lettuce, mignonette, raw
Pea, green, frozen, boiled, drained
Broccoli, fresh, boiled, drained
Brussels sprout, fresh, boiled, drained
Cabbage, bok choy, stir-fried without oil
Cauliflower, boiled, drained
Snowpea, boiled, drained
Cabbage, savoy, boiled, drained
Salad, coleslaw, commercial

Orange vegetables

Carrot, baby, peeled, raw
Carrot, mature, peeled, boiled, drained
Carrot, baby, peeled, boiled, drained
Pumpkin, butternut, peeled, boiled
Pumpkin, peeled, baked

Legumes

Dhal (legume curry), Indian restaurant-style

Bean, red, kidney, canned, drained

Lentil, dried, boiled, drained

Baked beans, canned in tomato sauce

Baked beans, canned in tomato sauce, salt reduced

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

Sprout, alfalfa, raw

Avocado, raw

Beetroot, canned, drained

Capsicum, green, raw

Capsicum, red, raw

Celery, raw

Cucumber, common, unpeeled, raw

Cucumber, lebanese, unpeeled, raw

Mushroom, common, raw

Onion, mature, white skinned, peeled, raw

Tomato, common, raw

Tomato, cherry, raw

Tomato, hydroponic, raw

Tomato, whole, canned in tomato juice, boiled, drained

Tomato, whole, canned in tomato juice, boiled

Sprout, bean, raw

Mushroom, straw, Asian, canned in brine, drained

Capsicum, green, stir-fried without oil

Capsicum, red, stir-fried without oil

Celery, stir-fried without oil

Squash, button, boiled, drained

Mushroom, common, stir-fried without oil

Onion, mature, brown skinned, peeled, stir-fried without oil

Onion, mature, white skinned, peeled, stir-fried without oil

Zucchini, green skin, boiled

Fruit

Juice, apple, shelf stable, no added vitamin c

Juice, apple, shelf stable, added vitamin c

Juice, lemon
Juice, orange, home squeezed
Juice, orange, added vitamin c
Juice, orange, no added vitamin c
Banana, lady finger or sugar, peeled, raw
Orange, navel (washington), peeled, raw
Strawberry, raw

Mandarin (imperial), peeled, raw

Orange, valencia, peeled, raw

Orange, navel (all varieties), peeled, raw

Nectarine, unpeeled, raw

Peach, unpeeled, raw

Plum, unpeeled, raw

Apricot, dried

Pineapple (cayenne), peeled, raw

Grape, black muscatel, raw

Grape, thompson seedless or sultana, raw

Mango, peeled, raw

Kiwifruit, hayward, peeled, raw

Melon, rockmelon (cantaloupe), peeled, raw

Melon, watermelon, peeled, raw

Apple, red delicious, unpeeled, raw

Apple, red skin, unpeeled, raw

Apple, green skin, unpeeled, raw

Pear, packhams triumph, unpeeled, raw

Banana, cavendish, peeled, raw

Sultana

Wholegrain cereals

Wheat bran, unprocessed

Rice, brown, boiled, no added salt

Oats, rolled, boiled, no added salt

Bread, from wholemeal flour

Bread, from wholemeal flour, toasted

Bread, from wheat flour, added dried fruit, toasted

Bread roll, from wholemeal flour

Bread, mixed grain

Bread, mixed grain, toasted

Bread roll, mixed grain

Biscuit, savoury, wholemeal wheat flour, crispbread

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

Breakfast cereal, wheat bran, flakes, sultanas, added vitamins B1, B2, B3, B6 & folate, Fe & Zn

Breakfast cereal, mixed grain flakes (wheat, oats), added dried fruit, added vitamins B1, B2, B3 & folate & Fe

Breakfast cereal, mixed grain (wheat, corn, rice & oat), flakes, added dried fruit & nuts, added vit B1, B2, B3, C & folate, Ca & Fe

Muesli, untoasted or natural style, unfortified

Refined cereals

Pasta, white wheat flour, boiled from dry, with added salt

Pasta, white wheat flour, boiled from dry, no added salt

Rice, white, boiled, no added salt

Bread, from white flour

Bread, from white flour, toasted

Bread, flat (pita or Lebanese), white

Bread, from white flour, added fibre

Bread, from white flour, added fibre, toasted

Bread roll, from white flour

Crumpet, from white flour, toasted

Muffin, English style, from white flour, toasted

Breakfast cereal, flakes of corn, added vitamins B1, B2, B3, C & folate, Fe & Zn

Breakfast cereal, puffed or popped rice, 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

Pasta, vegetable filled, fresh, boiled, without added sauce

Poultry

Chicken, breast, lean, baked

Chicken, thigh, lean, baked

Chicken, barbecued, with skin

Chicken, breast, lean, skin & fat, baked

Chicken, breast, lean, grilled

Oily fish

Salmon, Atlantic, grilled

Salmon, red, canned in brine, drained

Salmon, pink, canned in brine, drained

Sardine, canned in tomato sauce

Salmon, Atlantic, steamed or poached

Non oily fish

Tuna, canned in water, added salt, drained

Tuna, flavoured, canned in water, added salt, drained

Prawn, king (large size), flesh only, purchased cooked

Fish, crumbed, purchased frozen, baked

Fish finger, crumbed, purchased frozen, grilled

Egg

Egg, chicken, whole, hard-boiled

Egg, chicken, whole, poached

Egg, chicken, whole, fried, peanut oil

Omelette, chicken egg, added butter

Egg, chicken, scrambled, no added fat

Red meat

Beef, sirloin steak, fully-trimmed, grilled

Beef, mince, low fat (lean/heart smart), dry fried (2008)

Beef, mince, regular, dried fried (2006)

Beef, casserole cuts, fully-trimmed, cooked

Lamb, leg roast, fully-trimmed, roasted

Low fat dairy

Milk, cow, fluid, skim (~0.15% fat)

Milk, cow, fluid, reduced fat (1.5%), increased Ca, folate & vitamin D

Milk, cow, fluid, reduced fat (1.5%), added Ca, Mg, Zn & vitamin D

Milk, cow, fluid, skim (~0.15% fat), added milk solids

Milk, cow, fluid, reduced fat (1%)

Yoghurt, low fat (<0.5%), apricot pieces or flavoured

Yoghurt, low fat (<0.5%), strawberry pieces or flavoured

Yoghurt, low fat (<0.5%), fruit pieces or flavoured, intense sweetened

High fat dairy

Cheese, cheddar, processed

Cheese, cheddar, regular fat

Cheese, swiss

Cheese, parmesan, shaved

Cheese, cheddar, reduced fat (~25%)

Unsaturated fats and oils

Margarine, polyunsaturated

Margarine, polyunsaturated, reduced salt (sodium = 300 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 (70% fat)

Margarine spread, polyunsaturated (70% fat), reduced salt (sodium = 380 mg/100g)

Margarine spread, polyunsaturated, reduced fat (60% fat), reduced salt

Oil, blend of polyunsaturated vegetable oils

Oil, soybean

Oil, sunflower

Oil, grapeseed

Oil, maize

Appendix 6: Effects of cooking on nutrients and contaminants

The effects of various cooking styles on grass carp (*Ctenopharynyodon Idellus*) fillets (fish with intermediate fat content of ~2%) were recently reported in an article by Zhang et al. (2013). The authors investigated the effects of six cooking styles including boiling, steaming, microwaving, grilling, pan-frying and deep-frying (both in soybean oil, a pre-dominantly polyunsaturated n-6 oil). There was significant moisture reductions in the fillets with steaming (3%), microwaving (11%), grilling (16%), pan-frying (37%) and deep-drying (42%). There was a small non-significant increase in moisture with boiling. Protein content increased significantly where there was significant moisture loss. There was a small and non-significant fat content reduction with boiling and steaming possibly due to leaching of the oil into the water. Fat content increased in microwaved, grilled, pan-fried and deep-fried fillets due to loss of water during cooking and absorption of the frying oil in the case of pan-frying and deep-frying. Boiling, steaming, microwaving and grilling only marginally affected fatty acids profile whereas pan-frying and deep-frying significantly changed the fatty acids composition and reflected those in the frying oil. The n-3/n-6 ratio also did not change with boiling, steaming, microwaving and grilling but reduced significantly with pan-frying and deep-frying.

In another 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). Total lipid content increased the most by deep-frying through the addition of sunflower oil but the percentage change was much lower than that observed in the study by Zhang et al. (2013). 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.

In an Australian study by Mooney et al. (2002), cooking (including pan frying, deep frying , grilling, steaming and microwaving) did not affect LCn3PUFA content in blue-eye trevalla, spikey dogfish or gummy shark. Deep-frying showed the highest fat content followed by pan-frying. The saturated fat/mono-unsaturated fat/poly-unsaturated fat profile of the samples reflected the composition of the cooking oil.

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.

Appendix 7: 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 100g) 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 8: 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 9: 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.