Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids in pregnant women living in New Zealand

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Abstract

Background/Aims: Adequate intakes of omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFAs) are required for fetal growth, brain development and to support a healthy pregnancy. This study aimed to investigate dietary intakes and food sources of n-6 and n-3 PUFAs in a cohort of New Zealand (NZ) pregnant women.

Method: Pregnant women (n=596) in their third trimester of pregnancy from throughout NZ completed an online validated FFQ to assess PUFA intakes over the past three months. Individual and combined intakes of the main PUFAs (linoleic acid, LA; alpha linolenic acid, ALA; arachidonic acid, AA; eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA) were compared with dietary recommendations using frequency summary statistics.

Results: Estimated median [25th, 75th percentile] intakes were: 11,580 [8,840, 15,760]mg/d LA (recommended 10,000mg/d), 1,300 [790, 2,120]mg/d ALA (recommended 1,000mg/d), 90 [60, 110]mg/d AA (upper limit 800mg/d), 180 [90, 460]mg/d total n-3 LC-PUFA (EPA plus DHA) (recommended 500mg/d), 60 [30, 190]mg/d EPA (recommended 220mg/d, and 110 [50, 250]mg/d DHA (recommended 200mg/d), with 30.9% of participants consuming more than 200mg/d DHA. Participants taking PUFA supplements (19.6%) had median intakes of 370 [210, 530]mg/d DHA, with 79.5% meeting DHA recommendations. Participants taking PUFA supplements were 16.5 times more likely to meet recommendations for DHA compared to participants not taking supplements. For participants not taking PUFA supplements (80.4%), DHA intakes were 90 [50, 160]mg/d and only 19% met the recommendations. Across all women fish and seafood were the main contributors of DHA (84.8%) and EPA (82.1%) intakes, yet only 9.5% and 12.2% of women consumed canned fish or fresh/frozen fish respectively at least twice per week. Over half of participants reported intakes of poultry (63.1%) and beef (60.8%) at least twice per week. Red meats and poultry

(36.8%) alongside eggs (23.3%) were important sources of AA intakes. Fats and oils largely contributed to LA (43.2%) and ALA (55.7%) intakes.

Conclusion: The majority of pregnant women did not meet the recommended intakes for DHA, which may be in part due to low fish/seafood intakes. Women taking PUFA supplements were more likely to meet these recommendations. These findings highlight the need for nutrition advice on the benefits of consuming n-3 LC-PUFA rich foods such as fish/seafood during pregnancy.

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This thesis would not have been completed without the support of several people which I would like to acknowledge here. Firstly, I would like to thank all the pregnant women who donated their precious time to complete this study. Without these women this thesis would not exist.

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Finally, to my lovely partner, who inspired me to chase my dreams and that we have only one life to get it right. Thanks for doing all the delicious cooking and looking after the house chores during the final stages of writing up my thesis. I will be forever grateful for your patience, love and support during my studies.

Table of Contents

List of Tables

List of Figures

List of Appendices

Abbreviations

AA - Arachidonic Acid

AFFSA - French Food Safety Agency

AI - Adequate Intake

ALA - Alpha-Linolenic Acid

AMDR - Acceptable Macronutrient Distribution Range

Aust-PUFA FFQ - Australian Polyunsaturated Fatty Acids Food Frequency Questionnaire

CNS - Central Nervous System

DHA - Docosahexaenoic Acid

DHQ - Diet History Questionnaire

DPA - Docosapentaenoic Acid

EFA - Essential Fatty Acid

EPA - Eicosapentaenoic Acid

FABMpm - Membrane Fatty Acid Binding Proteins

FABPs - Fatty Acid Binding Proteins

FADS - Fatty Acid Desaturase

FAO & WHO - Food and Agriculture Organization of the United Nations and World Health Organization

FAT - Fatty Acid Transport Proteins

FFA - Free Fatty Acids

FFQ - Food Frequency Questionnaire

FR - Food Record

GDM - Gestational Diabetes Mellitus

GH - Gestational Hypertension

HBP - High Blood Pressure

HDL - High-Density Lipoprotein

IQ - Intelligence Quotient

ISSFAL - International Society for the Study of Fatty Acids and Lipids

IUGR - Intrauterine Growth Restriction

LA - Linoleic Acid

LC-PUFA - Long Chain Polyunsaturated Fatty Acid

LDL - Low-Density Lipoprotein.

LXR - Liver X Receptor

n-3 - Omega-3

n-6 - Omega-6

NHMRC - National Health and Medical Research Council

NNS NZ – New Zealand National Nutrition Survey

NRV - Nutrient Reference Values

NOAEL – No Observed Adverse Effect Level

NZ – New Zealand

NZ-PUFA FFQ - New Zealand Polyunsaturated Fatty Acids Food Frequency Questionnaire

PE - Preeclampsia

PERILIP - Perinatal Lipid Intake Working Group

PND - Postnatal Depression

PPARs - Peroxisome Proliferator-Activated Receptors

PTWI - Provisional Tolerable Weekly Intakes

PUFA - Polyunsaturated Fatty Acids

RCT - Randomised Control Trial

RDI - Recommended Dietary Intake

SDT - Suggested Dietary Target

SGA - Small for Gestational Age

SREBP - Sterol Regulatory Element-Binding Protein

TAGs - Triacylglycerols

UL - Upper Level of Intake

VLDL - Very Low-Density Lipoprotein

Chapter One - Introduction

My sister in her last trimester of pregnancy. One of many pregnant women who inspired me to conduct this research.

1.1 Background

The 1,000 days spanning from conception to two years of life is a critical period of time when optimal nutrition can impact on lifelong health and development, with pregnancy being a crucial part of this journey (Black et al.; Christian, Mullany, Hurley, Katz, & Black, 2015). A constant supply of key nutrients such as omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFAs) during pregnancy is essential to fetal growth, development of the immune and central nervous systems (Innis & Friesen, 2008; Koletzko et al., 2008), for maintenance of a healthy gestation and normal parturition (Allen & Harris, 2001; Lewin et al., 2005; Makrides, 2009b). However, dietary intakes of n-6 and n-3 PUFAs in developed countries may be inadequate for optimal health outcomes (Bernard et al., 2013; Cosatto, Else, & Meyer, 2010; Denomme, Stark, & Holub, 2005; Donahue et al., 2009; Friesen & Innis, 2009, 2010; Jia et al., 2015; Thomas, Ghebremeskel, Lowy, Crawford, & Offley-Shore, 2006).

Omega-6 PUFAs, represented by linoleic acid (LA) and the long chain (LC) PUFA arachidonic acid (AA), are widely spread in most foods consumed in developed countries (Calder et al., 2010a; Stanley et al., 2007). In contrast n-3 PUFAs are found only in a few foods such as fish and seafood, the richest sources of LC-PUFAs (eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA), and in a minority of vegetable sources that mainly supply alpha-linolenic acid (ALA) (Gebauer, Psota, Harris, & Kris-Etherton, 2006; Ratnayake & Galli, 2009; Sanders, 2014). Pregnant women may decrease their intakes of fish and seafood due to corncerns regarding food safety and fetotoxic effects of environmental contaminants (Bloomingdale et al., 2010; Mozaffarian & Rimm, 2006). Although country specific advisory recommendations suggest that pregnant women can safely eat certain fish and seafood on a weekly basis as a means of increasing their intake of n-3 LC-PUFAs (Bonham et al., 2008; Kris-

Etherton, Grieger, & Etherton, 2009), many women misinterpret the advice and assume that avoiding fish and seafood is a safer option (Smith & Sahyoun, 2005). This suggests that these women may not be fully aware of the important roles of n-3 LC-PUFAs during pregnancy (Emmett, Akkersdyk, Yeatman, & Meyer, 2013; Sinikovic, Yeatman, Cameron, & Meyer, 2009).

Both LA and ALA are essential fatty acids (EFA) as the human body is not capable of synthesising them endogenously, and therefore they must be obtained in the diet (Burdge & Calder, 2006; Ratnayake & Galli, 2009). These EFA are mostly required for the synthesis of LC-PUFAs, which are important for normal growth, development and physiological functioning of the human body (Cetin, Alvino, & Cardellicchio, 2009; Dutta-Roy, 2000; Jordan, 2010; Le, Meisel, de Meijer, Gura, & Puder, 2009). Although, metabolic adaptations that occur during pregnancy can upregulate maternal ability to convert EFA into LC-PUFAs (Giltay, Gooren, Toorians, Katan, & Zock, 2004), this pathway is limited and may be insufficient to make up for the increased demands imposed by fetal growth and development (Burdge, 2006). In addition, the developing fetus is unable to synthesise LC-PUFAs because of their immature and most likely inactive pathways, therefore the fetus is dependent on maternal LC-PUFA supplies (Koletzko et al., 2008). Thus, LC-PUFAs, particularly DHA and AA, are considered conditionally essential during pregnancy and for this reason they must be obtained from dietary sources (Cunnane, 2000).

To meet fetal PUFA requirements during pregnancy, maternal metabolism undergoes a series of metabolic adaptations, which result in increased circulating levels of n-6 and n-3 LC-PUFAs (Herrera & Ortega-Senovilla, 2014). From the maternal circulation, these fatty acids are selectively taken up by the placenta and transferred to the developing fetus (Brenna & Lapillonne, 2009; Campbell, Gordon, & Dutta-Roy, 1998; Haggarty,

Ashton, Joynson, Abramovich, & Page, 1999; Ruyle, Connor, Anderson, & Lowensohn, 1990). Because of this selective PUFA uptake it is suggested that fetal requirements are met at the expense of maternal requirements thereby leaving the mother at risk of poor PUFA status up until delivery (Al, Van Houwelingen, & Hornstra, 1997; Bonham et al., 2008). Maternal PUFA status may take up to a year to fully recover, which may have implications for maternal and fetal status in subsequent pregnancies (Hornstra, 2000; Makrides & Gibson, 2000; Zeijdner, Houwelingen, Kester, & Hornstra, 1997).

Extensive amounts of DHA and AA are rapidly incorporated into the developing brain, retina and fat tissues of the fetus (Stillwell & Wassall, 2003). Within the central nervous system DHA promotes flexibility of synaptic membranes, neurotransmission, and regulation of gene expression, all of which will support the development and coordination of cognitive functions (Innis, 2007a; Innis & Friesen, 2008; Uauy & Dangour, 2006). Arachidonic acid is an important secondary messenger and plays a crucial role in fetal growth and maturation of numerous physiological functions, including the gastrointestinal tract and immune system (Hadders-Algra, Bouwstra, Van Goor, Dijck-Brouwer, & Muskiet, 2007; Innis, 2003, 2007b). Consequently, the developing fetus is reliant on maternal provision of LC-PUFAs and hence an inadequate supply of these key nutrients can impact perinatal growth (Mitchell et al., 2004; Olsen et al., 1986; Van Eijsden, Hornstra, Van Der Wal, Vrijkotte, & Bonsel, 2008) and development of neurological (Hibbeln et al., 2007; Innis & Friesen, 2008), and immune functions (Krauss-Etschmann et al., 2007; Kremmyda et al., 2011; Sausenthaler et al., 2007).

In addition to supporting the growth and development of the fetus there is emerging evidence that the intakes of n-3 LC-PUFAs are inversely associated with a range of adverse outcomes during pregnancy. These

include gestational diabetes mellitus (Feskens, Bowles, & Kromhout, 1991; Poniedzialek-Czajkowska, Mierzynski, Kimber-Trojnar, Leszczynska-Gorzelak, & Oleszczuk, 2014), gestational hypertension and preeclampsia (Genuis & Schwalfenberg, 2006; Williams et al., 2006), and maternal mood disorders (Golding, Steer, Emmett, Davis, & Hibbeln, 2009; Hibbeln et al., 2003; Sontrop, Avison, Evers, Speechley, & Campbell, 2008; Vaz et al., 2013). However, evidence remains inconclusive as randomised control trials have failed to show a dose-response level that would prevent the development of these adverse effects during pregnancy (Imhoff-Kunsch, Briggs, Goldenberg, & Ramakrishnan, 2012; Mozurkewich et al., 2013; Poniedzialek-Czajkowska et al., 2014). However, improved gestational duration and decreased risk of early preterm delivery have been reported in several systematic reviews and meta-analysis (Giuseppe, Roggi, & Cena, 2014; Larqué, Gil-Sánchez, Prieto-Sánchez, & Koletzko, 2012; Makrides, Duley, & Olsen, 2006; Salvig & Lamont, 2011; Szajewska, Horvath, & Koletzko, 2006).

Although observational studies have identified an association between intakes of at least two weekly servings of fish during pregnancy and improved measures of neurodevelopment in infants and children, meta-analyses of several PUFA supplementation trials report evidence is inconsistent to explain improved measures of neurodevelopment (Giuseppe et al., 2014; Gould, Smithers, & Makrides, 2013; Imhoff-Kunsch et al., 2012). However, evidence suggests that n-3 LC-PUFA supplementation of mothers with low baseline PUFA status had greater beneficial effects on measures of motor activity, visual acuity, and cognitive functions in term infants compared to mother with normal PUFA status (Simmer et al., 2009). Positive associations were also reported between maternal n-3 LC-PUFA supplementation and reduced risk of several allergic conditions (Klemens, Berman, & Mozurkewich, 2011; Kremmyda et

al., 2011). However, research investigating the relationship between n-3 LC-PUFAs and atopic disease is an emerging area of research which is still in its early stages.

Based on the current evidence, many international organisations recommend that pregnant women should aim to achieve at least 200mg of DHA per day, with combined DHA plus EPA recommendations ranging from 300 to 500mg per day (AFFSA, 2010; Food and Agriculture Organization of the United Nations and World Health Organization, 2010; Koletzko et al., 2008; Simmer et al., 2009; Simopoulos et al., 2000). These recommended levels can be obtained from the intakes of at least two servings of fish per week (Koletzko, Cetin, & Brenna, 2007a). In New Zealand (NZ) and Australia, the National Health and Medical Research Council (NHMRC) recommend a daily adequate intake (AI) of 115mg of combined DHA, DPA and EPA for pregnant women. This recommendation was considered to be adequate for pregant women based of the available evidence at the time and the usual mean intake of women without apparent PUFA deficiency with an additional 25% to cover pregnancy requirements (Ministry of Health, 2006). However, the NHMRC also set a suggested dietary target (SDT) of 430mg per day EPA, DPA plus DHA for prevention of chronic disease, which has no distinction between pregnant or non-pregnant women and is consistent with recommendations from international organisations (NHMRC, 2006). Therefore, it may be prudent that pregnant women in NZ aim to meet these SDTs.

Many pregnant women in other developed countries are not meeting the recommendated intakes of n-3 LC-PUFAs during pregnancy (Bernard et al., 2013; Cosatto et al., 2010; Denomme et al., 2005; Donahue et al., 2009; Friesen & Innis, 2009, 2010; Jia et al., 2015; Thomas et al., 2006). In NZ, there is limited information about dietary intakes and food sources of n-

6 and n-3 PUFAs in pregnant women and it is unknown whether they are meeting recommended intakes.

1.2 Purpose of the study

According to worldwide research, many pregnant women are failing to meet the recommended intakes for n-6 and n-3 PUFAs (Bernard et al., 2013; Cosatto et al., 2010; Denomme et al., 2005; Donahue et al., 2009; Friesen & Innis, 2009, 2010; Jia et al., 2015; Thomas et al., 2006). In New Zealand there is limited information about food sources and dietary intakes of n-6 and n-3 PUFAs in pregnant women. Hence, it is unknown whether dietary recommendations for these key nutrients are being met. A validated food frequency questionnaire (FFQ) has been recently adapted and validated to assess n-6 and n-3 PUFA intakes in the NZ adult populations (Ingram, Stonehouse, Russell, Meyer, & Kruger, 2012). This FFQ will be used in this study, to investigate dietary intakes and food sources of n-6 and n-3 PUFAs in pregnant women living in NZ.

Results from this study are expected to provide a snapshot of current food sources and dietary intakes of n-6 and n-3 PUFAs in this population group. These findings may provide the justification for further studies and interventions during pregnancy that focus on the prevention of inadequate intakes of PUFAs.

1.3 Aim

To investigate dietary intakes and food sources of omega-6 and omega-3 PUFAs in a convenience sample of pregnant women living in New Zealand.

1.3.1 Objectives

1. To assess dietary intakes of omega-6 and omega-3 PUFAs in pregnant women.

2. To compare omega-6 and omega-3 PUFAs intakes of pregnant women with recommended intakes.

3. To compare omega-3 PUFA intakes between participants according to the consumption of PUFA supplements.

4. To determine the food sources of omega-6 and omega-3 PUFAs in pregnant women.

1.4 Thesis structure

This chapter of the thesis contextualises the study and highlights the importance of the research. Chapter two provides a review of the current literature relating to omega-6 and omega-3 PUFAs, including their metabolism, key roles, requirements during pregnancy, recommended intakes and barriers to achieving the recommended levels as well as the evidence-based research showing the impacts of PUFAs on birth outcomes. This chapter also explores worldwide studies that have investigated food sources and dietary intakes in pregnant women, and methods used to determine dietary intakes of PUFAs.

The methodology used for this research is described in chapter three. This chapter explains all procedures used for recruitment, determination of sample size and study population, research tools, data management and statistical analysis used in this study.

Dietary intakes of n-6 and n-3 PUFAs are described and compared to national and international PUFA recommendations for pregnant women, within the results chapter (Chapter four). Other results including food

sources of n-6 and n-3 PUFAs and study population characteristics will be also included in this chapter.

The findings of this study are discussed in further detail in chapter five. This chapter also presents how the findings of this study contribute to the research literature and how they compare with findings from other studies investigating food sources and dietary intakes of n-6 and n-3 PUFAs in pregnant women. Chapter five also includes a summary of findings, as well as strength and limitations of this study. Final conclusions and recommendations for future research are provided.

1.5 Researchers' contributions

Researcher	**Contributions**
Michele Eickstaedt	Main researcher and author of this thesis. Applied for ethics, designed and conducted the research, developed online questionnaires (demographics, diet and health questionnaire on Survey Monkey), recruited participants, sorted and statistically analysed data, interpreted and discussed the results.
Dr Cath Conlon	Main academic supervisor. Applied for ethics, helped designing the research protocols and questionnaires, assisted with interpretation of results, and revised the thesis
Dr Kathryn Beck	Academic supervisor. Assisted with for ethics application and designing the research protocols and questionnaires. Assisted with sample calculation, statistical data analysis, interpretation of results, and revised the thesis.
Owen Mugridge	Developed the study webpage and assisted with developing the online questionnaires (Survey Monkey).
PC Tong	Assisted with data handling.
Matt Levin, Peter Jeffery and Steve Chalmers	Helped with all technical issues related to the online PUFA database and PUFA FFQ.

Chapter Two - Literature Review

My beloved nephew Henrique, when he was one month old.

2.1 Introduction

To appreciate the role of omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFAs) during pregnancy, it is important to understand how these nutrients influence physiological functions that maintain health integrity. Therefore, this chapter will present an overview of the metabolism and key roles of n-6 and n-3 PUFAs in the body. Literature searches were conducted between June 2014 and December 2015 using electronic databases Medline (OvidSP) (Ovid MEDLINE® 1946 to Present update), Web of Science (1900 – 2014), Scopus and Google Scholar for articles published in English. Key terms used PUFA, fatty acids, omega-3, omega-6, docosahexaenoic, dietary intakes, pregnancy, fetal development, perinatal growth, cognitive functions, postnatal depression, gestational diabetes mellitus and preeclampsia. Bibliographic references from papers published by key authors were also used to identify other important papers.

2.2 Overview of omega-6 and omega-3 polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFAs) are a class of lipids represented by two major families known as omega-6 (n-6) and omega-3 (n-3), which are widely found in the diet and human body (Dutta-Roy, 2000; Ratnayake & Galli, 2009). These PUFAs are made of large carbon chains with a methyl group (H_3C) at one end and a carboxyl group (COOH) at the other (Germann & Stanfield, 2005; Patterson, Wall, Fitzgerald, Ross, & Stanton, 2012; Ruxton, Reed, Simpson, & Millington, 2007). Each pair of carbons in the chain are either connected by single bonds, which are saturated with four atoms of hydrogen, or double bonds, which are formed in the absence of two hydrogen atoms (Mann & Truswell, 2012). The presence of two or more double bonds is the reason for the 'Polyunsaturated' terminology used to classify these fatty acids (Germann & Stanfield, 2005; Ruxton et

al., 2007). Figure 2.1 rpresents the structure of a polyunsaturated fatty acid chain and the location of the double bonds.

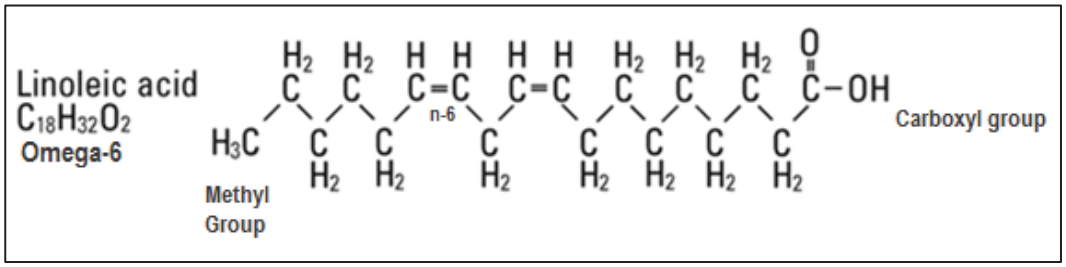

Figure 2.1 – Structure and position of double bonds in a polyunsaturated fatty acid commonly present in human's diet and body. Adapted from: (Mann & Truswell, 2012).

According to the location of the first double bond in the carbon chain, PUFAs are classified as either an n-6 or n-3 fatty acid (Cetin et al., 2009; Schmitz & Ecker, 2008). For example, n-3 fatty acids have the first double bond located at the third carbon of the hydrocarbon chain (counting from the methyl group end), whereas n-6 fatty acids have the first double bond located at the sixth carbon position (see Figure 2.1) (Adkins & Kelley, 2010; Holman, 1992). Moreover, the number of carbons and double bonds of a PUFA will also determine their nomenclature. The most prominent PUFA in human metabolism contain between 18 to 22 carbon atoms and two to six double bonds. These include alpha-linolenic acid (ALA, C18:3, n-3), eicosapentaenoic acid (EPA, C20:5, n-3), docosahexaenoic acid (DHA, C22:6, n-3), linoleic acid (LA, C18:2, n-6) and arachidonic acid (AA, C20:4, n-6) (Ratnayake & Galli, 2009; Stipanuk & Caudill, 2013) (Figure 2.2).

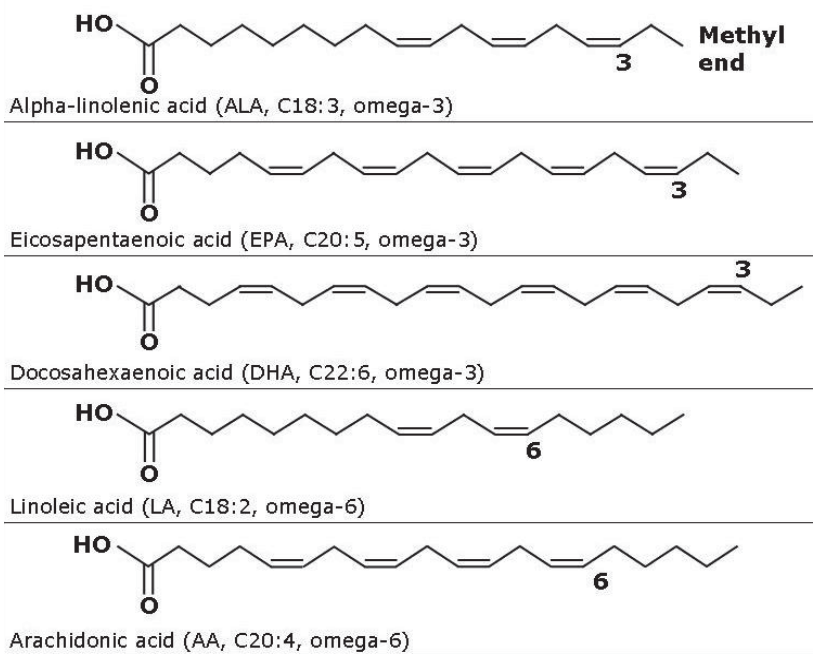

Figure 2.2 - Names and structure of polyunsaturated fatty acids relevant to human metabolism. Adapted from: (Ratnayake & Galli, 2009).

2.2.1 Essential fatty acids and long chain polyunsaturated fatty acids

Linoleic acid (LA) and α-Linolenic acid (ALA) are essential fatty acids (EFA), mostly required for the synthesis of long chain (LC) PUFAs, important for normal growth, development and physiological functioning of the human body (Cetin et al., 2009; Dutta-Roy, 2000; Jordan, 2010; Le et al., 2009). Humans are not able to synthesise EFA as they lack the enzymes required to produce the double bonds present in LA and ALA (Flachs, Rossmeisl, & Kopecky, 2014; Ratnayake & Galli, 2009). Therefore, these EFA must be obtained from dietary sources to maintain good health and prevent deficiency (Stipanuk & Caudill, 2013).

Both ALA and LA are comprised of an 18-carbon chain, which can be further converted into their corresponding 20- and 22-carbon LC-PUFAs. While LA can be converted into AA, ALA can be converted into EPA, DPA and DHA (Brenna, Salem, Sinclair, Cunnane, & Issfal, 2009; Cetin et al., 2009) (Figure 2.3).

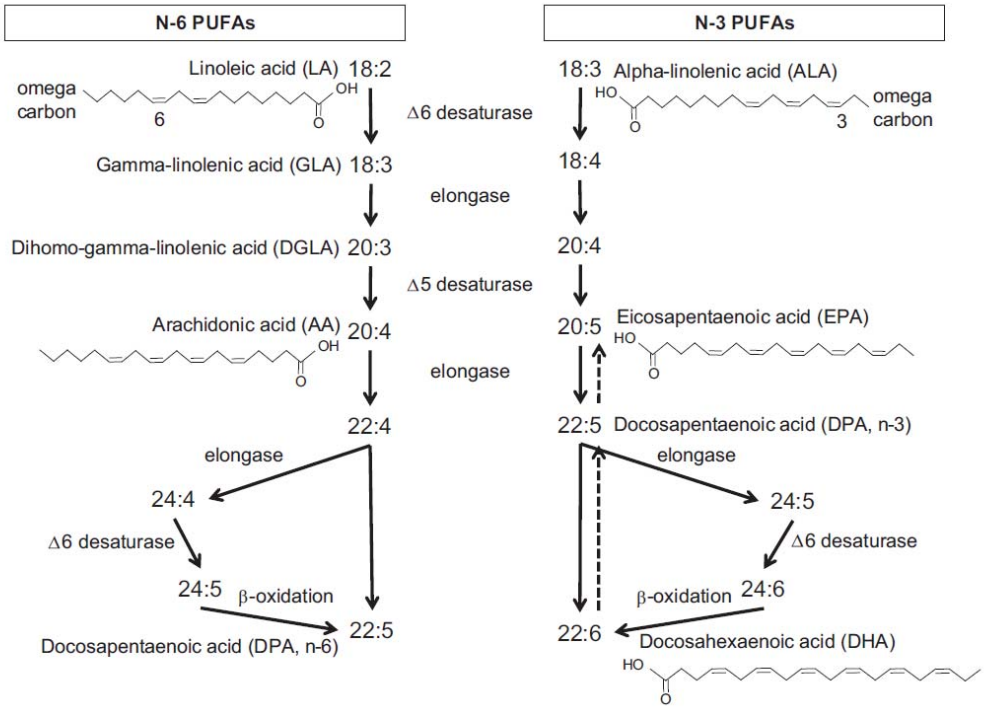

Figure 2.3 – Synthesis long chain polyunsaturated fatty acid from parent essential fatty acids. Source: (Liu, Green, John Mann, Rapoport, & Sublette, 2015).

The liver is the major site hosting the synthesis of LC-PUFAs (Burdge & Calder, 2006), which also occurs to a minor extent in other sites such as the human heart, placenta, brain, pancreas and kidney (Cho, Nakamura, & Clarke, 1999). The conversion process involves a series of enzymatic actions including, the introduction of extra double bonds (desaturation), and inclusion of two extra carbon atoms to the hydrocarbon chain (elongation) (Burdge & Calder, 2006; Innis, 2003; Schuchardt, Huss, Stauss-Grabo, & Hahn, 2010). Following elongation, a subsequent β-oxidation reaction takes place to shorten the very-long-chain intermediates formed during the elongation reaction (Adkins & Kelley, 2010).

The enzymatic pathway for the synthesis of LC-PUFAs is shared by both ALA and LA, therefore these fatty acids compete for the same enzymes (Adkins & Kelley, 2010; Burdge & Calder, 2006; Schuchardt et al., 2010).

Consequently, increased dietary intakes of one will impact on the metabolism of the other (Dutta-Roy, 2000; Flachs et al., 2014; Schmitz & Ecker, 2008; Zamaria, 2004). Although the desaturase enzyme (Δ6-desaturase) exhibits a higher preference for ALA, LA is widely available in the human diet, thereby negatively affecting the endogenous synthesis of n-3 LC-PUFAs (Calder et al., 2010a). The requirements for n-6 PUFAs seem to be fully satisfied with either endogenous synthesis or dietary intakes (Koletzko et al., 2007a; Stipanuk & Caudill, 2013).

Conversion rates for n-3 PUFAs are suggested to be extremely low, with estimated rates as low as 0.2% for EPA, 0.13% for DPA and 0.05% for DHA (Burdge & Calder, 2006). However, conversion rates were estimated to be up to 21% for EPA and 9% for DHA in women of childbearing age. The latter is attributed to the effect of female hormones such as oestrogen, which seems to up-regulate the activity of desaturase and elongase enzymes (Brenna & Lapillonne, 2009; Burdge & Calder, 2005; Extier et al., 2010; Williams & Burdge, 2006). The increased capacity to synthesize LC-PUFAs is of particular importance during pregnancy when both mother and fetus have higher demands particularly for DHA (Burdge & Calder, 2006). Despite this, it is still uncertain whether endogenous synthesis supplies adequate LC-PUFAs to meet both maternal and fetal requirements (Calder et al., 2010a; Cunnane, 2000). Thus LC-PUFAs, particulartly DHA may be considered conditionally essential during pregnancy, and therefore are required to be obtained from exogenous sources (Calder et al., 2010a; Cunnane, 2000; Le et al., 2009).

Several factors may impact on the synthesis of LC-PUFAs. These include stress hormones; increased intakes of *trans* and saturated fatty acids; low intakes of cofactors such as magnesium, iron, calcium, copper, zinc and B vitamins; high intakes of sucrose; low intakes of protein; inflammation, and smoking (Agostoni et al., 2008; Marangoni et al., 2004). Also, some

individuals may have polymorphisms in the fatty acid desaturase (FADS) gene cluster (responsible for the expression of desaturase enzymes), which limit the ability to convert EFA into LC-PUFAs (Lattka, Illig, Koletzko, & Heinrich, 2010; Marangoni et al., 2004; Saunders, Davis, & Garg, 2012; Schuchardt et al., 2010; Scientific Advisory Committee on Nutrition (SACN), 2004; Zamaria, 2004). These factors are important influences on the synthesis of LC-PUFA which need to be taken into consideration when investigating the PUFA status of individuals (Zamaria, 2004).

2.2.2 Metabolism of polyunsaturated fatty acids

Most naturally occurring fatty acids found in the human diet are derived from triacylglycerols (TAGs) (over 90%), with a small portion being derived from phospholipids (Amate, Gil, & Ramirez, 2002; Mu & Porsgaard, 2005). Triacylglycerols are hydrolised into free fatty acids (FFA) and monoglycerides (glycerol backbone with one fatty acid molecule) during the digestive process (Mann & Truswell, 2012). Free fatty acids and monoglycerides are then mostly absorbed into the enterocyte in the small intestine (~95%) (Armand, 2007). Once absorbed into the enterocytes, fatty acids and monoglyceride will be re-esterified into TAGs, and further incorporated into chylomicrons. The latter is a water soluble lipoprotein transporter comprised of cholesterol and TAGs (Stremmel, Pohl, Ring, & Herrmann, 2001). Chylomicrons are then released into the interstitial fluid, then to the lymphatic system, and eventually reaching the blood circulation (Burdge & Calder, 2005; Germann & Stanfield, 2005). The absorption mechanism is schematised in Figure 2.4.

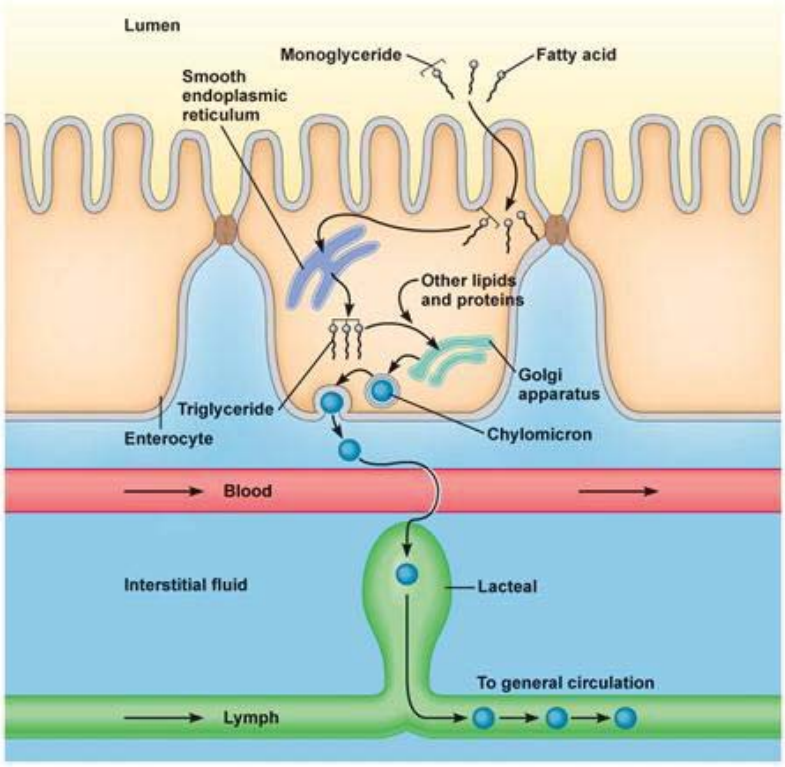

Figure 2.4 – Absorption of fatty acids. Source: (Germann & Stanfield, 2005).

Once in the blood circulation, chylomicrons are attacked by lipoprotein lipases that hydrolyse most of TAGs releasing FFAs (Burdge & Calder, 2006; Glatz, Luiken, & Bonen, 2010). These FFAs will then bind to albumin and remain circulating in the blood until cellular uptake occurs (Liu et al., 2015). Subsequent cellular uptake of fatty acids is dependent upon the cell's metabolic demands (Stremmel et al., 2001). The latter is mediated by fatty acid binding proteins (FABPs), which may also influence the fatty acid's transport, sequestration and intracellular metabolic utilisation (Glatz et al., 2010; Liu et al., 2015). Within the cells, EFA and LC-PUFAs will be used for the production of energy (β–oxidation) and incorporation into membrane phospholipid structure (Burdge & Calder, 2005; Green, Orr, & Bazinet, 2008). Furthermore, if fatty acids are not immediately utilised they can be re-esterified into TAGs and kept in cell storage pools for later utilisation (Burdge & Calder, 2006; Liu et al., 2015).

2.3 Key roles of polyunsaturated fatty acids

The primary role of LA and ALA is the synthesis of LC-PUFAs (Bourre, 2004). In addition, ALA is an important source: it crosses the blood brain barrier where most of it is oxidised, therefore facilitating fuel supply to the brain through ketogenesis (Freemantle et al., 2006). Furthermore, LA is involved in the synthesis of ceramides, which forms a protective skin barrier against fluid loss (Cunnane, 2000; Muskiet et al., 2006). It remains unclear whether ALA plays any other specific functional roles other than being a substrate for the synthesis of LC-PUFAs (Cunnane, 2000; Innis, 2003).

Omega-6 and omega-3 LC-PUFAs are important for forming multicellular organisms and maintaining their integrity (Arnoldussen & Kiliaan, 2014; Burdge & Calder, 2005; Calder, 2012; Cetin et al., 2009; Jordan, 2010; Muskiet et al., 2006; Stipanuk & Caudill, 2013). As key structural components, these LC-PUFAs influence the membrane's permeability, flexibility, turnover and renewal (Innis, 2007a). By influencing the membrane's physical properties, LC-PUFAs also impact the function of membrane protein complexes. These include membrane-binding receptors and transporters, ion channels, and other essential signalling pathways (Calder, Kremmyda, Vlachava, Noakes, & Miles, 2010b; Muskiet et al., 2006; Schmitz & Ecker, 2008). Protein complexes influence a range of biochemical mechanisms inside the cell as well as interactions between the cell and the extracellular environment (Bourre, 2004; Innis, 2003, 2007a; Kidd, 2007; Muskiet et al., 2006; Ramakrishnan, Imhoff-Kunsch, & DiGirolamo, 2009; Stillwell & Wassall, 2003). Biochemical mechanisms influenced by membrane phospholipid PUFA composition include information processing, signal transduction, synthesis of eicosanoids, β-oxidation and gene regulation circuits (Adkins & Kelley, 2010; Innis, 2003, 2007a; Le et al., 2009; Muskiet et al., 2006; Saltert & Tarling, 2007;

Schmitz & Ecker, 2008; Serhan, Chiang, & Van Dyke, 2008). These key processes influence several metabolic and physiologic pathways responsible for the maintenance of health integrity (Cetin et al., 2009).

Eicosanoids are synthesised as a normal response to physiological or pathological stimuli, such as an injury or acute infection (Calder, 2006). For example, n-6-derived prostaglandins and leukotrienes are eicosanoids that are crucial for establishing a homeostatic state after a pathogen insult, via modulation of both induction and resolution of inflammation (Ricciotti & FitzGerald, 2011). These prostaglandins and leukotrienes are also vital for the development of fetal thymus, an important organ of the immune system (Dutta-Roy, 2009). In addition, prostaglandins are also crucial on the onset of parturition as well as curbing both fetal movements and breathing during delivery (Challis, 1998; Challis et al., 2002; Keelan et al., 2003; Olson, 2003; Slater, Zervou, & Thornton, 2002). However, increased intakes of n-6 PUFAs combined with acute infections during pregnancy may produce large amounts of prostaglandins, potentially causing premature labour (Allen & Harris, 2001). In addition, increased dietary intakes of n-6 PUFAs may up-regulate the synthesis of n-6-derived eicosanoids, which can influence exacerbated cell proliferation, pro-aggregator, and inflammatory effects (Patterson et al., 2012; Simopoulos, 2006). These may influence the development of inflammatory-related diseases (Patterson et al., 2012; Ratnayake & Galli, 2009; Schmitz & Ecker, 2008; Serhan, Dalli, Colas, Winkler, & Chiang, 2015), including arthritis, age-related macular degeneration, cognitive dysfunctions, diabetes and coronary heart disease (CHD) (Adkins & Kelley, 2010; Burdge & Calder, 2006; Mozaffarian & Wu, 2011; Simopoulos, 2008).

Eicosanoids derived from EPA and DHA mainly exert anti-inflammatory effects that counterbalance the effects of AA-derived eicosanoids (Le et al., 2009; Schmitz & Ecker, 2008). Important functions of the n-3 LC-PUFA-

derived eicosanoids include anti-inflammatory, inflammation resolving, antioxidant, anti-platelet aggregation, vasodilation, anti-arrhythmic and neuroprotective effects (Adkins & Kelley, 2010; Calder, 2006; Serhan, 2010; Serhan et al., 2008). During pregnancy, eicosanoids derived from n-3 LC-PUFAs influence an increased uterine and placental vascular tone, which improves blood flow and the supply of nutrients and oxygen to both the placenta and fetus (Crawford, 2000). Docosapentaenoic acid (DPA) are also substrates for the synthesis of important eicosanoids with strong anti-platelet aggregation effects, however more studies are required to establish these effects (Kaur, Cameron-Smith, Garg, & Sinclair, 2011; Mann, O'Connell, Baldwin, Singh, & Meyer, 2010).

Both n-6 and n-3 LC-PUFAs can down or up-regulate the expression of certain genes involved in the metabolism of lipids and carbohydrates; partitioning of metabolic fuel; thermoregulation; cell differentiation; growth, and inflammatory response (Adkins & Kelley, 2010; Davidson, 2006; Deckelbaum, Worgall, & Seo, 2006; Muskiet et al., 2006; Sampath & Ntambi, 2004).

The effects of LC-PUFAs modulate the expression of genes that coordinate metabolic effects, such as increased glucose utilisation and partitioning of fatty acids towards oxidation instead of accumulation in TAG storage (Davidson, 2006; Flachs et al., 2014; Sampath & Ntambi, 2004, 2005). However, n-3 LC-PUFAs can more effectively activate the expression of genes involved in the above mechanisms compared to n-6 PUFAs (Patterson et al., 2012). Therefore increased dietary intakes of n-3 LC-PUFA may have an important role in the prevention or treatment of metabolic disorders, such as hyperlipidaemia, diabetes and cardiovascular disease (Calder, 2012; Mozaffarian & Wu, 2011; Muskiet et al., 2006; Patterson et al., 2012; Ristic-Medic & Vucic, 2013; Simopoulos, 2008).

Most of the beneficial effects exerted by n-3 and n-6 PUFAs seem to derive from their ability to influence changes on cell membrane properties, gene expression and synthesis of eicosanoids (Adkins & Kelley, 2010; Morse, 2012). The compilation of these effects impact on a range of important physiological responses including glucose uptake and utilisation; synthesis and clearance of LDL cholesterol and TAG; platelet aggregation, cell differentiation and proliferation; endothelial cell motility, and inflammatory responses (Burdge & Calder, 2006; Muskiet et al., 2006). Thus, a balanced intake of n-6 and n-3 PUFAs is crucial for optimal health (Russo, 2009).

Other protective effects attributed mainly to EPA and DHA in a range of health conditions have been investigated. These include cancer (Patterson & Georgel, 2014; Wang et al., 2014), bone health disorders (Högström, Nordström, & Nordström, 2007; Salari, Rezaie, Larijani, & Abdollahi, 2008), reproductive problems (Afeiche et al., 2014; Aksoy, Aksoy, Altinkaynak, Aydin, & Ozkan, 2006), and a range of cognitive disorders such as poor cognitive performance, age-related cognitive decline, Alzheimer's disease (Freund-Levi et al., 2006; Quinn et al., 2010; Sydenham, Dangour, & Lim, 2012), children's attention-deficit/hyperactivity disorder (ADHD) (Joffre, Nadjar, Lebbadi, Calon, & Laye, 2014), depression, mood disorders (Mozurkewich et al., 2013; Parker et al., 2006), and many psychiatric disturbances (Amminger et al., 2010; Mossaheb et al., 2012). Increased intakes of n-3 LC-PUFAs have been associated with improved cognitive development in infants (Koletzko et al., 2008) and an excellent recent review has critically evaluated the evidence from randomised controlled trials on the effects of LC PUFA's on cognitive functioning in children and adults (Stonehouse, 2014).

Although a considerable number of studies reported beneficial effects of increased intakes of n-3 PUFAs in numerous health conditions, greater effects were observed in individuals with lower intakes of these PUFAs

(Stonehouse, 2014). Therefore, positive findings cannot be generalized to the wider population. For this reason more well-designed studies are warranted to establish a n-3 LC-PUFA dose-response that can guarantee beneficial effects for both mental and physical health of all population groups (Burdge & Calder, 2005; Kornsteiner, Singer, & Elmadfa, 2008; Szajewska et al., 2006).

2.4 Omega-6 and omega-3 polyunsaturated fatty acids requirements during pregnancy

Because of the important biological and physiological effects of n-6 and n-3 LC-PUFAs (refer to Section 2.3), these nutrients are considered particularly important during pregnancy when their supply is in high demand (Haggarty, 2004; Jensen, 2006). In addition to meeting normal physiological demands for LC-PUFAs, increased amounts of these fatty acids are required during pregnancy to support the constant formation and growth of new tissues such as the placenta, mammary glands, uterus, expanded plasma volume and red blood cell mass, and most importantly the developing fetus (Faupel-Badger, Hsieh, Troisi, Lagiou, & Potischman, 2007; Innis, 2005; Kind, Moore, & Davies, 2006; Morse, 2012).

Extensive amounts of DHA and AA are rapidly incorporated into the developing brain, retina and fat tissues of the fetus (Stillwell & Wassall, 2003). Docosahexaenoic acid is involved in numerous roles within the central nervous system, such as flexibility and fluidity of synaptic membranes, neurotransmission, regulation of gene expression, and neuroprotective effects (Innis, 2007a). Arachidonic acid is an important secondary messenger and plays a crucial role in fetal growth and maturation of numerous physiological functions, including gastrointestinal tract and immune system (Hadders-Algra et al., 2007; Innis, 2003, 2007b).

While pregnant women may possess an increased ability to convert EFA into LC-PUFAs, this pathway is still limited and may be insufficient to make up for the increased pregnancy LC-PUFA requirements (Burdge, 2006). Therefore, EPA and DHA are considered conditionally essential during pregnancy and for this reason they must be obtained either from dietary sources or supplementation (Cunnane, 2000). In addition, the developing fetus is unable to synthesise LC-PUFAs because of their immature and most likely inactive brain and liver desaturase enzymes (Koletzko et al., 2008). Consequently, to meet the increased perinatal demands of LC-PUFA, the fetus relies on the mother for an effective and constant supply of these fatty acids via placental transfer (Dutta-Roy, 2009; Innis, 2005).

2.4.1 Placental transfer of polyunsaturated fatty acids

The placenta is a transient vascular organ that plays a crucial role in selecting and transferring key nutrients required for fetal growth and homeostasis as well as directing fetal waste products for elimination (Burton & Fowden, 2015; Cetin & Alvino, 2009; Dutta-Roy, 2009). Substantial placental growth is observed during the early stages of pregnancy which continues steadily up until term (Klingler, Demmelmair, Larque, & Koletzko, 2003). Approximately 88% of placental structural membrane phospholipids are comprised of PUFAs, with predominantly larger amounts of AA compared to DHA (Bitsanis, Ghebremeskel, Moodley, Crawford, & Djahanbakhch, 2006; Dutta-Roy, 2009; Klingler et al., 2003). Therefore, an adequate maternal LC-PUFA intake, stores and metabolism are required to support optimal placental growth and development (Cetin et al., 2009; Oey et al., 2003; Shekhawat et al., 2003).

In this regard, it is suggested that the placenta can not only influence the mobilisation of PUFAs from maternal stores to the circulation, but also increase PUFA uptake from maternal circulation depending on its own

metabolism as well as fetal needs (Grisaru-Granovsky, Samueloff, & Elstein, 2008; Herrera & Amusquivar, 2012). Although the mechanisms by which the placenta up-regulates PUFA uptake remain unclear, it is suggested that placental hormones may play important roles in fine tuning the maternal metabolism in order to ensure a constant supply of nutrients for both placenta and the fetus (Burton & Fowden, 2015).

Placental PUFA uptake and transfer seems to be an ultra-selective process that follows a descending order of PUFA preference. This preference order starts from highly unsaturated and longer chained fatty acids to the less unsaturated and smaller chained fatty acids (e.g. DHA>AA>ALA>LA) (Brenna & Lapillonne, 2009; Campbell et al., 1998; Haggarty et al., 1999; Ruyle et al., 1990). Besides controlling uptake and transfer of PUFAs to the fetus, the placental metabolism of PUFAs is suggested to be a substantial source of prostaglandins, important for maintenance and progression of a normal pregnancy as well as maturation of the immune system in the fetus (Section 2.3) (Challis, 1998; Challis et al., 2002; Keelan et al., 2003; Olson, 2003; Slater et al., 2002).

In brief, placental PUFA uptake, metabolism and transfer to the fetus are schematised in Figure 2.5. The placenta structure is composed of villous trophoblasts cells, with the brush border membrane facing the maternal side while the basal membrane faces the fetal side. A range of placental binding and transport proteins control the flow of fatty acids between maternal and fetal circulation (Cetin et al., 2009). Fatty acid transport proteins (FAT) are located in the membrane of the trophoblast cells, from where they conduct the transfer of free fatty acids (FFAs) from the maternal to fetal circulation. Fatty acid binding proteins (FABMpm) present in the membrane of trophoblast cells sequester LC-PUFAs from the maternal circulation, which are transferred into the cell and subsequently hydrolysed by placental lipases into FFAs (Dutta-Roy, 2009; Koletzko,

Larque, & Demmelmair, 2007b). Within the trophoblasts cells, FFAs are subsequently partioned into: β–oxidation for energy production; placental structural components; placental metabolism including synthesis of prostaglandins; lipid storage, and transfer via FAT to the basal membrane for further release into the fetal circulation (Cetin & Alvino, 2009; Cetin, Parisi, Berti, Mando, & Desoye, 2012; Herrera & Amusquivar, 2012). Specific fetal proteins such as α-fetoprotein as well as albumin bind FFAs in fetal circulation (Herrera, 2002). Following this, FFAs are taken up by fetal liver, for further re-esterification into TAGs and released back into the fetal circulation from where they reach the target tissues (Cetin et al., 2009).

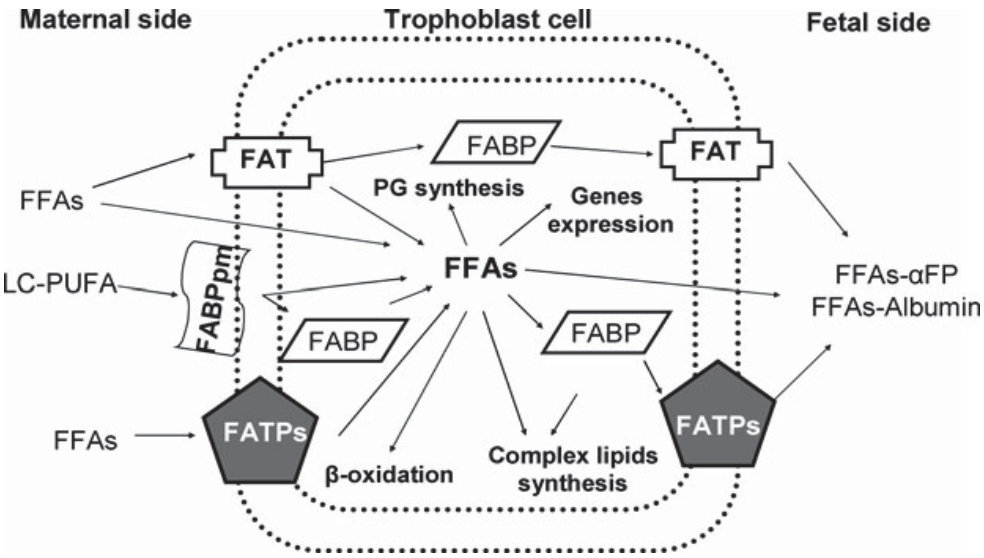

Figure 2.5 – Schematic model for placental PUFA uptake, metabolism and transfer to the fetus. Source (Cetin et al., 2009). **Abbreviations:** FABPpm, plasma membrane fatty acid-binding protein; FABP intracellular fatty acid-binding protein, FAT, fatty acid transport protein; PG, Prostaglandins.

Placental uptake and translocation of LC-PUFAs seem to be tightly regulated by a complex interplay between FABPpm, FABP, placental lipases and FAT (Hanebutt, Demmelmair, Schiessl, Larqué, & Koletzko, 2008; Herrera & Ortega-Senovilla, 2014). Interestingly, LC-PUFAs modulate expression of genes that activate these placental proteins

(Saltert & Tarling, 2007). For instance, LC-PUFAs are ligands for peroxisome proliferator-activated receptors (PPARs), which seem to influence placental uptake, transport and accumulation of PUFAs (Schaiff et al., 2005). Other nuclear transcription factors modulated by LC-PUFAs (e.g. Sterol regulatory element-binding protein – SREBP and liver X receptor - LXR) are suggested to up-regulate maternal endogenous synthesis of fatty acids and TAGs for further placental uptake (Weedon-Fekjaer, Duttaroy, & Nebb, 2005).

In summary, all these combined mechanisms, by which the placenta up-regulates the mobilisation of LC-PUFAs from maternal sources towards selective placental uptake and transfer to the fetus, form a perfect milieu that facilitates the accumulation of LC-PUFAs into the fetus at the expense of maternal supplies (Poniedzialek-Czajkowska et al., 2014). This phenomenon is denominated biomagnification, when substantial amounts of LC-PUFAs and little EFA are selectively transferred to the fetus leaving the maternal plasma with very low LC-PUFAs and high EFA levels (Berghaus, Demmelmair, & Koletzko, 1998; Haggarty, 2002; Montgomery, Speake, Cameron, Sattar, & Weaver, 2003).

It is possible that alterations in the gene cluster of proteins involved in the placental uptake, metabolism and translocation of PUFAs may cause metabolic changes that influence adverse outcomes (Hanebutt et al., 2008). These may include complications that range from placental dysfunction, reduced or exaggerated fetal growth, to more serious outcomes such as embryonic death (Cetin et al., 2002; Hanebutt et al., 2008). For instance, disruptions of placental fatty acid oxidation, such as faulty or low expression of enzymes involved in this process may affect energy production within the human placenta. The latter can impair placental function and consequently compromise fetal growth and development (Oey et al., 2003). In addition, placental metabolism and

transfer of LC-PUFAs may also be compromised by other pregnancy-related complications (Hanebutt et al., 2008), such as obesity, preeclampsia (PE), and gestational diabetes mellitus (GDM) (Bitsanis et al., 2006; Catalano, 2010; Cetin et al., 2012; Gauster et al., 2011; Leddy, Power, & Schulkin, 2008). These complications may influence changes on placental lipases and transport proteins activities, which can affect the amount of PUFA transferred to the fetus, and consequently impact on fetal growth and development (Gauster et al., 2007; Magnusson, Waterman, Wennergren, Jansson, & Powell, 2004).

2.4.2 Maternal metabolic adaptations

Appropriate fetal growth and development are reliant on adequate amounts of nutrients flowing through the placenta (Cetin, Alvino, Radaelli, & Pardi, 2005). The placenta influences a number of maternal metabolic adaptations that secure a continuous supply of nutrients to sustain fetal demands throughout the different stages of pregnancy (Dutta-Roy, 2009; Herrera, 2002). For instance, during the first half of pregnancy, placental-mediated hormones will fine tune maternal metabolism towards building up nutrient stores (Burton & Fowden, 2015). This is considered an anabolic stage, marked by hyperphagia, increased fat deposition and body weight gain (Herrera, 2002; Lain & Catalano, 2007). The nutrient stores formed during this anabolic stage will be accessed at a later stage of pregnancy, when increased nutrients supply is required to support exponential fetal growth and development (Cetin et al., 2012). From the second trimester onwards, a catabolic state starts to shape maternal metabolism, and major changes are observed in lipid and glucose metabolism in order to facilitate placental and fetal growth (Herrera & Ortega-Senovilla, 2014). Figure 2.6 schematizes the adaptation of maternal metabolism throughout pregnancy.

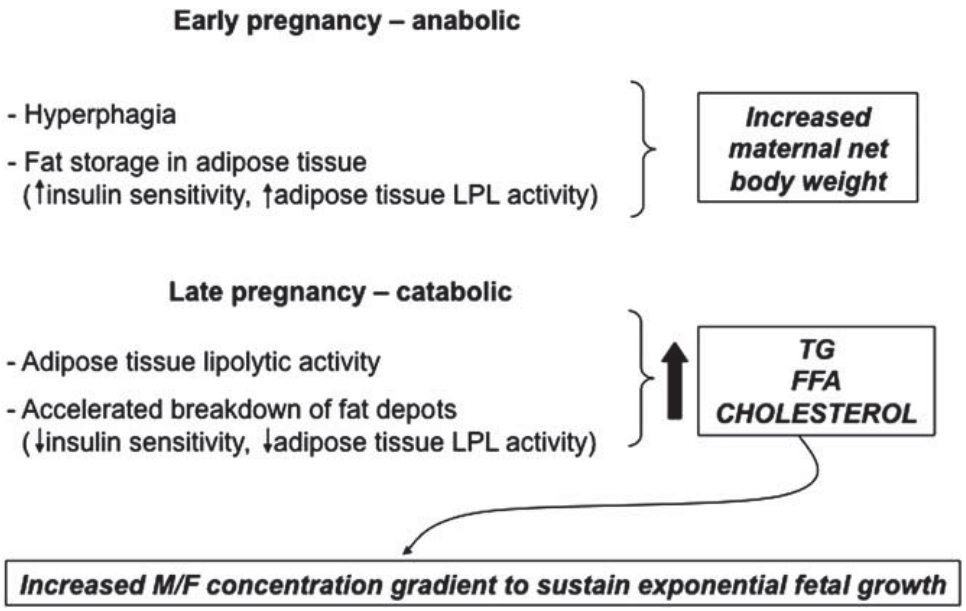

Figure 2.6 – Adaptations of maternal metabolism in favor of fetal growth and development. Source: (Cetin et al., 2012). **Abbreviations:** LPL, lipoprotein lipase; TG, triacylglycerol; FFA, free fatty acid.

Maternal metabolic adaptations throughout pregnancy are thought to be mediated by several placental hormones. These include progesterone, oestrogen, cortisol, growth hormone, leptin, lactogen and prolactin (Catov et al., 2007; Dutta-Roy, 2009; Herrera & Ortega-Senovilla, 2014). For instance, oestrogen levels seem to increase steadily during pregnancy up until birth, which may up-regulate the synthesis of LC-PUFAs (Giltay et al., 2004). Although the conversion of EFA to LC-PUFAs may be at increased rates during the progression of pregnancy, this process remains a minor pathway (Burdge & Calder, 2006; Burdge, Sherman, Ali, Wootton, & Jackson, 2006; Williams & Burdge, 2006). In addition, maternal conversion synthesis of LC-PUFAs may be further limited by polymorphisms in the FADS gene cluster (see Section 2.2.1) (Lattka et al., 2013). Increased leptin release is also observed during pregnancy. This hormone can up-regulate lipase activity, which increases lipolysis in maternal fat stores. As a result, free fatty acids (FFAs) will be available for further placental uptake and transfer to the fetus (Grisaru-Granovsky et al., 2008; Hanebutt et al.,

2008; Krauss-Etschmann et al., 2007). In addition, lactogen steadily increases during pregnancy and is suggested to induce insulin release and influence insulin resistance (Barbour et al., 2007).

With progression of pregnancy, maternal metabolic adaptations, will result in a well pronounced hyperlipidaemia, which include all lipid classes and keep on increasing up until delivery (Kirwan et al., 2002; Wada et al., 2010). In addition, insulin sensitivity will decrease by over half (50 - 70%) during the last stages of pregnancy compared to non-pregnant women (Butte, 2000; Nelson, Matthews, & Poston, 2010). Increased insulin resistance will also influence lipolysis, resulting in increased levels of FFA and TAGs in maternal circulation (Wada et al., 2010). From the maternal circulation, FFA and TAGs will be taken up by the liver for the synthesis of very low density lipoprotein (VLDL - highly impregnated with TAG). These VLDLs will then be released into the circulation for further placental uptake (Herrera, 2002). Figure 2.7 presents the major metabolic adaptations that occur with the progression of pregnancy.

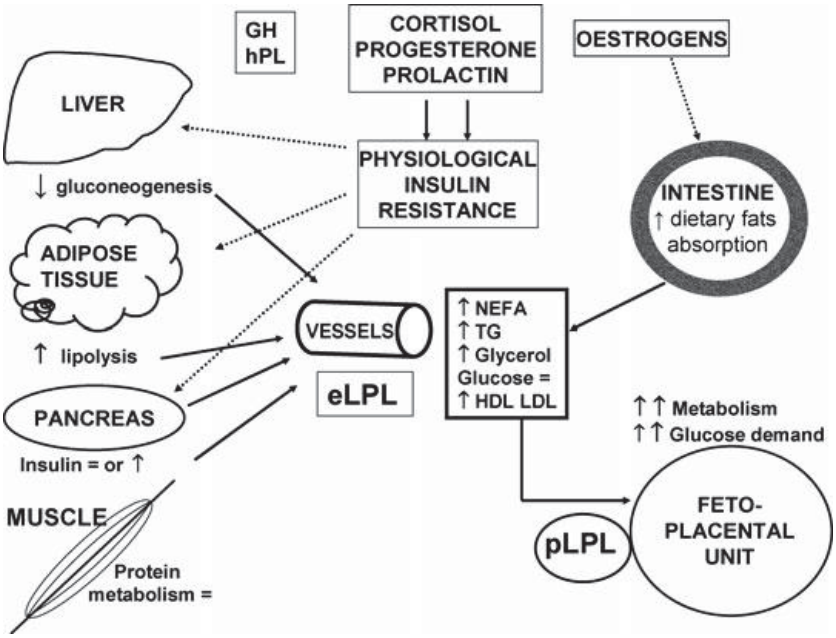

Figure 2.7 – Maternal metabolism adaptation during the third trimester of gestation – a catabolic state. Source: (Cetin et al., 2009). **Abbreviations**: eLPL, endothelial lipoprotein lipase; pLPL, placental lipoprotein lipase; NEFA, non-esterified fatty acids; TG, triacylglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

In summary, a combination of factors determines how well fetal PUFA requirements are met during the third trimester of pregnancy. These factors include the mother's health status and dietary quality; maternal fat stores formed during early pregnancy; an enhanced ability to convert EFA into LC-PUFAs; maternal endocrine status, metabolism and partitioning of PUFAs in storage, utilisation and circulation; and a perfectly functioning placenta (Haggarty, 2004; Williams & Burdge, 2006).

2.4.3 Maternal and fetal requirements and tissue incorporation

Maternal PUFA status is critical to secure optimal supply to the developing fetus. Therefore, maternal dietary intake must be sufficient to satisfy the mother's requirements as well as those of her fetus (Morse, 2012). During the first few weeks of pregnancy, fetal PUFA requirements seem to be low compared to the increased maternal fat deposition that occurs as a result of metabolic adaptations (Brenna & Lapillonne, 2009). However, PUFA requirements for the mother, the placenta and the fetus steadily increase from halfway through towards the final weeks of pregnancy (Herrera & Amusquivar, 2012; Morse, 2012; Otto, van Houwelingen, Badart-Smook, & Hornstra, 2001a).

The third trimester of pregnancy is a stage of rapid brain growth and extensive development, as well as formation of fat storage in the fetus (Joffre et al., 2014; Makrides, Smithers, & Gibson, 2010b). Therefore, an increased supply of LC-PUFAs is of particular importance during this stage (Stillwell & Wassall, 2003; Williams & Burdge, 2006). During this stage, substantial amounts of DHA are accreted in the fetal brain more efficiently and in preference to other PUFAs (Koletzko et al., 2007a). Docosahexaenoic acid is profoundly associated with fetal cognitive development during the last trimester of pregnancy (Uauy & Dangour, 2006). Therefore, infants that are born preterm may miss out on the full LC-

PUFA accretion during the last trimester of pregnancy, which may result in negative health and development outcomes (Fleith & Clandinin, 2005; Makrides et al., 2010b; Uauy & Dangour, 2006).

As opposed to DHA, little attention has been given to EPA since this PUFA does not seem to accumulate in fetal brain and retina. However, this fact does not discard the importance of EPA in the synthesis of eicosanoids which are important for maternal cardiovascular health and suppression of the inflammatory response (Klemens et al., 2011; Ryan et al., 2010).

In normal term healthy pregnancies, approximately 65% of the DHA is accumulated in fetal adipose tissues, while 30% is accreted in muscle mass, 3.9% in the brain, and as little as 0.7% in the liver (Brenna & Lapillonne, 2009). The vast stores of DHA in the adipose tissues are suggested to be enough to sustain the newborn fatty acid demands during the first three months of life. Therefore, these PUFA stores can be used as a buffer for the infant, during the changeover from placental to oral DHA supply (Burdge & Calder, 2006; Correia, Balseiro, Correia, Mota, & de Areia, 2004).

A total of 600g of PUFA is estimated to be transferred from maternal supplies to the fetus throughout a normal term pregnancy (over nine months), and this is translated to an average daily supply of 2.2g of PUFAs (Bourre, 2004; Uauy & Dangour, 2006). Autopsy studies have estimated that a daily amount of approximately 70mg of n-3 PUFA, mainly DHA, and 552mg of n-6 PUFA are accumulated in the brain and other tissues during the third trimester of pregnancy (Clandinin, Chappell, Heim, Swyer, & Chance, 1981; Clandinin et al., 1980; Innis, 2005).

Although these accretion rates may serve as the basis for establishing PUFA recommended intakes during pregnancy, they do not take into account any estimations for the mother's own PUFA needs for the

maintenance of normal physiological functions beyond placental and fetal requirements (Innis, 2003). Literature suggests that maternal PUFA accretion in adipose and placental tissues are substantially higher than the accretion demands imposed by the fetus (Brenna & Lapillonne, 2009; Makrides, 2009a). Therefore, studies have proposed that pregnant women may need to consume as much as 300mg/day DHA to provide for their needs as well as those demanded by fetal and placental PUFA accretion (Connor, Lowensohn, & Hatcher, 1996). However, an optimal level of dietary LC-PUFAs during pregnancy has not yet been identified. Thus, further studies are required to establish PUFA recommended intakes for an optimum health outcomes for both the pregnant women and their developing fetus (Flock, Harris, & Kris-Etherton, 2013).

2.5 Implications of omega-6 and omega-3 polyunsaturated fatty acids on maternal and fetal health outcomes

Both n-6 and n-3 LC-PUFAs exert fundamental roles as membrane structural components, in the synthesis of eicosanoids, and as a functional regulator in the expression of genes involved in cell differentiation and growth. These key roles are essential for normal fetal growth and development (Dutta-Roy, 2000; Uauy & Dangour, 2006; Uauy, Hoffman, Peirano, Birch, & Birch, 2001). Increased amounts of these LC-PUFAs, especially DHA, are required during the last trimester of pregnancy when fetal growth and brain development are accelerated (refer to Section 2.4.3).

Fetal LC-PUFA requirements appear to be met at the expense of maternal status, which may become relatively depleted up until birth. This discrepancy is of serious concern if maternal dietary intakes are insufficient (Al et al., 1997; Bonham et al., 2008). For example, pregnant women following diets poor in DHA-rich foods, such as diets excluding fish and seafood or exclusively vegetarian diets, are at risk of insufficient intakes of

these fatty acids (Hornstra, 2000; Makrides & Gibson, 2000). However, the literature suggests that maternal n-3 LC-PUFA status will decline during the last trimester of pregnancy, independent of increased intakes of fish and seafood. This fact, confirms that the rates of DHA accretion in fetal tissues are extremely high during the last trimester of gestation, which decreases maternal PUFA status (Bonham et al., 2008). Furthermore, maternal PUFA status can further decrease due to longer gestations, short birth intervals, increasing parity, and multiple pregnancies, and may only fully recover six months after delivery. This may have implications for maternal and fetal status in subsequent pregnancies (Hornstra, 2000; Makrides & Gibson, 2000; Zeijdner et al., 1997). Therefore, maternal dietary intakes of LC-PUFAs need to be adequate in order to meet both maternal and fetal requirements while preserving maternal PUFA stores (Abu-Saad & Fraser, 2010; Makrides & Gibson, 2000).

An accumulating number of studies demonstrate that an inadequate supply of n-6 and n-3 LC-PUFAs, during perinatal development has adverse effects on fetal structural and functional development, as well as somatic growth (Uauy & Dangour, 2006). These may result in long-term complications that can be important determinants of health and morbidity throughout life (Dutta-Roy, 2000; Innis, 2007a).

The most researched health effects associated with low LC-PUFA intakes focus on n-3 LC-PUFAs and include intrauterine growth and development of cognitive, visual and immune functions, which are mostly investigated after birth in infants and/or children (Brenna & Lapillonne, 2009). Similarly, a substantial number of studies have investigated associations of low n-3 LC-PUFAs with preterm birth, preeclampsia, gestational diabetes, and post-natal depression (Food and Agriculture Organization of the United Nations and World Health Organization, 2011; Jensen, 2006).

2.5.1 Fetal cognitive and visual development

Background

About 60% of the dry weight of the brain tissue is fat, where AA and DHA are the most abundant structural fatty acids (Klemens et al., 2011). As key structural components of phospholipid membranes, both AA and DHA play important roles in the neurogenesis, myelination, and synaptogenesis processes during fetal development (Uauy & Dangour, 2006). These roles include the formation and differentiation of cells as well as modulation of the transcription of genes responsible for the activation of lipid-binding proteins, secondary messengers, ion channels and signal reception (Innis, 2007b; Lukiw & Bazan, 2008; Uauy & Dangour, 2006). These actions are crucial in the process of neural message transmission and maturation of the central nervous system (CNS) functions (Innis & Uauy, 2003).

The speed of all of these processes is increased during the third trimester of pregnancy, thereby demanding large amounts of LC-PUFAs (Georgieff, 2007; Morse, 2012; Ryan et al., 2010). Substantial amounts of DHA are accumulated in the cerebral cortex (~40%), in membranes of synaptic communication centres and photoreceptors of the retina (~60%) (Dutta-Roy, 2000; Singh, 2005). Within the cortex, DHA mostly accumulates in the hippocampus and frontal lobes, which are areas of the brain responsible for attention, working and long-term memory, emotions, and inhibitory control (Gould, Makrides, Colombo, & Smithers, 2014). As a substantial component of the retina, DHA is crucial for the development, maturation and integrity of visual acuity (Innis & Friesen, 2008; Jacques et al., 2011). Within the photoreceptors of the retina, DHA enables the visual transduction system to convert the light received by the retina into visual images in the brain (Morse, 2012; Uauy et al., 2001). Moreover, n-3 LC-PUFAs seem to be important for maturation and protection of hearing (Bourre, 2004). Therefore, the developing brain is particularly vulnerable to

insufficient maternal n-3 LC-PUFA supply, and inadequate supply may negatively affect many functional outcomes (Burdge & Calder, 2006; Georgieff, 2007; Jordan, 2010).

Arachidonic acid is also largely present in the developing brain where it is crucial for the activation of secondary messengers (Kidd, 2007). Second messengers facilitate the transmission of signals between the membranes micro domains and the inner cell working mechanisms that are important for fetal growth and development (Klemens et al., 2011; Lukiw & Bazan, 2008).

Animal studies

Animal studies have established that low DHA intakes and maternal status can impact the structure of the developing fetal brain, which causes impaired neurogenesis and altered metabolism of several neurotransmitters, membrane receptor and protein activities; reduced gene expression, and impaired performance tasks of learning, memory and reduced visual acuity (Chalon, 2006; Innis, 2007a; Klemens, Salari, & Mozurkewich, 2012; Makrides et al., 2010b; McCann & Ames, 2005; Novak, Dyer, & Innis, 2008). Rat models demonstrated that maternal DHA dietary deprivation resulted in depletion of DHA from areas of the brain associated with affection and memory (Levant, Ozias, & Carlson, 2007). Decreased DHA in the hippocampus was also reported, and associated with altered dopamine and serotonin levels in certain brain areas, which increased the hypothalamic-pituitary axis response to stress, and disrupted sleeping patterns (Levant et al., 2008).

Human observational studies

In humans, pregnant women with low DHA status can give birth to infants with suboptimal DHA status (Hornstra, 2000; Otto et al., 2001a). Early post-mortem studies suggested that poor fetal DHA status influenced long-

term motor, cognitive, visual and behavioural problems. These include a disrupted sleep-wake cycle (Cheruku, Montgomery-Downs, Farkas, Thoman, & Lammi-Keefe, 2002), reduced visual acuity (Birch et al., 2005; Innis & Friesen, 2008; Makrides, Neumann, Byard, Simmer, & Gibson, 1994; Uauy et al., 2001), slower language development, reduced learning and attention, lower school performance and antisocial behaviours (Heird & Lapillonne, 2005; Hibbeln et al., 2007; Klemens et al., 2012; Novak et al., 2008).

In contrast, an increased supply of DHA to the developing fetus is associated with improved visual acuity and cognitive function (Dunstan, Simmer, Dixon, & Prescott, 2008; Uauy et al., 2001). Numerous cohort studies have identified associations between maternal intake of fish and seafood (important sources of DHA), and enhanced neurological development in children (Klemens et al., 2012; Morse, 2012). These studies including 341 to 25,466 mother-child pairs, found at least two servings of fish were positively associated with better motor, cognitive and behaviour development at 18 months (Oken et al., 2008a); higher intelligence quotient (IQ), behaviour and verbal development at 8 years of age (Hibbeln et al., 2007); higher child developmental scores at 15 and 18 months of age (Daniels, Longnecker, Rowland, Golding, & Team, 2004), and improved children's visual-motor abilities at 38 months (Oken et al., 2008b). In addition, higher consumption of fish and seafood by pregnant women, demonstrated beneficial effects on psychomotor performance at 11 months and better visual acuity performance at 6 months (Jacobson et al., 2008) and at school age (11.3 years) (Jacques et al., 2011). One to two serves of oily fish per week during the last trimester of pregnancy was associated with a reduced risk of hyperactivity and higher IQ scores in 9 year old children (n=217) (Gale et al., 2008). However, the same study also reported that intakes higher than three serves of oily fish per week did

not show further benefits. Thus, it is possible that the beneficial effects of oily fish intakes in this study may have been conterbalanced by increased exposure to environmental contaminants. Another study found that maternal intakes of other seafood, such as squid and shellfish, had an inverse association with measures of child neurodevelopment, which may be in part due to environmental contaminants present in these sources (Mendez et al., 2009).

Biomarker studies

Biomarker studies have shown strong associations between increased umbilical cord blood LC-PUFA composition and later neurological development (Lattka et al., 2013). It was reported that neurologically abnormal infants had lower DHA, AA and EFA composition in their cord blood compared to neurologically normal infants between 10 and 14 days after birth (Dijck-Brouwer et al., 2005). Maternal and cord DHA status significantly correlated with psychomotor scores and intra-daily variability of activity, and several measures of maturation of sleeping patterns at six months (Zornoza-Moreno et al., 2014). However, a study showed that maternal fish oil supplementation (500mg/d DHA plus 150mg/d EPA) had no significant differences in cord blood composition of DHA and AA of children diagnosed neurologically abnormal versus normal children at age four years (Escolano-Margarit et al., 2011). This study also reported that children with optimal neurological performance at age 5.5 years had significantly higher DHA concentrations in cord blood than children with suboptimal performance.

Randomised control trials, systematic reviews and meta-analysis

Randomised control trials (RCTs) have used a range of n-3 LC-PUFA doses and concentrations, and higher doses showing no evidence of harm to either mothers or their offspring (Morse, 2012). In 72 pregnant women

who were randomly assigned to consume 2,200mg/d of DHA or placebo from the second trimester of pregnancy until delivery, improved hand-eye coordination was observed in children (at 2.5years) born to supplemented mothers (Dunstan et al., 2008). However, a follow-up of these children (n=50) at 12 years old found no significant differences for measures of cognition, language and motor skills (Meldrum, Dunstan, Foster, Simmer, & Prescott, 2015). Another study supplied 300mg DHA in muesli bars consumed five times per week in pregnant women (n=15), from week 24 until delivery, with results showing improved problem solving in infants at nine months (Judge, Harel, & Lammi-Keefe, 2007). Further studies demonstrated DHA supplementation (800mg/d) from mid-pregnancy until delivery had no significant effects on measures of attention and working memory and inhibitory control in children aged 27 months (n=185) (Gould et al., 2014). Infants girls (60-days old) born to mothers (n=68) with low median DHA intakes had visual acuity below average compared to infants of mothers (n=67) receiving DHA supplementation (400mg/d) (Innis & Friesen, 2008). A later study found that supplementation with 800mg DHA plus 100mg EPA per day from mid-pregnancy had no effects on infant visual acuity measured at four months (n=182) (Smithers, Gibson, & Makrides, 2011). A large trial which provided either 800mg/d DHA or a placebo from mid-pregnancy until birth, found no significant difference bretween groups in cognitive and language development in 694 children at 18 months of age (Makrides et al., 2010a).

Although many trials found positive effects of maternal n-3 LC-PUFAs supplementation on measures of neurological development in infants and children, other studies have failed to show any positive results (Dunstan et al., 2008; Innis & Friesen, 2008; Judge et al., 2007; Makrides et al., 2010a; Meldrum et al., 2015; Smithers et al., 2011). These studies used a range of different tests, mostly designed to detect abnormal neurological

development of children, as well as different treatments during pregnancy (Cheatham, Colombo, & Carlson, 2006). Also, many trials had small samples sizes (15 to 185 participants), which may decrease the power of their findings. Therefore, it is impossible to generalise these findings to the wider population (Makrides et al., 2010a). Furthermore, meta-analysis and systematic reviews have concluded that there is not enough evidence to establish the link between maternal intakes of n-3 LC-PUFA supplements and improved children's cognitive and visual development. A recent meta-analysis, which included eleven studies (n=5,272), did not find consistent results to support the beneficial effects of maternal n-3 LC-PUFA supplementation and improved cognitive or visual development (Gould et al., 2013). Although, while there is no conclusive evidence of beneficial effects of dietary intakes/supplementation of n-3 LC-PUFA, there is good evidence showing positive associations of these PUFAs and visual acuity, motor activity, and general improved cognitive functions in term infants (Simmer et al., 2009).

2.5.2 Measures of intrauterine growth

Background

Intrauterine growth is normally determined using measures of birth weight, length, and head circumference. Infants born weighing less than 2,500g are classified as low birth weight, and below the 10^{th} percentile of the reference population are considered small for gestational age (SGA). Low birth weight and small for gestational age can be a result of intrauterine growth restriction (IUGR) (Van Eijsden et al., 2008).

The mechanisms elucidating IUGR are unclear, however impaired placental functionality, due to poor growth and development, seems to be associated with this discrepancy (Cetin et al., 2002; Hanebutt et al., 2008). Poor placental functionality can negatively impact on the rate of nutrients

and oxygen being transferred to the fetus, which may decrease fetal growth and development (Cetin & Alvino, 2009; Hanebutt et al., 2008; Sibley et al., 2005). This may happen in part due to alterations in lipases and the activity of transport proteins (refer to Section 2.4.1), which can impact on decreased supply of PUFAs to the fetus (Gauster et al., 2007). Placental lipase activity is suggested to be 47% less active in IUGR compared to normal pregnancies (Magnusson, Waterman, Wennergren, Jansson, & Powell, 2004). Therefore, the fetus with IUGR tends to have lower tissue concentration of PUFAs compared to fetuses with normal intrauterine growth rates at a similar gestational age (Hanebutt et al., 2008; Krauss-Etschmann et al., 2007). Consequently, IUGR infants are at higher risk premature birth, death, including disabilities and increased susceptibility to developing chronic diseases (e.g. diabetes, hypertension and cardiovascular diseases) later in life (Barker, Eriksson, Forsén, & Osmond, 2002; Cetin et al., 2002; Visentin et al., 2014)

Observational studies

Literature suggests that maternal n-3 LC-PUFAs status and dietary intakes of fish and seafood can influence measures of intrauterine growth in uncomplicated pregnancies (Olsen et al., 1986; Van Eijsden et al., 2008). A retrospective cohort in NZ, investigating maternal nutritional risk factors associated with SGA neonates (n=1,714) found that increased intake of fish was significantly associated with lower risk of giving birth to low birth weight neonates. In addition, this study also reported that despite low general fish consumption among participants (median intakes of 0.5 serves per week), even a modest fish intake was associated with a lower risk of having low birth weight neonates (Mitchell et al., 2004). A more recent study found that pregnant women (n= 2398) eating fish twice per week or more had a lower risk of having SGA infants compared to those eating fish less than once a month (Guldner, Monfort, Rouget, Garlantezec, & Cordier,

2007). Further studies also found a positive association of maternal fish and seafood intakes and birth size (Brantsaeter et al., 2012; Imhoff-Kunsch et al., 2012). However, a systematic review including 14 observational studies (n=114,006) found mixed results between fish intake (two to four servings per week) and measures of perinatal growth (Imhoff-Kunsch et al., 2012).

A Dutch cohort (n=3,380) found no consistent associations between maternal fish intakes and measures of fetal growth, other than a positive association between shellfish intake and low birth weight (Heppe et al., 2011). Another study assessed data from the Danish National Birth Cohort (n=44,824 between 12–30 weeks gestation) found that pregnant women having more than two servings of oily fish per week (≥60g/day) had a significantly higher risk of giving birth to SGA infants compared to women consuming less than one serving of fatty fish per week (≤5g/day). However, these may be due to high levels of environmental contaminants commonly present in oily fish. No negative associations were found for lean fish (Halldorsson, Meltzer, Thorsdottir, Knudsen, & Olsen, 2007).

Similarly, a Norwegian study (n=62,099) found that high maternal intakes of fatty fish (≥60g/day) had no or inverse associations with measures of birth size, but positive associations were reported between the intakes (≥60g/day) of lean fish and all measures of birth size, as well as shellfish and birth weight (Brantsaeter et al., 2012). Other studies have reported an inverse association between high intakes of seafood (over one serving per week) and SGA, however this association was observed among certain seafood species considered high in environmental contaminants (e.g. shellfish, crustaceous and canned tuna) (Guldner et al., 2007; Mendez et al., 2010; Oken, Kleinman, Olsen, Rich-Edwards, & Gillman, 2004). Thus, these studies suggest that the type and quantity of fish and seafood

consumed during pregnancy can impact on fetal measures of intrauterine growth.

Randomised control trials, systematic reviews and meta-analysis

Data from a large Australian trial (n=2,320) indicated that pregnant women supplemented with 800mg/d DHA from mid-pregnancy until birth had infants with mean birth weight 68g heavier and 35% less cases of low birth weight than control groups (Makrides et al., 2010a). A more recent trial (n=350), not included is this meta-analysis, found that pregnant women supplemented with 600mg/d DHA from mid-pregnancy gave birth to infants with higher birth weight (172g), head circumference (0.5cm) and infant length (0.7cm) compared to controls (Carlson et al., 2013). However, a meta-analysis of 15 large RCTs (n=1,456 to 7,038), which used supplementation containing DHA plus EPA (11 trials) or DHA alone (two trials) (doses ranging from 80 to 2,200mg/d), or EPA plus DHA-fortified foods (three trials), found no significant differences in birth length, head circumference, and risk of SGA or IUGR between groups (Imhoff-Kunsch et al., 2012).

Other meta-analyses and systematic reviews have revealed no significant differences in birth weight or SGA between supplemented groups versus controls (Horvath, Koletzko, & Szajewska, 2007; Makrides et al., 2006; Szajewska et al., 2006). Horvath et al. (2007) included four trials (n=1264) in their meta-analysis, which supplemented women who had high-risk pregnancies with either higher EPA concentrations compared to DHA (two trials), only EPA or n-6 PUFAs. No significant differences were observed in birth weight and SGA risk between supplemented and control groups. Szajewska and colleagues (2006) assessed six RCTs (n=1,278) that supplemented pregnant women with DHA alone (150 to 200mg/d) or DHA (920 to 1,183mg/d) and EPA (803 to 1,280mg/d) combined, with findings showing no significant difference in birth weight between groups. However,

data from four RCTs, included in the above meta-analysis, indicated that n-3 LC-PUFA supplementation may increase head circumference, but with small effect size (Helland et al., 2001; Malcolm, McCulloch, Montgomery, Shepherd, & Weaver, 2003; Smuts, Borod, Peeples, & Carlson, 2003a; Smuts et al., 2003b). Trials (n=2755) systematically reviewed by Makrides et al. (2006) supplemented pregnant women with marine oils (EPA plus DHA ranging from 133 to 3,000mg/d) from mid-pregnancy until birth, with data showing a small increase (~54g) in birth weight. However, a more recent meta-analysis including three important trials (n=1,187) which supplemented pregnant women with 2,700mg/d EPA plus DHA reported that birth weight was 71g higher for supplemented versus non-supplemented groups (Salvig & Lamont, 2011).

In summary, no significant differences in birth length, head circumference or risk for SGA or IUGR were reported within these meta-analyses. Only a slight increase in birth weight in participants receiving supplements has been observed, which was likely to be associated with an increase in gestational duration (Imhoff-Kunsch et al., 2012; Makrides et al., 2006).

2.5.3 Pregnancy duration and preterm birth

Background

Preterm births affecting up to 10% of pregnancies in developed countries (Salvig & Lamont, 2011). An infant born before born before 37 weeks of gestation is considered preterm, while early preterm is born before 32 weeks of gestation (very preterms are born between 28 and 32 weeks and extremely preterm are born before 28 weeks) (Ramel & Georgieff, 2014; World Health Organization, 2012). Being preterm is one of the major risk factors for both short- and long-term negative outcomes, including poor growth and neurological development, and increased chance of morbidities later in life (Morse, 2012). Adequate intakes on n-6 and n-3 LC-PUFAs are

suggested to influence pregnancy length and thereby the birth of either term or preterm infants (Allen & Harris, 2001; Olson, 2003).

Omega-6-derived eicosanoids, such as lipoxygenase and prostaglandins (refer to section 2.3.1), are crucial for the process of normal parturition. These eicosanoids are suggested to trigger cervical ripening, myometrial contractility, initiation of labour as well as curbing both fetal movements and breathing during delivery (Challis, 1998; Challis et al., 2002; Keelan et al., 2003; Olson, 2003; Slater et al., 2002). Intra-amniotic injections of AA may be used as means of inducing labour. However, it has been proposed that increased intakes of n-6 PUFAs may influence higher production of n-6 derived eicosanoids, which may lead an increased pro-inflammatory status and early onset parturition (Allen & Harris, 2001). On the other hand, increased intakes of n-3 LC-PUFAs have been suggested as down regulators of the onset of spontaneous labour. This may be in part due to n-3 LC-PUFA synthesis of eicosanoids that inhibit the ripening of the cervix, and relax the uterine smooth muscle cells (Giuseppe et al., 2014; McGregor et al., 2001). Thus, increased intakes of n-3 LC-PUFA may be required for the synthesis of eicosanoids that will counterbalance the effects of n-6 PUFA derivatives (Jacobson et al., 2008).

Observational studies

A review of observational studies investigating maternal fish and seafood intakes and gestational duration reported inconsistent results (Imhoff-Kunsch et al., 2012). While some studies suggest positive associations such as four days increase in gestational length (Guldner et al., 2007; Olsen et al., 2006), other studies found no associations (Imhoff-Kunsch et al., 2012). Intakes of fish and seafood were not associated with length of gestation or risk of preterm birth in a large cohort of women (n=2,109) (Oken, Kleinman, Olsen, Rich-Edwards, & Gillman, 2004).

Randomised control trials, systematic reviews and meta-analysis

Accumulating evidence indicates that supplementation with n-3 LC-PUFA may improve gestation length and decrease the risk of early preterm births (Giuseppe et al., 2014; Larqué et al., 2012). A recent trial randomly assigned 350 pregnant women to receive either 600mg/d DHA or a placebo from mid-pregnancy, with findings showing that women had 2.9 days longer gestations and fewer early preterm infants when supplemented compared to controls (P<0.05) (Carlson et al., 2013).

Horvath et al. (2007) concluded that LC-PUFA supplementation (3,000mg/d EPA or 900mg/d DHA plus 1,300mg/d EPA) significantly decreased the numbers of early preterm delivery in higher risk pregnancies. Findings from the meta-analysis conducted by Szajewska et al. (2006) revealed a mild increase of 1.6 days in the duration of pregnancy (P=0.01) and reduced risk of early preterm by 31% in women receiving n-3 LC-PUFA supplementation. Data from three high-quality trials included in a systematic review conducted by Makrides and colleagues (2006) indicated that pregnant women receiving marine oil supplements had a mean gestation length that was 2.6 days longer and 61% lower risk of early preterm delivery compared to controls. Findings from a more recent systematic review and meta-analysis of three n-3 LC-PUFA (2,700mg/d EPA plus DHA) supplementation trials reported that gestation length was 4.5 days higher in women taking n-3 PUFA (n=516) supplements versus controls (n=405) (P<0.05), and less preterm births were observed in supplemented (8.9%) compared to control groups (16.3%), with a similar trend for early preterm births (Salvig & Lamont, 2011).

In the meta-analysis conducted by Imhoff-Kunsch et al. (2012), differences between supplemented versus non-supplemented women were not significant in gestational duration, but lower risk of preterm (P=0.08) and early preterm (P=0.01) births were observed in supplemented women.

Data from an Australian trial (n=2,320) included in this meta-analysis stood out, showing that women supplemented with 800mg/d DHA from mid-pregnancy until birth reduced the number of early preterm births by more than 50% compared to controls (*P*=0.03). In contrast, DHA supplemented women in this trial also had more post-term births, which required birth induction and/or caesareans, compared to controls (*P*=0.01) (Makrides et al., 2010a).

In summary, evidence from a few well-designed large trials indicate that maternal supplementation with n-3 fatty acids increase gestation length and reduce the risk of preterm deliveries and its associated complications. These findings are supported by the present systematic review and meta-analysis (Giuseppe et al., 2014; Larqué et al., 2012; Makrides et al., 2006; Salvig & Lamont, 2011; Szajewska et al., 2006). However, many studies have shown conflicting results (Imhoff-Kunsch et al., 2012), which suggests that more research is needed to support the recommendation of n-3 LC-PUFA as measures of preventing the early onset of parturition (Salvig & Lamont, 2011).

2.5.4 Immune function

Background

The incidence of allergic diseases has dramatically increased in developed countries over the past years (Klemens et al., 2011). At the same time, interest in the roles of n-3 and n-6 LC-PUFAs in the development and maturation of fetal immune functions has increased (Calder et al., 2010a). Their important roles include the synthesis of lipid mediators and modulation of gene expression, both crucial in the development of the innate immune system (Calder, 2008; Demmelmair, von Rosen, & Koletzko, 2006). Thus, adequate supply of LC-PUFAs is required for optimal development of fetal innate system, which will provide immediate

response against antigens (e.g. allergen, virus and bacteria) that cause infection in the offspring later in life (Prescott & Clifton, 2009).

Literature suggests that dietary intakes high in n-6 PUFAs and low n-3 LC-PUFAs during pregnancy may increase offspring susceptibility to developing allergic diseases (Blümer & Renz, 2007; Demmelmair et al., 2006; Sausenthaler et al., 2007). An increased maternal n-6 PUFA intakes and supply to the fetus, may influence pro-inflammatory effects that may predispose the developing immune system to exaggeratedly react upon exposure to antigens (Calder, 2009; Dunstan et al., 2003). Similarly, the mother's inflammatory profile, including recurrent inflammation and allergic disturbances during pregnancy may also shape fetal immune system (Denomme et al., 2005). Yet, it is important to acknowledge that adequate amounts of n-6 PUFA are required for the development of important components in the immune system, such as maturation of the thymus in the fetus (Dutta-Roy, 2009).

Observational studies

Accumulating data from observational studies suggests that increased maternal intakes of n-3 LC-PUFA during pregnancy have been associated with protection against the development of allergic diseases in childhood (Krauss-Etschmann et al., 2007; Sausenthaler et al., 2007). This may happen because both DHA- and EPA- derived lipid mediators elicit effects that oppose the pro-inflammatory actions of AA-derived eicosanoids (refer to Section 2.3) (Calder, 2013).

Associations between fish intakes during pregnancy and improved immune functions have been investigated (Fitzsimon et al., 2007; Saadeh, Salameh, Baldi, & Raherison, 2013). A systematic review of fourteen epidemiological studies found that in nine studies maternal oily fish intakes, ranging from "at least once monthly" to "once or more per week", provided

children with immune protection (ranging from 25 to 95%) against developing asthma, food allergies, atopic dermatitis and eczema in children (1 to 18 years) (P<0.05 for all) (Kremmyda et al., 2011). However, it is important to consider that the type of fish in maternal diet can influence different outcomes (e.g. fish PUFA concentrations and levels of environmental contaminants) (Salam, Li, Langholz, & Gilliland, 2005).

Randomised control trials, systematic reviews and meta-analysis

Studies have also shown that n-3 LC-PUFA supplementation during pregnancy can modify immune responses in infants and may reduce subsequent infant allergy (Dunstan et al., 2003; Warstedt, Furuhjelm, Duchen, Fälth-Magnusson, & Facerás, 2009). This is aligned with findings from a meta-analysis indicating that maternal fish oil supplementation in pregnancy (ranging from 200 to 3,700mg/d n-3 LC-PUFA) is associated with immunologic alterations in cord blood which may extend up until adolescence. Data from four RCTs (n=1,072) included in this meta-analysis indicated that maternal supplementation with fish oil during pregnancy is associated with protective effects against common allergic conditions, including food allergies and atopic dermatitis in infants, as well as possible reduced risk of eczema, and asthma and hay fever throughout childhood to adolescence (Kremmyda et al., 2011). Another systematic review and meta-analysis (four trials; n=802) also suggested that maternal supplementation with n-3 LC-PUFAs (650 to 3,700g/d n-3 LC-PUFA) during pregnancy had protective effects against response to skin prick tests for egg allergy in infants up to 12 months and development of asthma during childhood (Klemens et al., 2011).

Findings from these studies reveal that pregnancy is a unique ‘window of opportunity’, when the amounts of n-6 and n-3 PUFAs supplied to the developing immune system will influence how its phenotype will be established (D'Vaz, 2012). In addition, accumulating data show the

potential effects of n-3 LC-PUFAs from supplementation and/or fish intakes during pregnancy and measures of improved immune response in children (Klemens et al., 2011; Kremmyda et al., 2011). Thus, an adequate supply of n-6 and n-3 PUFAs, with increased n-3 LC-PUFAS, is crucial for proper immune system development (Calder et al., 2010a). However, further research is required to confirm the role of maternal supplementation and/or dietary intakes of these fatty acids, and dose responses associated with their immuno-modulatory effects.

2.5.5 Gestational diabetes mellitus

Increased insulin resistance is one of the many natural adaptations of maternal metabolism (refer to Section 2.4.2) that occur to fulfil the increased nutrient demands imposed by the developing fetus during pregnancy (Dutta-Roy, 2009; Herrera, 2002). With progression of pregnancy, insulin sensitivity decreases between 50 and 70% (Butte, 2000), which in turn causes increased circulation of metabolic fuels, such amino acids, glucose and fatty acids (Kirwan et al., 2002; Nelson et al., 2010; Wada et al., 2010).

However, exaggerated changes to maternal glucose metabolism may lead to gestational diabetes mellitus (GDM). While the mechanisms regulating glucose metabolism during pregnancy are not clear, it is suggested that body fat mass and oxidative stress are positively associated with the etiology of GDM (Lappas et al., 2011; Nelson et al., 2010). Thus, overweight or obese women may have higher risk of developing GDM, although women with pre-pregnancy insulin resistance or previous GDM are also at increased risk (Catalano, 2010; Catalano & Ehrenberg, 2006).

Gestational diabetes mellitus is one of the most common metabolic disorders affecting approximately 7% of pregnant women worldwide (Lawrence, Contreras, Chen, & Sacks, 2008; Poniedzialek-Czajkowska et

al., 2014). Negative outcomes associated with GDM include increased risk of mortality, adverse birth outcomes (e.g. preeclampsia), and if not well managed this metabolic condition may persist after birth increasing the chances of developing type 2 diabetes (Bartha, Martinez-Del-Fresno, & Comino-Delgado, 2003; Ben-Haroush, Yogev, & Hod, 2004; Thomas et al., 2006).

In GDM, the activity of placental lipases is increased, resulting in higher supply of FFA to the fetus. Also, exaggerated amounts of glucose and amino acids are also made available in the circulation of GDM mothers (Nelson et al., 2010). Consequently, this increased supply of nutrients will contribute to excessive fetal growth (Bitsanis et al., 2006; Gauster et al., 2011; Lindegaard, Damm, Mathiesen, & Nielsen, 2006; Wijendran et al., 2000). Perinatal overgrowth may result in macrosomic or large for gestational age offspring, which may increase the chances of maternal and fetal trauma, shoulder dystocia, caesarean sections and longer hospitalisation periods (Catalano et al., 2012; Landon et al., 2011). Moreover, infants born to GDM mothers are at increased risk of becoming obese and developing metabolic syndrome later in life (Catalano et al., 2009; Sullivan & Grove, 2010).

Although GDM may increase the availability of fatty acids and other nutrients for placental transfer to the fetus, it is also suggested to impair maternal DHA metabolism. This metabolic dysruption may result in decreased DHA status in both mothers and their newborn. Moreover, maternal and newborn DHA statuses are further decreased in overweight and obese women who develop GDM (Min, Ghebremeskel, Lowy, Thomas, & Crawford, 2004; Pagán et al., 2013). Consequently, low DHA will be accreted in fetal tissues, which may impact on negative infant and children neurological and immune development (refer to Section 2.5.1).

Studies have been conducted to elucidate the benefit of n-3 LC-PUFA supplementation in preventing and treating GDM. Animal studies have shown supplementation with n-3 LC-PUFA improves peripheral glucose uptake (Lardinois & Starich, 1991). Delayed onset of diabetes was observed in individuals diagnosed with glucose intolerance when treated with n-3 LC-PUFA supplementation (Feskens et al., 1991). However, it was suggested in a cohort of pregnant women (n=1,733) that early pregnancy n-3 LC-PUFA intakes of 300mg/d and over were associated with increased risk of developing GDM (Radesky et al., 2008). In addition, in a RCT (n=2,399) supplementing pregnant women with 800mg/d DHA, from mid-pregnancy until delivery, no effects of n-3 LC-PUFAs in the prevention of GDM was observed (Zhou et al., 2012). Although LC-PUFAs exert important effects in resolving pro-oxidative/inflammatory states, further research is needed to clarify how the latter could prevent the onset of GDM (Poniedzialek-Czajkowska et al., 2014).

2.5.6 Preeclampsia and gestational hypertension

Background

Normal gestation is marked by a series of metabolic adaptations that are tailored to ensure that both maternal and fetal energy and nutrients needs are met (refer to Section 2.4.2). These adaptations include increased insulin resistance, hyperlipidaemia, hyperdynamic circulation, increased oxidation of fatty acids for constant energy production, and increased markers of inflammation (Bellamy, Casas, Hingorani, & Williams, 2007; Makrides & Gibson, 2000).

Therefore, pregnancy can be a transient period when certain metabolic adaptations may be disrupted, contributing to the pathogenesis of gestational hypertension (GH) and/or preeclampsia (PE). Gestational hypertension is diagnosed when a pregnant woman develops high blood

pressure (HBP) (≥140/90mmHg), while PE consists of HBP plus the presence of protein (≥300mg) in the urine and increased markers of oxidative stress (Mehendale et al., 2008; Olafsdottir et al., 2006; Stewart, Conrad, & Jeyabalan, 2013).

Preeclampsia and GH are estimated to affect approximately 6% of pregnancies in developed countries (Stewart et al., 2013). These disturbances are associated with many negative birth outcomes, including IUGR, premature births, medicalisation, induced labuor, cesarean section, prolonged hospitalisation, as well as maternal and infant mortality (Genuis & Schwalfenberg, 2006). In addition, these can cause vasoconstriction and endothelial tissue damage, affecting the liver, heart, brain, kidneys and placenta (Makrides, 2008; Makrides & Gibson, 2000; Stewart et al., 2013; Zhou et al., 2012). Many studies have suggested the association between PE and a higher risk of metabolic syndrome and heart diseases later in life (Bellamy et al., 2007; Melchiorre, Sutherland, Liberati, & Thilaganathan, 2011).

Increased rates of GH and PE are observed in women, previously diagnosed with PE, obesity, nulliparous, multiple pregnancy, diabetic, hypertensive, chronic inflammation conditions or in women aged less than 18 years or older than 35 years (Barton & Sibai, 2008; Catalano, 2010; Stewart et al., 2013).

Although the causes are still not known, n-3 LC-PUFAs seem to be inversely associated with the driving mechanisms of GH and PE (Genuis & Schwalfenberg, 2006; Williams et al., 2006). This may be due to the effects of EPA- and DHA-derived eicosanoids, which play important anti-inflammatory and vaso-dilative roles (refer to Section 2.3) that may counteract the mechanisms leading to GH and PE (Makrides, 2008).

Observational studies

An inverse association of n-3 LC-PUFAs with GH and PE was suggested by observational studies conducted in countries with a low incidence of GH and PE and high intakes of marine foods rich in n-3 LC-PUFA. For example, Inuits in Northwest Canada, who had a high consumption of marine mammals and fish had very few cases of GH compared to women with lower consumption of these foods (Gerrard, Popeski, Ebbeling, Brown, & Hornstra, 1990; Popeski, Ebbeling, Brown, Hornstra, & Gerrard, 1991). Other studies showed that women with low n-3 LC-PUFA status were up to 7.6 times more likely to develop GH and PE compared to women with higher n-3 LC-PUFA (Huiskes et al., 2009; Mehendale et al., 2008; Williams, Zingheim, King, & Zebelman, 1995). Similar effects were also shown in Asian women (n=75), who had a higher incidence of GH with lower plasma levels of n-3 LC-PUFA at mid-pregnancy (P=0.02) (Lim et al., 2015). The same study also reported that an increase of 1% in total n-3 LC-PUFA in plasma was associated with 24% lower GH risk. Further studies have confirmed that lower intakes of n-3 LC-PUFAs and fish were related with the development of PE (Oken et al., 2007; Olsen et al., 1992; Olsen et al., 1986).

Randomised control trials, systematic reviews and meta-analysis

Fish oil supplementation has been proposed as a potential means of preventing and/or treating GH and PE (Zhou et al., 2012). Findings from a meta-analysis conducted by Imhoff-Kunsch et al. (2012) (five RCTs; n=2,625) indicated no impact of n-3 LC-PUFA (up to 3,000mg/d) supplementation on GH and PE. Similar findings were reported in the meta-analysis of Szajewska et al. (2006) (two RCTs, n=328), Makrides et al. (2006) (five RCTs, n=1,831), and Horvath et al. (2007) (one RCTs, n=321).

However RCTs within meta-analysis have reported inconsistent findings and more RCTs are required to establish dose-response and when n-3 LC-PUFA supplementation should be undertaken as means of preventing or treating GH and PE.

2.5.7 Mood disorders and depression

Background

Maternal n-3 LC-PUFA decline during the last trimester of pregnancy due to increased fetal demands for DHA brain and adipose tissue accretion (Bonham et al., 2008). As a result, the mother may take up six months to fully recover her n-3 LC-PUFAs stores, which in turn may be dependent on dietary intakes and bioavailability of these fatty acids (Hornstra, 2000). Failure to recover n-3 LC-PUFA stores may occur due to inadequate dietary intakes, subsequent pregnancies within less than a year interval and multiparity (Al et al., 1997; Hornstra, 2000; Makrides & Gibson, 2000; Zeijdner et al., 1997). Low maternal DHA stores have been suggested as a risk factor for postnatal depression (PND) (Makrides, Crowther, Gibson, Gibson, & Skeaff, 2002; Otto, De Groot, & Hornstra, 2003). The latter is suggested based on the important roles that DHA plays in the brain, which seem to influence serotonin levels and regulate mood (Makrides, 2009a; McNamara & Carlson, 2006).

Observational studies

An ecological analysis including data from over 16 countries showed that both low maternal intakes of fish and seafood and low DHA contents in breast milk were inversely associated with PND (P<0.0001) (Hibbeln, 2002). Studies also support an inverse associations between n-3 LC-PUFA intakes from marine sources (≥320mg/d n-3 LC-PUFA) and maternal anxiety (Vaz et al., 2013) and depressive symptoms (Golding et al., 2009; Hibbeln et al., 2003; Sontrop et al., 2008).

Randomised control trials, systematic reviews and meta-analysis

To date, many RCTs investigating the effects of n-3 LC-PUFAs intakes on maternal episodes of mood disorders and depression have had methodological limitations including open-labelled design, small sample sizes, and lack of control groups (Larqué et al., 2012; Makrides, 2008). An meta-analysis including seven RCTs (n=612), in which pregnant women were randomly assigned to receive n-3 LC-PUFA supplementation (ranging from 200mg/d DHA to 4,000mg/d EPA plus DHA) or placebo, showed no significant effect on symptoms of PND in both groups (Jans, Giltay, & Willem Van Der Does, 2010). However, from all of these studies, only one study (n=36) was double-blind, and data from this study showed beneficial effects of supplementation with n-3 LC-PUFAs (1,200mg/d DHA plus 2,200mg/d EPA) on symptoms of PND (Su et al., 2008).

More recently, double-blind RCTs have investigated the effects of n-3 LC-PUFA supplementation on the prevention of depression symptoms amongst pregnant women with increased risk for depression.

One study randomly assigning 126 pregnant women at risk of depression to either a DHA-rich supplement (900mg DHA and 180mg EPA), EPA-rich supplement (1060mg EPA and 274mg DHA) or placebo in early pregnancy, found that higher serum DHA concentrations between 34 and 36 weeks gestation were inversely related to the Beck Depression Inventory (BDI). However, there was no conclusive data to determine whether supplementation with n-3 LC-PUFAs can prevent depressive symptoms during and after pregnancy (Mozurkewich et al., 2013). Another study (n=2,399) reported no differences in reported depressive symptoms between supplemented (800mg/d DHA from mid-pregnancy until birth) and control women at six weeks and six months after birth (Makrides et al., 2010a).

It is possible that the fatty acid composition ratios of supplements used in many pregnancy trials could have influenced the contradicting results in the prevention of depression symptoms (Makrides et al., 2010a). Existing data suggests that EPA may be more effective in preventing mood disorders and PND symptoms compared to DHA, however most of the studies supporting this data suffered from poor methodological design and small sample sizes (Ramakrishnan et al., 2009).

Although existing data suggesting that an adequate maternal dietary intake to prevent n-3 LC-PUFAs depletion may be inversely associated with mood disorders and PND, there is no strong evidence supporting the effects of n-3 LC-PUFAs supplementation in the prevention of these disorders.

2.5.8 Summary of the evidence

Observational studies

Several observational studies have reported the benefits of consuming n-3 LC-PUFA from marine sources during pregnancy and positive maternal and fetal outcomes (Imhoff-Kunsch et al., 2012). These include protective effects against preeclampsia (PE) (Huiskes et al., 2009; Lim et al., 2015; Mehendale et al., 2008), PND (Golding et al., 2009; Hibbeln et al., 2003; Sontrop et al., 2008), mood disorders (Vaz et al., 2013), and early preterm birth (Olsen et al., 2006). In addition, many observational studies reported increased measures of intrauterine growth (Brantsaeter et al., 2012; Guldner et al., 2007; Mitchell et al., 2004; Van Eijsden et al., 2008), improved neurological development (Hibbeln et al., 2007; Morse, 2012; Oken et al., 2008a), visual acuity (Jacobson et al., 2008; Jacques et al., 2011), and stronger immune system (Dunstan et al., 2003; Fitzsimon et al., 2007; Saadeh et al., 2013; Warstedt et al., 2009) in the infants of mothers who consumed fish and seafood during pregnancy. Conversely, some studies have reported negative effects of maternal fish consumption during

pregnancy and measures of neurological development (Gale et al., 2008; Mendez et al., 2009) and intrauterine growth (Heppe et al., 2011; Mendez et al., 2010; Oken et al., 2004) due to increased exposure to environmental contaminants. Findings from a systematic review indicated inconsistent results for associations between fish intake (two to four servings per week) and measures of perinatal growth and preterm birth (Imhoff-Kunsch et al., 2012).

Despite consistent positive findings, it is not possible to establish a conclusive link between maternal fish and seafood intakes and improved neurodevelopment outcomes in children. It is possible that conflicting results may be due to environmental contaminants and a range of important nutrients in some sources (e.g. protein, vitamin D, selenium), which also influenced different health outcomes for both mothers and their developing fetuses (Salam et al., 2005; Vaz et al., 2013).

Randomised control trials, systematic reviews and meta-analysis

Studies investigating the effects of maternal n-3 PUFA supplementation on pregnancy outcomes have had mixed results. For instance, only a slight increase in birth weight in participants receiving supplements was observed, which was associated with an increased gestational length (Imhoff-Kunsch et al., 2012; Makrides et al., 2006). However, no significant differences in infant length, head circumference or risk for SGA or IUGR were reported in other RCTs (Horvath et al., 2007; Makrides et al., 2006; Szajewska et al., 2006). Improved gestational duration and decreased risk of early preterm delivery were reported in several systematic reviews and meta-analysis (Giuseppe et al., 2014; Larqué et al., 2012; Makrides et al., 2006; Salvig & Lamont, 2011; Szajewska et al., 2006). One meta-analysis also reported that n-3 LC-PUFA supplemented women had significantly increased risk of post-term birth, which often requires birth induction and/or caesarean interventions (Makrides et al., 2006). Interestingly, no

associations were reported between maternal n-3 LC-PUFA supplementation and prevention or improvement of GH, PE, GDM (Makrides et al., 2006; Poniedzialek-Czajkowska et al., 2014; Szajewska et al., 2006; Zhou et al., 2012), and PND (Jans et al., 2010). However, existing indication of positive effects of n-3 LC-PUFA on PND cannot be ignored (Simmer et al., 2009).

Inconsistent findings were also reported for measures of neurodevelopment (Giuseppe et al., 2014; Gould et al., 2013; Imhoff-Kunsch et al., 2012). Despite this, n-3 LC-PUFA supplementation of mothers with low baseline PUFA status showed great beneficial effects on measures of motor activity, visual acuity, and cognitive functions in term infants (Simmer et al., 2009). Positive associations were also reported between maternal n-3 LC-PUFA supplementation and reduced risk of several allergic conditions (Klemens et al., 2011; Kremmyda et al., 2011). Furthermore, no evidence of any potential harm to mothers or their children were reported for supplementation with doses up to 3,000mg n-3 LC-PUFAs per day (Makrides et al., 2006; Simmer et al., 2009).

Mixed and inconclusive findings may have resulted from study design and methodological failures (Makrides, 2009a). These include the type of supplement (e.g. dose, PUFA composition, type of oil and esterification), the duration of the treatment, large attrition rates, sample sizes, blindness of the trial, compliance rates, maternal baseline PUFA status, lack of sensitivity in many outcome tests (e.g. neurodevelopment tests), dietary intakes of PUFA and other factors that can influence maternal PUFA status (e.g. smoking, high intakes of n-6 PUFAs and saturated fats) (Gould et al., 2014; Lauritzen, Hansen, Jorgensen, & Michaelsen, 2001; Makrides, 2009a). These factors were not always considered by many studies, thereby influencing results with limited power. Another factor to be considered is the existence of genetic differences that may influence

maternal and fetal PUFA status and consequently the findings of many studies (Schaeffer et al., 2006; Xie & Innis, 2008). Trials have also used supplements with a range of doses and ratios of n-3 LC-PUFAs making it hard to distinguish between the effects DHA versus EPA on maternal and fetal health outcomes (Akabas & Deckelbaum, 2006; Karr, Alexander, & Winningham, 2011).

In summary, some good evidence shows the potential benefit of maternal n-3 LC-PUFA intakes from marine and supplementation sources on maternal and fetal health outcomes, while other studies also show controversial results. Although evidence indicates positive effects of maternal n-3 LC-PUFA supplementation, no dose response has yet been established. In addition, greater benefits are observed in mothers with low baseline levels (Simmer et al., 2009). Thus, existing evidence for almost all studied maternal and fetal health outcomes is not conclusive, and require further larger well-designed trials to establish dose-responses and support the recommendation of n-3 LC-PUFA intakes during pregnancy as means of improving birth outcomes.

2.6 Recommendations for omega-6 and omega-3 PUFA intake during pregnancy

Current n-6 and n-3 PUFA recommendations for pregnant women are designed to cover the estimated amount of PUFA accretion in both maternal and fetal tissues throughout pregnancy, while maintaining maternal homeostasis, and optimal fetal growth and development (Brenna & Lapillonne, 2009; Innis, 2003; Koletzko et al., 2007a). These recommendations are often derived from scientific consensus conferences, where experts in the field join discussion panels to review the current existing evidence on PUFA intakes and birth outcomes in order to establish

recommendations (Flock et al., 2013; Koletzko et al., 2007a; Koletzko et al., 2008; Simmer et al., 2009; Simopoulos et al., 2000).

However, current evidence is not conclusive and lacks power to support the development of recommended dietary intakes (RDIs) that will meet the needs of n-6 and n-3 PUFAs of most pregnant women (Flock et al., 2013; Simopoulos et al., 2000). Therefore, recommended intakes for PUFAs are normally established as adequate intakes (AIs), which are an estimate based on daily intakes of apparently healthy individuals that are assumed to be adequate, based on observational and trial studies (NHMRC, 2006). In addition, AIs can be established using a scientific judgment approach when inadequate relevant data is available (Whelan, Jahns, & Kavanagh, 2009).

In 2006, the National Health and Medical Research Council (NHMRC) published the Nutrient Reference Values (NRVs) for Australia and New Zealand. The NRVs include recommended AIs for EFA (LA and ALA) and total n-3 LC-PUFAs (combined EPA, DPA plus DHA), but not for n-6 LC PUFAs (AA) (NHMRC, 2006). The recommendations for pregnant women were established based on AIs for non-pregnant and non-breastfeeding women of reproductive age (16 – 40 years), with an additional 25% to cover for the average increase in body weight during pregnancy. An Upper Level of Intake (UL) of 3,000mg/day was also established for total n-3 LC-PUFAs for children, adolescents and adults. In addition, Suggested Dietary Targets (SDT) values were set for the prevention of chronic disease in the adult population, with total n-3 LC-PUFA intakes of 430 mg/day being recommended for adult women (Ministry of Health, 2008b; NHMRC, 2006).

A range of recommendations for n-6 and n-3 PUFAs are observed for other countries, with stablished values available mostly for ALA, LA and combined EPA and DHA. Individual values for EPA and DHA are also

recommended in some countries. However, there are no specific recommended levels established for DPA other than within combined total n-3 LC-PUFAs (EPA, DPA plus DHA) (NHMRC, 2006). Table 2.1 presents the current national and international recommendations for n-6 and n-3 PUFA's intakes during pregnancy.

Table 2.1 – Recommended intakes of omega-6 and omega-3 polyunsaturated fatty acids for pregnant women

Organisation / Year	Organisation Type	Recommendations / Milligrams per day (mg/d)						Reference
		LA	AA	ALA	EPA	DHA	EPA + DHA	
Nutrient Reference Values for Australia and New Zealand - NHMRC (2006)[1]	Authoritative Organisation	10,000	-	1,000	-	-	Total (DHA+EPA+DPA) 110 (14-18yr) 115 (19-50yr) SDT 430 UL 3000	(NHMRC, 2006)
Australian Scientific Consensus Workshop - (2009)[2]	Expert Scientific Organisation	-	-	-	-	200	500	(Simmer et al., 2009)
Dietary Guidelines Advisory Committee - USDA (2010)[1]	Expert Scientific Organisation	13,000	-	1,400	-	-	250	(United States Department of Agriculture (USDA), 2010)

Organisation / Year	Organisation Type	Recommendations / Milligrams per day (mg/d)						Reference
		LA	AA	ALA	EPA	DHA	EPA + DHA	
International Society for the Study of Fatty Acids and Lipids - ISSFAL (2000)[1]	Expert Scientific Organisation	4,440 UL 6,670	-	1,600	≥220	≥300	≥650	(Simopoulos et al., 2000)
European Food Safety Agency – EFSA Europe, (2012)[1]	Authoritative Organisation	UL 10,000	-	-	-	100 – 200	≥250	(European Food Safety Authority, 2012)
Food and Agriculture Organization of the United Nations – FAO and World Health Organization - WHO (2010)	Authoritative Organisations	≥6,400**	UL-AMDR 800 NOAEL	≥1,300**	-	≥200* UL-AMDR 1,000	300* UL-AMDR 2,000 NOAEL	(Food and Agriculture Organization of the United Nations and World Health Organization, 2010)
World Association of Perinatal Medicine Dietary Guidelines	Expert Scientific Organisation	-	-	-	-	200	-	(Koletzko et al., 2008)

Organisation / Year	Organisation Type	Recommendations / Milligrams per day (mg/d)						Reference
		LA	AA	ALA	EPA	DHA	EPA + DHA	
Working Group (2008)[2]								
Consensus Recommendation PERILIP (2007)[2]	Expert Scientific Organisation	-	-	-	-	>200[#] UL 1,000	UL 2,700	(Koletzko et al., 2007a)
AFFSA (2010)[3]	Authoritative Organisation	8,800	-	2,250	250	250	500	(AFFSA, 2010)

Keys and abbreviations: * Average nutrient requirement based on minimum adult acceptable macronutrient distribution range (AMDR) plus an increment for energy demands of pregnancy; ** These values were calculated based on the total average energy requirement in pregnancy (2,300Kcal/d) and the minimum intake level (AI) for essential fatty acids to prevent deficiency symptoms estimates as 2.5% energy LA and 0.5% energy ALA (Food and Agriculture Organization of the United Nations and World Health Organization, 2010); [1] Adequate intake (AI); [2] Consensus recommendations from Expert Groups; [3] French population reference intakes encompass the physiological requirements for almost the entire population; [#] This recommended intake of DHA can be met by consumption of one to two portions of fish per week, including at least one weekly serving of oily fish. LA, linoleic acid; ALA, alpha-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; NOAEL, no observed adverse effect level in randomized control trials; SDT, suggested dietary target; UL, upper tolerable intake level; UL-AMDR, upper limit of acceptable macronutrient distribution range; PERILIP, Perinatal Lipid Intake Working Group; AFFSA, The French Food Safety Agency.

Most organisations and expert groups represented in Table 2.1 seem to focus consistently on recommendations for n-3 PUFAs with only a minority suggesting dietary intake values for n-6 PUFAs. For instance, the only recommended value for n-6 LC-PUFA (AA) were established as a UL (Elmadfa & Kornsteiner, 2009b). These may be due to the fact AA is not considered an essential fatty acid for healthy individuals who consume a usual diet that provides at least 2.5% of total energy intake as LA (Food and Agriculture Organization of the United Nations and World Health Organization, 2010). The diet of developed countries normally provides increased amounts of LA, which in turn influences the endogenous production of AA in sufficient amounts for all humans aged 6 months and older (European Food Safety Authority, 2009). In addition, there is no evidence suggesting that pregnant women have insufficient dietary intake of n-6 PUFAs, or that they would benefit from increasing their dietary intakes of these PUFAs (Koletzko et al., 2007a; Koletzko et al., 2008; Plourde & Cunnane, 2007).

To date there is no RDIs established for DHA and EPA due to the lack of conclusive evidence consistent dose-response relationships between nutrient intake and health benefits (Flock et al., 2013; Food and Agriculture Organization of the United Nations and World Health Organization, 2010). Current recommendations for these LC-PUFAs, particularly for DHA, tend to be set based on theoretical calculations that are justified by extensive research showing the benefits of sufficient DHA and EPA dietary intakes during pregnancy (Blumfield, Hure, Macdonald-Wicks, Smith, & Collins, 2012; Jordan, 2010; Koletzko et al., 2008). Therefore, several organisations and expert groups have proposed consensus recommendations suggesting that pregnant women should aim to achieve daily intakes of at least 200mg of DHA, 220mg of EPA and 250mg of combined EPA and DHA (AFFSA, 2010; European Food Safety

Authority, 2012; Food and Agriculture Organization of the United Nations and World Health Organization, 2010; Koletzko et al., 2007a; Koletzko et al., 2008; Simmer et al., 2009; Simopoulos et al., 2000; United States Department of Agriculture (USDA), 2010).

In Australia, a consensus recommendation of 200mg for DHA per day is suggested for pregnant women (Simmer et al., 2009), which is consistent with the recommendations of most of the organisations and expert groups presented in Table 2.1. However, recommendations from the NHMRC (Australia and New Zealand) have set an AI that combines DHA, EPA and DPA, at a range of 110 to 115mg per day (NHMRC, 2006). When this AI for combined n-3 LC-PUFA was established there was no current dose-response set for the beneficial effects of EPA, DPA and DHA individually, therefore it was impossible to distinguish individual requirements for each of these PUFAs. Despite this, the NHMRC acknowledged the importance of increasing the intakes of n-3 LC-PUFAs for prevention of chronic diseases, thereby suggesting a SDT of 430mg/day for adult women for optimal health. The SDT for n-3 LC-PUFAs are based on the 90th centile of intake levels of Australian and New Zealand adult women, which was attributed as a safe and beneficial intake to prevent chronic diseases (NHMRC, 2006). This SDT is almost four times higher than the AI recommended for pregnant women and slightly lower than consensus recommendations of 500mg/day for pregnant women (AFFSA, 2010; Simmer et al., 2009). In addition, SDTs do not differentiate between non-pregnant, pregnant and/or breastfeeding women (NHMRC, 2006). Therefore, it may be reasonable that women may also try to achieve these SDTs values during pregnancy.

Some experts suggest that daily recommendations ranging between 200 to 300mg/day DHA plus EPA during pregnancy may be conservative, as these values mirror the minimum values recommended for prevention of

chronic diseases in the general population (Calder et al., 2010a; Flock et al., 2013; Food and Agriculture Organization of the United Nations and World Health Organization, 2010; Jensen, 2006; Mozaffarian & Rimm, 2006). In addition, most studies investigating the effects of n-3 LC-PUFAs on maternal and fetal health outcomes, have used high daily doses such as 3,000mg EPA and DHA combined or 1,000mg DHA per day. These high doses are suggested to be over 20 times higher than the normal dietary intakes for these PUFAs (Cetin & Koletzko, 2008; Innis, 2005; Koletzko et al., 2007a; Makrides et al., 2006). These high doses have shown no toxicological effects or increased risk of unwanted effects. Potential adverse effects may include bleeding complications which may lead to maternal anaemia; reduced cytokine production and reduced vitamin E bioavailability which in turn may increase lipid peroxidation (Elmadfa & Kornsteiner, 2009b; Koletzko et al., 2008; Makrides et al., 2006; Sanders, 2009b). Thus, n-3 LC-PUFA intakes up to 3,000mg/day have been considered as a safe tolerable UL for adults (IOM, 2005; NHMRC, 2006). However, more recently a FAO/WHO expert consultation released a report where they defined that n-3 LC-PUFA intakes above the UL of the Acceptable Macronutrient Distribution Range (AMDR) set as 2,000mg/day may increase the risk of unwanted effects (Food and Agriculture Organization of the United Nations and World Health Organization, 2010). Therefore, high intakes of EPA and DHA from marine foods or supplements should not exceed the recommended UL in order to avoid any adverse effects (Bourre, 2007; IOM, 2005; Makrides, 2009a).

In summary, evidence-based consensus statements from international expert groups and health organisations have established that pregnant women should aim to achieve at least 200mg DHA and an average of 300mg of combined EPA and DHA on daily basis. These recommendations are currently considered sufficient to fulfil the LC n-3

PUFA needs during pregnancy for maintenance of maternal homeostasis as well as fetal growth and brain development (AFFSA, 2010; Food and Agriculture Organization of the United Nations and World Health Organization, 2010; Koletzko et al., 2008; Simmer et al., 2009; Simopoulos et al., 2000). The recommended AI for n-3 LC-PUFAs from the NHMRC (110 and 115mg of combined DHA, EPA and DPA) seem to be conservative compared to other international recommendations. However, unless evidence from dose-response trials showing beneficial effects of n-3 LC-PUFA on birth outcomes are consistently positive, recommended intakes for these important fatty acids will remain within the conservative ranges (Blumfield et al., 2012; Brenna & Lapillonne, 2009).

2.7 Food sources of omega-6 and omega-3 polyunsaturated fatty acids

In most developed countries, approximately 90% of the total dietary intake of PUFAs is comprised by n-6 PUFAs (Calder et al., 2010a), with LA contributing about 95% of this (Stanley et al., 2007). The main sources of n-6 PUFAs include meats, eggs, processed foods, most vegetable oils, nuts and seeds (Muskiet et al., 2006). Red meats, poultry, eggs and dairy products are rich sources of AA (Saunders et al., 2012), while fish from marine and fresh waters represent minor sources of AA (Ratnayake & Galli, 2009).

In contrast, n-3 PUFAs are found in very few plant-derived sources that provide ALA, such as flaxseed, chia seeds, walnuts, some vegetable oils (e.g. rapeseed and canola oil), and green leafy vegetables like spinach and kale (Gebauer et al., 2006; Ratnayake & Galli, 2009; Sanders, 2014). Approximately 90% of the n-3 PUFA in the diet of people in developed countries is comprised by ALA (Stanley et al., 2007). Long chain n-3 PUFAs are present to a lesser extent since its main food source, marine-

derived foods, are not part of the everyday diets in many developed countries. Marine-derived foods such as fish, seafood and seaweed are richest sources of EPA and DHA, with minor contributions to DPA (Sanders, 2014). Oily fish (>5% fat), such as salmon, mackerel, sardines, and tuna, are considered the richest sources of EPA and DHA (Jordan, 2010; Markhus et al., 2013). Major DPA contributions are supplied by terrestrial animal-derived foods such as dairy, meat, poultry and eggs (Rahmawaty, Charlton, Lyons-Wall, & Meyer, 2013a). These foods also contribute to EPA and DHA intakes (Russell & Bürgin-Maunder, 2012), but to a much smaller extent compared to fish and seafood, which are suggested to have up to 15 times higher concentrations of n-3 LC-PUFAs than terrestrial animal derived foods (Howe, Buckley, & Meyer, 2007; Howe, Meyer, Record, & Baghurst, 2006). Other sources of EPA and DHA include fortified food products, such as eggs, milk and spreads (Whelan et al., 2009). Table 2.4 shows the fatty acid composition of some of the most common food sources of n-3 and n-6 PUFAs consumed in New Zealand.

Table 2.2 - Omega-6 and omega-3 polyunsaturated fatty acids composition of common food sources in New Zealand

Food Source	LA	ALA	AA	EPA	DPA	DHA
			mg/100g			
Fish and seafood						
Salmon, Atlantic, raw	376	85	72	-	48	313
Salmon, king, New Zealand, raw	372	46	-	218	106	295
Hoki, flesh, grilled	26	2	17	41	14	164
Kahawai, flesh ,grilled	94	23	-	480	-	1240
Trevally, flesh, grilled	76	19	-	174	-	420
Snapper, flesh, grilled	26	9	-	153	76	259
Tarakihi, flesh, grilled	40	1	34	39	19	231
Flounder, flesh, grilled	75	4	28	133	44	92
Eel, flesh, raw	-	-	-	30	-	270
Tuna, in brine, canned, drained, composite	33	1	29	33	4	259
Tuna, Albacore, flesh, raw	-	-	-	250	-	943
Mussel, Green, steamed	-	25	-	550	17	289
Milk and alternatives						
Milk, fluid, whole	56	21	33	3	3	-
Cheese, Edam	208	184	0	-	-	-
Soy milk	1,789	303	0	-	-	-
Eggs	0	0	0	-	-	-
Egg, whole ,raw	646	20	13	-	-	7
Egg, free range, raw	1,154	60	23	-	8	8
Meat						
Beef,mince,raw,7%fat	0	61	0	13	15	2
Pork, mince, raw, 9.4%fat	980	90	60	0	0	30
Sheep, lamb, mince, lean & fat ,raw	220	117	30	17	20	10
Chicken, flesh, raw	1,130	90	40	-	9	10
Fat and oils						
Margarine,Mono,55% fat, Olivani Light	12,119	1,701		-	-	-
Margarine,Poly,50% fat, Flora Light	19,049	1,487		-	-	-
Butter, unsalted	1,658	553		79	79	79
Peanut butter	12,127	-		-	-	-
Flaxseed oil	16,891	58,402		-	-	-
Rice bran oil	27,099	934		-	-	-
Canola oil	18,400	10,000		-	-	-
Sunflower oil	59,400	300		-	-	-
Soybean oil	55,100	7,200		-	-	-
Sesame oil	40,700	500		-	-	-
Rapeseed oil	63,256	1,170		-	-	-

Abbreviations: LA, linoleic acid; ALA, alpha-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
Source: (Foodworks, 2009; Plant and Food Research, 2014)

The fatty acid composition of animal food sources can vary between different species and is substantially reliant upon the environment and type of feed available (Bourre, 2004; Kartikasari, Hughes, Geier, Makrides, & Gibson, 2010). For instance, the fatty acid composition of animals that are wild, free-range and able to feed from natural sources may present increased levels of n-3 PUFAs (Michaelsen et al., 2011). Fish species from the ocean and very cold waters tend to present higher concentrations of fat and therefore higher levels of n-3 LC-PUFAs compared to species from fresh waters with warmer temperatures, (Michaelsen et al., 2011). Interestingly, a study found that many of the fish mostly consumed in Australia (e.g. Barramundi, Skate, Southern Bluefin Tuna and Coral Trout) are poor sources of n-3 LC-PUFAs, as these fish contain lower fat contents due to warm water temperatures and food sources available in their ecosystem (Soltan & Gibson, 2008).

However, the fatty acid composition of farmed fish, as well as other animals can be modified by the type of feed supplied to these animals (Soltan & Gibson, 2008). Conventional meat, poultry, dairy and fish farming have been increasingly based on corn or other grains/cereals feed, which supply a LA rich and ALA poor feed, thereby resulting in animal-derived food products with decreased amounts of n-3 and higher amounts of n-6 PUFAs (Holman, 1998; Sanders, 2014).

In countries like Australia and New Zealand, the majority of cattle and sheep farms tend to be mainly grass-fed (Howe et al., 2007). Existing data suggests that meat products from grass-fed animals have higher n-3 PUFAs, particularly DPA levels compared to grain-fed meat from other countries such as the United States (Droulez, Williams, Levy, Stobaus, & Sinclair, 2006).

The price and availability of food can influence purchasing decisions of consumers and food manufacturers. For instance, vegetable oils that are richer in ALA are offered with fewer options and more expensive prices compared to LA rich oils that are widely available with competitive prices (Michaelsen et al., 2011). Moreover, fish and seafood are more expensive compared to other protein rich food sources (Mitchell et al., 2004). Therefore, the cost of food ingredients will be an important determinant of the fatty acids composition of the diets of individuals.

2.8 Contribution of food sources to omega-6 and omega-3 polyunsaturated fatty acids

In many countries, the greatest sources of n-3 LC-PUFA are seafood and fish, while seed oils, poultry, eggs and red meats meat are the biggest contributors to n-6 PUFA intakes (Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006). Products derived from terrestrial animals are the major sources of AA, whereas most vegetable oils and fats substantially contribute to LA intakes (Meyer et al., 2003).

Fish and seafood are considered the major sources of DHA and EPA, therefore their consumption is often used as a measure to determine dietary intakes of n-3 LC-PUFA (Sontrop et al., 2008). Terrestrial animal sources (e.g. poultry and red meats) are also suggested to contribute to n-3 LC-PUFA intakes, with substantial contributions to DPA compared to small contributions to EPA and DHA intakes (Howe et al., 2007). However, contributions to n-3 LC-PUFAs intakes from fish and seafood are suggested to be up to 5 to 15 times higher than contributions from terrestrial animal sources, and mainly account for EPA and DHA intakes (Howe et al., 2006).

Current dietary patterns in many developed countries include substantial amounts of terrestrial animal-derived foods and small amounts of fish and

seafood (Meyer, 2011; Plourde & Cunnane, 2007). These dietary patterns are observed in countries like the United Kingdom (UK), where adults' weekly consumption of total terrestrial animal meats (including delicatessen meats and sausages) was estimated to be 1,126g compared to 210g of total fish and seafood. Still, fish and seafood contributed to 76% of total n-3 LC-PUFA (EPA, DPA plus DHA) intakes, followed by poultry (12.3%), red meats (5.7%) and eggs (3.4%) (Givens & Gibbs, 2006). Even lower intakes of fish, such as 140g/week were estimated in the adults population by the UK Advisory Committee on Nutrition and Food Standards Agency (United Kingdon Scientific Advisory Committee on Nutrition, 2004). Low fish intakes were also found in pregnant women in the UK (n=88), where the majority of participants reported consuming less that two servings of fish per week (54% GDM participants and 81% non-GDM) (Thomas et al., 2006).

Although fish and seafood intakes are considered low in many other European countries (Astorg et al., 2004; Linseisen et al., 2003), they are still the main sources of n-3 LC-PUFAs intakes. In Belgium, data from a two day estimated food record found fish and seafood contributed to 87% of EPA, 66% of DPA and 80% of DHA intakes among 461 women of childbearing age, while meat, poultry and eggs together contributed to over half of AA intakes. Interestingly, total fish and seafood (66%) contributions to DPA intakes were higher than that from meat, poultry and eggs combined (31.2%). Fats and oils were the main contributors to LA (31%) and ALA (45%) (Sioen et al., 2006). A more recent study using a non-validated FFQ confirmed that fish and seafood are still the main sources of EPA and DHA among 414 Belgian adult women (aged 18 to 39 year) (Sioen, Devroe, Inghels, Terwecoren, & De Henauw, 2010). Findings from a cohort study conducted in 1,335 women in their last trimester of pregnancy in France found that over half the participants

(63%) consumed less than two servings of fish per week, which contributed to just over half of DHA intakes (54%) (Bernard et al., 2013). Further European cohorts indicated that the most common sources of DHA intake in Germany, Spain and Hungary were fish, poultry and eggs (Franke et al., 2008).

In Canada, fish and seafood consumption is considered relatively low amongst pregnant women (Friesen & Innis, 2010). Findings from a study suggested that mean fish and seafood intakes were less than two servings per week amongst women (n=55) in their last trimester of pregnancy. Yet, fish and seafood were found to be the main food sources for DHA (~80%) and EPA (65%), while meat and poultry were the main contributors of AA (~80%) intakes, with important contributions to EPA (32%) and DHA (9%) intakes. Eggs were also important sources of AA (27%) and DHA (10%) (Innis & Elias, 2003). A later study confirmed that fish and seafood were the major contributors of EPA (66%) and DHA (76%) intakes, and meat and poultry (51%), as well as eggs (30%), were the main sources for AA intakes in pregnant women (n=204). Notwithstanding, dairy products (15% of EPA intakes), and eggs (15% of DHA intakes) were also important contributors to n-3 LC-PUFA intakes. Total PUFA intakes were mainly comprised by LA (84.2%), which also represented 99% of the total n-6 PUFA intakes (Friesen & Innis, 2009). Another Canadian study included seaweed alongside fish and seafood as the main food sources contributing to 79% of total n-3 LC-PUFAs (87% EPA, 59% DPA plus 81% DHA) intakes in pregnant women. This study was part of the Alberta Pregnancy Outcomes and Nutrition (APrON) cohort, which used a 24-h recall to identify the main food sources of n-3 LC-PUFAs in 600 pregnant women at each gestational trimester. Results also indicate that poultry (14%) and meat (11%) were important contributors to DPA (Jia et al., 2015).

A retrospective analysis from the Australian National Nutrition Survey from 1995 (NNS95) using a reliable and comprehensive FFQ to investigate dietary PUFA intakes, including n-3 LC-PUFA contributions from meats, suggested that meat sources contribute to almost half of the total n-3 LC-PUFA intakes estimated for Australian adults (Howe et al., 2006). Although the consumption of red meats and poultry in Australia is suggested to be six times higher compared to fish and seafood consumption (Meyer et al., 2003), contributions to n-3 LC-PUFAs from fish and seafood were still slightly higher (48%) than meat (43%) (Howe et al., 2006). In addition, earlier studies reported that meat, poultry and game products were the main contributors of AA intakes (70%), followed by fish and seafood (27%) (Meyer et al., 2003). More recently, findings from the Australian Longitudinal Study on Women's Health (ALSWH) suggest that women who are trying to conceive (n=454), pregnant (n=606) or who gave birth less than 12 months ago (n= 829) have fish intakes of 31g, 28.8g and 27.8g per day respectively, which are below the recommended levels (42 – 64g/day, equivalent to 2 to 3 150g fish serves per week) for optimal maternal and infant health outcomes (Food Standards Australia and New Zealand (FSANZ), 2011; Taylor, Collins, & Patterson, 2014). Consumption of meat products was found to be higher in pregnant women (174g/day) compared to non-pregnant women (109g/day) according to findings from a study conducted by Cosatto and colleagues (2010). This study also reported that pregnant women ate less fish and seafood (35g/day) than non-pregnant women (51g/day).

Findings from the NZ National Nutrition Survey (NNS) 2008/09 showed that the main contributors to total PUFA intakes in females aged 19-30 (n=634) and 31-50 (n=746) years were respectively: butter and margarine (8.8 and 10.3%); bread (7.0 and 8.0%) and bread-based dishes (7.0 and 6.1%); potatoes, taro and kumara (78.2 and 6.6%); vegetables (5.6 and

6.6%); poultry (6.9 and 5.7%); all red meats (beef, veal, pork, lamb, mutton and other meats) (9.5 and 8.6%); and fish and seafood (4.5 and 6.0%). The majority of women reported the intakes of red meats (88.9 and 90.1%, for ages 19 to 30 and 31 to 50 years respectively), poultry (91.7 and 88.1%) at least once per week compared to less than half of women who reported the intakes of fresh/frozen (32.2 and 40.4%), canned (23.3 and 34.7%), and battered (12.2 and 13.7%) fish and seafood at least once per week (University of Otago, 2011; University of Otago & Ministry of Health, 2011). In the NNS 1997 (n=4636 adults aged 15 and over), although there was no further analysis to determine the contribution of food sources to daily intakes of total and individual PUFAs, findings suggest a higher proportion of people chose consuming beef/veal (54%), beef mince (45%) and poultry (42%) at least once a week compared with fish (15%) and shellfish (6%) (Ministry of Health, 1999). These findings suggest that NZ's standard diet is similar to Australia and other developed countries like the United States and the UK, with high intakes of red meats and poultry, and low fish and seafood intakes. However, it is unknown whether the NZ adult population, especially pregnant and childbearing-aged women are meeting the recommended n-3 LC-PUFAs levels for optimal health outcomes.

The opposite is observed in populations such as the Japanese, the Inuit people from Nunavik in Canada and people of the Republic of Seychelles, who consume high amounts of fish and seafood with minimal intakes of red meats and poultry as part of their standard diets. The majority of the Japanese population (95%) consume fish and seafood at least once per week (Iso et al., 2006), with an average intake approximately four times higher than other developed countries, which is likely to contribute to their increased n-3 LC-PUFAs intakes (Miyagawa et al., 2014). Consumption of fish in the Seychelles is considered high, with pregnant women consuming

an average of nine fish meals per week, the equivalent to approximately 500g of fish weekly (Bonham et al., 2008). While the Inuit people have increased intakes of fish and marine mammals, and therefore their total daily n-3 LC-PUFA intakes are substantially high (2,115mg of EPA + DHA) (Dewailly, Blanchet, Gingras, Lemieux, & Holub, 2003).

Low intakes of fish and seafood is an issue of particular concern for pregnant women as these foods are the main sources of n-3 LC-PUFA, and required at greater amounts for normal fetal growth and development (see Section 2.5).It is concerning that dietary data available for many developed countries, show that fish intake among pregnant and non-pregnant women to be below the two weekly servings recommended (Koletzko et al., 2007a).

2.9 Dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids

Dietary intakes of n-3 LC-PUFAs are thought to be approximately 80% lower than what they used to be over two centuries ago (Genuis & Schwalfenberg, 2006). Also, current dietary intakes are considered to be insufficient for optimal health outcomes (Elmadfa & Kornsteiner, 2009a; Hibbeln et al., 2006; Meyer, 2011). The latter is a reflection of the agricultural and industrial revolutions that happened over 10,000 years ago, when remarkable changes started shaping the dietary patterns of developed countries. Substantial amounts of seeds, grains, dairy products, vegetable oils and processed foods became important components of the modern western diet (Cordain et al., 2005; Simopoulos, Leaf, & Salem, 1999). In addition, small farms and food producers were taken over by mass-scale food and agricultural industries, which employed the use of cheaper grains based animal feed and vegetable oils in food manufacturing, causing an increased n-6 and reduced n-3 PUFAs

composition of animal products as well as processed foods (Blasbalg, Hibbeln, Ramsden, Majchrzak, & Rawlings, 2011). The modern western diet reflect these changes with high amounts of omega-6 PUFAs, saturated fatty acids, *trans* fatty acids, and refined carbohydrates, and poor in intakes of omega-3 PUFA (Janssen & Kiliaan, 2014; Muskiet et al., 2006). A schematic summary of the major changes in diet composition followed after the agricultural and industrial revolutions are presented in Figure 2.8.

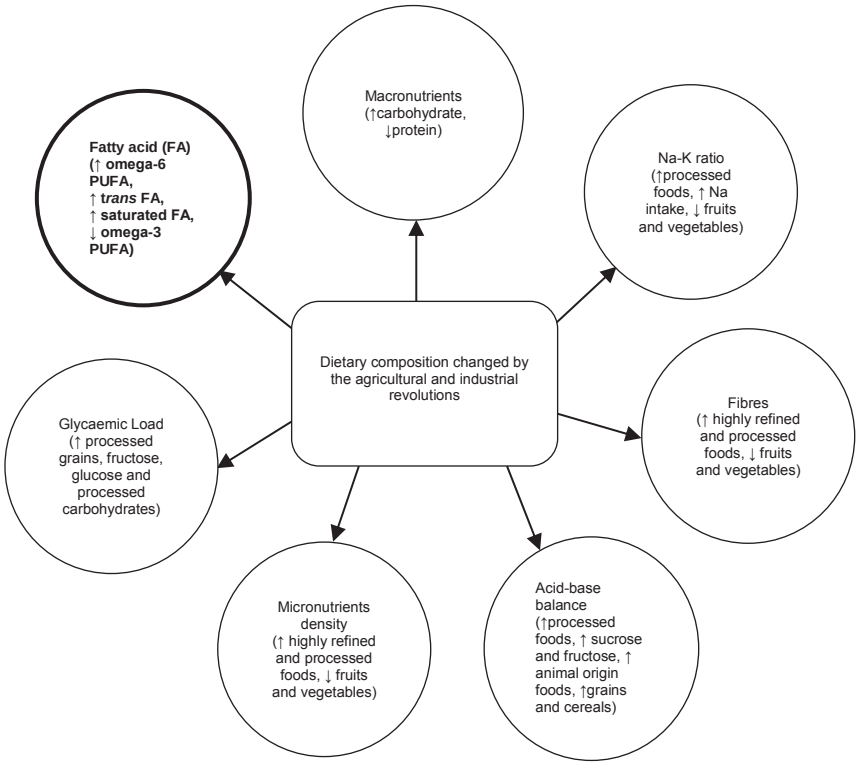

Figure 2.8 - The major dietary composition changes happening over the past two centuries. Adapted from Muskiet et al. (2006) and Cordain et al. (2005).

A study by Hibbeln and colleagues, (2006), showed that many populations around the world are not achieving the recommended intakes of 500mg/day for total n-3 LC-PUFAs (EPA plus DHA) suggested by the International Society for the Study of Fatty Acids and Lipids (ISSFAL; Simopoulos et al., 2000) (refer to Figure 2.9). Findings of this study were based on commodity data for domestic food supply and food

disappearance data, available for populations of 38 countries including New Zealand (Hibbeln et al., 2006).

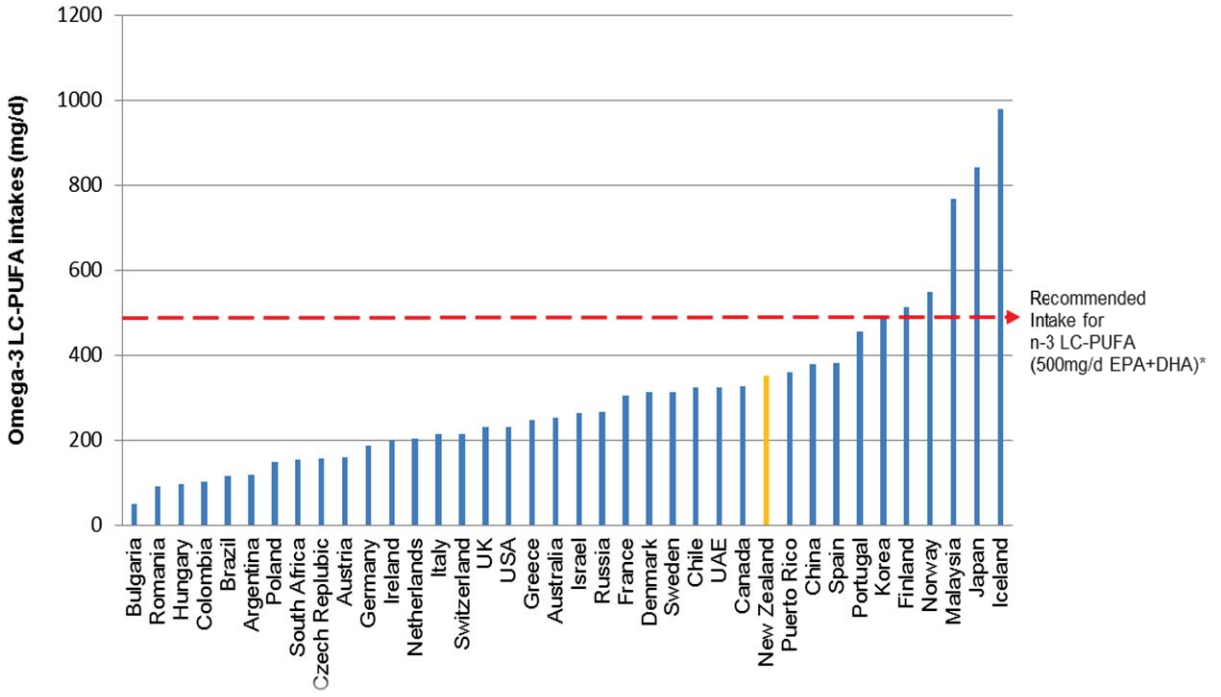

Figure 2.9 – Intakes of long chain omega-3 polyunsaturated fatty acids (mg/d) of various populations worldwide. Figure adapted from Hibbeln et al. (2006).

*International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommendations for long chain omega-3 polyunsaturated fatty acid intakes (Simopoulos et al., 2000).

These dietary intake data aligns with results from a more recent systematic investigation on global consumption of major dietary fats, assessing dietary survey data derived from 266 countries worldwide (83% had representative samples). Surveys were conducted in adults aged 16 and over (n=1,630,069) in 1990 and 2010. Some limitations were identified, including more data being available for 2010 compared to 1990, different data collection methods that lead to differences in reported intakes between countries, and data for all fatty acids not being available for some countries. However, findings from this comprehensive investigation revealed that in 20 years, there was an overall increase in n-

3 and n-6 PUFA intakes. An increase in n-3 LC-PUFA intakes (25mg/day) was observed in populations from Pacific Islands, Iceland, South Korea, Mediterranean countries, and Japan. Despite this increase in n-3 LC-PUFA intakes, mean global intakes seem to remain below optimal levels. Nearly 80% of the countries included in this investigation had mean total n-3 LC-PUFA intakes below 250mg/day, which does not meet the recommendations for healthy adults of 350 to 500mg/day (Micha et al., 2014; Scorletti & Byrne, 2013; Simopoulos et al., 2000).

However, limitations must be acknowledged when comparing global PUFA intakes, as these may be influenced by over- or under-estimations of the true dietary intake of PUFAs. Differences between countries may be caused by cultural dietary diversity, differences in the socio-demographic characteristics of the population, distinctive data collection methods and food composition databases, as well as the country–specific variety and availability of PUFA rich food sources (Meyer, 2011). Despite these limitations, the available data suggests that many populations may be easily achieving the recommended levels for n-6 PUFAs while recommendations for n-3 LC-PUFAs are far from being met (European Food Safety Authority, 2009; Food and Agriculture Organization of the United Nations and World Health Organization, 2010; Kornsteiner et al., 2008; Muskiet et al., 2006; Ratnayake & Galli, 2009; Whelan et al., 2009).

2.9.1 Studies investigating dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids in pregnant women overseas

While existing data shows that n-3 LC-PUFAs intake of women in developed countries is generally low (Ervin, Wright, Wang, & Kennedy-Stephenson, 2004; Gebauer et al., 2006; Howe et al., 2006; Linseisen et al., 2003; Sioen et al., 2010; Sioen et al., 2006), there is little data showing that these women change their dietary habits to enhance their n-3 PUFA intakes upon becoming pregnant (Bosaeus et al., 2015; Makrides, 2009a).

In contrast, dietary studies have supported the idea that pre-pregnancy dietary habits remain unaltered during pregnancy (Crozier, Robinson, Godfrey, Cooper, & Inskip, 2009; Fowler, Evers, & Campbell, 2012; Hure, Young, Smith, & Collins, 2009; Morrison et al., 2012; Wilkinson, Miller, & Watson, 2009). Moreover, an increasing number of studies analysing diet quality prior to and during pregnancy reported that women are failing to meet the recommended levels of n-3 LC-PUFAs (Bernard et al., 2013; Cosatto et al., 2010; Crozier et al., 2009; De Vriese, De Henauw, De Backer, Dhont, & Christophe, 2001; De Vriese et al., 2002; Denomme et al., 2005; Donahue et al., 2009; Fawzi, Rifas-Shiman, Rich-Edwards, Willett, & Gillman, 2004; Fowler et al., 2012; Friesen & Innis, 2009, 2010; Hure et al., 2009; Innis & Elias, 2003; Jia et al., 2015; Lakin et al., 1998; Loosemore, Judge, & Lammi-Keefe, 2004; Meyer, 2011; Oken et al., 2004; Oken et al., 2007; Otto et al., 2001a; Sioen et al., 2010; Sontrop et al., 2008; Stark et al., 2005; Thomas et al., 2006; Wu, Dyer, King, & Innis, 2013).

A systematic review and meta-analysis of in developed countries found pregnant women were not meeting their country-specific recommendations for all nutrients and energy (Blumfield et al., 2012). Dietary intakes for PUFAs were also below the recommended levels. Although this meta-analysis included ninety observational studies (n=126,242), only four studies had representative samples, with the remaining having small sample sizes. Other limitations that may influence the estimations of nutrient intake, making it difficult to accurately interpret this data included: unsatisfactory description of methods and participant characteristics; dietary analysis of pregnant women at diverse gestational ages; the use of different dietary assessment methods and food composition databases, and the large difference in time when studies were conducted (between years 1961 – 2009) (Blumfield et al., 2012).

Despite these limitations, findings from this meta-analysis are a good indication that pregnant women are not achieving their local dietary recommendations.

There are not many studies investigating dietary intakes of n-6 and n-3 PUFAs in pregnant women. Often, studies combine dietary assessment from multiple nutrients. For this review, a total of twenty studies reporting n-6 and n-3 PUFA intakes in pregnant women were identified and are presented in Table 2.3. The majority of studies investigated the intakes of PUFAs in pregnant women during their last trimester (Denomme et al., 2005; Donahue et al., 2009; Friesen & Innis, 2009, 2010; Innis & Elias, 2003; Loosemore et al., 2004; Sioen et al., 2010), while a few studies conducted their dietary PUFA assessment during the first trimester (Fawzi et al., 2004; Oken et al., 2007; Otto et al., 2001a; Wu et al., 2013), second trimester (Cosatto et al., 2010; Olsen et al., 2007; Sontrop et al., 2008), or two or more different time-points over the course of pregnancy (De Vriese et al., 2002; Jia et al., 2015; Otto et al., 2001a; Stark et al., 2005).

When combining data from fifteen studies reporting total PUFA intakes in pregnant women, mean intakes ranged from 9,800 to 17,700mg/d. Mean LA intakes ranged between 8,000 and 15,600mg/d in seventeen studies. Dietary data for mean ALA intakes from twenty studies ranged from 823 to 2,100mg/d. Arachidonic acid had mean intakes ranging between 20 to 289mg/d in seventeen studies. Data for total n-3 LC-PUFAs (EPA, DPA plus DHA) was available in two studies in pregnant women, with mean intakes ranging from 336.2mg/d in Australia and 450mg/d in Canada. Mean intakes of total EPA plus DHA were available for eight studies and ranged between 85 to 328mg/d. Eicosapentaenoic acid and DPA had mean dietary intakes ranging between 8 to 200mg/d and 17 and 102mg/d, respectively, in healthy pregnant women. These studies are all presented in Table 2.3.

Most importantly, Table 2.3 also presents dietary intake data for DHA, which ranged from 59 to 320mg/d in normal pregnant women. Mean DHA intakes below 200mg/d were reported in studies from Australia, the United States, Canada, the United Kingdom (UK), Scotland, France and the Netherlands. For instance, findings from a Canadian cohort (n=600) suggest that three-quarters of pregnant women (73%) did not meet the recommendations for DHA, which were 10.6 and 11.1 times more likely to be met by women taking 3 LC-PUFA supplements (30%) (Jia et al., 2015). Another Canadian study found that mean n-3 LC-PUFA intakes were 85mg/d in pregnant women (n=2,421), with nearly half of pregnant women (49.6%) having n-3 LC-PUFA intakes even lower than that (Sontrop et al., 2008). In Australia, pregnant women taking part in a cross-sectional study had median DHA (75mg/d) and total n-3 LC-PUFA (235mg/d) intakes below consensus recommendations (DHA, 200mg; EPA plus DHA, 500mg/d) from ISSFAL (Simopoulos et al., 2000) and the National Heart Foundation of Australia (Colquhoun, Ferreira-Jardim, Udell, & Eden, 2008), with only 9% achieving 200mg/d of DHA (Cosatto et al., 2010).

In contrast, countries such as Denmark and Japan presented mean DHA intakes above 300mg/d (Miyake et al., 2007; Otto et al., 2001a). A few studies conducted in small groups of Belgian pregnant women (n=26 and 30), also reported mean high DHA intakes (≥280mg/d), which could have been potentially influenced by the proximity to the coastal area where the studies were conducted, thereby encouraging increased intakes of fish and seafood (De Vriese et al., 2001; De Vriese et al., 2002).

High intakes of DHA were also reported in a European cohort, of pregnant women from Spain, Germany and Hungary, with reported median DHA intakes between 235 and 413mg/d (Franke et al., 2008). However, a study conducted in pregnant women (n=822) living in a Spanish Mediterranean area reported that more than half of participants had insufficient intakes of

n-3 PUFAs (data not included in Table 2.3) (Rodriguez-Bernal et al., 2013). These conflicting results may be explained by the higher educational levels of participants in the European cohort.

Other studies reported data for women with gestational diabetes mellitus (GDM), which ranged from 31mg/d DHA in the United States to 200mg/d in the UK (Loosemore et al., 2004; Thomas et al., 2006). Higher intakes of DHA found in women with GDM (n=44) in the UK may be explained by the fact that these women were provided with dietary advice once diagnosed with the condition (Thomas et al., 2006). However, in the United States pregnant women diagnosed with GDM (n=14) also received dietary advice, yet rarely reported the consumption of foods rich in n-3 LC-PUFAs (Loosemore et al., 2004). A Scottish study from Lakin and colleagues (1998) reported that mean DHA intakes of 270mg/d in omnivorous type one diabetic women (n=5). In contrast, values as little as 9mg/d DHA were reported amongst vegetarian pregnant women (n=4) in this same study (Lakin et al., 1998).

Two studies in the United States reported dietary data for DHA at delivery, with mean intakes showing a decrease from 110 to 80mg/d from gestational week 29 to delivery, and 81 to 59mg/d from gestational week 24 to delivery (Donahue et al., 2009; Stark et al., 2005).

Table 2.3 - Studies investigating dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids in pregnant and non-pregnant women

Country and year	Population (age and gestational age)	Design	Dietary assessment method	Total PUFA mg/d	LA mg/d	ALA mg/d	AA mg/d	EPA mg/d	DPA mg/d	DHA mg/d	Total LC n-3 PUFA EPA+DHA mg/d	Reference
Australia, 2009	n=94 28±5 years 20±5 weeks gestation	Cross-sectional	Validated FFQ	-	-	-	-	72[a]	75	75	235*	(Cosatto et al., 2010)
Australia, 2003	n=606 27.2±1.5 years Gestational age not reported	Young women cohort part of the Australian Longitudinal Study on Women's health (ALSWH, n=7,486)	74-item Validated FFQ assessing diet over the past 12 months	-	-	1,100±440	-	-	-	-	336±379*	(Taylor et al., 2014)
Canada, 2011	n=222 32.7±5.0 years 16 weeks gestation	Prospective cohort	Interview-administered 500-item FFQ at 16 weeks gestation	-	13,400± 5,700	1,700±900	93±44	61±65	-	110±96	-	(Wu et al., 2013)
Canada, 2010	n=105 33±3.9 years 36 weeks gestation	Cross-sectional	Interview-administered 500-item Validated FFQ	14,700± 7,890	12,200± 6,910	1,690±1,160	120± 58.3	81.1±78.3	-	146±161	-	(Friesen & Innis, 2010)

Country and year	Population (age and gestational age)	Design	Dietary assessment method	Total PUFA mg/d	LA mg/d	ALA mg/d	AA mg/d	EPA mg/d	DPA mg/d	DHA mg/d	Total LC n-3 PUFA EPA+DHA mg/d	Reference
Canada, 2010	n=600 31.6 (17 – 44) years 1st, 2nd and 3rd trimesters	Cohort at each trimester of pregnancy – Alberta Pregnancy Outcomes and Nutrition Cohort (APrON)	24-h recall and supplement intake questionnaire, conducted by a trained interviewer	**1st trimester** (n=129) 13,000± 7,000 **2nd trimester** (n=567) 13,000± 8,000 **3rd trimester** (n=502) 14,000± 8,000	11,000± 6000 11,000± 6,000 11,000± 7,000	1,200±800 1,300±1,200 1,400±1,200	104±110 106±157 113±157	135±259 141±351 172±389	24±44 31±92 40±98	159±268 187±461 237±508	318±517* 359±828 450±924	(Jia et al., 2015)
Canada, 2009	n=204 33±3.9 years 36 weeks gestation	Randomised Control Trial 400mg/d DHA or corn/soybean oil	Interview-administered Validated FFQ conducted at 16 and 36 weeks of pregnancy	**3rd trimester** 13,300±700 #	11,900±610	1,600±1,000	118±59.1	85.1±86.7	-	160±169	-	(Friesen & Innis, 2009)
Canada, 2005	n=20 28.6±0.8 years 27.7±1.5 weeks gestation	Cross-sectional	3-d dietary record, 3-d duplicate food portion collection and direct quantitation of dietary fatty acids analysis	9,800± 900	8,000±750	1,300±160	99±19	35±19	-	82±33	-	(Denomme et al., 2005)

Country and year	Population (age and gestational age)	Design	Dietary assessment method	Total PUFA mg/d	LA mg/d	ALA mg/d	AA mg/d	EPA mg/d	DPA mg/d	DHA mg/d	Total LC n-3 PUFA EPA+DHA mg/d	Reference
Canada, 2002 - 2005	n=2421 30.1±5.1 years 10 – 22 weeks gestation	Cross-sectional investigating depressive symptoms in relation to fish consumption and n-3 LC-PUFA intakes	Validated FFQ with 4 additional questions about fish and seafood consumption conducted by a trained interviewer by telephone	-	-	-	-	-	-	-	85.1	(Sontrop et al., 2008)
Canada, 1998	n=55 32.5 years (20 – 40 years) 35 weeks gestation	Cohort	Interview-administered Validated 500-item FFQ conducted at 28 and 35 weeks of pregnancy	13,400±600	11,200±400	1,600±100	121±7	78±2	-	160±20	-	(Innis & Elias, 2003)
United States, 2004	Control n=31 25±5.7 years GDM n=14 31.25±5.1 years 3rd trimester	Longitudinal and intervention. Intervention group (GDM) received prescribed diet for management of diabetes.	Interview-administered 24-h recall conducted at 30 – 34 and 34 – 38 weeks of pregnancy. Average intake from repeated recalls is presented.	**Control** 13,170± 6,200 **GDM** 16,060± 7,480	11,560± 5,440 14,360± 6,560	1,340±660 1,520±880	155±87 134±70	16±21 12±9	- -	68±100 31±19	120±200 50±33	(Loosemore et al., 2004)

Country and year	Population (age and gestational age)	Design	Dietary assessment method	Total PUFA mg/d	LA mg/d	ALA mg/d	AA mg/d	EPA mg/d	DPA mg/d	DHA mg/d	Total LC n-3 PUFA EPA+DHA mg/d	Reference
United States, 1999 - 2002	n=1540 Participants with normal blood pressure 32±5.2 years 1st trimester	Cohort	Self-administered semi-quantitative FFQ. validated for n-3 PUFA but not for n-6 PUFA	12,988± 3,651	11,748± 3,112	934±385	93±33	-	-	-	184±316	(Oken et al., 2007)
United States, 1999 - 2002	n=1,666 32.3±4.8 years 3rd trimester	Prospective cohort	140-item Self-administered validated FFQ completed at 29 weeks of pregnancy and delivery	**29 weeks** 13,420± 3,680 **At delivery** [b] -	12,140± 3,240 -	990±400 -	90±30 -	60±80 40±60	- -	110±90 80±90	160±170 120±140	(Donahue et al., 2009)
United States, 2000	n= 157 African American 24.5±5.2 years 2nd and 3rd trimester	Prospective cohort	Validated 111-item, self-administered FFQ completed at 24 weeks and delivery	**24 weeks** 19,000± 4,800 **At delivery** 17,700± 4,400	16,800± 4,400 15,600± 4,100	1,680±410 1,550±320	115±63 102±57	39±61 26±42	- -	81±94 59±59	119±152 85±100	(Stark et al., 2005)
United States, 1997 - 1999	n=75 African American 30±6.1 years n= 132 Caucasian 32.5±4,0 years 1st trimester	Validation study	179-item FFQ adapted from the one Nurse's Health Study.	- -	- -	**African American** 1,040±400 **Caucasian** 940±440	- -	- -	- -	- -	260±290 180±160	(Fawzi et al., 2004)

Country and year	Population (age and gestational age)	Design	Dietary assessment method	Total PUFA mg/d	LA mg/d	ALA mg/d	AA mg/d	EPA mg/d	DPA mg/d	DHA mg/d	Total LC n-3 PUFA EPA+DHA mg/d	Reference
Belgium, 2009	n=414 27.7±6.1	Cross-sectional	FFQ (not validated)	-	-	-	-	-	-	-	**All women** (n=414) 276±362 **Pregnant** (n=18) 328±323	(Sioen et al., 2010)
Belgium, 2001	n=30 30 years (25 – 37 years) Weeks 15 and 35 of pregnancy	Prospective cohort	Interview-administered Validated 180-item FFQ (completed at 6-22 and 32-40 weeks pregnancy)	**1st trimester** 14,900± 5,900 **3rd trimester** 15,400± 6,500	12,600± 5,400 13,100± 5,900	1,260±500 1,410±580	130±40 130±40	180±110 150±100	- -	310±180 280±190	- -	(De Vriese et al., 2002)
Belgium, 2001	n=26 30 years (25 – 37 years) Weeks 15 and 35 of pregnancy	Validation of FFQ against 7-d estimated food record	Interview-administered 180-item FFQ completed between 6 - 22 weeks and 32 – 40 weeks of pregnancy	**1st trimester** 15,900± 6,200 **3rd trimester** 16,100± 6,400	12,900± 5,700 13,700± 5,900	1,300±530 1,500±550	130±40 130±40	170±110 150±90	- -	300±200 300±190	- -	(De Vriese et al., 2001)
Denmark, 1999	n=54,344 2nd trimester	Prospective cohort (Danish National Birth Cohort)	Self-administered 360-items Semi-quantitative FFQ completed at week 25 gestation. Dietary reported over 1 month.	-	9,500±3,200	2,100±800	90±50	130±120	60±30	320±260	-	(Olsen et al., 2007)

Country and year	Population (age and gestational age)	Design	Dietary assessment method	Total PUFA mg/d	LA mg/d	ALA mg/d	AA mg/d	EPA mg/d	DPA mg/d	DHA mg/d	Total LC n-3 PUFA EPA+DHA mg/d	Reference
France, 2003	n=1335 29.2±4.8 years 1st week after delivery	Cohort -EDEN mother-child study	Not Validated 124-item semi-quantitative FFQ completed a few days after delivery	**Breastfeeding** (n=997) 11,400± 4,980 **Not breastfeeding** (n=338) 10,900± 4.820	9,600±4,440 9,200±4,260	878±344 823±338	151±79 153±87	81±57 71±53	- -	170±111 148±990	- -	(Bernard et al., 2013)
Netherlands, 2000	n=20 30.7±0.6 (SEM) 10 weeks gestation	Cohort investigating changes in maternal fatty acids profile during early pregnancy	Self-administered Validated FFQ completed at enrollment and week 10 of gestation	15,690±900	13,170±900	1,020±90	20±0.00	80±30	20±10	140±50	-	(Otto et al., 2001a)
Scotland, 1998	n=10 Omnivore 28±6 years n=4 Vegetarian 27±3 years n=5 Diabetic omnivore 29±7 years 3rd trimester	Cross-sectional	Self-administered validated 173-item FFQ complete between 38 and 42 weeks of pregnancy	**Omnivore** 11,563± 3,050 **Vegetarian** 12,042± 2,899 **Diabetic omnivore** 13,026± 4,411	9,554±2,751 10,446± 2,662 10,564± 3,524	1,218±327 1,492±202 1,405±444	198±51 42±17 289±41	143±126 8±5 180±186	102±22 17±10 125±8	173±154 9±5 270±275	- - -	(Lakin et al., 1998)

Country and year	Population (age and gestational age)	Design	Dietary assessment method	Total PUFA mg/d	LA mg/d	ALA mg/d	AA mg/d	EPA mg/d	DPA mg/d	DHA mg/d	Total LC n-3 PUFA EPA+DHA mg/d	Reference
Europe, 200 - 2003	<u>Spain</u> n=62 30.1±4.9 years <u>Germany</u> n=97 33.5±3.5 years <u>Hungary</u> n=152 29.4±4.8 years Weeks 20 and 30 of pregnancy	Three European cohorts	24-item FFQ completed at weeks 20 and 30 of pregnancy	<u>Spain</u> **Week 20** 29,500 [24,200-40,600][c] **Week 30** 29,500 [24,000-38,700] <u>Germany</u> **Week 20** 20,100 [16,500-31,100] **Week 30** 21,000 [15,800-27,200] <u>Hungary</u> **Week 20** 39,000 [29,600-51,900] **Week 30** 37,400 [27,900-56,100]	-	-	-	-	-	<u>Spain</u> **Week 20** 413 [297-327] **Week 30** 403 [325-494] <u>Germany</u> **Week 20** 235 [154-244] **Week 30** 259 [136-363] <u>Hungary</u> **Week 20** 315 [231-444] **Week 30** 317 [239-458]		(Franke et al., 2008)

Country and year	Population (age and gestational age)	Design	Dietary assessment method	Total PUFA mg/d	LA mg/d	ALA mg/d	AA mg/d	EPA mg/d	DPA mg/d	DHA mg/d	Total LC n-3 PUFA EPA+DHA mg/d	Reference
United Kingdom, 2006	n=44 Control 28.02±5.8 years 39.3±1.4 weeks gestation n=44 GDM 31.25±5.3 years 38.5±1.1 weeks gestation	Intervention study. Intervention group (GDM) received dietary counseling every 2 weeks.	4-d food record (consecutive days including 1 weekend) day). Dietary data available for both groups after intervention.	**Control** 13,550± 5,570 **GDM** 14,290± 7,150	11,710± 5,180 12,210± 6,590	1,330±560 1,420±770	120±130 282±174	110±220 160±250	- -	130±140 200±170	- -	(Thomas et al., 2006)
Japan, 2002	n=1002 <29 (37.9%), 29-31 (29.8%) and 32+ (32.2%) years <15 (35.6%), 15-20 (32.8%) and 21+ (31.5%) weeks gestation	Cross-sectional	Self-administered validated 147-item FFQ completed at <15, 15-20 and 21+ weeks gestation	-	-	-	-	200±200	-	300±200	-	(Miyake et al., 2007)

Data expressed as mean±SD unless otherwise specified. * Total EPA + DPA + DHA; NHANES; [#] Ramdom assignment to supplenet of placebo did not impact on dietary intakes between groupd and therefore intakes are presented for all participants; [a] median fatty acid intakes; [b] FFQ applied at delivery was composed of nine questions designed to assess the frequency of consumption of major food sources of n-3 and *trans* fatty acids over the past month (not validated), [c] medians and interquartile ranges (P25-P75) **Abbreviations**: GDM, gestational diabetes mellitus; LA , linoleic acid ; ALA, alpha-linolenic acid: AA, arachidonic acid ; EPA, eicosapentaenoic acid ; DPA, docosapentaenoic acid ; DHA, docosahexaenoic acid.

Limitations of studies

The majority of the studies included in Table 2.3 used food frequency questionnaires (FFQs), while a few used 24-hour recalls and food records (FR), one study which used duplicate food collection with direct quantification of PUFA intakes (Denomme et al., 2005). Limitations referent to these dietary assessment methods cannot be discarded as resulting dietary data could be over- and/or under-estimated (Thompson & Subar, 2013). In addition, some of these methods may have not accounted for intakes of PUFA supplements. Dietary assessment methods and their strengths and limitations will be further explored in Section 2.10.

Other important limitation to be considered includes sample sizes, which were small for a few studies. In addition, the differences between s participants' characteristics from each study (including age, educational level, gestational age, country and area of residence, ethnic background, and household income), and the different seasons and years when studies were conducted could also potentially impact on true dietary intakes of PUFAs (Lee & Nieman, 2010).

Despite limitations, dietary data derived from studies in Table 2.3 show a range of dietary DHA and total n-3 LC-PUFA intakes suggesting that many pregnant women are not achieving the international consensus recommendations of daily 200mg of DHA and 500mg of total n-3 LC-PUFAs (refer to Section 2.6). In addition, the majority of studies have described their participants' PUFA intakes using the mean, which may over-estimate true dietary intakes due to possible outliers (Field, 2009). Therefore, PUFA intakes derived from these studies may be even lower if described using the medians. Yet, it is concerning that some studies also reported mean DHA intakes below 60mg/d, which suggests that some pregnant women are not even achieving the daily estimated value for fetal tissue accretion during the last trimester (~70mg/d - refer to Section 2.4.3) (Makrides, 2009a).

2.9.2 Studies investigating dietary intakes of polyunsaturated fatty acids in New Zealand in pregnant women

Only a few studies have investigated dietary intakes of energy and macronutrients including total PUFA intakes in pregnant women in New Zealand. An early study conducted in Dunedin pregnant women (n=95), used the 3-day weighed food record method to assess maternal dietary intakes at each trimester of pregnancy. Findings from this study showed that median intakes of total fat have increased from the first (91,000mg/day) towards to the last trimester (94,000mg/day), while total PUFA median intakes have decreased from 12,000mg/day in the first and second trimesters to 10,000mg/day during the last trimester (McKenzie-Parnell, Wilson, Parnell, Spears, & Robinson, 1993). More recently, Watson and colleagues conducted two studies using 24-h recall and 3-day food records at 4 and 7 months of pregnancy to investigate maternal dietary intakes, with results showing median intakes of total PUFA between 10,000 and 11,000mg/day (n=369) (Watson & McDonald, 2014), and mean intakes ranging from 11,000 to 12,000mg/day (n=403) (Watson & McDonald, 2010) in pregnant women from northern NZ.

Further studies reporting PUFA intakes in NZ included the National Adult Nutrition Surveys (NNS) from 1997 and 2008/09. Both NNS used the 24-hour dietary recalls coupled with the dietary history questionnaire (DHQ) (2008/09) or a FFQ in addition to the DHQ (1997) to assess dietary intakes and food sources in adults aged 15 years and over. Results from the NNS-1997 showed women aged 25 to 44 years (n=1964) had mean intakes of total PUFA of 11,000mg/d (Ministry of Health, 1999). In the NNS 2008/09, mean intakes of total PUFA were 9,900 and 10,500mg/d in women aged 19 to 30 (n=434) and 31 to 50 (n=745) years respectively (University of Otago, 2011; University of Otago & Ministry of Health, 2011). Despite the availability and good quality data from the NZ NNS 1997 and 2008/09, there were no reports detailing dietary intakes of individual fatty acids.

Limited existing data report the intakes of individual PUFAs in NZ. These include data from a review in which estimated PUFA intakes in adults were 15,000mg/day for LA, 1,000mg/day for ALA and less than 200mg/day for total DHA and EPA (Eyres, 2000a, 2000b). At these levels, total DHA and EPA are below the suggested dietary targets (SDTs - 610mg/d men and 430mg/d women) recommended for prevention of chronic diseases (NHMRC, 2006).

It is unclear why previous studies have only investigated total PUFAs intakes with no interest in individual fatty acids. Perhaps, the lack of a robust dietary assessment tool capable of estimating dietary intakes of individual fatty acids was the reason. However, in 2012 a robust FFQ was adapted and validated to estimate PUFA intakes in healthy adults in NZ. Median dietary intakes were estimated for 48 adults in the validation study, with values being 10,200mg/d for LA, 1,900mg/d for ALA, 90mg/d for AA, 170mg/d for EPA, 60mg/d for DPA and finally 220mg/d for DHA. Values for DHA were high, which could be due to the intakes of PUFA supplements, which were accounted for in this particular FFQ. However, this is only a speculation as no further data on the intakes of PUFA supplements intake were made available for this validation-focused study (Ingram et al., 2012).

2.9.3 Barriers to achieving recommended intakes of omega-3 polyunsaturated fatty acids

Studies support that increased dietary intakes of n-3 LC-PUFAs during pregnancy are important for optimal maternal and fetal outcomes (refer to Section 2.5). However, an increasing number of studies reported that pregnant women are not meeting the recommended levels for n-3 LC-PUFAs (see Section 2.9.1). This may be an effect of socioeconomic, individual, cultural, ethical and environmental factors, that can influence the mother's dietary PUFA intakes, and consequently impact on maternal

and fetal health outcomes (Abu-Saad & Fraser, 2010; Gebauer et al., 2006; Thompson et al., 2010).

The cost of the richest n-3 LC-PUFA food sources, such as fish or fortified products (e.g. eggs), can be relatively expensive, therefore creating a significant barrier to obtaining adequate intakes of n-3 LC-PUFAs (Mitchell et al., 2004; Pauga, 2009; Troxell et al., 2005). Evidence shows that women with low socioeconomic status and education levels may be more likely to have a diet that does not contain such food sources compared to women with higher socioeconomic status (Carder & Lewis, 1999; Darmon & Drewnowski, 2008; Donahue et al., 2009; Gibbs, Rymer, & Givens, 2010; Rodriguez-Bernal et al., 2013).

Some individuals may not be able to achieve the recommended levels n-3 LC-PUFAs within their diets for different reasons, which may include being allergic to fish and seafood, following a diet that excludes important sources of n-3 LC-PUFA (e.g. vegan or vegetarian), and disliking or avoiding fish consumption due to sustainability issues (Bauch, Lindtner, Mensink, & Niemann, 2006; Kris-Etherton et al., 2009).

Individuals following a vegetarian or vegan dietary patterns, for personal or religious (e.g. hinduism) reasons, are at risk of not achieve the recommended levels for n-3 LC-PUFAs for several reasons including, low conversion rates of ALA to n-3 LC-PUFAs; an increased intake of LA from grains and nuts which further suppresses the synthesis of n-3 LC-PUFA, and exclusion of important food sources from their diets, including meat, eggs, fish and seafood (Haggarty, 2004; Kornsteiner et al., 2008; Lakin et al., 1998). For instance, vegetarian diets including eggs and dairy products are suggested to provide only 20mg/d of DHA (Sanders, 2009a). Furthermore, vegetarian and/or vegan women were shown to have lower DHA levels in tissues, blood, and breast milk compared to omnivorous women (P<0.001) (Sanders, 2009a).

Exposure to potential environmental contaminants, including methylmercury, is the primary concern of eating fish and seafood, as this heavy metal can cause particular harm to the developing brain of the growing fetus and children at large exposure levels (Gebauer et al., 2006). Toxic mercury levels, set as Provisional Tolerable Weekly Intakes (PTWI) of >1.6μg/kg body weight (Food Standards Australia and New Zealand, 2011), are associated with infants and children impaired cognitive and neurological development (Clarkson, Magos, & Myers, 2003; Oken et al., 2005), hyperactive disorders and poor social behaviours (Poniedzialek-Czajkowska et al., 2014; Sagiv, Thurston, Bellinger, Amarasiriwardena, & Korrick, 2012). However, higher fish consumption (more than two servings per week) was associated with better infant cognition when mercury levels were ≤1.0μg/kg body weight during pregnancy (Hibbeln et al., 2007; Oken et al., 2005). Similar studies found that increased intakes of fish and seafood were associated with improved child language development (Strain et al., 2012). Many studies have considered the risk-benefit of fish and seafood consumption, suggesting that detrimental effects of contaminants are exceeded by the beneficial effects of PUFAs and other nutrients that are essential for normal fetal growth and neurodevelopment (Al-Ardhi & Al-Ani, 2008; Costa, 2007; Davidson et al., 2011; European Food Safety Authority, 2012; Hibbeln et al., 2007; Markhus et al., 2013; Mozaffarian & Rimm, 2006; Strain et al., 2008; Strain et al., 2012).

Based on the evidence, many organisations have established advice for the risk of consuming certain fish and seafood with high levels of contaminants in vulnerable populations, which include children and pregnant and lactating women (Smith & Sahyoun, 2005). At the same time, pregnant women are recommended to increased their n-3 LC-PUFA intakes from fish and seafood to improve maternal health and fetal brain development (Hibbeln et al., 2007; Koletzko et al., 2008; Oken et al., 2005), which creates mixed messages and confusion within individuals

(Smith & Sahyoun, 2005). Moreover, messages about contaminants in fish and seafood can be negatively perceived (Sioen et al., 2010). These may result in a decreased intake of fish and seafood amongst pregnant women who may not be fully aware of the importance of n-3 LC-PUFA and other nutrient contents of these food sources for optimal birth outcomes (Emmett et al., 2013; Sinikovic et al., 2009).

Decreasing fish and seafood intakes to avoid methylmercury exposure can compromise the intakes of n-3 LC-PUFAs and other crucial nutrients that are important during pregnancy (Daniels et al., 2004; Lund, 2013; Taylor et al., 2014). A recent study demonstrated that fish intake is positively associated with vitamin D and choline intakes, which are also important nutrients for a healthy gestation and optimum fetal development (Wu et al., 2013). Other studies suggest that dietary intakes of two 150g servings of fish (e.g. Tuna, Atlantic and Australian Salmon) per week can fulfil pregnant women's AI (NHMRC, 2006) by: 915% for n-3 LC-PUFAs, 68% for Vitamin D, 19% for Vitamin E, 12% for iodine and up to 45% of the RDI for Selenium (Taylor et al., 2014).

It is suggested that most fish and seafood consumed in Australia and NZ are within the safe mercury limits for the general population (Love, Rush, & McGrath, 2003; Taylor et al., 2014). The maximum concentration allowed in fish in Australia and NZ are set between 0.5 to 1.0mg/kg (Food Standards Australia and New Zealand, 2011). Levels of mercury exposure from fish and seafood in males and females aged 25 years and over in NZ were estimated to be between 0.19 to 0.34µg/kg body weight per week (based on fish/seafood intakes of 245g per week), which are well below the 1.6µg/kg PTWI levels (Ministry of Primary Industries, 2011). In addition, even if pregnant women consume two 150g servings of fish with mean levels of mercury of 0.343mg/kg, as found in wild tuna caught in Australia for example, their exposure to this contaminant will still be within the PTWI (Taylor et al., 2014). Therefore, when fish and seafood are

selected and consumed according to recommendations from local food safety authorities, pregnant women can safely achieve the beneficial effects of important nutrients in these food sources without reaching mercury toxic levels (Mahaffey et al., 2011; Smith & Sahyoun, 2005).

2.9.4 Achieving the recommendations

Without an adequate nutritional supply of n-3 PUFA rich foods, mothers can experience lower levels of these fatty acids during pregnancy (Ramakrishnan, 2011). Fortunately it is likely that the initiation of enhanced n-3 LC-PUFA intake at any stage in pregnancy can at least partially make up for previously low intakes prior to conception and in the first weeks of gestation, unlike other nutrients such as folate, for which intake around the time of conception is crucial (Brenna & Lapillonne, 2009; Cao, Schwichtenberg, Hanson, & Tsai, 2006).

Some research suggests that reducing LA intakes to 7g/d and increasing ALA intakes to 2g/d may be a way of achieving a desirable and balanced intake of n-3 PUFAs or a better n-6:n-3 ratio (Eyres, 2000a; Simopoulos et al., 1999). However, LA is a predominant constituent of mainly commonly consumed foods, including spreads, oils and most takeaway and processed foods (Meyer et al., 2003). In addition, substituting LA rich oils with of ALA-rich oils such as walnut, canola and flaxseed can be expensive (Wood, Mantzioris, Gibson, & Muhlhausler, 2013) and not necessarily improve n-3 LC-PUFA status of individuals for several reasons previously discussed (see Section 2.2). Therefore reducing LA intakes does not appear to be a practical and sustainable approach to improving n-3 PUFA intakes and further accumulation of membrane phospholipids (Baum et al., 2012). In addition, controversies regarding the importance of the n-6:n-3 ratio continue to reign in the research field as both n-6 and n-3 PUFAs yield important functions in protecting health integrity. Thus, n-6:n-3 ratio may not be an useful concept, and it may even distract attention

away from increasing absolute intakes of n-3LC-PUFAs (Stanley et al., 2007). Instead, increasing dietary intakes of n-3 LC-PUFA rich foods may be the most effective way of improving DHA and EPA body stores while preventing the effects of their deficiencies (Gebauer et al., 2006; Meyer, 2011).

Consensus recommendations of 200mg/day of DHA for pregnant women can be met by consuming one to two weekly 115g servings of oily fish such as salmon, herring and mackerel (European Food Safety Authority, 2010; Koletzko et al., 2007a; Koletzko et al., 2008; Simmer et al., 2009). However, pregnant women who intend to achieve n-3 LC-PUFA recommendations from fish and seafood are advised to follow their country specific food safety advisories in regards of potential environmental contaminants present in certain species of fish and seafood (Kris-Etherton et al., 2009).

Current guidelines from Food Standards Australia and New Zealand (FSANZ (2011)) recommend weekly intakes of two to three 150g serving of fish species with lower mercury levels, as indicated in Table 2.4.

Table 2.4 - Number of weekly serves of fish and seafood that can be safely consumed in New Zealand

Pregnant women and women planning pregnancy
2 – 3 serves* per week of canned tuna, sardines, salmon, mackerel, eel, warehou, kahawai, skipjack tuna, whitebait, blue cod, tarakihi, john dory, hoki, flounder, monkfish, mussels[1] or any other fish and seafood not listed below[2]
OR
1 serve per week of Orange Roughy (Sea Perch) or Catfish and no other fish that week
OR
1 serve per fortnight of Shark (Flake), Billfish (Swordfish / Broadbill and Marlin) or trout caught in geothermal regions, and no other fish that fortnight

Key: * 1 serve equals 150g for adults and older children are equivalent to approximately two frozen crumbed fish portions. Canned fish are sold in diverse sizes, average size can have ~170g while snack size can have ~95g. 1 Approximately 10 medium size mussels. 2 Limit or avoid the consumption of Bluff oysters and queen scallops due to high cadmium concentrations. Adapted from (Food Standards Australia and New Zealand (FSANZ), 2011; Miller, Pearce, & Bettjeman, 2014; Ministry of Health, 2006; Ministry of Primary Industries, 2013)

Other dietary sources of DHA include meats, poultry and eggs. For instance, 100g of lean red meat can contribute to 30mg DHA. A standard egg normally contains 45mg DHA, which can increase up to 195mg if chickens are fed with n-3 PUFA rich foods (Makrides, 2008; Meyer, 2011). Further strategies for achieving recommended levels, such as consuming other DHA rich foods, fortified foods (at least 60mg total EPA and DHA per serve) and/or omega-3 supplements can be very important (Garg, Wood, Singh, & Moughan, 2006; Gebauer et al., 2006; Meyer et al., 2003).

2.9.5 Supplements

Supplementation has been proposed as a way of pregnant women meeting n-3 LC-PUFA recommendations (Sanders, 2009b). A range of improved birth outcomes and the use of n-3 LC-PUFA supplementation have been explored in depth in Section 2.5. Most benefits were observed for high doses of n-3 LC-PUFAs (up to 3,000mg/d) supplementation during pregnancy, with no evidence of adverse effects for either mothers or their offspring (Carlson et al., 2013; Horvath et al., 2007; Makrides & Gibson, 2007; Szajewska et al., 2006).

Evidence also suggests that women with lower n-3 LC-PUFA status may benefit from n-3 LC-PUFA supplementation (Simmer et al., 2009). Moreover, studies suggested that maternal n-3 LC-PUFA supplementation can prevent complete depletion of maternal fatty acid stores imposed by the increased fetal demands in the last trimester (Bonham et al., 2008). A study investigating maternal n-3 LC-PUFA status acording to participants number of pregnancies found no association between these variables in women who consumed fish oil supplement, suggesting that adequate intakes of n-3 LC-PUFA especially throughout the last trimester can prevent DHA depletion (Dunstan et al., 2004). Yet a dose response has not yet been established, it was suggested that pregnant women (n=48) with low intakes of fish can improve their DHA status by up to 50% when

supplemented with doses as low as 200mg/day in the last trimester of pregnancy (Bergmann et al., 2008).

Studies investigated the bioavailability of n-3 LC-PUFAs from fish versus supplements. It was suggested that n-3 LC-PUFAs from fish are more effectively incorporated into plasma lipids than when administered in capsules (Elvevoll et al., 2006). The bioavailability of n-3 LC-PUFAs may be influenced by the different matrices present in fish flesh and fish oil capsules (Stonehouse et al., 2011; Visioli, Risé, Barassi, Marangoni, & Galli, 2003). However, studies have shown that tissues incorporation of n-3 LC-PUFAs from both fish and fish oil supplements happen at similar rates (Stonehouse et al., 2011).

Even though supplements may seem to be a helpful alternative for achieving the recommended intakes of n-3 LC-PUFAs, there are also some disadvantages to be considered. These include increased susceptibility to oxidation, lack of regulations to secure high-quality products and sustainability issues. For instance, fish oils contain highly unsaturated n-3 fatty acids that are very unstable and prone to oxidation. Although health implications from consuming oxidised fish oil supplements are unclear, oxidised lipids can trigger the oxidation of other fatty acids leading to a chain reaction that can reach the fatty acids in the membrane and cause cellular damage, which may give origin to disease (Albert, Cameron-Smith, Hofman, & Cutfield, 2013). A recent NZ study testing fish oil supplements showed that only 8% on 32 supplements had the fatty acids composition in accordance with what was described in their labeled contents and were below the recommended limits of oxidation levels (Albert et al., 2015). However, this study did not use validated and certified tests, which can compromise the reliability of their findings.

In addition, fish oil supplementation can also be a very expensive source not only financially but environmentally. Most fish oils are extracted from

large amounts of fish, which is not necessarily a sustainable and environmentally friendly process (McMichael & Butler, 2005). Therefore, other sustainable sources for n-3 LC-PUFA supplements have been explored, including algae-based as a potential solution for individuals who are unable to fulfil their recommended levels of n-3 LC-PUFAs with dietary intakes (Gebauer et al., 2006; Sioen et al., 2010).

2.10 Dietary assessment methods used to investigate omega-6 and omega-3 polyunsaturated fatty acids intakes

Overview of dietary assessment methods

There are several different methods available to assess dietary intakes of n-6 and n-3 PUFAs. The most commonly used methods include 24-hour recalls, food records (FRs, estimated and weighed), diet history questionnaire (DHQ), dietary screeners and food frequency questionnaires (FFQ) (Thompson & Subar, 2013). Other methods include direct quantification of dietary PUFA intakes from duplicated food collection over a short period of time (e.g. 3 days) and biochemical assays of biomarkers of nutrients exposure (Lee & Nieman, 2010). However, these other methods are less convenient methods. Each dietary assessment tool has its strengths and limitations presented in Table 2.5.

Table 2.5 - Methods commonly employed to investigate dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids

Method and Description	Strengths	Limitations
24h recall Provides detailed information on the foods and beverages consumed over the past 24 hours, based on portion sizes, brands, recipes, preparation methods and time of consumption.	Easily and quickly administered (approximately 20 minutes). Low respondent burden. Low cost. Multiple separate recalls days (at least 3 days) can provide a good assessment of foods consumed regularly. Can provide good estimates of dietary intakes of most nutrients in participants. Open-ended questions increase specificity. Can be self-administered or conducted by a trained interviewer.	Single day's dietary data cannot determine usual dietary intakes for individuals. Not appropriate to measure intake of foods that vary from day to day (e.g. fish and seafood), and that may vary with seasonal changes (e.g. summer versus winter). Reliant on respondent's memory. May require a trained interviewer with knowledge of locally and culturally eaten foods. Prone to over- and under-reporting. Data entering and analysis can be labour and time consuming.
Food record (FR) Provides detailed dietary intake data, including information on time of intake, brand, recipe quantity and portion size. Respondents record all foods and beverages at the time of consumption for a period ranging from 1 to 7 day. Foods and beverages can be estimated using standard household measures (e.g. cups, tablespoons), or weighed (weighed food record - WFR). Participants can add complementary information on consumed foods (e.g. food packages or photographs) if necessary. Respondents should be trained to accurately record their dietary intakes.	Weighed FR is considered the "gold standard" method for dietary assessment. Self-administered. Does not rely upon respondents memory. Multiple days FR can provide dietary intake details that are more representative of usual intakes. Open-ended responses increase specificity. Can provide good estimates of dietary intakes for most nutrients and foods. May indicate information on eating habits (e.g. mood and food relationships).	Large research burden. Data entry and analysis can be labour intensive and time consuming. Can be expensive. Perceived portion sizes and domestic measures may vary between individuals (e.g. estimated sizes of fruits or cups). These may over- or under-estimate dietary intakes. Dietary intakes may be inaccurate if respondents do not record foods and beverages upon consumption. Incomplete records may increase as more days of record are kept, which affects the validity of dietary data in the later days of a 7-days FR. Both FR and WFR present high participant burden. Requires certain literacy skills and time demanding.

Method and Description	Strengths	Limitations
Food frequency questionnaire (FFQ) Determines usual dietary intakes of energy and/or nutrients based on the frequency to which foods and beverages are consumed over an extended period of time. It can be tailored for specific nutrients (e.g. PUFAs). Frequency of food intakes may include consumption over the day, week, month, and year. *Non-quantitative FFQ* – is a simple FFQ that only asks the frequency of which foods and beverages are consumed. Cannot determine nutrient intakes. *Semi-quantitative FFQ* - indicates the portion sizes for foods to better guide respondents on their answers. *Quantitative FFQ* – respondents describe their portion sizes (e.g. small, medium large) based on a defined portion size.	Quantitative and semi-quantitative FFQs can be used to estimate intakes of energy and nutrients. Practical to rank individuals according to their dietary intakes. Useful to investigate associations of diet and disease. May include open-ended questions, which increase specificity. Low researcher and respondent burden. Time and cost saver. Can be interview- or self-administered (higher quality data when FFQ is administered by interviewer). Flexibility of completing the FFQ in the field or it can be mailed to respondents. More recent FFQs are available online and linked to food composition databases, which speeds data collection and analysis. Useful in studies with large sample sizes.	Reliant on respondent's memory and ability to complete the FFQ. Some FFQs may take longer to complete (e.g. > 30 minutes), increasing respondent's burden. Dietary intakes may vary with season. Closed-ended questions may decrease specificity. Non-blinded FFQs may lead individuals to adjust their answers. Dietary intakes may be compromised when foods are grouped into broad categories. Defined portion sizes and foods may not be meaningful to some respondents. Quantitative FFQ can be burdensome for respondents. Prone to under- and over-estimation of dietary intakes. Not suitable for cross-cultural comparisons. May lack the inclusion of some items that are exclusively eaten by certain ethnic groups, which can under-estimate dietary intakes.
Diet history questionnaire (DHQ) Used to assess respondent's usual dietary intakes over an extended period of time. Data collection usually involves 3 steps: 1) usual dietary patterns 2) cross-check on step 2 (FFQ or 24-hour recall) 3) 3-day FR	Determines usual dietary intakes. Can be used to gather data on all nutrient intakes. Provides detailed information about foods and beverages consumed. Can identify eating patterns and seasonal changes. Conducted by interviewer. Open-ended questions increase specificity.	Time consuming. Requires data coding which can be difficult and expensive. Requires trained interviewers, with knowledge of locally and culturally eaten foods. Large respondent and researcher burden. Susceptible to over- and/or under-reporting.

Method and Description	Strengths	Limitations
Dietary screeners Brief questionnaire tailored to identify whether individuals are at risk inadequate dietary intakes of specific nutrients (e.g. fat, sodium, dietary fibre, fruits). Can be a simplified food frequency questionnaire containing an average of 15 to 30 food items.	Quickly completed (approximately 10 minutes). Can be self-administered. Low cost and minimal respondent burden. May include open-ended questions, which increase specificity. Ideal for research that does not require quantitative accuracy of dietary intakes or assessment of the whole diet. Useful for clinical settings and epidemiological studies that investigate associations between diet and disease.	Reliant on respondent's memory. Dietary intakes may vary with season. Closed-ended questions can decrease specificity. Mostly provides qualitative data and therefore cannot measure usual intakes of the population. Focus only in a limited part of the diet (e.g. fruits, fish/seafood, n-3 LC-PUFA intakes).
Biochemical assay of biomarkers Biomarkers can measure exposure to nutrients from short- to long-term periods depending on the type of sample and biochemical assay employed. For example, the omega-3 index is a valid biomarker of n-3 LC-PUFA status. This test investigates the concentrations of DHA and EPA in erythrocyte membranes with results indicating the total percentage of n-3 LC PUFA. Whereas plasma phospholipids reflect intakes over the past few days and adipose tissue levels reflect long-term PUFA intakes. Common biomarkers used to assess PUFA intakes include, blood (plasma and erythrocyte phospholipid), umbilical cord blood, adipose tissue, breast milk.	Can provide a more accurate measure of long-term nutrient intake than can other dietary assessment tools. Can be used to determine change in nutrient status in intervention studies. Can be a better indicator of the association between nutrient intakes and risk of certain diseases (e.g. high LDL-cholesterol and the risk of cardiovascular diseases) . The Omega-3 index is a promising biomarker for cardiovascular disease and may be a potential predictor of other health conditions. Provide a validity check for other dietary assessment tools such as FFQs.	Can be expensive as well as invasive. May not be culturally appropriate due to religious and cultural beliefs. Only a limited number of biomarkers have been investigated, validated and standardised. No current cut-off levels have been established for fatty acid composition in plasma and tissues to which optimal health can be secured. Sensitive to many modifying factors other than dietary intakes, such as, age, smoking, genetic variants, and physiological factors (e.g. disease, obesity, pregnancy), individual inter-variability, gender, and rates of RBC erythrocyte phospholipid turnover.

Method and Description	Strengths	Limitations
Direct quantification of nutrient intake Identical portions of all food and beverages consumed are collected over the same period of time and kept in refrigerated. Researchers combine and blend all duplicate foods to homogeneity. The mix is then divided into equal parts that represent the numbers of days taken to collect the duplicates, with one part analysed biochemically.	Allows direct quantification of usual dietary intakes. Low researcher burden. Suitable for cross-sectional and cohort studies with small sample sizes.	Expensive. High participant burden. Under-estimation of usual dietary intakes can be a problem. For example, participants may feel guilty of wasting expensive or favourite foods, and may not collect duplicates for all foods and beverages consumed.

Source: (Baylin, Kabagambe, Siles, & Campos, 2002; Blumfield et al., 2012; Fawzi et al., 2004; Gibson, 2005; Harris, Varvel, Pottala, Warnick, & McConnell, 2013; Lee & Nieman, 2010; Potischman, 2003; Thompson & Subar, 2013)

Further methods used to measure dietary intakes include household food inventory, supermarket checkout records and food disappearance data. These methods are burdenless for participants and are based on the number of individuals in a household and food availability, purchasing and consumption within each household. These data can provide good information about foods eaten in a household and general countrywide dietary data. The latter can be useful for the development of FFQs. However, these methods overestimate dietary intakes and are not valid for estimation of individual dietary intakes as foods may not be evenly distributed in households and food wastage is not accounted for. In addition, foods obtained from private vegetable gardens, small animal farms and fishing are not be accounted for food disappearance data and supermarket checkout records (Emmett, 2009).

More recently, photography and video records have been used for collection of dietary intakes. These methods are considered valid, time effective, and well accepted with low respondent burden. However, large expenses, difficulty in distinguish and processing food images as well as high researcher burden are the biggest limitations of these methods (Lee & Nieman, 2010).

Collecting dietary intake data can be challenging, and each dietary assessment tool has its strengths and limitations. Nevertheless, the nature of the study investigating dietary intakes will determine the most appropriate tool to be used in the investigation. For instance, major factors to be considered when selecting the appropriate dietary assessment tools include the study design, sample size, characteristics of the target population (e.g. ability to communicate, education, age and culture), budget, time frame and available resources (Lee & Nieman, 2010). In addition, it is important that the selected method has been previously validated and tested for reproducibility in the target population in order to improve the quality and accuracy of the dietary data collected (Blumfield et al., 2012). Another important point to acknowledge is that all dietary assessment

methods will always present some limitations (Øverby, Serra-Majem, & Andersen, 2009).

As described in Table 2.5, 24-hour recalls and FRs (estimated and weighed) provide good estimates of dietary intakes for most nutrients and foods. The WFR is considered the 'gold standard' method to assess dietary intake due to its high level of precision in recording real dietary intakes (Lee & Nieman, 2010). However, data collection using WFR can be time-consuming and burdensome for participants, which may lead to low compliance (Lee & Nieman, 2010). In addition, these methods may yield extremely low or high intakes of particular nutrients as data is derived from the consumption of foods over a very short period of time such as the past 24 hours or a few days only. Therefore, these methods may be unable to give a real picture of the usual nutrient intakes especially when these are mainly provided by food sources that are not normally consumed on daily basis, such as fish and seafood (Innis & Elias, 2003; Lee & Nieman, 2010; Sioen et al., 2006). Although the use of a 24-hour recalls is ideal for large population groups, assessing dietary intakes for individual PUFAs may require multiple 24h recalls or FRs of a wide range of days (Fawzi et al., 2004).

Food frequency questionnaire to assess PUFA intakes

It has been calculated that at least 30 days of dietary data are required to estimate the individual PUFA intakes in women aged 18 years and over (Nelson, Black, Morris, & Cole, 1989). Therefore, FFQs may be the best approach to estimate usual dietary intakes of individual PUFAs. This method can be considerably fast, low cost and enable the collection of food and nutrient intakes over longer periods (e.g. weeks, months or year) (Thompson et al., 2010). As FFQs can be tailored to specifically collect data on dietary intake of individual PUFAs, these tools may be the most practical and primary method of choice in large epidemiological studies investigating PUFA intakes and its relation to health-related outcomes (Gibson, 2005).

A systematic review of dietary assessment methods used in studies investigating n-3 PUFA intakes identified good or acceptable correlations between FFQ estimates and blood or subcutaneous lipids (*r* ranged from 0.40 to 0.60) as well as good accordance with other methods such as 24-hour recalls and DHQs (Øverby et al., 2009). A number of studies found that fish and seafood intakes were reported with great reliability when using a validated FFQ (Daniels et al., 2004; Davidson et al., 2008; Mendez et al., 2009; Oken et al., 2008a). Further studies using FFQs to determine EPA and DHA intakes of pregnant women found that self-reported fish intakes had good correlation with erythrocyte phospholipid levels of DHA (*r* range from 0.37 to 0.63) and EPA (*r* range from 0.44 to 0.58) (Innis & Elias, 2003). In addition, FFQs appears to be valid tools to estimate nutrient intakes and to rank pregnant women according to their nutrient intakes (Brantsæter, Haugen, Alexander, & Meltzer, 2008; Erkkola et al., 2001; Fawzi et al., 2004; Hibbeln et al., 2007; Ingram et al., 2012; Lyu, Hsu, Chen, Lo, & Lin, 2014; Meyer, Swierk, & Russell, 2013; Mouratidou, Ford, & Fraser, 2006).

However, FFQs are not free of limitations and a major source of error is due to restrictions imposed by an individual's perception of pre-defined portion sizes and foods, which may not be meaningful to some respondents. It is also suggested that foods consumed near the time of completing a FFQ predominantly remain in the memory of respondents, and therefore responses tend to be based on these more recently consumed foods (Fowke et al., 2004).

Other important factors that can impact on an individual's responses include the length of time, the cognitive effort required to complete the dietary assessment tool, the method used to collect the data (e.g. online or hardcopy), and the layout in which the tool is presented (Rolstad, Adler, & Rydén, 2011). For example, the length of the FFQ may range from 10 to over 180 items, and this will impact on the time required for its completion. Completion times longer than

30 minutes may pose a barrier for respondents completing a FFQ (De Vriese et al., 2001). Similarly, the number of days included in the FR can impact on participant compliance and burden, leading to incomplete records. However, it is suggested that self-administered online dietary assessment tools may give respondents the convenience to access the tool from any location with internet access (Gibson, 2005; Subar et al., 2012). In addition, online dietary assessment tools can be linked to food composition databases and programmed to automatically calculate dietary intakes of nutrients, thereby reducing researcher burden (Gibson, 2005).

The use of food composition databases to estimate dietary intakes of nutrients relies on the completeness of these databases (Lee & Nieman, 2010). The major limitations associated with food composition databases are that new foods and ingredients are constantly added to the diets of individuals making these databases out of date. Also, food composition databases may not account for nutrient losses due to food storage and preparation resulting in an over-estimation of dietary intakes (Lee & Nieman, 2010). Thus, these limitations may reduce the validity of dietary assessment tools that are based on food composition databases (Blumfield et al., 2012).

2.10.1 The semi-quantitative New Zealand polyunsaturated fatty acids food frequency questionnaire

The New Zealand semi-quantitative PUFA food frequency questionnaire (NZ-PUFA FFQ) is a reasonably short self-administered tool designed to capture the usual intake of PUFAs in healthy adults in NZ. This tool was adapted from the Australian-PUFA FFQ (Aus-PUFA FFQ), a validated self-administered online tool which assesses usual dietary intake of the 38 most common sources of PUFAs, including supplements and fortified foods, in the Australian adult population (Swierk, Williams, Wilcox, Russell, & Meyer, 2011). Using the Aus-FFQ as a model, the NZ-PUFA FFQ included items that provide ≥0.1g PUFA/100g of food, however a number of foods were excluded, included or

adjusted according to their relevance to the New Zealand diet and food availability. Foods assumed to have negligible amounts of n-3 PUFAs, such as fruits and certain vegetables were not included in the FFQ. The NZ-PUFA FFQ includes 36 items, with a range of NZ specific cuts meats, sausage/delicatessen meats, fish/ seafood, eggs, fats/oils/spreads, vegetables, breads, cereals, nuts, desserts, takeaway foods and PUFA supplements (Ingram et al., 2012).

This dietary assessment tool uses pictures and standard household measures as defined portion sizes for each item and respondents are asked to report the consumption frequency on a range from 'never' to up to 'daily intakes' over the past three months. In addition, open-ended questions are available to allow the identification of items not included in the FFQ, such as other specific brands and types of foods, n-3 fortified products and PUFA supplements that may be commonly consumed by respondents (Ingram et al., 2012).

The length of the NZ-PUFA FFQ is considered reasonably short and completion time varies between 10 to 15 minutes. In addition, this FFQ was designed as an online tool built on a program called ASP.net (version1.1; Microsoft) which is linked to a NZ-PUFA database designed to automatically calculate the daily dietary intakes of each individual PUFA (LA, ALA, AA, EPA, DPA and DHA) (Ingram et al., 2012). The development of the NZ-PUFA database was also based on the Australian PUFA database (Sullivan, Brown, Williams, & Meyer, 2008; Sullivan, Williams, & Meyer, 2006; Swierk et al., 2011), with the process described elsewhere (Ingram et al., 2012). In summary, PUFA values of NZ specific foods replaced the different values of similar foods available in the Australian database for as many foods as possible. In the end, the NZ-PUFA database was composed of 47% of food values based on NZ specific analytical data and NZ fatty acid composition tables (Plant and Food Research, 2014).

From these, 86% of the values for the major food sources of omega-3 PUFA were derived from NZ-specific analytical data (Ingram et al., 2012).

Ingram and colleagues (2012) conducted a study to validate the NZ-PUFA FFQ for the NZ healthy adult population (n=48) against erythrocyte fatty acids measures and 3-day WFR. Results showed good agreement between the estimated intakes derived from the NZ-PUFA FFQ and the WFR for LA, ALA, AA and total PUFA. However, the NZ-PUFA FFQ estimated significantly higher intakes of EPA, DPA, DHA and total n-3 LC-PUFAs compared to the estimated values from the WFR. Despite this, statistical analysis using the method of triads resulted in a high level of validity, with 68% of the variance in dietary intakes of EPA, DPA, DHA and total n-3 LC-PUFAs being detected by the NZ-PUFA FFQ (Ingram et al., 2012). Overall, results from this validation study showed that the NZ-PUFA FFQs is a valid and robust tool to estimate PUFA intakes in healthy adults in NZ.

It is important to acknowledge the existence of inter-population variability in dietary patterns, which may be pronounced in pregnant women due to numerous dietary taboos and food safety restrictions. Studies have shown that FFQs are valid and reliable tools to estimate dietary intakes in pregnant women (Fawzi et al., 2004; Lyu et al., 2014). Comparisons between FFQ responses from pregnant and non-pregnant women (n=600) showed no differences in mean dietary intakes of energy and macronutrients, which support the validity of FFQs previously validated for adult women for use in pregnant women (Kaplan et al., 2014). In addition, many women of childbearing age change their diets minimally upon becoming pregnant (Crozier et al., 2009; Fowler et al., 2012; Hure et al., 2009; Morrison et al., 2012; Wilkinson et al., 2009). As the NZ-PUFA FFQ has been found to valid and reliable to measure dietary intakes of PUFA in healthy adult women, it is likely that this tool is a valid method to estimate PUFA intakes of pregnant women.

2.11 Summary of the literature review

Omega-6 and n-3 PUFAs are key structural and functional components of all cells in the body. During pregnancy, these key nutrients are required at increased amounts to support the constant formation and growth of maternal tissues, the placenta and the development of the fetus. Thus, adequate dietary intakes of n-6 and n-3 PUFAs are required to sustain not only fetal and placental demands, but also the mother's own physiological needs.

Omega-6 is widely available in the diet of developed countries, with vegetable oils, red meats and poultry representing the major food sources for this PUFA family. Omega-3 is found in fewer foods, particularly fish and seafood (DHA and EPA), and some in limited vegetable sources (e.g. green leafy vegetables, canola oil, linseed and chia seeds). Dietary patterns in developed countries have been shown to supply insufficient amounts of n-3 LC-PUFAs for optimal health. Several studies conducted in pregnant women in developed countries have used validated FFQs to investigating dietary intakes of PUFA. Findings from these studies are consistent, showing increased n-6 PUFA intakes while failing to meet consensus recommendations for total and individual n-3 LC-PUFAs.

Dietary data from the past NNSs (1997, 2008/09) suggests that dietary patterns of the NZ population are aligned with the diets of other developed countries, where intakes of red meats and poultry are predominantly higher that the intakes of fish and seafood. This suggests that dietary intakes in NZ are possibly higher in n-6 PUFAs and low in n-3 LC-PUFAs. However, it is unknown whether the NZ adult's population, including pregnant women are meeting the recommended n-6 and n-3 PUFAs levels for optimal health outcomes. Only recently a FFQ (NZ-PUFA FFQ) to assess the intakes of individual PUFAs was designed and validated for the NZ adult population (Ingram et al., 2012).

Therefore, investigating dietary intakes and food sources of n-6 and n-3 PUFAs in pregnant women in NZ using the NZ-PUFA FFQ is timely.

Henrique, seven months and full of joy…

3.1 Introduction

This chapter will provide an overview of the study design, including detailed description of the methods.

3.2 Study design

This cross-sectional study was designed to ascertain the dietary intakes and food sources of omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFAs) in a cohort of pregnant women living in New Zealand (NZ). An online validated food frequency questionnaire (FFQ) was used to conduct this investigation. The study process is presented in Figure 3.1.

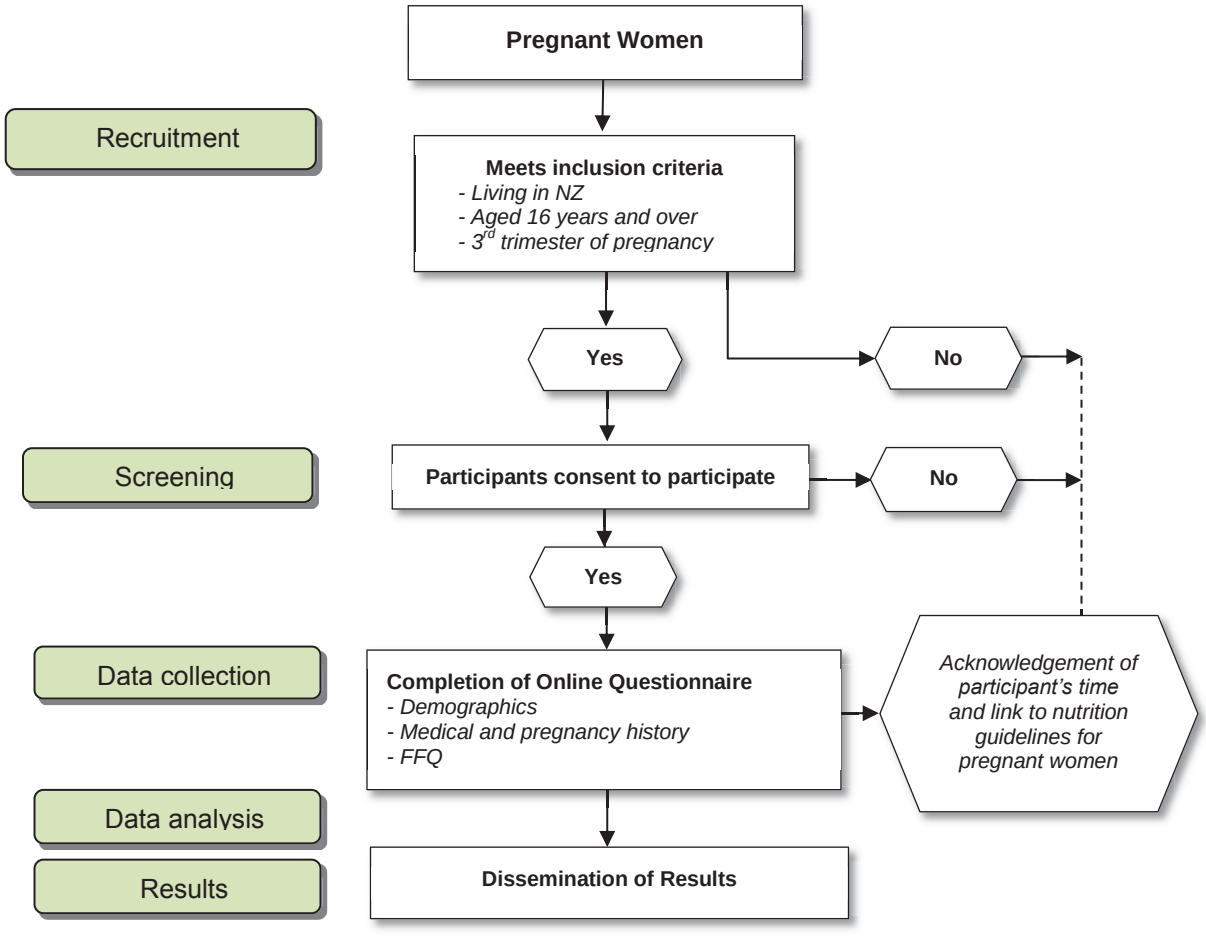

Figure 3.1 - Study process flow chart.

3.3 Ethical approval

This project was reviewed and approved by the Massey University Human Ethics Committee (MUHEC): Northern, Application 14/027 (Appendix 1).

3.4 Study population and eligibility

A convenience sample of 450 pregnant women was determined as an appropriate number to determine the mean value of docosahexaenoic acid (DHA) to within ± 20mg based on a 5% significance level (Mathers, Fox, & Hunn, 1998).

Pregnant women were eligible to take part in this study if they were in their third trimester (28 weeks and over) of pregnancy, living in NZ and aged 16 years and over.

3.5 Recruitment and screening

Participants were recruited from September 2014 to March 2015. Convenience and snowball sampling techniques were employed to facilitate the recruitment process and to obtain the study population. Recruitment strategies included word-of-mouth; face-to-face contact and the distribution of informative flyers and posters (Appendix 2) in maternal health care centres and other community venues' notice boards (e.g. baby show, parent centres, church, library, cafes). Information about the study was also shared via the press (Appendix 3), social networking media (e.g. Facebook and Twitter) and newsletters. Informative emails (Appendix 4) were sent out to a number of organisations that included Massey University staff members, District Health Boards, midwives and other workplaces that care for pregnant women throughout NZ (see Appendix 5). In addition, two agencies (Bounty and ReachMe) sent out emails to increase the number of participants during the final stages of recruitment (n=120).

Participants were screened for their eligibility to take part in the study over the phone and via email if they had contacted the researcher. Some participants were also screened in person during field recruitment. Eligibility was confirmed at the start of the online questionnaire. All participants were provided with an online or hardcopy of the information sheet (Appendix 6) about the study. Participants consented to take part in the study either online or hardcopy agreement.

3.6 Questionnaire

An online questionnaire was developed to collect data for this study using a safe web-based survey provider (Survey Monkey®). This online questionnaire was composed of questions designed to collect information related to participants' socio-demographic characteristics, and health and pregnancy history. On completion of these questions participants were directed to the NZ-PUFA food frequency questionnaire (FFQ) which is hosted at the Massey University website domain. The online questionnaire (Appendix 7) took approximately 15 to 20 minutes to complete. In these questionnaires, questions requiring personal information were not compulsory, whereas all questions related to the primary outcome of this study (e.g. dietary intakes and food sources) were compulsory.

Socio-demographic information

Nine socio-demographic questions were designed to enable a description of study participants. Data was collected on participants' location in NZ, ethnicity, educational level, household income and number of people living in the same household.

Health and pregnancy history

Eleven questions were designed to gather data on participants' overall health, pregnancy history and dietary patterns which may have influenced their PUFA dietary intakes during pregnancy (see Appendix 7).

Assessment of dietary intakes and food sources of PUFAs

Food sources and dietary intakes of PUFAs were assessed using the semi-quantitative NZ-PUFA FFQ. This FFQ is a robust online dietary assessment tool that was developed and validated to estimate dietary intakes of total and individual PUFAs over the past year in the NZ healthy adult population (Ingram et al., 2012). This tool was designed to calculate the average daily intake for each of the PUFAS in grams (g) per participant. Intake of the following PUFAs were determined: Arachidonic Acid (AA), Alpha-linolenic Acid (ALA), Linoleic Acid (LA), Docosapentaenoic Acid (DPA), Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA).

The NZ-PUFA FFQ assessed the frequency of consumption and quantity for 36 items (foods, beverages and supplements), within several options, including 'never', 'monthly', 'weekly' and 'daily' intakes. Defined portion sizes were represented using pictures of different serving sizes or by numbers of pieces eaten at each time. Questions were divided into main sections for fats and oils; milk; bread and breakfast cereal; eggs; fish and seafood; PUFA supplements; meats; pasta; snacks and deserts, and takeaway foods. After each section, the NZ PUFA FFQ automatically estimated the number of weekly portions reported by the respondent. Respondents were then asked to confirm if the estimated portions were correct, and they could go back and change their answers if needed. Equations were linked to each available answer from a set of multiple choices, regarding portion size and intakes frequency, for each question in the FFQ. These equations were set to automatically calculate the PUFA contributions from each item in the FFQ according to participant's responses.

Calculations of PUFA intakes were based on data from the NZ-specific fatty acid database (NZ-PUFA database), also developed by Ingram and colleagues (2012). In addition, open ended questions were included to identify other specific brands and types of foods, n-3 fortified products and PUFA supplements commonly consumed by participants.

Although the validation for the NZ-PUFA FFQ assessed PUFA intakes over the past 12 months, the present study chosen to assess the intakes of pregnant women over the past three months for two main reasons. Firstly, the aim of this study was to investigate PUFA intakes during pregnancy only and at a stage when pregnant women have stable dietary patterns. After 16 weeks of pregnancy, women are usually past dealing with morning sickness and food cravings, as well as having adjusted dietary intakes according to food and nutrition guidelines (Fawzi et al., 2004). Secondly, this study aimed to assess dietary intakes that were close to or during the last trimester of pregnancy, when accretion of LC-PUFAs, particularly DHA, are increased (Kuipers et al., 2012). In addition, the NZ-PUFA FFQ was adapted from an Australian version (Aust-PUFA FFQ), which was designed to cover the intakes of the past three months. Validation studies for both PUFA FFQs showed good validity and reproducibility (Ingram et al., 2012; Swierk et al., 2011), however higher validity coefficients were found for the three months' timeframe in the Aust-PUFA FFQ (Swierk et al., 2011).

3.7 Data collection

The majority of data was collected anonymously via online questionnaire from September 2014 to March 2015. For participants who requested a hardcopy of the questionnaire, contact details were required for postage. These contact details were not recorded or linked to any study documentation. All data collected via hardcopy and online questionnaires, was loaded and stored in

computer files, which were password protected, access-restricted, and used exclusively for the purpose of the study.

After completion of the online questionnaire all participants were thanked for their participation in the study. Participants, who alternatively informed their email address at the start of the questionnaire, were sent an email to thank them for their participation in the study (Appendix 8) and a link to the Ministry of Health *Eating for Health Pregnant Women* leaflet (Appendix 9). Information about when and how to access the results of this study was also provided in this email. The same information was sent in hardcopies to participants who completed a hardcopy version of this study.

3.8 Data handling

All data, including hardcopies, was loaded into an Excel spreadsheet and then transferred to statistical analysis software (SPSS – Statistics Software version 21.0, IBM Incorporation, New York, USA).

Once loaded into SPSS data was checked for outliers before starting statistical analysis. Outliers were closely investigated and corrected if necessary. For example, in questions where participants had to report the quantity (number of portions or pieces) of foods they consumed (e.g. questions related to takeaways), some participants indicated the weight in grams of the food consumed rather than the number of portions (e.g. 200g portion rather than 1 piece). As the NZ-PUFA database was set up to calculate the intakes of PUFAs based on the number of portions, a large number such as 200g was identified as 200 portions, resulting in an outlier. All cases reporting portion sized in grams rather than pieces were adjusted to match the portion sizes pre-defined in NZ-PUFA FFQ.

In addition, a number of participants selected ‘other’ supplements as their answer of choice for intake of PUFA supplements. For those who indicated the

type and brand of supplements, their PUFA intakes were adjusted based on the PUFA contents of the reported supplement. Nutritional information for PUFA supplements, not available in the NZ-PUFA database, was obtained from the manufacturers' original website.

3.9 Data analysis

Following data cleaning, statistical analyses were performed. Variables were tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests as well as visual inspection of normality plots. Descriptive statistics for participants' characteristics were presented using mean±SD, or median (25^{th}, 75^{th} percentiles) for continuous data or frequency summary statistics for categorical data.

3.9.1 Description of participants

Frequency summary statistic tests were used to describe the proportion of participants born in New Zealand versus overseas, length of time in New Zealand, which area participants lived, ethnicity, education level, household income, number of people living in the same household, gestational age, number of children and other pregnancy related issues (e.g. hypertension, morning sickness). Participants' age was presented in years as median (25^{th}, 75^{th} percentiles).

The Mann-Whitney test and chi-square analysis were undertaken to report differences between participants who completed the questionnaire versus participants who did not complete the questionnaire.

3.9.2 Participants dietary characteristics

Frequency summary statistics tests were also used to describe dietary characteristics of participants, including current diet, dietary changes reported

within current pregnancy, inclusion or exclusion of any particular foods as well as consumption of PUFA supplements.

3.9.3 Dietary intakes of polyunsaturated fatty acids

Median PUFA intakes of participants were visually compared with recommended values and frequency tests were performed to determine the proportion of participants meeting the recommended levels for PUFAs.

The estimated dietary intakes of PUFAs were compared with recommendations from the National Health and Medical Research Council, (NHMRC, 2006), the Joint FAO & WHO expert report (Food and Agriculture Organization of the United Nations and World Health Organization, 2010), the International Society for the Study of Fatty Acids and Lipids (ISSFAL) (Simopoulos et al., 2000), the Australian Scientific Consensus Workshop (Simmer et al., 2009) and Perinatal Lipid Intake Working Group (PERILIP) (Koletzko et al., 2007a). The selected recommended intakes for each PUFA are described in Table 3.1.

Table 3.1 - Selected recommended intakes of polyunsaturated fatty acids for pregnant women in this study

Recommendation (mg/d)		Organisation	Reference
LA	AI - 10,000	NHMRC / New Zealand and Australia	(NHMRC, 2006)
	AI - ≥6,400[#]	FAO & WHO	(Food and Agriculture Organization of the United Nations and World Health Organization, 2010)
ALA	AI - 1,000	NHMRC / New Zealand and Australia	(NHMRC, 2006)
	AI - 1,600	ISSFAL	(Simopoulos et al., 2000)
AA	UL - 800	FAO & WHO / Global Body	(Food and Agriculture Organization of the United Nations and World Health Organization, 2010)
EPA	AI - ≥220	ISSFAL	(Simopoulos et al., 2000)
DHA		FAO & WHO*	(Food and Agriculture Organization of the United Nations and World Health Organization, 2010)
	200	PERILIP**	(Koletzko et al., 2007a)
		Australian Scientific Consensus Workshop**	(Simmer et al., 2009)
Total n-3 LC-PUFA (EPA+DPA +DHA)	AI - 115 SDT - 430 UL – 3,000	NHMRC / New Zealand and Australia	(NHMRC, 2006)
Total EPA+DHA	300**	FAO & WHO	(Food and Agriculture Organization of the United Nations and World Health Organization, 2010)
	500*	Australian Scientific Consensus Workshop	(Simmer et al., 2009)

[#]This value was caculated based on the total average energy requirement in pregnancy (2,300Kcal/d) and the minimum intake level for essential fatty acids to prevent deficiency symptoms estimates as 2.5% energy LA (Food and Agriculture Organization of the United Nations and World Health Organization, 2010); * Average nutrient requirement based on minimum adult acceptable macronutrient distribution range (AMDR) plus an increment for energy demands of pregnancy; ** Consensus recommendations. **Abbreviations**: mg/d; milligrams per day AI, adequate intake; UL, upper tolerable intake level; SDT, suggested dietary target; LA, linoleic acid; ALA, alpha-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n-3 LC-PUFA, omega-3 long chain polyunsaturated fatty acid.

In addition, participants were divided into two groups – those taking and not taking PUFA supplements, and the Chi-square test and odds ratio used to determine the likelihood of achieving recommended DHA intakes during pregnancy. Further frequency tests were used to determine the contributions from supplements to total and individual PUFAs intakes, and to compare these

to contributions from fish and seafood in participants taking supplements (n=117).

3.9.4 Food sources for polyunsaturated fatty acids

The proportion and contribution of different food sources to the total daily intakes of PUFAs were calculated using frequency statistics. The contribution of each food source was expressed as mean g/d for total and individual PUFAs, and then calculated as a percentage of the total mean intake of each PUFA within the total population sample. These mean values did not include contributions from PUFA supplements.

Main food sources resulting from the FFQ responses were combined into nine main food groups according to their similarities in nutritional composition, based on the work by Astorg et al. (2004). Further analysis was performed to determine the proportion and contribution of these food groups to total and individual PUFAs. In addition, the consumption of fish and seafood as well as meats amongst participants was determined by frequency tests.

3.10 Funding

The School of Food and Nutrition, Massey Institute of Food Science and Technology, College of Health provided financial support for this study.

Chapter Four - Results

He is a little man now. “Never stop developing and perfecting your abilities, even if you have already achieved significant visible results.” *Sunday Adelaja*

4.1 Attrition rates

Of 999 who expressed an interest in taking part in the study, 827 were eligible to participate, 231 withdrew and 596 of these women completed the study (completion rate of 72%) (Figure 4.1). To complete this study, participants had to respond to a range of questions referent to their demographics, health during pregnancy and dietary intakes characteristics, and submit their answers to the research database. However the majority of participants completed the study online, it is important to aknowledge that a small proportion (n=4; 0.7%) of participants also completed hardcopies of the questionnaire.

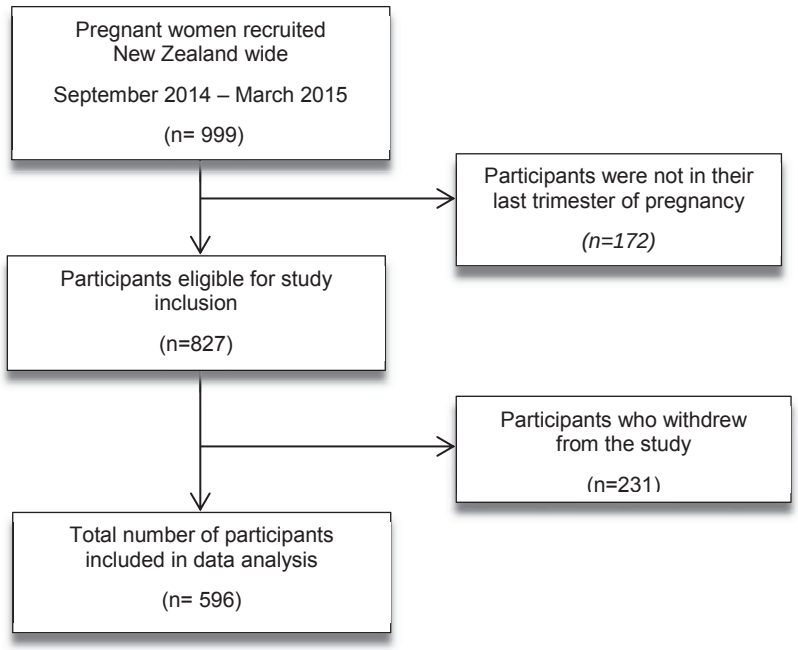

Figure 4.1 - Flow of participants throughout the study

Participants who withdrew from the study provided consent and completed the initial questions on demographics and health during pregnancy but did not complete the questions regarding dietary intake. Using this data it was possible to detect significant differences between participants who completed the study and those who withdrew from the study. Participants who withdrew from the

study had a median [25th, 75th percentile] age (29 [27, 34] years) that was two years younger than the age (31 [28, 35] years) of participants who completed the study (P<0.001, small effect size r= -0.14). Further comparisons between participants who completed versus those who did not complete revealed women born overseas were more likely to complete the questionnaire (76.7%) compared with women born in NZ (70.9%) (P=0.02). In addition, other European women (91.9%) were more likely to complete the study compared with New Zealand-European (79.6%), Asian (75.0%), Pacific Island (71.4%), Māori (68.3%), and other (54.2%) ethnic groups (P<0.001). Women with university degrees (80.5%) were more likely to complete the questionnaire than women with secondary, primary and lower levels of education (60.8%) (P<0.001). A greater proportion of women from households with higher incomes ($60,000 to $99,999 (83.8%) and $100,000 plus (81.4%)) were more likely to complete the questionnaire compared to women with household incomes below $59,999 (63.4%) ($P$<0.001). Participants with gestational age between 28 and 32 weeks (75.7%) were more likely to complete the questionnaire than participants of gestational age (33 - 37 weeks (71.7%), 38 - 40 weeks (63.8%) and 40 weeks plus (63.8%) (P=0.024).

Only results from women who completed the study are presented in this chapter, which is divided into three main sections including description of the study population, and dietary intakes and food sources of n-6 and n-3 PUFAs.

4.2 Description of the study population

Participants who completed the study (n=596) were located throughout NZ. Figure 4.2 shows the proportion of participants from each region.

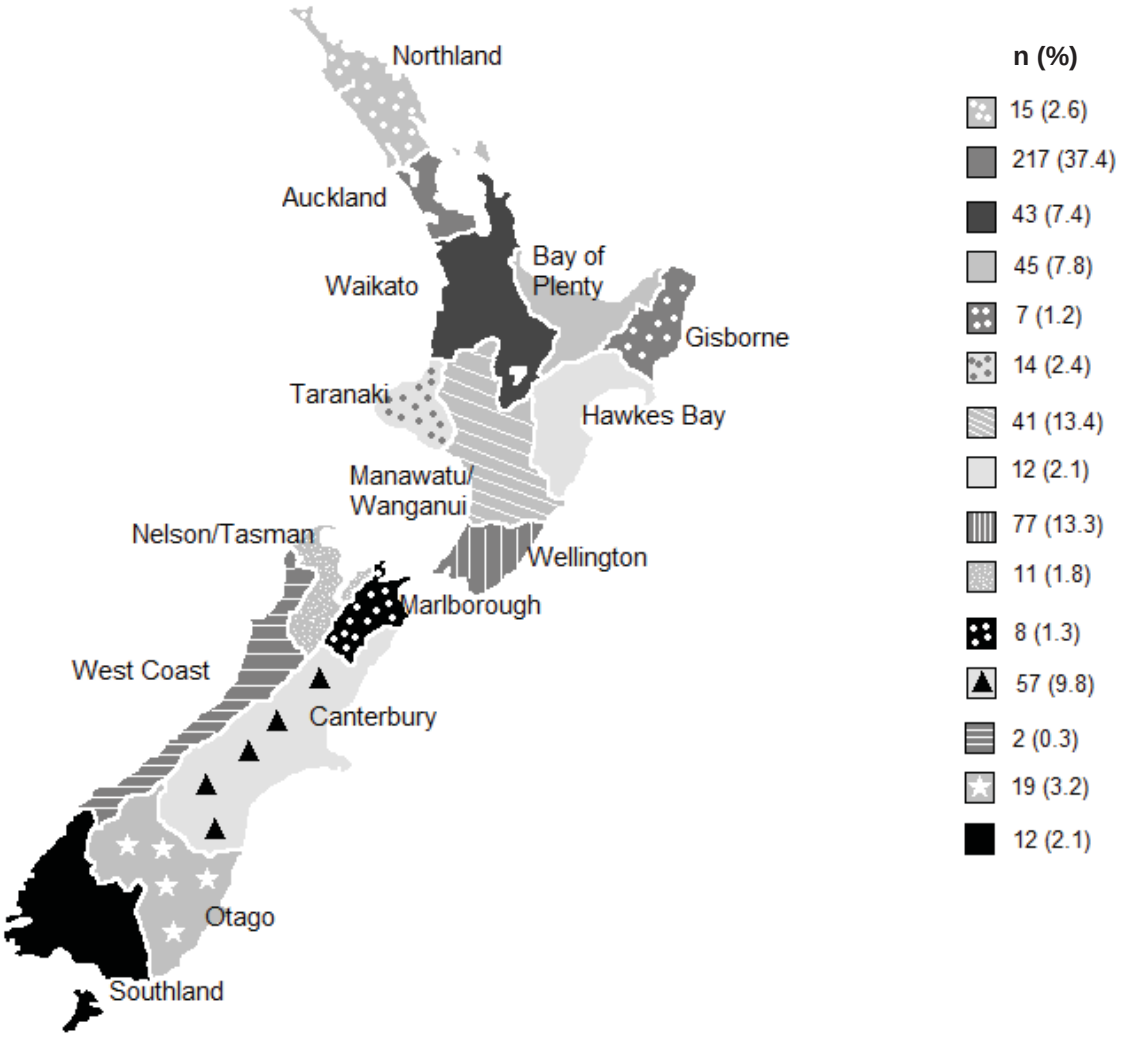

Figure 4.2 - Distribution of participants throughout New Zealand

The pattern legend indicates the proportion (n (%)) of participants from 15 different regions in NZ. 16 participants had missing data for this question.

Further characteristics of the participants who completed this study are detailed in Table 4.1. Participants had a median [25th, 75th percentile] age of 31 [28, 35] years. The majority of the participants were New Zealand-European (74.3%), followed by Māori (9.4%), other European (5.7%) and Pacific Island (3.4%) ethnicity. The majority of participants (77.2%) were born in NZ, and most (79.8%) of those who were born overseas, had been in NZ for longer than 5 years.

The majority of the participants were well educated and had a high (in excess of $100,000/year) household income. Most participants lived in households with two to three other people (see Table 4.1).

As one of the study's criteria, women had to be 28 weeks gestation or more to take part in the study. Over half of the participants (50.8%) were between 28 and 32 weeks of pregnancy, with the remainder between 33 and 37 weeks (35.9%), 38 and 40 weeks (10.9%), and 40 weeks gestation and over (2.3%). The majority of women (75.9%) had planned their pregnancy, and 37.2% of the women were primagravida (pregnant for the first time). During the pregnancy 71% of women had experienced morning sickness, 3.9% hypertension and 3.4% had gestational diabetes mellitus.

Table 4.1 - Characteristics of the study population

Characteristics	n= 596
Age (Years)	
Median [25th, 75th percentile]	31 [28, 35]
Ethnicity n (%)[a]	
New Zealand-European	442 (74.3)
Māori	56 (9.4)
Other European	34 (5.7)
Pacific Island	20 (3.4)
Indian	13 (2.2)
Chinese	10 (1.7)
Southeast Asian	8 (1.3)
Other	12 (2.0)
Country of birth n (%)	
NZ	460 (77.2)
Other	136 (22.8)
Length of time in NZ n (%)*	
Less than 5 years	27 (20.1)
5 to 9 years	35 (26.1)
10 to 19 years	42 (31.3)
20 years plus	30 (22.4)
Highest educational level n (%)	
Primary School	3 (0.5)
Secondary School	157 (26.3)
University Degree	268 (45.0)
Postgraduate Degree	120 (20.1)
Prefer not to answer	13 (2.2)
Other	31 (5.2)
None	4 (0.7)
Household income n (%)[a]	
Under $10,000	9 (1.5)
$10,000 - $19,999	14 (2.4)
$20,000 - $39,999	34 (5.7)
$40,000 - $59,999	63 (10.6)
$60,000 - $79,999	100 (16.8)
$80,000 - $99,999	88 (14.8)
$100,000 plus	222 (37.4)
Prefer not to answer	64 (10.8)

Characteristics	n= 596
Gestational age n (%)	
Between 28 – 32 weeks	303 (50.8)
Between 33 – 37 weeks	214 (35.9)
Between 38 – 40 weeks	65 (10.9)
40 weeks and over	14 (2.3)
Current pregnancy planned n (%)[b]	
Yes	448 (75.9)
No	142 (24.1)
Number of pregnancies including current pregnancy n (%)[b]	
1	222 (37.2)
2	180 (30.5)
3	104 (17.6)
4 or more	84 (14.3)
Number of children n (%)[c]	
None	289 (48.7)
1	192 (32.3)
2	77 (13.0)
3	26 (4.4)
4	5 (0.8)
5	3 (0.5)
6 or more	2 (0.3)
Number of other people living in the same household including the participant n (%)[c]	
1	22 (3.7)
2	260 (43.9)
3	184 (31.1)
4	86 (14.5)
5	30 (5.1)
6 or more	10 (1.7)
Current pregnancy health related issues	
Morning sickness [d]	418 (71.0)
Hypertension [b]	23 (3.9)
Gestational diabetes mellitus [b]	20 (3.4)

* n = 134 (missing data for 2 participants)

Missing data for: [a] 2 participants; [b] 6 participants; [c] 4 participants; [d] 7 participants.

4.2.1 Participant's dietary characteristics

Most of the participants followed an omnivorous diet (96.1%), with a few vegetarians (3.6%) and vegans (0.3%). Less than half of participants reported having made a change to their diet (39.1%) since falling pregnant. Nearly one third of all participants (32.3%) indicated they had included additional foods and 83.8% had excluded certain foods during their current pregnancy. The most commonly included additional foods were fruits and vegetables (15.1%), dairy (10.2%), meats (6.9%) and fish and seafood (6.2%). Other additional foods reported by 8.6% of participants included: iron rich foods such as offal, molasses and fortified products such as breakfast cereal; fibre rich foods (e.g. prunes, wholemeal foods; probiotic rich foods (e.g. kefir beverages and yoghurt); seaweed and spirulina, and herbal beverages and teas such as raspberry leaf, liquorice, ginger and nettle. The majority of participants (75.3%) who excluded certain foods from their diets reported avoiding higher risk foods during pregnancy, as recommended in the guidelines for pregnant women (Ministry of Health, 2008a; Ministry of Primary Industries, 2013). Approximately 19% of participants indicated avoiding fish and seafood during pregnancy. Other excluded foods commonly reported (14.9%) were eggs, peanuts, caffeinated and herbal beverages, and spicy foods. Further details about participants' dietary characteristics are presented in Table 4.2. The consumption of n-3 PUFA fortified products was indicated by 7.6% (n=45) of the participants, with the most commonly reported additional PUFA-rich foods including: fortified margarine and vegetable spreads; LSA (ground linseed, sunflower seeds and almond), flaxseeds, chia seeds, seaweed, yoghurt and other seeds and vegetable oils (e.g. Bestow oil).

Table 4.2 - Participants' dietary characteristics

Participants responses (n=596)	**n (%)**
Type of diet followed[a]	
Omnivorous	568 (96.1)
Vegetarian	21 (3.6)
Vegan	2 (0.3)
Dietary changes reported within pregnancy	
Yes	233 (39.1)
No	363 (60.9)
Included additional foods during current pregnancy	191 (32.3)[b]
Fruits and vegetables	90 (15.1)
Dairy	61 (10.2)
Meats	41 (6.9)
Fish and seafood	37 (6.2)
Nuts and seeds	32 (5.4)
Breads and cereals	24 (4.0)
Other	51 (8.6)
Excluded foods during current pregnancy	496 (83.8)[b]
Higher risk foods to avoid when pregnant*	449 (75.3)
Fish and seafood	114 (19.1)
Takeaways and processed foods	38 (6.4)
Sugary foods and beverages	37 (6.2)
Dairy	7 (1.2)
Other	89 (14.9)
Consumption of n-3 PUFA fortified products	
Yes	45 (7.6)
No	551 (92.4)

* New Zealand Guidelines sourced from Food Safety in Pregnancy (Ministry of Primary Industries, 2013) and Eating for Healthy Pregnant Women (Ministry of Health, 2008a). Missing data for: [a] 5 participants, [b] 4 participants. **Abbreviation**: PUFA, polyunsaturated fatty acid.

4.3 Dietary intakes of polyunsaturated fatty acids

The mean intakes of total PUFA, and individual n-6 and n-3 PUFA were found to be skewed and transformation could not produce normal distribution. Therefore, the median and the 25th and 75th percentiles will be used to describe participants PUFA intakes. Dietary intakes of total and individual PUFAs are presented in Table 4.3. The intakes are expressed in milligrams per day (mg/d). Daily PUFA intakes are also presented separately for participants taking and not taking PUFA supplements.

Table 4.3 - Intakes of individual polyunsaturated fatty acids

	Total PUFA	**LA**	**ALA**	**AA**	**EPA**	**DPA**	**DHA**	**EPA + DHA**	**Total n-3 LC-PUFA***
				mg/d					
All participants (n = 596)									
Mean	15,360	13,240	1,620	90	**160**	50	**200**	**360**	**410**
Std. Deviation	7,450	6,890	1,130	50	260	40	250	500	530
25th percentile	10,780	8,840	790	60	30	30	50	90	120
Median	13,670	11,580	1,300	90	**60**	40	**110**	**180**	**220**
75th percentile	18,340	15,760	2,120	110	190	60	250	460	520
Participants not taking supplements (n = 479)									
Mean	14,950	12,940	1,630	90	**90**	50	**140**	**240**	**280**
Std. Deviation	7,410	6,890	1,140	40	140	40	200	340	380
25th percentile	10,380	8,650	810	60	20	20	50	70	100
Median	13,070	11,330	1,310	90	**50**	40	**90**	**140**	**180**
75th percentile	17,650	15,290	2,130	110	100	60	160	240	300
Participants taking supplements (n = 117)									
Mean	17,040	14,460	1,570	100	**440**	50	**430**	**870**	**920**
Std. Deviation	7,380	6,800	1,110	60	410	40	310	630	700
25th percentile	11,880	9,900	770	70	200	30	210	440	480
Median	15,250	13,040	1,260	90	**350**	40	**370**	**680**	**770**
75th percentile	21,380	17,880	2,130	120	520	70	530	1,090	1,140

* Sum of EPA, DPA plus DHA. **Abbreviations**: AA, arachidonic acid; ALA, alpha-linolenic acid; DPA, docosapentaenoic acid; LA, linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

4.3.1 Individual polyunsaturated fatty acid intakes and current recommendations

Median daily intakes of combined and individual PUFAs were compared with recommended adequate intakes (AIs) and suggested dietary intakes (SDTs) from the National Health and Medical Research Council (NHMRC) for Australia (AU) and NZ (NHMRC, 2006), the Joint FAO & WHO expert report (Food and Agriculture Organization of the United Nations and World Health Organization, 2010), the Australian Scientific Consensus Workshop report (Simmer et al., 2009), and international working groups such as Perinatal Lipid Intake Working Group (PERILIP) (Koletzko et al., 2007a) and the International Society for the Study of Fatty Acids and Lipids (ISSFAL) (Simopoulos et al., 2000) (see Table 4.4).

Median [25th, 75th percentile] intake of linoleic acid (LA) for all participants was 11,580 [8,840, 15,760] mg/d, which was clearly above the recommended intakes from NHMRC (10,000mg/d) and FAO & WHO (6,400mg/d). Over half of participants met the recommendations from NHMRC (64.4%) as well as FAO & WHO (92.3%).

Participants had a median intake of 1,300 [790, 2,120]mg/d of alpha-linolenic acid (ALA) which was above the AI from NHMRC (1,000mg/d). This AI was met by 64% of participants.

Upper limits (UL) were the only recommendations available for arachidonic acid (AA). Participant's median intakes of AA were 90 [60, 110]mg/d, visibly below the UL (800mg/d) established by FAO & WHO. All participants (100%) intakes of AA were below the ULs.

Median intakes of eicosapentaenoic acid (EPA) were 60 [30, 190]mg/d, which were considerably below the recommended levels from ISSFAL (≥220mg/d), with just 23.2% of participants meeting these recommendations.

Recommendations from the Australian Scientific Consensus Workshop, PERILIP and FAO & WHO for docosahexaenoic acid (DHA) are set as 200mg/d. The median DHA intakes of all participants were 110 [50, 250]mg/d, with 31% of participants meeting the recommendations. In addition, further frequency tests revealed that approximately 33% of participants had DHA intakes below the 70mg/day estimated as daily DHA accretion in fetal tissues during the last trimester of pregnancy (Innis, 2005).

The current AI for total n-3 LC-PUFA (EPA, DPA plus DHA) from NHMRC for pregnant women in AU and NZ are 110mg/d for women aged 14 to 18 years and 115mg/d for women aged 19 years and over. All participants in this study had a median total n-3 LC-PUFA intake of 220 [120, 520]mg/d, that was twice as much as the AI from NHMRC. Approximately three-quarters of participants (76%) met the AI for total n-3 LC-PUFAs. The NHMRC also recommends a SDT of 430mg/d for total n-3 LC-PUFAs for all adult women for maintenance of optimal health, however less than one-third of participants (29.9%) met this SDT. The NHMRC suggests an UL of 3,000mg/d for total n-3 LC-PUFAs, which was exceeded by five participants (0.8%) with intakes ranging from 3,220mg to 4,700mg/d.

Total median EPA plus DHA intakes (180 [90, 460]mg/d) were below the Australian Scientific Consensus Workshop recommended dietary intakes (500mg/d) and the FAO & WHO recommendations (300mg/d). Only 23.2% of the participants met the Australian Scientific Consensus recommendation and slightly more 34.9% met the FAO & WHO recommendation (Table 4.4).

Table 4.4 - Omega-6 and omega-3 polyunsaturated fatty acid intakes versus recommended levels of all participants (n = 596)

Recommendation mg/d		Daily intakes mg/d*	n (%) meeting recommendations
LA			
NHMRC[a]	AI - 10,000	11,580 [8,840, 15,760]	384 (64.4%)
FAO & WHO[b]	AI - ≥6,400[#]	11,580 [8,840, 15,760]	550 (92.3%)
ALA			
NHMRC	AI - 1,000	1,300 [790, 2,120]	383 (64.3%)
ISSFAL[c]	AI - 1,600	1,300 [790, 2,120]	225 (37.8%)
AA			
FAO & WHO	UL - 800	90 [60, 110]	596 (100%)[d]
EPA			
ISSFAL	AI - ≥220	60 [30, 190]	138 (23.2%)
DHA			
Australian Scientific Consensus Workshop [Δ, e], PERILIP[Δ, f] and FAO & WHO[+]	200	110 [50, 250]	184 (30.9%)
Total n-3 LC-PUFA (EPA+DPA+DHA)	AI – 115[g]	220 [120, 520]	457 (76.7%)
NHMRC	SDT - 430	220 [120, 520]	178 (29.9%)
	UL - 3,000	220 [120, 520]	591(99.2%)
Total EPA+DHA			
Australian Scientific Consensus Workshop	500[Δ]	180 [90, 460]	138 (23.2%)
FAO & WHO	300[+]	180 [90, 460]	208 (34.9%)

* Values are reported as median [25th, 75th percentile] unless otherwise indicated; [#] This value was calculated based on the total average energy requirement in pregnancy (2,300Kcal/d) and the minimum intake level (AI) for essential fatty acids to prevent deficiency symptoms estimates as 2.5%E LA (Food and Agriculture Organization of the United Nations and World Health Organization, 2010); [Δ] Consensus recommendations from Expert Scientific Groups; [+] Average nutrient requirement based on minimum adult acceptable macronutrient distribution range (AMDR) plus an increment for energy demands of pregnancy; [a] Adequate Intake (AI) from National Health and Medical Research Council (NHMRC, 2006); [b] Joint FAO & WHO (Food and Agriculture Organization of the United Nations and World Health Organization, 2010); [c] International Society for the Study of Fatty Acids and Lipids (ISSFAL) (Simopoulos et al., 2000); [d] 100% of participants had AA intakes below the tolerable upper intake level or upper limit (UL); [e] Australian Scientific Consensus Workshop Recommendations (Simmer et al., 2009); [f] Perinatal Lipid Intake Working Group (PERILIP) (Koletzko et al., 2007a); [g] AI for pregnant women aged 19 – 50 years. **Abbreviations**: AI, adequate intake; SDT, suggested dietary target; AA, arachidonic acid; ALA, alpha-linolenic acid; LA, linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

The proportion of participants meeting the recommendations for each individual PUFA was determined using frequency tests.

Participants intakes according to the intakes of PUFA supplements

Table 4.5 details PUFA intakes versus recommended levels for participants taking PUFA supplements compared with participants not taking PUFA supplements. Both groups were visibly above the recommended levels for LA. For both groups, ALA median intakes were also above the AIs from the NHMRC, but below to the recommended levels from ISSFAL. Intakes of AA were below the recommended UL for participants taking PUFA supplements and participants not taking PUFA supplements.

The median EPA intake of participants taking PUFA supplements was 350 [200, 520]mg/d, clearly above the recommended levels (≥220mg). However, participants not consuming PUFA supplements were below recommendations, with median EPA intakes of 50 [20, 100]mg/d. Over 71% of participants taking PUFA supplements were above the recommended levels for EPA, while only 11.3% of participants not taking PUFA supplements met the recommendations.

Participants not taking PUFA supplements had a median DHA intake of 90 [50, 160]mg/d, which was less than half of the recommended intake (200mg), with the majority (81%) of these participants not meeting the recommendations for DHA. In contrast, participants taking PUFA supplements had a median DHA intake of 370 [210, 530]mg/d, with over 79% of these participants meeting the recommended intakes. Further statistical analysis suggested that participants taking PUFA supplements were significantly more likely to meet DHA recommendations (χ^2= 161.22, P<0.001) than participants not taking PUFA supplements. The odds for participants meeting the recommended levels for DHA were 16.52 times more likely if taking PUFA supplements compared to participants not taking PUFA supplements.

Participants from both groups had a median total n-3 LC-PUFA (EPA, DPA plus DHA) intake that met the AI of 115mg/d recommended by the NHMRC. In participants not taking PUFA supplements median intakes were 180 [100,

300]mg/d, while participants taking PUFA supplements had a median total n-3 LC-PUFA intake of 770 [480, 1,140]mg/d. Nearly all (99%) of participants taking PUFA supplements compared to 71.2% of participants not taking supplements met the recommendations for total LC n-3 PUFA. However, only 17.3% of participants met the SDT recommendations from the NHMRC compared to 81.2% of participants taking PUFA supplements. Upper tolerable limits for total LC n-3 PUFA were exceeded by three participants taking PUFA supplements (average intake was 3,900mg/d), and two participants not taking PUFA supplements (average intake was 4,000mg/d).

Just over two-thirds (70.1%) of participants consuming PUFA supplements met the recommendations of the Australian Scientific Consensus Workshop for total combined EPA and DHA (500mg/d), with median intakes of 680 [440, 1,090]mg/d. Participants not taking PUFA supplements had a median combined EPA and DHA intake of 140 [70, 240]mg/d, which was considerably below recommendations. Only 11.7% of these participants met these recommendations. Recommendations from FAO & WHO (300mg/d) are slightly lower than the ones from the Australian Scientific Consensus Workshop and only 21.5% of participants not taking supplements met these recommendations.

Table 4.5 - Omega-6 and omega-3 polyunsaturated fatty acid intakes versus recommended levels (participants taking PUFA supplements versus participants not taking PUFA supplements)

Recommendation mg/d		Daily intakes mg/d*	n (%) meeting recommendations
Participants taking PUFA supplements (n=117)			
LA			
NHMRC[a]	AI - 10,000	13,040 [9,900, 17,880]	88 (75.2%)
FAO & WHO[b]	AI - ≥6,400[#]	13,040 [9,900, 17,880]	112 (95.7%)
ALA			
NHMRC	AI - 1,000	1,260 [770, 2,130]	71 (60.7%)
ISSFAL[c]	AI - 1,600	1,260 [770, 2,130]	43 (36.8%)
AA			
FAO & WHO	UL - 800	90 [70, 120]	117 (100%)[d]
EPA			
ISSFAL	AI - ≥220	350 [200, 520]	84 (71.8%)
DHA			
Australian Scientific Consensus Workshop[Δ, e], PERILIP[Δ, f] and FAO & WHO[+]	200	370 [210, 530]	93 (79.5%)
Total n-3 LC-PUFA (EPA+DPA+DHA)	AI -115[g]	770 [480, 1,140]	116 (99.1%)
NHMRC	SDT - 430	770 [480, 1,140]	95 (81.2%)
	UL - 3,000	770 [480, 1,140]	114 (97.4%)
Total EPA + DHA			
Australian Scientific Consensus Workshop	500[Δ]	680 [440, 1,090]	82 (70.1%)
FAO & WHO	300[+]	680 [440, 1,090]	105 (89.7%)

Recommendation mg/d		Daily intakes mg/d*	n (%) meeting recommendations
Participants not taking PUFA supplements (n=479)			
LA			
NHMRC[a]	AI - 10,000	11,330 [8,650, 15,290]	296 (61.8%)
FAO & WHO[b]	AI - ≥6,400[#]	11,330 [8,650, 15,290]	438 (91.4%)
ALA			
NHMRC	AI - 1,000	1,310 [810, 2,130]	312 (65.1%)
ISSFAL[c]	AI - 1,600	1,310 [810, 2,130]	182 (38.0%)
AA			
FAO & WHO	UL - 800	90 [60, 110]	479 (100%)[d]
EPA			
ISSFAL	AI - ≥220	50 [20, 100]	54 (11.3%)
DHA			
Australian Scientific Consensus Workshop[Δ, e], PERILIP[Δ, f] and FAO & WHO[+]	200	90 [50, 160]	91 (19.0%)
Total n-3 LC-PUFA (EPA+DPA+DHA)	AI -115[g]	180 [100, 300]	341 (71.2%)
NHMRC	SDT - 430	180 [100, 300]	83 (17.3%)
	UL – 3,000	180 [100, 300]	477 (99.6%)
Total EPA + DHA			
Australian Scientific Consensus Workshop	500[Δ]	140 [70, 240]	56 (11.7%)
FAO & WHO	300[+]	140 [70, 240]	103 (21.5%)

* Values are reported as median [25th, 75th percentile] unless otherwise indicated; [#] This value was calculated based on the total average energy requirement in pregnancy (2,300Kcal/d) and the minimum intake level (AI) for essential fatty acids to prevent deficiency symptoms estimates as 2.5%E LA (Food and Agriculture Organization of the United Nations and World Health Organization, 2010); [Δ] Consensus recommendations from Expert Scientific Groups; [+] Average nutrient requirement based on minimum adult acceptable macronutrient distribution range (AMDR) plus an increment for energy demands of pregnancy; [a] Adequate Intake (AI) from National Health and Medical Research Council (NHMRC, 2006); [b] Joint FAO & WHO (Food and Agriculture Organization of the United Nations and World Health Organization, 2010); [c] International Society for the Study of Fatty Acids and Lipids (ISSFAL) (Simopoulos et al., 2000); [d] 100% of participants had AA intakes below the tolerable upper intake level or upper limit (UL); [e] Australian Scientific Consensus Workshop Recommendations (Simmer et al., 2009); [f] Perinatal Lipid Intake Working Group (PERILIP) (Koletzko et al., 2007a); [g] AI for pregnant women aged 19 – 50 years. **Abbreviations**: AI, adequate intake; SDT, suggested dietary target; AA, arachidonic acid; ALA, alpha-linolenic acid; LA, linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

The proportion of participants meeting the recommendations for each individual PUFA was determined using frequency tests.

Polyunsaturated fatty acid intakes of vegetarian and vegan participants

Table 4.6 shows PUFA intakes of participants following vegan and vegetarian dietary patterns. Few participants followed vegetarian (n=21, 3.6%) or vegan (n=2, 0.3%) dietary patterns. One vegetarian participant reported the use of PUFA supplements and her intakes were EPA (939mg/d), DHA (676mg/d) and total n-3 LC-PUFAs (1,649mg/d) were well above the recommended levels. In contrast, vegetarian participants not taking supplements (n=20) had median intakes of EPA (39mg/d), DHA (65mg/d) and total n-3 LC-PUFAs (143mg/d) below recommended levels, with only 10% of those meeting the recommended intakes for DHA. Vegan participants did not consume PUFA supplements and their median intakes were: EPA, 10mg/d, DHA, 35mg/d, and total n-3 LC-PUFAs, 59mg/d.

Table 4.6 - Omega-6 and omega-3 polyunsaturated fatty acid intakes of vegetarian and vegan participants

	Total PUFA	LA	ALA	AA	EPA	DPA	DHA	Total n-3 LC-PUFA
				mg/d				
Vegetarian taking PUFA supplements (n=1)								
Daily Intakes	16,030	13,471	799	111	939	34	676	1,649
Recommended intake	-	10,000[1]	1,000[2]	UL 800[3]	≥220[4]	-	200[5]	500[5]
n (%) meeting recommended intakes	-	1 (100%)	0 (0%)	1 (100%) *	1 (100%)	-	1 (100%)	1 (100%)
Vegetarian not taking PUFA supplements (n=20)								
Daily Intakes[#]	11,164 [8,010,14,070]	9,312 [6,970, 12,950]	1.325 [752, 2,100]	63 [42, 96]	39 [10, 100]	33 [15, 50]	65 [17, 106]	143 [43, 270]
Recommended intake	-	10,000[1]	1,000[2]	UL 800[3]	≥220[4]	-	200[5]	500[5]
n (%) meeting recommended intakes	-	7 (35%)	15 (75%)	20 (100%)*	1 (5%)	-	2 (10%)	3 (15%)
Vegans not taking PUFA supplements (n=2)								
Daily Intakes[#]	18,734 [15,831,21,640]	17,600 [14,110, 21,012]	1.025 [463, 1,600]	92 [70, 114]	10 [7, 12]	15 [11, 18]	35 [31, 38]	59 [50, 70]
Recommended intake	-	10,000[1]	1,000[2]	UL 800[3]	≥220[4]	-	200[5]	500[5]
n (%) meeting recommended intakes	-	2 (100%)	1 (50%)	2 (100%)*	0 (0%)	-	0 (0%)	0 (0%)

[1, 2] Nutrient Reference Values for Australia and New Zealand (NHMRC, 2006); [3] Joint FAO & WHO (Food and Agriculture Organization of the United Nations and World Health Organization, 2010); [4] International Society for the Study of Fatty Acids and Lipids (ISSFAL) (Simopoulos et al., 2000); [5] Australian Scientific Workshop Consensus Recommendations (Simmer et al., 2009), Perinatal Lipid Intake Working Group (PERILIP) (Koletzko et al., 2007a) & Joint FAO & WHO; [#] Values are reported as median [25th, 75th percentile]; * 100% of participants had AA intakes below the tolerable upper intake level or upper limit (UL). **Abbreviations**: AA, arachidonic acid; ALA, alpha-linolenic acid; DPA, docosapentaenoic acid; LA, linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

4.4 Food sources for polyunsaturated fatty acids

The total mean dietary PUFA intake was mainly comprised of n-6 PUFAs (86.7%), to which LA contributed the majority (99.3%) compared with AA (0.7%). Total n-3 PUFAs contributed to 13.6% of total PUFA intakes, and was comprised mostly of ALA (87.0%), with minor amounts of EPA (4.4%), DPA (1.9%) and DHA (6.8%). Figure 4.3 shows the proportion of contributions from n-6 and n-3 PUFAs and each individual fatty acid to total PUFA intakes.

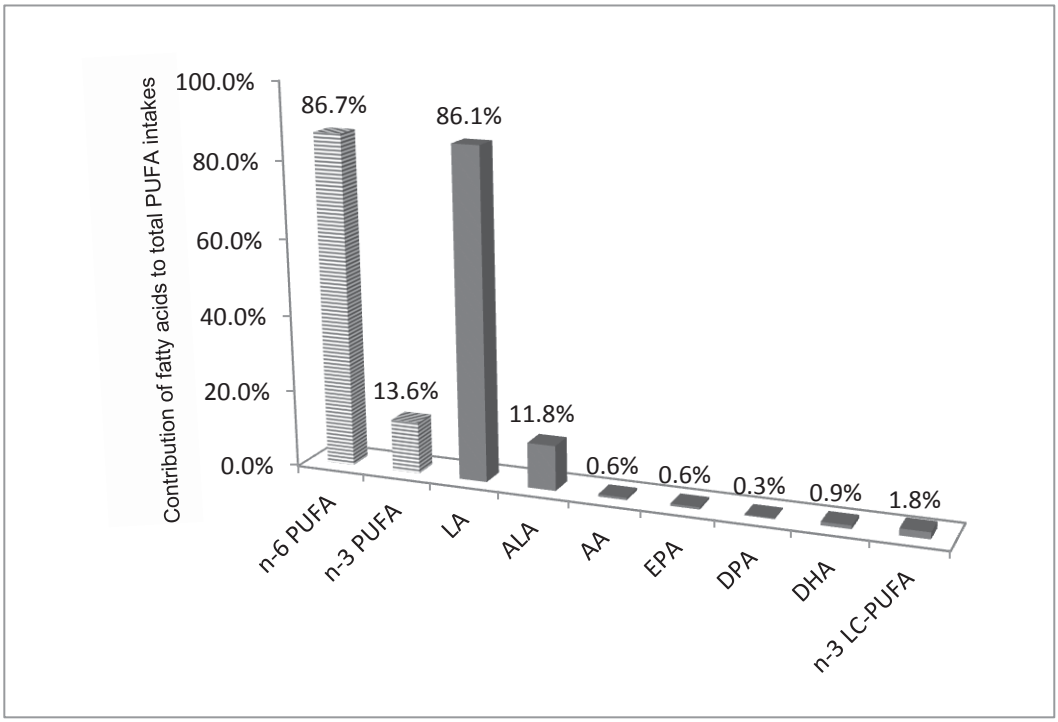

Figure 4.3 – Contributions (%) of n-6 and n-3 PUFAs and individual fatty acids to total mean intake of PUFAs (n=596)

Abbreviations: n-6, omega-6; n-3, omega-3; AA, arachidonic acid; ALA, alpha-linolenic acid; DPA, docosapentaenoic acid; LA, linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

The major food sources contributing to total polyunsaturated fatty acid intakes were fats and oils (oil, butter plus margarine) (43.4%) followed by nuts and seeds (21.2%), vegetables (7.2%) and bread (7.2%). The same group of foods were also the major contributors to LA intakes, with fats and oils (43.2%) as the

major source, followed by nuts and seeds (24.1%), vegetables (7.9%) and bread (6.0%). A large proportion of ALA intake was contributed by fats and oils (55.0%), bread (18.5%) and bacon (16.1%). Vegetables contributed to approximately 4% of ALA intakes. Eggs (23.9%), poultry (16.2%), bacon (16.1%), beef (12.2%) and fresh/frozen fish (11.8%) substantially contributed to AA intakes. The main sources for EPA intakes were fresh/frozen fish (28.4%) and canned fish (18.8%), followed by beef (5.6%) and shellfish & seafood (3.0%). Fresh/frozen fish (33.6%), beef (22.0%), canned fish (11.2%), bacon (10.0%) and chicken (7.5%) where the major contributors to DPA intakes. The main contributors to DHA were fresh/frozen fish (36.7%) and canned fish (24.0%), with minor contributions from other food sources such as eggs (3.8%), shellfish & seafood (2.8%) and bacon (1.0%). The main food sources and their contributions to total PUFA intakes are detailed in Table 4.7.

Table 4.7 - Contribution of food sources to n-6 and n-3 polyunsaturated fatty acid intakes

Food source	Total PUFA*		LA		ALA		AA		EPA		DPA		DHA		Total n-3 PUFA	Total n-6 PUFA
	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	mg/d
Bacon	456	3.00	405	3.06	257	16.06	14.5	15.76	2	2.04	5	10.00	4.5	3.00	268.5	419.5
Ham	16.4	0.11	12.8	0.10	0.9	0.06	1	1.09	0.99	1.01	0.06	0.11	0.23	0.15	2.17	13.8
Deli Meats/Salami/Luncheon	63.04	0.41	58	0.44	4.7	0.29	0.01	0.01	0	0.00	0	0.01	0	0.00	4.71	58.01
Sausages	125	0.82	101	0.76	14.5	0.91	5.28	5.74	2.1	2.14	0.9	1.80	1.38	0.92	18.88	106.28
Beef	157	1.03	89	0.67	36	2.25	11	11.96	9	9.18	11	22.00	0.32	0.21	56.32	100
Lamb	36.3	0.24	18.01	0.14	14.34	0.90	2.49	2.70	0	0.00	1.4	2.80	0.18	0.12	15.92	20.5
Chicken	228	1.50	197	1.49	10.9	0.68	14.6	15.87	0	0.00	3.75	7.50	2	1.33	16.65	211.6
Pork	75.7	0.50	637	4.81	5	0.31	4.6	5.00	0.4	0.41	0.75	1.49	1.13	0.8	7.28	641.6
Veal	1.8	0.01	0.79	0.01	0.3	0.02	0.3	0.33	0.17	0.18	0.21	0.42	0.03	0.02	0.71	1.09
Other Meat	15.9	0.10	11.7	0.09	1.62	0.10	0.76	0.82	0.23	0.24	1.53	3.05	0.07	0.05	3.45	12.46
Egg	180	1.18	144	1.09	5.7	0.36	21.47	23.33	0.04	0.04	1.5	3.00	7.6	5.07	14.84	165.5
Fresh/Frozen Fish	196	1.29	37	0.28	12.5	0.78	10.6	11.52	45.5	46.43	16.8	33.60	73.44	48.96	148.24	47.6
Canned Fish	383	2.52	265	2.00	30.9	1.93	2.7	2.93	30	30.61	5.6	11.20	48	32.00	114.5	267.7
Fish Paste	0.8	0.01	0.43	0.00	0.06	0.00	0.02	0.02	0.12	0.12	0.03	0.05	0.17	0.12	0.38	0.45
Shellfish and Seafood	12.2	0.08	0.37	0.00	0.23	0.01	0.46	0.50	4.8	4.90	0.65	1.30	5.55	3.70	11.23	0.83
Bread	1,097.5	7.21	798	6.03	299	18.69	0	0.00	0	0.00	0	0.00	0	0.00	299	798
Breakfast cereal	351	2.31	329	2.49	21	1.31	0	0.00	0	0.00	0	0.00	0	0.00	21	329
Pasta	103.8	0.68	72.6	0.55	31.1	1.94	0	0.00	0	0.00	0	0.00	0	0.00	31.1	72.6

Food source	Total PUFA*		LA		ALA		AA		EPA		DPA		DHA		Total n-3 PUFA	Total n-6 PUFA
	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	mg/d
Milk	216	1.42	176	1.33	39	2.44	0	0.00	0.12	0.12	0.12	0.24	0.12	0.08	39.35	176
Oil and Butter	4,440	29.17	3,929	29.70	513.7	32.11	0	0.00	0	0.00	0	0.00	0	0.00	513.7	3,929
Margarine / Vegetable spread	2,163.9	14.22	1,786	13.50	377.6	23.60	0	0.00	0	0.00	0	0.00	0	0.00	377.6	1,786
Nuts and Seeds	1,945	12.78	1,909	14.43	36	2.25	0	0.00	0	0.00	0	0.00	0	0.00	36	1,909
Peanut Butter	1,287	8.46	1,287	9.73	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	1,287
Snacks and Desserts	268	1.76	242	1.83	26	1.63	0	0.00	0	0.00	0	0.00	0.03	0.02	26.03	242
Takeaway Foods	284	1.87	252	1.90	30.5	1.90	1	1.09	0.27	0.27	0.2	0.40	0.53	0.35	31.45	253
Vegetables	1,108	7.28	1,046	7.91	61.8	3.86	0	0.00	0	0.00	0	0.00	0	0.00	61.8	1,046
Total	15,210		13,800		1,830		90		100		50		150		2,120	13,890

* Mean intakes do not include contributions from PUFA supplements. Proportion (%) of the total intake and grams per day of each FA contributed by each food is presented as the mean of the whole population sample (n=596). **Abbreviations**: AA, arachidonic acid; ALA, alpha-linolenic acid; DPA, docosapentaenoic acid; LA, linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

Food sources presented in Table 4.7 were combined into nine main food groups based on their similarities in nutritional composition (Astorg et al., 2004). For instance, fats & oils group included vegetable oils, butter, lard and margarine. This food group was the main contributor for LA (43.2%) and ALA (55.7%) intakes. Meat, poultry & eggs, were the main contributor for AA (60.0%) intakes, with substantial contributions to DPA (40.3%) intake as well. Intakes of AA were also complemented by the delicatessen meats & sausages (22.6%) and the fish & seafood (14.9%) groups. The main contributor to DHA intake was the fish & seafood group (84.8%), which included all fresh, frozen and canned fish as well as shellfish and fish paste. This group contributed substantially to EPA (82.1%) and DPA (46.2%). The cereal products group, including breads and pasta, was responsible for approximately 21.9% of ALA and 9.1% of LA intakes. Only minor contributions (less than 4.0%) to individual PUFA intakes were observed for takeaway foods, snacks & desserts, nuts & seeds, and milk. Figure 4.4 shows the contributions from each food groups to the mean intakes of each fatty acid.

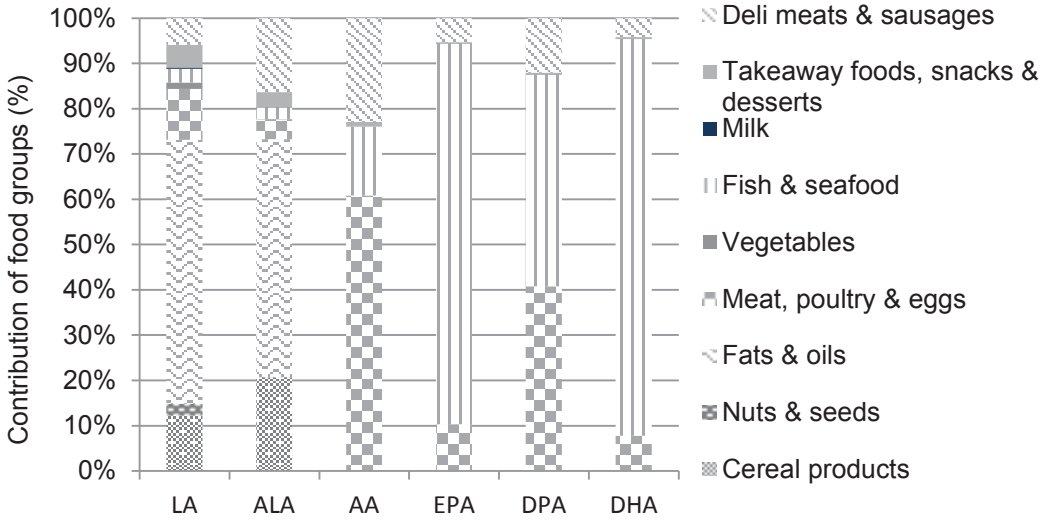

Figure 4.4 - Contributions (%) of food groups to estimated daily intakes of individual PUFAs within this study population (n=596) **Abbreviations**: AA, arachidonic acid; ALA, alpha-linolenic acid; DPA, docosapentaenoic acid; LA, linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

4.4.1 Consumption of fish and seafood

The consensus statement of Perinatal Lipid Intake Working Group (PERILIP) suggests that pregnant women can meet the recommendation of 200mg DHA by consuming one to two portions of fish per week, including oily fish (Koletzko et al., 2007a). Interestingly in this study, a large proportion of participants indicated 'never' or 'less than once per month' for their intakes of canned fish (54.7%), fresh/frozen fish (26.4%) and shellfish and seafood (82.7%). One third of participants reported consuming fresh/frozen fish one to three times per month (37.4%), while 36.3% reported intakes of once per week or over. Table 4.8 shows the frequency of fish and seafood consumption amongst participants.

Table 4.8 - Frequency of fish and seafood intake

Frequency (n = 596)	Canned Fish n (%)	Fresh/Frozen Fish	Shellfish & Seafood
Never	207 (34.7)	79 (13.3)	362 (60.7)
Less than once per month	119 (20.0)	78 (13.1)	131 (22.0)
1 to 3 times per month	130 (21.8)	223 (37.4)	77 (12.9)
Once per week	84 (14.1)	143 (24.0)	17 (2.9)
2 times per week	38 (6.4)	57 (9.6)	5 (0.8)
3 to 4 times per week	17 (2.9)	15 (2.5)	3 (0.5)
Once per day	1 (0.2)	0 (0.0)	1 (0.2)
2 or more times per day	0 (0.0)	1 (0.2)	0 (0.0)

Frequency of fish and seafood intakes amongst all participants (n=596).

4.4.2 Consumption of meat

Over half of participants reported consuming chicken (63.1%) and beef (60.8%) at least twice per week. Participants also reported the intake of lamb (28.7%), pork (20.0%), bacon (27.1%) and sausages (22.2%) at

least once per week. Table 4.9 shows that a higher proportion of participants reported the consumption meats of at least once per week.

Table 4.9 - Frequency of meat consumption

Frequency (n = 596)	Chicken n (%)	Beef	Lamb	Pork	Bacon	Sausages
Never	30 (5.0)	45 (7.6)	105 (17.6)	177 (29.7)	90 (15.1)	101 (16.9)
Less than once a month	9 (1.5)	15 (2.5)	132 (22.1)	144 (24.2)	114 (19.1)	127 (21.3)
1 to 3 times per month	54 (9.1)	49 (8.2)	188 (31.5)	156 (26.2)	231 (38.8)	236 (39.6)
Once per week	127 (21.3)	125 (21.0)	134 (22.5)	96 (16.1)	119 (20.0)	110 (18.5)
2 times per week	240 (40.3)	228 (38.3)	34 (5.7)	20 (3.4)	34 (5.7)	19 (3.2)
3 to 4 times per week	128 (21.5)	127 (21.3)	3 (0.5)	2 (0.3)	7 (1.2)	3 (0.5)
Once per day	8 (1.3)	6 (1.0)	0 (0.0)	1 (0.2)	1 (0.2)	0 (0.0)
2 or more times per day	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Frequency of meat intakes amongst all participants (n=596).

4.4.3 Contribution of supplements to polyunsaturated fatty acid intakes

A total of 117 participants reported taking PUFA supplements. These participants had a median [25th, 75th percentile] age of 33 [28, 36] years, which was two years older than the age (31 [28, 35] years) of participants not taking PUFA supplements (P<0.013, small effect size r= -0.10). The majority of participants taking supplements were highly educated (76.7% - university degree). These participant were more likely to be taking PUFA supplements compared to participants below secondary education levels (P=0.019). Household income, country of birth and ethnicity had no statistically significant impact on the consumption of PUFA supplements (P>0.05).

Polyunsaturated fatty acids supplements included in this study were mainly marine- and plant-based oils and capsules, comprised of n-6 and/or n-3 PUFAs. The proportion and contribution in milligrams from

which supplements contributed to the mean daily intakes of PUFAs is described in Table 4.10. In participants taking supplements, the intake of PUFA supplements contributed to 4.3% of total PUFA, 59.7% of DHA and 73.3% of EPA daily estimated intakes. Consumption of PUFA supplements had substantial contributions to the mean intakes of EPA (440±410mg/d) and DHA (430±310mg/d), which were notably higher than the intakes from participants not taking PUFA supplements for these same fatty acids (90±140mg/d EPA and 140±200mg/d DHA – see Table 4.3).

Table 4.10 - Contribution of PUFA supplements to n-6 and n-3 PUFA intakes in participants taking PUFA supplements (n=117)

	Total PUFA		LA		ALA		AA		EPA		DPA		DHA	
	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	%	mg/d	%
Intake of PUFA supplements	730	4.30 [a]	50	0.35	110	6.72	0.77	0.79	320	73.34	0.00	0.00	260	59.67
Total PUFA intake	17,040± 7,380*		14,460± 6,800*		1,570± 1,110*		100± 60*		440± 410*		50± 40*		430± 310*	

[a] Contribution (%) to estimated daily intake for each individual PUFA for participants taking PUFA supplements (n=117); * mean±SD in mg/d. **Abbreviations**: AA, arachidonic acid; ALA, alpha-linolenic acid; DPA, docosapentaenoic acid; LA, linoleic acid; DHA, docosahexaenoic acid, EPA, eicosapentaenoic acid.

Further analysis was performed to compare the contribution of fish and seafood versus PUFA supplements to each individual FA for participants taking PUFA supplements. Fish and seafood contributed 13.4% and 21.0% of EPA and DHA daily intakes compared to 73.3% and 59.7% of supplement contributions to EPA and DHA daily intakes respectively (see Figure 4.5).

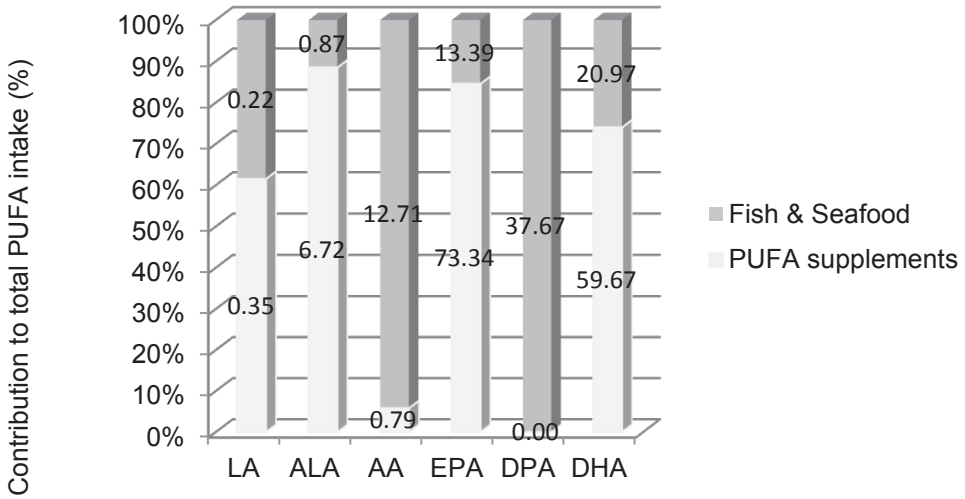

Figure 4.5 – Contributions (%) of PUFA supplements versus fish & seafood in participants taking PUFA supplements (n=117)

Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; DPA, docosapentaenoic acid; LA, linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

Chapter Five - Discussion and conclusions

Henrique, when he was learning to sit up. This is just the beginning of many brilliant years to come.

5.1 Introduction

This study is the first to investigate dietary intakes and food sources of omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFAs) in pregnant women living in New Zealand (NZ). This was assessed using a food frequency questionnaire (FFQ) previously validated for the NZ population.

This chapter brings the main findings from this study into the context of the existing literature exploring dietary intakes and food sources of n-6 and n-3 PUFAs in pregnant women living in other developed countries. Discussion of the relevance of the results, methodological considerations as well as recommendations for future research and final conclusions will be included in this chapter. In addition, strengths and limitations of this study will be discussed.

5.2 Summary of findings

The majority of pregnant women (69.1%) in this study were not meeting the international consensus recommendations of 200mg/d of docosahexaenoic acid (DHA) during pregnancy (Perinatal Lipid Intake Working Group - PERILIP, Joint FAO & WHO and the Australian Scientific Consensus Workshop). The median [25th, 75th percentile] DHA intakes for the study population were 110 [50, 250]mg/d. However, further analysis of subgroups revealed that median DHA intakes of participants taking PUFA supplements (370 [210, 530] mg/d) were substantially higher than the intakes of participants not taking supplements (90 [50, 160]mg/d). It was estimated that PUFA supplements accounted for over half of the DHA intakes (59.7%) within these participants (n=117). Recommended intakes for DHA were met by 79.5% of participants taking PUFA supplements, who were also 16.5 times more likely to meet these recommendations than participants not taking PUFA supplements. In contrast, only 19% of

participants not taking PUFA supplements met recommended intakes for DHA.

Similarly for EPA, only a small proportion of all women (23.2%) met the International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommended levels (AI ≥220mg/d). Participants taking PUFA supplements had median EPA intakes (350 [200, 520]mg/d) that were notably higher than the intakes of participants not taking supplements (50 [20, 100]mg/d). Most participants taking PUFA supplements (71.8%) achieved the recommended intakes from ISSFAL (Simopoulos et al., 2000) compared to only 11.3% of participants not taking PUFA supplements.

Recommendations for total n-3 LC-PUFAs (EPA plus DHA) from the Australian Scientific Consensus Workshop (500mg/d) were achieved by 70.1% of participants taking PUFA supplements (680 [440, 1,090]mg/d, EPA+DHA), with 11.7% of participants not taking supplements meeting these recommendations (140 [70, 240]mg/d, EPA+DHA). However, most participants (99% of participants taking supplements versus 71.2% of the participants not taking supplements) were able to meet the adequate intake (AI) of 115mg/d for total n-3 LC-PUFAs (EPA, DHA plus docosapentaenoic acid - DPA) recommended by the National Health and Medical Research Council (NHMRC, 2006). The NHMRC also have a Suggested Dietary Target (SDT) of 430mg/d recommended for prevention of chronic diseases in adult women, which does not distinguish between pregnant and non-pregnant women. However, the SDT was met by only 17.3% of participants not taking PUFA supplements compared to 81.2% of participants taking PUFA supplements. Although median DPA intakes in this cohort (40 [30, 60]mg/d) contributed to the intakes of total n-3 LC-PUFAs, individual intakes could not be compared with recommendations

due to the lack of established values. Over half of participants (64%) were able to meet the AI for alpha-linolenic acid (ALA) (1,000mg/d) recommended by NHMRC, with median intakes (1,300 [790, 2,120]mg/d) above these recommendations.

Median intakes of linoleic acid (LA) and arachidonic acid (AA) were 11,580mg/d and 90mg/d respectively. Meeting the recommendations for these n-6 PUFAs was not a concern for the majority of the participants, with over 91% of women meeting the recommendations for LA (AI ≥6,400mg/d) and all (100%) below the UL of 800mg/d for AA (FAO & WHO). Likewise, most participants were able to meet recommendations from the NHMRC for LA (AI 10,000mg/d) (64.4%). Omega-6 PUFAs contributed predominantly to total PUFA intakes (86.7%).

The major food sources contributing to LA intakes were fats and oils (43.2%), nuts and seeds (24.1%), and all meats and eggs (12.7%). Meat, poultry and eggs were also the main contributors to AA intakes (60.0%), with some contributions from delicatessen meats and sausages (22.6%), as well as fish and seafood (15.0%).

Main food sources of ALA were fats and oils (55.7%), breads and pasta (21.9%), as well as delicatessen meats and sausages (17.3%). Meat, poultry and eggs also contributed to a substantial proportion of DPA (40.3%) and less than 10% of EPA and DHA intakes. Fish and seafood were the main contributors of n-3 LC-PUFAs (DHA, 84.78%; EPA, 82.06%; DPA, 46.16%). Despite this, reported dietary patterns indicated that intakes of fish and seafood were not common in this study population. Only 9.5% and 12.2% of women consumed canned fish or fresh/frozen fish at least twice per week.

5.2.1 Dietary intakes of polyunsaturated fatty acids and current recommendations

The present study estimated the dietary intakes of total PUFAs and individual key fatty acids (see Table 4.3). Although dietary intakes - particularly for n-3 LC-PUFAs - were clearly skewed, the means instead of medians will be used for comparison purposes given that most studies reported the means. Mean dietary intakes of total PUFAs in this study population were estimated as 15,360±7,450mg/d. These intakes are within the intake range of 10,900 to 17,700mg/d observed in pregnant women from Canada (Friesen & Innis, 2009, 2010; Jia et al., 2015), the United States (Donahue et al., 2009), France (Bernard et al., 2013), the Netherlands (Otto et al., 2001a), Belgium (De Vriese et al., 2001; De Vriese et al., 2002), Scotland (Lakin et al., 1998) and the United Kingdom (Thomas et al., 2006). Total PUFA intake values were mainly derived from FFQs and food records, with the exception of a few studies using 4-day food record, 24-h recall and direct PUFA quantification of food duplicates. The study using 4-day food records included records of consecutive days which were completed by a small sample of 44 healthy pregnant women in the United Kingdom, with findings aligning with the present study (Thomas et al., 2006). A Canadian study ascertained total PUFA intakes (9,800mg/d) that were lower than the levels observed in other countries, based on direct quantitation of duplicate food collections over a three days period (Denomme et al., 2005). Despite the small sample size of only 20 participants, findings from this study may suggest that suggests that the dietary assessment methods used in other studies could have over-estimated dietary intakes of total PUFAs. However, the majority of FFQs used in the above studies were validated (De Vriese et al., 2002; Donahue et al., 2009; Friesen & Innis, 2009, 2010; Lakin et al., 1998; Otto et al., 2001a), and the 24-h recall was administered by a trained interviewer (Jia et al., 2015), thereby strengthening their findings.

Although the present study was the first to investigate the intakes of individual PUFAs in pregnant women, other studies in NZ, have estimated intakes of total PUFAs alongside a range of nutrients. These studies estimated a lower median daily intake of total PUFA of 10,000 to 11,000mg/d in pregnant women living in Dunedin (n=95) (McKenzie-Parnell et al., 1993) and the upper North in NZ (n=369) (Watson & McDonald, 2014). Mean values ranging from 11,000 to 12,000mg/day for total PUFA intakes were also reported in an earlier study conducted in pregnant women (n=403) (Watson & McDonald, 2010). Total PUFA intakes ranged from 9,900 to 11,000mg/d in non-pregnant women participating in the National Nutrition Surveys (NNS) in 1997 (n=1,964) and 2008/09 (n=1,179) (Ministry of Health, 1999; University of Otago, 2011; University of Otago & Ministry of Health, 2011). A possible reason for these lower mean total PUFA intakes may be the different dietary assessment tools employed to investigate PUFA intakes in the different studies. For instance, the present study used a validated PUFA FFQ, whereas the NNSs used a 24h recall, and the other studies used 3-d food records (McKenzie-Parnell et al., 1993) or in addition to a 24h recall (Watson & McDonald, 2010, 2014). Another reason could be the lack of a robust and validated tool capable of assessing total and individual PUFA intakes taking into account both dietary and supplementary sources. Thus, a strength of the current study is being the first to use a NZ-validated FFQ to assess dietary intakes of individual fatty acids in pregnant women, based on the consumption of important food sources as well as supplements containing PUFA. However, it is important to acknowledge that the present study was not a representative group of the NZ population whereas both NNSs had representative samples. Thus, dietary intakes may only be relevant to similar population groups.

In the present study, most pregnant women met the recommended intakes for n-6 PUFAs. In fact, mean LA intakes (13,240±6,890mg/d) were above the adequate intakes (AI) (10,000mg/d) (NHMRC, 2006) and mean AA intakes (90±50mg/d) were almost nine folds below the suggested UL (800mg/d) (FAO & WHO). These reported mean dietary intakes are consistent with the levels observed in pregnant women in Canada (11,000 - 13,400mg/d LA, 93 – 113mg/d AA) (Jia et al., 2015; Wu et al., 2013), and the United States (11,748 – 15,600mg/d LA, 90 – 115mg/d AA) (Donahue et al., 2009; Oken et al., 2007; Stark et al., 2005). This data also supports that, similarly to the current study, meeting the recommendations for n-6 PUFAs does not appear to be an issue for pregnant women in developed countries. However, dietary intakes of LA that exceed recommended intakes may decrease the synthesis of n-3 LC-PUFAs as well as their incorporation into tissues, because both PUFA families share the same metabolic pathway where they over the same enzymes (Adkins & Kelley, 2010; Lattka et al., 2010). Although AA is required for normal fetal growth and development, it is a precursor of pro-inflammatory substances which may also be unfavourable during gestation (Adkins & Kelley, 2010). Pregnant women in this study were not at risk of consuming large amounts of AA, however as intakes of LA were above recommended levels, they may benefit from reducing their intakes of LA.

Mean dietary intakes of ALA in this current study were estimated as 1,620±1,130mg/d, with 64% of women meeting the AIs recommended by NRVs (1,000mg/d). Similar mean intakes were estimated for pregnant women in Canada (1,600 – 1,700mg/d) (Friesen & Innis, 2009, 2010; Innis & Elias, 2003; Jia et al., 2015; Wu et al., 2013), the United States (1,550 – 1,680mg/d) (Stark et al., 2005), and Belgium (1,410 – 1,500mg/d) (De Vriese et al., 2001; De Vriese et al., 2002). Dietary ALA intakes below recommended levels were reported in France (878mg/d) (Bernard et al.,

2013) and the United States (990mg/d) (Donahue et al., 2009). Although mean ALA intakes in the current study were above recommendations, this does not necessarily translate to a higher synthesis of n-3 LC PUFAs. Even though pregnant women may possess a higher ability to convert ALA into EPA and DHA at suggested rates of 21% and 9% respectively (Williams & Burdge, 2006), these conversion rates are likely to be limited by increased intakes of saturated and n-6 fatty acids, genetic polymorphisms affecting the synthetic pathway as well as obesity and poor dietary patterns and lifestyles (Lattka, Illig, Koletzko, & Heinrich, 2010; Marangoni et al., 2004; Saunders et al., 2012; Schuchardt et al., 2010). However, it is interesting to estimate what the converted values would possibly be in the present study regardless of limiting factors. Therefore, based on the mean ALA intakes of 1,620mg/d, and conversion rates suggested above, approximately 340mg/d of EPA and 145mg/d of DHA could potentially be added to the daily intakes of this population.

Less than one-third of all women in the present study met the international recommended intakes for EPA (22.3%) and DHA (30.9%) (ISSFAL, PERILIP, Joint FAO & WHO and the Australian Scientific Consensus Workshop). When dividing women into groups according to whether they took PUFA supplements or not, mean DHA intakes of participants not taking PUFA supplements (n=479) were estimated as 140±200mg/d, with only 19% meeting the consensus recommendations of 200mg/d of DHA (PERILIP, Joint FAO & WHO and the Australian Scientific Consensus Workshop) (Food and Agriculture Organization of the United Nations and World Health Organization, 2010; Koletzko et al., 2007a; Simmer et al., 2009). Mean EPA intake for participants not taking supplements were estimated as 90±140mg/d, with only 11.3% of women meeting the ISSFAL recommended AI (≥220mg/d).

Estimated mean EPA and DHA intakes in participants not taking PUFA supplements in the present study are consistent with the estimated mean intake ranges observed in other developed countries such as France (EPA, 90±140mg/d; DHA, 148±990mg/d), the United Kingdom (EPA, 110±220mg/d; DHA, 130±140mg/d), the Netherlands (EPA, 80±30mg/d; DHA, 140±50mg/d) and Canada (EPA, 85.1±86.7mg/d; DHA, 160±169mg/d) (Bernard et al., 2013; Friesen & Innis, 2009; Otto et al., 2001a; Thomas et al., 2006). Higher intakes of n-3 LC-PUFAs were observed in countries like Belgium and Denmark. Two Belgian studies conducted in small samples of pregnant women estimated mean intakes of 150mg/d for EPA and 250 to 300mg/d for DHA (De Vriese et al., 2001; De Vriese et al., 2002). However, it is possible that these findings were associated with increased fish intakes, given that the Belgium studies were conducted in an area close to the coastline. In Denmark, higher consumption of fish and seafood (86.3% of women consumed 1 – 2 fish serves per week and 11% consumed ≥3 fish serves per week) influenced high mean intakes of EPA (130±120mg/d) and DHA (320±260mg/d) of pregnant women (n=54,344) participating in the Danish National Birth Cohort (Oken et al., 2008a; Olsen et al., 2007).

Higher intakes of n-3 LC-PUFAs are expected in countries such as Japan, where traditionally there is a high consumption of seafood and fish. For instance, daily n-3 LC-PUFA intakes in middle-aged Japanese women (n=79) were estimated to be 314mg/d for EPA and 571mg/d for DHA (Kuriki et al., 2003). However, mean n-3 LC-PUFA intakes in pregnant women (n=1002) were estimated as 200 and 300mg/d for EPA and DHA respectively (Miyake et al., 2007), which could be a consequence of shifting dietary patterns from the traditional Japanese to a more Westernised version (Kuriki et al., 2003).

In contrast, lower EPA and DHA intakes were reported by studies using FFQs to assess PUFA intakes. In an Australian study (n=94), the median intakes of EPA and DHA were estimated as 72 and 75mg/d respectively (Cosatto et al., 2010). In this study, only 9% of participants achieved the recommended intake for DHA (200mg/d, ISSFAL). This study used the Australian PUFA FFQ, which was used as the basis for the development of the NZ PUFA-FFQ. When comparing median EPA and DHA intakes between the Australian study and the present study (60mg/d EPA; 110mg/d DHA), it is clear that participants in the current study had lower intakes of EPA and higher intakes of DHA intakes. This could be due to only 5% of participants in the Australian study consumed PUFA supplements compared to 19.6% of participants in the present study. Low compliance with recommendations was also reported in a group of Canadian pregnant women (n=222), where only 17% of the women met the recommended intake of 200mg/d for DHA (Wu et al., 2013).

Even lower DHA intakes were reported in a study conducted in Scotland, where estimated intakes were as low as 9mg/d in a small sample of vegetarian pregnant (n=4) women (Lakin et al., 1998). Pregnant women in the current study following a vegetarian or vegan dietary pattern were at risk of not achieving the recommended levels for n-3 LC-PUFAs. In this study, vegetarian participants not taking PUFA supplements (n=20) had median intakes of EPA (39mg/d), DHA (65mg/d) and total n-3 LC-PUFAs (143mg/d) below recommended levels, with only 10% of those meeting the recommended intakes for DHA. Similarly, median intakes of vegan participants not taking PUFA supplements (n=2) were below recommendations for n-3 LC-PUFAs (EPA, 10mg/d, DHA, 35mg/d, and total n-3 LC-PUFAs, 59mg/d). In contrast, one vegetarian participant who reported the use of PUFA supplements showed intakes of 939mg/d EPA, 676mg/d DHA and 1,649mg/d total n-3 LC-PUFAs, which were well above

the recommended levels. Therefore, it may be reasonable that women following vegetarian/vegan dietary patterns consider the use of algae-based n-3 LC-PUFAs supplements (Gebauer et al., 2006; Sioen et al., 2010).

Studies using other methods to assess PUFA intakes also reported low DHA intakes in pregnant women. Mean DHA intakes among these studies were 82mg/d based on a direct PUFA quantitation of a three day food duplicate in Canada (Denomme et al., 2005), 68 to 110mg/d estimated by 24-h recalls in the United States (Donahue et al., 2009; Loosemore et al., 2004; Stark et al., 2005), and 130mg/d derived from a 4-d food record (Thomas et al., 2006). However, it is likely that these dietary assessment methods under-estimated the intakes of n-3 LC-PUFA. As the richest sources of n-3 LC-PUFAs, fish and seafood are not eaten on a daily-basis, which limits the likelihood of these food sources being consumed within the timeframe assessed by these dietary assessment methods (Innis & Elias, 2003; Lee & Nieman, 2010; Sioen et al., 2006).

In the Alberta Pregnancy Outcomes and Nutrition (APrON) study, 24h recall were used in a cohort of 600 pregnant women mean DHA intakes of 237mg/d were reported. Although these intake levels seem to be within the recommendations, 73% of pregnant women failed to meet the 200mg/d recommended for DHA. Therefore, it is likely that the high mean intakes observed were influenced by the high proportion of women (30%) taking PUFA supplements (Jia et al., 2015). Participants taking PUFA supplements were over ten times more likely to meet the recommended intakes compared to women not taking PUFA supplements (Jia et al., 2015).

Similarly in the current study, the estimated mean DHA intakes (200±250mg/d) were influenced by high DHA intakes of women taking

PUFA supplements (19.6%). Women taking PUFA supplements had notably higher mean DHA intakes (430±310mg/d) compared to women not taking supplements (80.4%) (DHA, 140±200mg/d). The consumption of PUFA supplements contributed to 59.7% of DHA intakes within this group. The 200mg/d recommended for DHA were met by 79.5% of women taking PUFA supplements, who were also 16.5 times more likely to meet these recommendations versus women not taking PUFA supplements. Likewise, taking PUFA supplements contributed to higher mean intakes of EPA amongst the entire study population. Participants taking PUFA supplements had a mean EPA intake of 440±410mg/d compared to 90±140mg/d estimated for women not taking supplements. Taking PUFA supplements accounted for 74.3% of EPA intakes, and within those taking PUFA supplements, 71.8% met the recommended levels. Although PUFA supplements can help pregnant women achieving recommended intakes for n-3 LC-PUFAs, the use of PUFA supplements is not endorsed by the current nutrition guidelines for pregnant women in NZ (Ministry of Health, 2006). However, if pregnant women intend to use PUFA supplements as a means of achieving n-3 LC-PUFA recommendations, they should be careful to not exceed the UL (3,000mg/d) recommended for these PUFAs (Ministry of Health, 2006). In this study, five participants have exceeded the ULs for n-3 LC-PUFAs (n=3 were taking PUFA supplements).

A consensus recommendation of 200mg/d for DHA has been established as adequate to cover the accretion rates in fetal tissues throughout pregnancy as well as maternal needs for normal physiological functions (Brenna & Lapillonne, 2009). Maternal dietary DHA intakes below 70mg/d may impact on fetal development, deplete maternal DHA status and compromise the supply for maternal physiological needs (Donahue et al., 2009; Innis, 2003; Makrides & Gibson, 2000). Further analysis showed 33% of participants had DHA intakes below 70mg/d.

Mean dietary intakes for total n-3 LC-PUFAs including EPA, DPA plus DHA in this study population were estimated as 410±530mg/d, with the majority of participants (76.7%) meeting the AI (115mg/d) suggested by NHMRC (2006). Although the AI for n-3 LC-PUFAs was met by the majority of participants regardless of whether they took PUFA supplements or not, it is important to highlight that this AI is low compared to other recommendations (300mg/d EPA plus DHA, FAO & WHO; 500mg/d EPA plus DHA, Australian Scientific Consensus Workshop). When the AI for n-3 LC-PUFA for pregnant women was established by the NHMRC there was no current dose-response levels established for beneficial effects of total and individual n-3 LC-PUFAs (EPA, DPA and DHA) during pregnancy. Therefore, AI for combined n-3 LC-PUFA intakes during pregnancy were based on dietary n-3 LC-PUFA intake levels of apparently healthy individuals with an additional 25% to cover body changes during pregnancy (Ministry of Health, 2006).

Despite this, the NHMRC acknowledge the importance of increasing the intakes of n-3 LC-PUFAs for prevention of chronic diseases. A SDT of 430mg/d of n-3 LC-PUFAs is recommended for adult women for optimal health, without distinction of whether they are pregnant or not (NHMRC, 2006). The SDTs were mostly met by participants taking PUFA supplements (81.2%) compared to only 17.3% of women not taking PUFA supplements. Participants not taking PUFA supplements had mean total n-3 LC-PUFAs (280±380mg/d) considerably lower compared with women taking PUFA supplements (920±700mg/d). Mean total n-3 LC-PUFAs intakes of women not taking PUFA supplements were consistent with intakes reported for Australian pregnant women (n=606), estimated as 336±379mg/d (Taylor et al., 2014).

Other studies reported the intakes of EPA plus DHA when reporting total n-3 LC-PUFAs (n=8), with mean intakes ranging from 85.1 to 328mg/d (Donahue et al., 2009; Fawzi et al., 2004; Friesen & Innis, 2009; Loosemore et al., 2004; Oken et al., 2007; Sontrop et al., 2008; Stark et al., 2005; Taylor et al., 2014). Mean values estimated for EPA plus DHA for women in the present study were 360±500mg/d, which are above the range of mean intakes estimated for pregnant women from most countries, with the exception of Japan. Mean total EPA plus DHA intakes in Japanese pregnant women (n=1002) were estimated as 500mg/d (Miyake et al., 2007). When comparing this study's mean EPA plus DHA intakes with recommendations from Australian Scientific Consensus Workshop (500mg/d) and FAO & WHO (300mg/d), most of participants taking PUFA supplements (870±630mg/d) were able to meet this recommendations (70.1% and 89.7% respectively). However, mean EPA plus DHA intakes (240±340mg/d) of pregnant women not taking PUFA supplement were well below the recommended 500 and 300mg/d, with only 11.7% and 21.5% of women meeting these levels respectively.

Participants in the present study had mean DPA intakes of 50±40mg/d, which were within the range observed in other studies (20 to 125mg/d) (Lakin et al., 1998; Olsen et al., 2007; Otto et al., 2001a). Although DPA may only contribute to a small percentage of total n-3 LC-PUFAs, it is of interest as there is growing interest in this fatty acid's link to health and disease (Kaur et al., 2011; Mann et al., 2010). While the role of DPA during pregnancy is not clear, pregnant women have an increased ability to convert DPA into DHA (Extier et al., 2010; Otto, van Houwelingen, Badart-Smook, & Hornstra, 2001b; Williams & Burdge, 2006). Thus it is possible that DPA may serve as a top up for the increased DHA demands imposed during pregnancy (Howe et al., 2006; Kaur et al., 2011).

5.2.2 Food sources for polyunsaturated fatty acids

When comparing information on food sources of PUFAs between studies from a range of countries, it is important to consider that each country may have unique dietary patterns and utilise different food composition databases (Blumfield et al., 2012). In addition, animal products can largely differ in their fatty acids composition, depending on the type of environment and feeding regime provided to the animal (Larsen et al., 2010). Moreover, the use of distinct dietary assessment tools within the few studies exploring food sources of PUFAs can influence the quality of results. Altogether these observations can impact on the accuracy regarding dietary intakes and food sources of PUFAs.

Omega-6 polyunsaturated fatty acids

This study found that n-6 PUFAs comprised 86.7% of the estimated intake of total PUFAs, which was comprised of 99.3% of LA and less that 1% of AA. Similar proportions of n-6 PUFA were reported by Friesen and Innis (2009) in 204 pregnant women living in Canada. In fact, n-6 PUFAs are reported as the dominant PUFAs in the diets of pregnant women in most developed countries (Janssen & Kiliaan, 2014; Meyer, 2011; Muskiet et al., 2006).

The most important food sources of n-6 PUFAs in this study population were fats and oils (43.2%) and nuts and seeds (24.1%), which were the major contributors to LA intakes, whereas meats, eggs, poultry and meat products mostly contributed to AA intakes (82.6%). Apart from nuts and seeds, these foods were also reported as the main sources of ALA and AA in pregnant women living in Belgium (De Vriese et al., 2002) and Canada (Friesen & Innis, 2009, 2010; Innis & Elias, 2003). Interestingly, the Canadian study also reported that dairy products provided 10% of AA intakes (Friesen & Innis, 2009). In the present study, the only dairy

product considered was milk, which only contributed to a small amount of LA (1.3%). Therefore, based on findings from the Canadian study, it is possible that the FFQ used in the current study may have under-estimated the intakes of n-6 PUFAs by not including other dairy products apart from milk. However, good agreement between the NZ-PUFA FFQ and a 3-d weighed food record was reported when measuring LA and AA, according to the validation study conducted by Ingram and colleagues (2012).

Omega-3 polyunsaturated fatty acids

Omega-3 PUFAs only contributed to 13.6% of total PUFA intakes. Total n-3 PUFA intakes were mostly comprised of ALA (11.8%) and small amounts of n-3 LC-PUFAs (1.8%). Important food sources contributing to ALA intakes included fats and oils (55.7%), breads and pasta (21.9%), and delicatessen meats and sausages (17.3%). Fish and seafood were the primary sources of DHA (84.8%), EPA (82.1%) and DPA (46.2%) intakes. Meat, eggs and poultry contributed to 40.3% of DPA intakes with very small contributions to EPA (10.04%) and DHA (7.55%). These food sources are consistent with pregnancy studies conducted in Belgium (De Vriese et al., 2002), Canada (Friesen & Innis, 2009; Innis & Elias, 2003; Jia et al., 2015), Germany, Spain and Hungary (Franke et al., 2008). However, the EDEN Mother-Child Cohort, conducted in 1,335 pregnant women in France reported that fish contributed to only 54% of DHA intakes (Bernard et al., 2013). In a Canadian study, findings from a 24-h recall were fairly consistent with the current study, with fish, seafood and seaweed contributing to 81% of DHA, 87% of EPA and 59% of DPA intakes (Jia et al., 2015). Findings from other studies in Canada reported that meat and poultry (32%) (Innis & Elias, 2003) and dairy products (15%) were also important sources of EPA, while eggs (15%) were good sources of DHA (Friesen & Innis, 2009). In the current study the only dairy item included in the FFQ was milk, which contributed 2.4% of ALA and

less than 1% of EPA, DPA and DHA intakes. In addition, eggs contributed to less than 6% of DHA intakes in the current study. These differences may be explained by different fatty acids composition of animal products from NZ and Canada, eggs being a common ingredient in many foods in Canada (Friesen & Innis, 2009), and the inclusion of dairy products in the Canadian study.

Overall, fish and seafood were consistently reported as the major food sources of n-3 LC-PUFAs across different countries. Although fish and seafood are not typically consumed on a daily basis, their contributions to n-3 LC-PUFAs are suggested to be up to 5 to 15 times higher than the contributions from meat products (Howe et al., 2006). However, the intakes of these important food sources were found to be low within our study population, with only 9.5%, and 12.2% of women consuming canned fish or fresh/frozen fish at least twice per week. These rates suggest that the majority of pregnant women in this study were not meeting the recommended intakes of two to three 150g servings of fish per week (Food Standards Australia and New Zealand (FSANZ), 2011). Low intakes of fish and seafood were previously reported in the NZ NNS, where the intake of fish at least once per week was reported by 15% of adults in 1997 and 41.6% of participants in 2008/09 (University of Otago & Ministry of Health, 2011; Ministry of Health, 1999). Further data analysis in the NNS 1997 also showed that weekly intakes of fish and shellfish amongst women were reported, respectively, by 36% and 21% of Pacific Island, 17% and 14% of Māori, and 14% and 3% of NZ European and other women (Ministry of Health, 1999). Altogether, data from this current study and the past NNSs show that a small proportion of pregnant and non-pregnant women in NZ are consuming fish and seafood on a weekly basis, which is most likely translated into low intakes of n-3 LC-PUFAs.

Fish and meat intakes

Currently, most countries have established recommended intakes for fish intake during pregnancy, which are aimed at achieving the recommended intakes of n-3 LC-PUFAs while preventing harmful intakes of contaminants. These recommended intakes range from two weekly servings of fish with at least one oily fish serving per week in Europe (115g per serve) (Koletzko et al., 2007a; Koletzko et al., 2008) and Canada (150g per serve) (Kris-Etherton, Innis, & Ammerican, 2007), to no more than two weekly servings of oily fish in the UK (140g per serve) and the United States (170g per serve) (IOM, 2005; United Kingdon Scientific Advisory Committee on Nutrition, 2004). Despite recommendations, low fish intake is consistent throughout different countries. In two different studies conducted in Canada, more than 40% of women in both studies consumed less than two servings of fish per week (<150g/week) (Friesen & Innis, 2009; Wu et al., 2013). In the United States, 90% of pregnant women (n=1,540) consumed less that two servings of fish per week (Oken et al., 2007). Also, in France 63% of pregnant women (n=1,335) consumed fewer than the recommended two servings of fish per week (Bernard et al., 2013). In the Netherlands, findings for a small group of pregnant women (n=20), which cannot be extended to the larger population, indicated that mean intakes of fish (16.33g/d), represented less than the recommended two fish meals per week (Otto et al., 2001a). Similarly, intake of less than two serves of fish per week were reported by the majority of pregnant women (54% of participants with gestational diabetes mellitus (GDM) and 81% of non-GDM participants) in a study in the United Kingdom (n=88) (Thomas et al., 2006). In this study, women in the GDM group had undergone nutritional counselling, which resulted in a higher proportion of these women consuming fish twice per week compared women without GDM.

Fish intakes at levels that provide the recommended 200mg/d of DHA were observed in Japan were mean fish intakes of 1002 pregnant women were estimated as 48.3g/d, the equivalent of two 160g servings of fish per week. In Denmark, a large proportion (86.3%) of women taking part in the Danish National Birth Cohort (n=54,344) reported consuming one to two fish serves per week, with 11% of remaining women consuming at least three fish serves per week (Oken et al., 2008a; Olsen et al., 2007).

In contrast to fish intakes, meats were consumed more often amongst women in this study. Over half of the participants reported the intakes of chicken (63.1%) and beef (60.8%) at least twice per week. These findings align with data from the NZ NNS 1997 and 2008/09. In the NNS 1997, dietary data show a higher proportion of women (19 to 44 years) choosing red meats (51%, beef/veal; 47%, beef mince; 51%, poultry) over fish (15%) and shellfish (8%) at least once a week (Ministry of Health, 1999). In the NNS 2008/09, most adult women (19 to 50 years) consumed red meats (60.0%) more than three times per week, and poultry (90.0%) at least once per week compared to only one-third of women who chose fresh/frozen (36.3%), canned (30.5%) and battered fish (13.0%) (University of Otago & Ministry of Health, 2011). Similar findings have been reported in pregnant women living in Australia, who consumed meat products (174g/d) more frequently compared to fish and seafood (35g/d) (Cosatto et al., 2010). In the United Kingdom, meat intakes were also reported to be almost five times higher that fish intakes (Givens & Gibbs, 2006).

It is suggested that meat intakes observed in developed countries are up to five times higher than fish intakes. At these intake levels, meat can contribute to as much n-3 LC-PUFAs as one serving of fish (e.g. 43%, meats versus 48%, fish) (Howe et al., 2006). However, fish and seafood

contribute mainly to EPA and DHA, whereas meat contributes to DPA intakes. Dietary patterns in developed countries appear to be based on increased amounts of terrestrial animal-derived meats and small quantities of fish and seafood (Meyer, 2011), with terrestrial animal-derived foods being important contributors to total dietary intakes of n-3 LC-PUFAs (Howe et al., 2006).

Although two studies reporting DPA intakes in pregnant women were identified (Cosatto et al., 2010; Jia et al., 2015), only one study evaluated the contributions from food sources to DPA intakes. Similarly to the current study (46.2%, contribution of fish and seafood to DPA), the APrON study, found that fish, seafood and seaweed contribute to 59% of DPA intakes in Canadian pregnant women (Jia et al., 2015). However, the intakes of DPA from meat products and poultry were considerably distinct, with contributions of 29.8% and 7.5% respectively in the present study compared to 14% and 11% in the APrON study. These differences may be a result of low intakes of meat products being reported within the 24-h recall used to evaluate PUFA intakes.

Nevertheless, since fish and seafood have always been considered superior sources of n-3 LC-PUFAs, it is possible that many earlier studies have specifically focused on the contributions of these sources to n-3 LC-PUFAs intakes. Consequently, studies conducted in other developed countries may not have investigated terrestrial animal-derived foods as potential sources of n-3 LC-PUFAs (Howe et al., 2006). For this reason, it is likely that dietary intakes of n-3 LC-PUFA were possibly under-estimated as they may not include potential contributions from meat and other animal-derived products (Lopez-Garcia et al., 2004; Meyer et al., 2003). Therefore, considering a range of food sources and dietary intakes of DPA is a strength of the present study.

Although this study did not investigate the reasons behind the low intakes of fish and seafood within this population, concerns of contaminants in fish are seem as a barrier to n-3 LC-PUFA intakes (Malde, Alvheim, Brunborg, & Graff, 2012; Oken et al., 2004; Rahmawaty, Charlton, Lyons-Wall, & Meyer, 2013b; Sioen et al., 2010; Smith & Sahyoun, 2005; Taylor et al., 2014). In addition, many studies suggested that fish and seafood intakes were positively associated with individuals socioeconomic and education levels (Abu-Saad & Fraser, 2010; Donahue et al., 2009; Friesen & Innis, 2009). However, in the present study, the majority of participants were tertiary educated with higher household incomes and still presented with low intakes of n-3 LC-PUFA.

Finally, some studies found low intakes of n-3 LC PUFAs were influenced by low PUFA supplement use (Denomme et al., 2005; Friesen & Innis, 2009; Innis & Elias, 2003; Oken et al., 2004). Findings from this present study support this idea as within the 19.6% of participants consuming PUFA supplements, 79% of these women met the recommended intakes for DHA. In contrast, the majority of pregnant women did not take PUFA supplements and 81% of these women failed to meet the recommended DHA intakes. This study also reported that women taking PUFA supplements were 16.5 times more likely to meet the daily recommended intakes of DHA. Similarly, an Australian study reported low consumption of fish oil supplements (5%) amongst a cohort of 94 pregnant women. In this cohort over half of all women had mean DHA intakes below 96mg/d, with only 9% meeting the recommended intake for DHA (Cosatto et al., 2010). Findings from the APrON study (n=600) showed that 30% of women reported intakes of PUFA supplements, with over half (60%) meeting the European Consensus recommendations for DHA (Jia et al., 2015).

5.2.3 Dietary patterns

A range of adjustments are required to be made in the diets of women who become pregnant. These are imposed due to increased energy and nutrients requirements, morning sickness and food cravings, and food safety issues during pregnancy. Therefore, food and nutrition guidelines are designed to help pregnant women to meet their dietary intake requirements during pregnancy (Ministry of Health, 2006).

In this study, most participants followed an omnivorous diet (96.1%) with a minority being either vegetarian (3.6%) or vegan (0.3%). Over one-third of women (39.1%) reported making some changes to their diets upon becoming pregnant. These rates seem to fall far behind the rates reported in other studies conducted in Canada (n=20) and Australia (n=857), where 53% and 63% of women respectively indicated having made some changes to their typical diet upon falling pregnant (Denomme et al., 2005; Malek, Umberger, Makrides, & Zhou, 2015). Perhaps, women in this study have misinterpreted the question about whether they have made changes to their diets upon becoming pregnant, as a large proportion of participants reported having excluded certain foods during pregnancy.

In fact, the majority of women (83.8%) in this study indicated they had excluded certain foods during their current pregnancy, which mainly included foods that are considered as higher risk foods (75.3%) during pregnancy (Ministry of Health, 2008a; Ministry of Primary Industries, 2013). Some participants also reported the exclusion of fish and seafood (19%), and other items (14.9%) such as eggs, peanuts, caffeinated and herbal beverages and spicy foods. Similarly, findings from the Growing Up in New Zealand birth cohort study (n=5664) indicated that up to 87% of pregnant women reported avoiding certain foods and beverages at some stage during pregnancy (Morton et al., 2014). Other studies also report a

high proportion of women avoiding or reducing the intakes of higher risk foods during pregnancy (Daniels et al., 2004; Lund, 2013; Malek et al., 2015; Taylor et al., 2014). Although there is a significant body of research which suggests women do change their diets during pregnancy there is evidence that not all women change their dietary habits during this period (Crozier et al., 2009; Fowler et al., 2012; Hure et al., 2009; Morrison et al., 2012; Scott, Campbell, & Davies, 2007; Wilkinson et al., 2009).

Less than half of participants (32.3%) reported adding certain foods to improve their diets since becoming pregnant. The foods included were fruits and vegetables (15.1%), dairy (10.2%), meats (6.9%) and fish and seafood (6.2%). Although some women (6.2%) reported adding fish and seafood into their diets, more women reported excluding it (19%). Avoiding or excluding fish during pregnancy can compromise the intakes of n-3 LC-PUFAs. The latter may lead to poor maternal supply to the developing fetus, unless it is supplemented or consumed from other fortified sources (Calder et al., 2010a; Cunnane, 2000; Le et al., 2009). However, consumption of n-3 PUFA fortified products was only reported by 7.6% of participants. The most commonly reported products were margarine and vegetable spreads, products derived from seeds that are rich in n-3 PUFAs, seaweed and yoghurt. Responses from participants suggest women in this cohort were not fully aware of what n-3 fortified products were. For example:

"I take Elevit with iodine supplements (not sure if it contains omega 3)"

"I take algae supplements, and eat ground flax seeds, chia seeds, cauliflower, blueberries... all rich in omega-3 plus heaps of other vitamins"

"I don't know what Omega-3 fortified products would be"

"Chia seeds every morning in my porridge"

"Give my free range chooks linseed to eat"
"Juice/yoghurt/eggs"

Moreover, it is possible that women in the study were not familiar with n-3 PUFA fortified products most likely due to their lower availability compared to other western countries such as Australia (Rahmawaty et al., 2013b). In addition, fortified products may not be popular as it is suggested that the cost of such products (e.g. eggs) can be relatively expensive, therefore creating a significant barrier to their consumption (Mitchell et al., 2004; Pauga, 2009; Troxell et al., 2005).

5.3 Discussion of study methods

5.3.1 Study design

This cross-sectional study was designed to provide a snapshot of current dietary intakes and food sources of n-6 and n-3 PUFAs in a cohort of pregnant women in NZ. A convenience sample of 450 pregnant women was determined as adequate for this study. Study volunteers were recruited over a period of six months via snowballing strategy, using advertising material (e.g. posters and flyers) as well as emails sent to different relevant organisations (see Appendix 5), social networking media, press, and a face to face approach for women attending antenatal care centres. The use of snowballing recruitment techniques and a convenience sample are considered limitations of this study design. These techniques alongside the study's open-label design possibly attracted volunteers that are generally interested in the topic being investigated. In addition, the open-label design may increase the risk of bias as participants may adjust their answers as well as dietary intakes in order to meet research expectations.

An online platform was used to host all aspects of this study. All steps were self-administered by each participant, who received clear written instructions at the start of the screening step. This online structure is a major strength for this study as it allowed the collection of nationwide data from a large number of participants (n=596) and also made the completion of the study more convenient for respondents. However, it was up to the participant to indicate whether they were eligible (living in NZ, in their last trimester of pregnancy and aged 16 years and over) to take part and there was no guarantee that respondents met the study criteria. Despite the online structure, this study also considered participants without internet access, who completed the study using hard copies of questionnaires.

A strength of this study was the gestational stage when PUFA intakes were assessed. This study ascertained the intakes of PUFA in pregnant women in their last trimester of pregnancy (for the previous three months) when accretion of LC-PUFAs, particularly DHA, are increased (Kuipers et al., 2012). In addition, during the last trimester of pregnancy women may have a more stable dietary pattern since they are long past the first trimester of pregnancy, when many women may be adjusting their dietary habits to meet with current guidelines or suffer common pregnancy ailments such as morning sickness and cravings which may impact on their dietary habits (Fawzi et al., 2004). Likewise, other studies also chosen to assess dietary PUFAs during the third trimester of pregnancy for the same reasons described above (Denomme et al., 2005; Donahue et al., 2009; Friesen & Innis, 2009, 2010; Innis & Elias, 2003; Loosemore et al., 2004; Sioen et al., 2010).

Finally, because this study aimed to provide a snapshot of PUFA intakes within a cohort of pregnant women, analysis of blood or tissue biomarkers of n-6 or n-3 PUFAs were not included. However, the previous validation

study for the same FFQ found this tool efficiently estimated PUFA intakes of 42 healthy adults compared to erythrocytes and 3-d WFR as reference methods for validation (Ingram et al., 2012).

5.3.2 Study population characteristics

In total, 596 pregnant women aged 31 [28, 35] years completed this study. These women were distributed within all fifteen regions of New Zealand, with a higher proportion of participants living in Auckland (37.4%). The nationwide distribution of participants and the substantial cohort size is considered a strength of this study.

Although this study cohort was not a representative sample of the NZ pregnant women population, it is interesting to see how it compares to other representative populations. For instance, over half of women (62.4%) in the present study had previously been pregnant and 37.2% were pregnant for the first time, compared to 60% being previously pregnant and 41.2% being pregnant for the first time among all women giving birth in NZ in 2012 (n=62,321) (Ministry of Health, 2015). Parity can inversely influence maternal and fetal PUFA status as fetal PUFA demands are met at the expenses of maternal stores, thereby leaving maternal PUFA status partially depleted towards the end of pregnancy (Al et al., 1997; Bonham et al., 2008). Therefore, having subsequent short-interval pregnancies (less than one year), increased birth order, as well as multiple pregnancies are suggested to influence negatively maternal and fetal PUFA status (Hornstra, 2000; Makrides & Gibson, 2000; Stewart et al., 2007; Zeijdner et al., 1997). Although, over half of women reported being previously pregnant, the current study did not gather information on inter-pregnancy intervals or multiple pregnancies. This meant the extent to which parity affected maternal and fetal PUFA status within this study population could not be determined.

Despite parity similarities to women giving birth in NZ, the population in the present study had a low representativeness of ethnic groups. The majority of this study population were New Zealand-European women (74.3%) with only a minority represented by Māori (9.4%) and Pacific (3.4%) women. However, women giving birth in NZ in 2012 were represented by 48.8% of New Zealand-European, 25.2% of Māori and 11.2% of Pacific women (Ministry of Health, 2015). Therefore, the latter is an indication that the findings of this study may not be extended to ethnic groups such as Māori and Pacific pregnant women. It is possible that if this study had a higher response of Māori and Pacific women, it may have resulted in increased mean intakes of n-3 LC-PUFAs. This concept is based on data from the adult NNS 1997, which reported higher weekly intakes of fish and seafood amongst Pacific Island (36%) and Māori (17%) compared to NZ European and other (14%) women (Ministry of Health, 1999). However, it is important to consider that these findings were limited to non-pregnant women of childbearing age. There is no existing data reporting fish and seafood intakes during pregnancy in Māori and Pacific groups.

In addition, this study included a large proportion of women with tertiary education (65.1%) and a high (in excess of $100,000/year) household income (37.4%), indicating that this study population over-represents women from high socioeconomic levels. However, over half of women giving birth in NZ in 2012 lived in lower decile areas (27.6% NZDep deciles 9 - 10 and 22.7% NZDep decile 7 - 8), according to New Zealand deprivation Index (Ministry of Health, 2015).

The increased proportion of women from higher socioeconomic levels may be a disadvantageous result of having employed the convenience snowball sampling technique to recruit volunteers for this study. This

selection bias was also observed in other studies investigating PUFA intakes during pregnancy in the United States (Donahue et al., 2009; Oken et al., 2007), Canada (Denomme et al., 2005; Friesen & Innis, 2009; Jia et al., 2015; Sontrop et al., 2008) Australia (Daley, Patterson, Sibbritt, & MacDonald-Wicks, 2015; Taylor et al., 2014) and Europe (Franke et al., 2008). It is suggested that individuals with high socioeconomic strata tend to be more highly educated, more health conscious and interested in nutrition, and, therefore more likely to complete the study (Livingstone & Black, 2003; Malek et al., 2015; Sinikovic et al., 2009). Therefore, it is not surprising that this study had a completion rate of 72%, and that participants with university degrees and higher household incomes (≥$60,000) were significantly more likely to complete the study than women with lower levels of education and lower household incomes (<$59,999). A higher level of education and health literacy may also lead participants to under-report unhealthy foods and/or over-report healthy foods (Livingstone & Black, 2003).

5.4 Dietary assessment methods

The dietary assessment method selected to measure PUFA intakes in this study population was the NZ-PUFA FFQ (Section 2.10.1), a semi-quantitative FFQ designed to measure the usual dietary intakes of n-6 and n-3 PUFAs of healthy adults in NZ. The NZ-PUFA FFQ has been validated and is a robust and reliable tool to estimate total and individual PUFA intakes, based on the most important sources of PUFAs in NZ, which also included PUFA supplements (Ingram et al., 2012). It has been identified that an FFQ specifically designed to assess PUFA intake is more accurate than a generic FFQ which assesses the entire diet and is therefore unlikely to accurately estimate specific nutrients such as fatty acids (Meyer et al., 2013). Therefore, using the NZ-PUFA FFQ is a strength of this study.

During pregnancy biomarkers of PUFA status may be less reliable due to an increase in maternal plasma volume (Faupel-Badger et al., 2007) which provides a good rationale for using a validated FFQ in this exploratory study.

This study investigated PUFA intakes over the past three months. Although, the validation study was based on the intakes of PUFAs for the period of 12 months, the researchers stated that the biomarker used as a reference method for validation (erythrocytes) correlates more strongly with a timeframe of three months compared to 12 months (Ingram et al., 2012). In addition, the NZ-PUFA FFQ was adapted from an Australian FFQ, which showed slightly greater validity coefficients based on the PUFA intakes during the past three months (Swierk et al., 2011). However, results from the validation study have shown the NZ-PUFA FFQ is a reliable tool, it estimated EPA, DPA and DHA intakes significantly higher than the estimated values from the WFR (Ingram et al., 2012). Therefore, it is possible that n-3 LC-PUFAs amongst participants in this study were even lower than the currently estimated values.

Advantages of the NZ-PUFA FFQ include the online structure, which gave respondents the convenience to access the tool from any location with internet (Gibson, 2005; Subar et al., 2012). Respondent burden was also reduced due to the fact that the NZ-PUFA FFQ is a relatively simple self-administered tool that can be completed considerably fast (within approximately 15 minutes). In addition, the self-administered and online structure of the FFQ also kept this research to minimal costs and minimised the burden of data entering and coding, which also reduced the chances of bias due to typing errors. Another advantage of the online structure is the linkage to the NZ PUFA databases, which is programmed to automatically calculate the dietary intakes of PUFAs for each

respondent (Ingram et al., 2012). In addition, answers to all dietary questions are required, and respondents are asked to confirm the number of portions for each food group (e.g. meats, takeaway foods) before moving forward. Furthermore the NZ PUFA-FFQ is sensitive to intakes of foods not eaten on a daily basis (e.g. fish and seafood) and infrequent use of PUFA supplements, thereby producing a more reliable estimate of the usual intakes of PUFAs in this study population (Innis & Elias, 2003).

Despite these advantages, the NZ-PUFA FFQ has not been validated for pregnant women in NZ. However, dietary data resulting from this study is comparable to findings from other studies using FFQs to assess PUFA intakes in pregnant women (Bernard et al., 2013; Friesen & Innis, 2009, 2010; Otto et al., 2001a). In addition, other studies have shown that FFQs are valid and reliable tools to estimate dietary intakes in pregnant women (Fawzi et al., 2004; Lyu et al., 2014). Moreover, even though participants in this study reported making a few changes to their dietary intakes to meet guidelines, existing literature supports that women of childbearing age do not change their diets upon becoming pregnant (Crozier et al., 2009; Fowler et al., 2012; Hure et al., 2009; Morrison et al., 2012; Wilkinson et al., 2009). Comparisons between FFQ responses from pregnant and non-pregnant women (n=600) showed no differences in mean dietary intakes of energy and macronutrients, which support the validity of FFQs previously validated for adult women for use in pregnant women (Kaplan et al., 2014).

Similar to other subjective dietary assessment methods, the NZ-PUFA FFQ is not free of limitations. This FFQ was not designed to assess energy intake as it was specifically tailored to measure PUFA intakes. Therefore, the level of under-reporting in this study is unknown. This tool is reliant upon the memory of respondents, who are not always able to recall all foods and every single ingredient of food consumed over the

previous three months (McCabe-Sellers, 2010; Thompson & Subar, 2013). Also, foods consumed near the time of completing of the FFQ can remain at the forefront of the memory of respondents, and therefore responses may be predominantly based on these more recently consumed foods (Fowke et al., 2004). In addition, estimations of dietary intakes may be inaccurate due to respondents misinterpretation of pre-defined portion sizes (De Vriese et al., 2001).

Another important consideration is that even though the NZ-PUFA database was recently developed (2012), it may not include new foods and ingredients, which are constantly added to the diets of individuals, making the databases out of date (Lee & Nieman, 2010). Participants may have consumed additional PUFAs from other food sources not included in the FFQ, thereby underestimating PUFA intakes.

Although the NZ-PUFA FFQ had open questions were participants could describe the intakes of other foods that are either good sources or fortified with n-3 PUFAs, not many participants completed this question. The few participants who responded to this question often did not provide quantities and frequencies for the indicated foods. For instance, the inclusion of linseed, chia seeds and seaweed was commonly reported among pregnant women in this study. However no details on frequency and quantity intake were available which made it impossible to include these items for further analysis. Linseeds and chia seeds are rich sources of ALA, while seaweed contains high levels of n-3 LC-PUFA. Other items that may contribute to PUFA intakes but were not included in the NZ-PUFA FFQ are dairy products and alternatives (e.g. cheese, cream, yoghurt and tofu) and other milk substitutes (e.g. coconut, almond and rice milk).

Another limitation is that the database cannot account for nutrient loss due to food storage and preparation, which may result in overestimation of dietary intakes (Lee & Nieman, 2010). For instance, some of the n-3 LC-PUFAs in fish can oxidise during cooking, due to extreme heat (Kołakowska, Domiszewski, & Bienkiewicz, 2006). Moreover, the composition of composite foods, such as takeaways and desserts, was based on assumptions made about the ingredients used in these foods, which may also produce over or under-estimation of dietary intakes (Thompson et al., 2010).

5.5 Conclusion

This is the first study providing information on the intakes of n-6 and n-3 PUFAs in a large cohort of pregnant women in NZ. Dietary intakes of PUFAs were determined using a validated online FFQ, which allowed this study to reach pregnant women from all regions of NZ. Findings from this study support that these women have low n-3 LC-PUFA intakes and failed to meet international consensus recommended levels. In contrast, meeting the recommendations for n-6 PUFAs was not of concern amongst pregnant women in this study.

The inability to meet the recommendations for n-3 LC-PUFAs was likely to be due to the low intake of fish and seafood in this study population. In this study population, fish and seafood were the most important food sources contributing over 80% of EPA and DHA intakes. Despite this, only a few women reported the intakes of fresh/frozen fish (12.2%), canned fish (9.5%) or seafood (4.4%) at least twice per week.

This study found that taking PUFA supplements significantly increased the chances of pregnant women achieving the recommendations for n-3 LC-PUFAs. Although, only 19% of women in this study reported taking PUFA supplements, statistical analysis showed that these women were 16.5 times more likely to meet the recommended intakes for DHA compared to those women not taking PUFA supplements. Although PUFA supplements may be important alternatives to increase n-3 LC-PUFAs, it is important to consider that they can be expensive and not all brands will be free of harmful contaminants and oxidised PUFAs. Therefore, when n-3 LC-PUFAs recommendations cannot be met by dietary intakes alone, the use of PUFA supplements should be considered with caution.

Most health authorities recommend at least 200mg/d of DHA and 300 to 500mg/d for total n-3 LC-PUFAs (EPA plus DHA) for pregnant women, while in NZ recommendations are less than half of the above values (115mg/d for total EPA, DHA plus DPA). The n-3 LC-PUFAs, in particular DHA, must be deposited in adequate amounts in the fetal brain and adipose tissues during the last trimester of pregnancy. It is estimated that nearly half of the recommended intakes for DHA (~70mg) are accreted in fetal tissues on daily basis, with the remaining designated to cover maternal physiological needs (Innis, 2005; Koletzko et al., 2008)). Findings from this study showed that despite a high level of education and household income, over 69% of participants were not achieving the recommended 200mg/d of DHA, and that 33% of them had DHA intakes below 70mg/d.

Existing evidence supports positive associations between the intakes of fish, or supplements providing n-3 LC-PUFA, during pregnancy and improvement in gestational duration, and optimal visual and cognitive development (Giuseppe et al., 2014; Imhoff-Kunsch et al., 2012; Larqué et al., 2012; Makrides et al., 2006; Salvig & Lamont, 2011; Simmer et al., 2009; Szajewska et al., 2006). Therefore, strategies to increase intakes of n-3 LC-PUFAs in pregnant women in NZ are recommended. It is suggested that pregnancy is a period when women are willing to receive and seek nutritional advice (Birdsall, Vyas, Khazaezadeh, & Oteng-Ntim, 2009), thus pregnant women could be targeted and educated on the importance n-3 LC-PUFAs and safe intakes of fish and seafood during pregnancy (Meyer, 2011). In addition, pregnant women could be screened for inadequate n-3 LC-PUFA intakes at an earlier stage of pregnancy in order to establish corrective actions, such as nutrition counselling (Bosaeus et al., 2015; Hautero et al., 2013; Thomas et al., 2006) or

alternatively, the use of n-3 LC-PUFA supplements to ensure adequate intakes of n-3 LC-PUFAs.

5.6 Recommendations for future research

1. Review and update the NZ-PUFA FFQ and database to include other items that are potential sources of n-6 and n-3 PUFAs. These may include dairy products (e.g. cheese, cream, and yoghurt), other milk alternatives (e.g. coconut, almond and rice milks), gluten free products, chia and linseeds, seaweed products, as well as newly released PUFA supplements and fortified products available in the NZ market.

2. Validate the FFQ in pregnant women in NZ, including women from different ethnic groups (e.g. Māori, Pacific and Asian).

3. Repeat the study in a representative sample of pregnant women in NZ.

4. Where appropriate consider using biomarkers of n-6 and n-3 PUFAs in a subgroup to overcome misreporting errors and better indicate dietary intakes of these PUFAs.

5. Compare dietary intakes of n-3 LC-PUFAs between pregnant women from different ethnic and socio-demographic backgrounds.

6. Explore factors that influence the bioavailability of n-3 LC-PUFAs amongst pregnant women. These may include the effects of genetic polymorphisms, obesity, dietary patterns and conditions during pregnancy such GDM which may compromise DHA synthesis.

7. Assess biomarkers of n-3 LC-PUFAs intakes during multiple parity and short interval periods between pregnancies in order to estimate the extent to which maternal n-3 LC-PUFA intakes/status can influence maternal and fetal n-3 LC-PUFA status.

8. Investigate the awareness amongst NZ pregnant women and maternity care providers in regards to the importance of increasing the intakes of n-3 LC-PUFAs during pregnancy.

9. Development of an n-3 LC-PUFA screening questionnaire that antenatal care providers can easily apply in women from the time the start visiting the lead maternity care services. Detecting pregnant women with inadequate intakes of n-3 LC-PUFAs in earlier stages is important to ensure these women will get the corrective action they need to improve their intakes and status of n-3 LC-PUFAs before reaching the last trimester of pregnancy.

10. To establish the roles and minimum daily requirements of AA and DPA (n-3 LC-PUFA) during pregnancy.

11. Explore barriers to dietary intakes of n-3 LC-PUFAs and strategies to improve dietary intakes of n-3 LC PUFAs amongst pregnant women.

12. Review current recommendations for n-3 LC-PUFAs in NZ.

References

Abu-Saad, K., & Fraser, D. (2010). Maternal nutrition and birth outcomes. *Epidemiologic reviews, 32*(1), 5-25.

Adkins, Y., & Kelley, D. S. (2010). Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. *Journal of Nutritional Biochemistry, 21*(9), 781-792.

Afeiche, M. C., Gaskins, A. J., Williams, P. L., Toth, T. L., Wright, D. L., Tanrikut, C., . . . Chavarro, J. E. (2014). Processed meat intake is unfavorably and fish intake favorably associated with semen quality indicators among men attending a fertility clinic. *Journal of Nutrition, 144*(7), 1091-1098. doi: 10.3946/jn.113.190173

AFFSA. (2010). *Opinion of the French Food Safety Agency on the update of French population reference intakes (ANCs) for fatty acids*. France. Retrieved from http://www.anses.fr/Documents/NUT2006sa0359EN.pdf

Agostoni, C., Riva, E., Giovannini, M., Pinto, F., Colombo, C., Rise, P., . . . Marangoni, F. (2008). Maternal smoking habits are associated with differences in infants' long-chain polyunsaturated fatty acids in whole blood: a case-control study. *Archives of Disease in Childhood, 93*(5), 414-418. doi: 10.1136/adc.2007.129817

Akabas, S. R., & Deckelbaum, R. J. (2006). Summary of a workshop on n-3 fatty acids: Current status of recommendations and future directions. *American Journal of Clinical Nutrition, 83*(6), 1536S-1538S.

Aksoy, Y., Aksoy, H., Altinkaynak, K., Aydin, H. R., & Ozkan, A. (2006). Sperm fatty acid composition in subfertile men. *Prostaglandins Leukotrienes and Essential Fatty Acids, 75*(2), 75-79. doi: 10.1016/j.plefa.2006.06.002

Al-Ardhi, F. M. M., & Al-Ani, M. R. (2008). Maternal fish consumption and prenatal methylmercury exposure: A review. *Nutrition and Health (Bicester), 19*(4), 289-297.

Al, M. D. M., Van Houwelingen, A. C., & Hornstra, G. (1997). Relation between birth order and the maternal and neonatal docosahexaenoic acid status. *European Journal of Clinical Nutrition, 51*(8), 548-553. doi: 10.1038/sj.ejcn.1600444

Albert, B. B., Cameron-Smith, D., Hofman, P. L., & Cutfield, W. S. (2013). Oxidation of marine omega-3 supplements and human health. *BioMed research international, 2013*.

Albert, B. B., Derraik, J. G., Cameron-Smith, D., Hofman, P. L., Tumanov, S., Villas-Boas, S. G., . . . Cutfield, W. S. (2015). Fish oil supplements in New Zealand are highly oxidised and do not meet label content of n-3 PUFA. *Scientific reports, 5*.

Allen, K. G. D., & Harris, M. A. (2001). The Role of n-3 Fatty Acids in Gestation and Parturition. *Experimental Biology and Medicine, 226*(6), 498-506.

Amate, L., Gil, A., & Ramirez, M. (2002). Dietary long-chain PUFA in the form of TAG or phospholipids influence lymph lipoprotein size and composition in piglets. *Lipids, 37*(10), 975-980. doi: 10.1007/s11745-006-0989-9

Amminger, G. P., Schäfer, M. R., Papageorgiou, K., Klier, C. M., Cotton, S. M., Harrigan M, S. M., . . . Berger, G. E. (2010). Long-chain ω-3 fatty acids for indicated prevention of psychotic disorders: A randomized, placebo-controlled trial. *Archives of General Psychiatry, 67*(2), 146-154. doi: 10.1001/archgenpsychiatry.2009.192

Armand, M. (2007). Lipases and lipolysis in the human digestive tract: where do we stand? *Current Opinion in Clinical Nutrition and Metabolic Care, 10*(2), 156-164. doi: 10.1097/MCO.0b013e3280177687

Arnoldussen, I., & Kiliaan, A. (2014). Impact of DHA on Metabolic Diseases from Womb to Tomb. *Marine Drugs, 12*(12), 6190-6212.

Astorg, P., Arnault, N., Czernichow, S., Noisette, N., Galan, P., & Hercberg, S. (2004). Dietary intakes and food sources of n-6 and n-3 PUFA in french adult men and women. *Lipids, 39*(6), 527-535. doi: 10.1007/s11745-004-1259-6

Barbour, L. A., McCurdy, C. E., Hernandez, T. L., Kirwan, J. P., Catalano, P. M., & Friedman, J. E. (2007). Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. *Diabetes care, 30*(Supplement 2), S112-S119.

Barker, D., Eriksson, J., Forsén, T., & Osmond, C. (2002). Fetal origins of adult disease: strength of effects and biological basis. *International Journal of Epidemiology, 31*(6), 1235-1239. doi: 10.1093/ije/31.6.1235

Bartha, J. L., Martinez-Del-Fresno, P., & Comino-Delgado, R. (2003). Early diagnosis of gestational diabetes mellitus and prevention of diabetes-related complications. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 109*(1), 41-44.

Barton, J. R., & Sibai, B. M. (2008). Prediction and prevention of recurrent preeclampsia. *Obstetrics & Gynecology, 112*(2, Part 1), 359-372.

Bauch, A., Lindtner, O., Mensink, G. B. M., & Niemann, B. (2006). Dietary intake and sources of long-chain n-3 PUFAs in German adults. *European Journal of Clinical Nutrition, 60*(6), 810-812. doi: 10.1038/sj.ejcn.1602399

Baum, S. J., Kris-Etherton, P. M., Willett, W. C., Lichtenstein, A. H., Rudel, L. L., Maki, K. C., . . . Block, R. C. (2012). Fatty acids in cardiovascular health and disease: A comprehensive update. *Journal of Clinical Lipidology, 6*(3), 216-234. doi: 10.1016/j.jacl.2012.04.077

Baylin, A., Kabagambe, E. K., Siles, X., & Campos, H. (2002). Adipose tissue biomarkers of fatty acid intake. *American Journal of Clinical Nutrition, 76*(4), 750-757.

Bellamy, L., Casas, J.-P., Hingorani, A. D., & Williams, D. J. (2007). Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *Bmj*.

Ben-Haroush, A., Yogev, Y., & Hod, M. (2004). Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. *Diabetic Medicine, 21*(2), 103-113.

Berghaus, T. M., Demmelmair, H., & Koletzko, B. (1998). Fatty acid composition of lipid classes in maternal and cord plasma at birth. *European Journal of Pediatrics, 157*(9), 763-768. doi: 10.1007/s004310050931

Bergmann, R. L., Haschke-Becher, E., Klassen-Wigger, P., Bergmann, K. E., Richter, R., Dudenhausen, J. W., . . . Haschke, F. (2008). Supplementation with 200 mg/day docosahexaenoic acid from mid-pregnancy through lactation improves the docosahexaenoic acid status of mothers with a habitually low fish intake and of their infants. *Annals of Nutrition and Metabolism, 52*(2), 157-166. doi: 10.1159/000129651

Bernard, J. Y., De Agostini, M., Forhan, A., de Lauzon-Guillain, B., Charles, M.-A., Heude, B., & Grp, E. M.-C. C. S. (2013). The dietary n6:n3 fatty acid ratio during pregnancy is inversely associated with child neurodevelopment in the EDEN Mother-Child Cohort. *Journal of Nutrition, 143*(9), 1481-1488. doi: 10.3945/jn.113.178640

Birdsall, K. M., Vyas, S., Khazaezadeh, N., & Oteng-Ntim, E. (2009). Maternal obesity: A review of interventions. *International Journal of Clinical Practice, 63*(3), 494-507. doi: 10.1111/j.1742-1241.2008.01910.x

Bitsanis, D., Ghebremeskel, K., Moodley, T., Crawford, M. A., & Djahanbakhch, O. (2006). Gestational diabetes mellitus enhances

arachidonic and docosahexaenoic acids in placental phospholipids. *Lipids, 41*(4), 341-346. doi: 10.1007/s11745-006-5104-8

Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., de Onis, M., . . . Uauy, R. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. *The Lancet, 382*(9890), 427-451.

Blasbalg, T. L., Hibbeln, J. R., Ramsden, C. E., Majchrzak, S. F., & Rawlings, R. R. (2011). Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *American Journal of Clinical Nutrition, 93*(5), 950-962. doi: 10.3945/ajcn.110.006643

Bloomingdale, A., Guthrie, L. B., Price, S., Wright, R. O., Platek, D., Haines, J., & Oken, E. (2010). A qualitative study of fish consumption during pregnancy. *The American journal of clinical nutrition, 92*(5), 1234-1240.

Blümer, N., & Renz, H. (2007). Consumption of ω3-fatty acids during perinatal life: Role in immuno-modulation and allergy prevention. *Journal of Perinatal Medicine, 35*(SUPPL. 1), S12-S18. doi: 10.1515/JPM.2007.031

Blumfield, M. L., Hure, A. J., Macdonald-Wicks, L., Smith, R., & Collins, C. E. (2012). Systematic review and meta-analysis of energy and macronutrient intakes during pregnancy in developed countries. *Nutrition reviews, 70*(6), 322-336.

Bonham, M. P., Duffy, E. M., Wallace, J. M. W., Robson, P. J., Myers, G. J., Davidson, P. W., . . . Strain, J. J. (2008). Habitual fish consumption does not prevent a decrease in LCPUFA status in pregnant women (the Seychelles Child Development Nutrition Study). *Prostaglandins, Leukotrienes and Essential Fatty Acids, 78*(6), 343-350.

Bosaeus, M., Hussain, A., Karlsson, T., Andersson, L., Hulthén, L., Svelander, C., . . . Holmäng, A. (2015). A randomized longitudinal dietary intervention study during pregnancy: effects on fish intake, phospholipids, and body composition. *Nutrition journal, 14*(1), 1.

Bourre, J.-M. (2004). Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during ageing. *The journal of nutrition, health & aging, 8*(3), 163-174.

Bourre, J.-M. (2007). Dietary omega-3 fatty acids for women. *Biomedicine & pharmacotherapy, 61*(2), 105-112.

Brantsaeter, A. L., Birgisdottir, B. E., Meltzer, H. M., Kvalem, H. E., Alexander, J., Magnus, P., & Haugen, M. (2012). Maternal seafood consumption and infant birth weight, length and head

circumference in the Norwegian Mother and Child Cohort Study. *British Journal of Nutrition, 107*(3), 436-444.

Brantsæter, A. L., Haugen, M., Alexander, J., & Meltzer, H. M. (2008). Validity of a new food frequency questionnaire for pregnant women in the Norwegian Mother and Child Cohort Study (MoBa). *Maternal and Child Nutrition, 4*(1), 28-43.

Brenna, J. T., & Lapillonne, A. (2009). Background Paper on Fat and Fatty Acid Requirements during Pregnancy and Lactation. *Annals of Nutrition and Metabolism, 55*(1-3), 97-122.

Brenna, J. T., Salem, N., Jr., Sinclair, A. J., Cunnane, S. C., & Issfal. (2009). alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukotrienes and Essential Fatty Acids, 80*(2-3), 85-91.

Burdge, G. C. (2006). Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukotrienes and Essential Fatty Acids, 75*(3), 161-168. doi: 10.1016/j.plefa.2006.05.013

Burdge, G. C., & Calder, P. C. (2005). Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reproduction Nutrition Development, 45*(5), 581-597. doi: 10.1051/rnd:2005047

Burdge, G. C., & Calder, P. C. (2006). Dietary alpha-linolenic acid and health-related outcomes: a metabolic perspective. *Nutrition Research Reviews, 19*(1), 26-52. doi: 10.1079/nrr2005113

Burdge, G. C., Sherman, R. C., Ali, Z., Wootton, S. A., & Jackson, A. A. (2006). Docosahexaenoic acid is selectively enriched in plasma phospholipids during pregnancy in Trinidadian women - Results of a pilot study. *Reproduction Nutrition Development, 46*(1), 63-67. doi: 10.1051/rnd:2005061

Burton, G. J., & Fowden, A. L. (2015). The placenta: a multifaceted, transient organ. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 370*(1663), 20140066.

Butte, N. F. (2000). Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. *The American Journal of Clinical Nutrition, 71*(5), 1256s-1261s.

Calder, P. C. (2006). n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *American Journal of Clinical Nutrition, 83*(6), 1505S-1519S.

Calder, P. C. (2008). The relationship between the fatty acid composition of immune cells and their function. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 79*(3), 101-108.

Calder, P. C. (2009). Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie, 91*(6), 791-795. doi: 10.1016/j.biochi.2009.01.008

Calder, P. C. (2012). The role of marine omega-3 (n-3) fatty acids in inflammatory processes, atherosclerosis and plaque stability. *Molecular Nutrition & Food Research, 56*(7), 1073-1080. doi: 10.1002/mnfr.201100710

Calder, P. C. (2013). Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *British Journal of Clinical Pharmacology, 75*(3), 645-662. doi: 10.1111/j.1365-2125.2012.04374.x

Calder, P. C., Dangour, A. D., Diekman, C., Eilander, A., Koletzko, B., Meijer, G. W., . . . Pietinen, P. (2010a). *Essential fats for future health. Proceedings of the 9th Unilever Nutrition Symposium, 26–27 May 2010.* Paper presented at the European journal of clinical nutrition.

Calder, P. C., Kremmyda, L.-S., Vlachava, M., Noakes, P. S., & Miles, E. A. (2010b, Aug). *Is there a role for fatty acids in early life programming of the immune system?* Paper presented at the Proceedings of the Nutrition Society.

Campbell, F. M., Gordon, M. J., & Dutta-Roy, A. K. (1998). Placental membrane fatty acid-binding protein preferentially binds arachidonic and docosahexaenoic acids. *Life Sciences, 63*(4), 235-240. doi: 10.1016/s0024-3205(98)00267-7

Cao, J., Schwichtenberg, K. A., Hanson, N. Q., & Tsai, M. Y. (2006). Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. *Clinical Chemistry, 52*(12), 2265-2272.

Carder, J., & Lewis, N. (1999). Omega-3 Fatty Acid Intakes in Midwestern Women of Child-Bearing Age from Different Income and Education Levels. *Journal of the American Dietetic Association, 99*(9), A88.

Carlson, S. E., Colombo, J., Gajewski, B. J., Gustafson, K. M., Mundy, D., Yeast, J., . . . Shaddy, D. J. (2013). DHA supplementation and pregnancy outcomes. *American Journal of Clinical Nutrition, 97*(4), 808-815. doi: 10.3945/ajcn.112.050021

Catalano, P. M. (2010). Obesity, insulin resistance, and pregnancy outcome. *Reproduction, 140*(3), 365-371. doi: 10.1530/rep-10-0088

Catalano, P. M., & Ehrenberg, H. M. (2006). The short- and long-term implications of maternal obesity on the mother and her offspring. *Bjog-an International Journal of Obstetrics and Gynaecology, 113*(10), 1126-1133. doi: 10.1111/j.1471-0528.2006.00989.x

Catalano, P. M., Farrell, K., Thomas, A., Huston-Presley, L., Mencin, P., de Mouzon, S. H., & Amini, S. B. (2009). Perinatal risk factors for childhood obesity and metabolic dysregulation. *The American journal of clinical nutrition*, ajcn. 27416.

Catalano, P. M., McIntyre, H. D., Cruickshank, J. K., McCance, D. R., Dyer, A. R., Metzger, B. E., . . . Hadden, D. R. (2012). The hyperglycemia and adverse pregnancy outcome study associations of GDM and obesity with pregnancy outcomes. *Diabetes care, 35*(4), 780-786.

Catov, J. M., Patrick, T. E., Powers, R. W., Ness, R. B., Harger, G., & Roberts, J. M. (2007). Maternal leptin across pregnancy in women with small-for-gestational-age infants. *American Journal of Obstetrics and Gynecology, 196*(6), 558.e551-558.e558.

Cetin, I., & Alvino, G. (2009). Intrauterine growth restriction: implications for placental metabolism and transport. A review. *Placenta, 30, Supplement*(0), 77-82.

Cetin, I., Alvino, G., & Cardellicchio, M. (2009). Long chain fatty acids and dietary fats in fetal nutrition. *The Journal of physiology, 587*(14), 3441-3451.

Cetin, I., Alvino, G., Radaelli, T., & Pardi, G. (2005). Fetal nutrition: A review. *Acta Paediatrica, 94*, 7-13. doi: 10.1080/08035320510043709

Cetin, I., Giovannini, N., Alvino, G., Agostoni, C., Riva, E., Giovannini, M., & Pardi, G. (2002). Intrauterine growth restriction is associated with changes in polyunsaturated fatty acid fetal-maternal relationships. *Pediatric Research, 52*(5), 750-755. doi: 10.1203/01.pdr.0000031923.07968.d5

Cetin, I., & Koletzko, B. (2008). Long-chain omega-3 fatty acid supply in pregnancy and lactation. *Current Opinion in Clinical Nutrition and Metabolic Care, 11*(3), 297-302. doi: 10.1097/MCO.0b013e3282f795e6

Cetin, I., Parisi, F., Berti, C., Mando, C., & Desoye, G. (2012). Placental fatty acid transport in maternal obesity. *Journal of Developmental Origins of Health and Disease, 3*(6), 409-414. doi: 10.1017/s2040174412000414

Challis, J. R. (1998). Molecular aspects of preterm labor. *Bulletin et memoires de l'Academie royale de medecine de Belgique, 153*(5-6), 263-270; discussion 270-263.

Challis, J. R., Sloboda, D. M., Alfaidy, N., Lye, S. J., Gibb, W., Patel, F. A., . . . Newnham, J. P. (2002). Prostaglandins and mechanisms of preterm birth. *Reproduction, 124*(1), 1-17.

Chalon, S. (2006). Omega-3 fatty acids and monoamine neurotransmission. *Prostaglandins Leukotrienes and Essential Fatty Acids, 75*(4-5), 259-269. doi: 10.1016/j.plefa.2006.07.005

Cheatham, C. L., Colombo, J., & Carlson, S. E. (2006). n−3 Fatty acids and cognitive and visual acuity development: methodologic and conceptual considerations. *The American Journal of Clinical Nutrition, 83*(6), S1458-1466S.

Cheruku, S. R., Montgomery-Downs, H. E., Farkas, S. L., Thoman, E. B., & Lammi-Keefe, C. J. (2002). Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-state patterning. *The American journal of clinical nutrition, 76*(3), 608-613.

Cho, H. P., Nakamura, M. T., & Clarke, S. D. (1999). Cloning, expression, and fatty acid regulation of the human Delta-5 desaturase. *Journal of Biological Chemistry, 274*(52), 37335-37339. doi: 10.1074/jbc.274.52.37335

Christian, P., Mullany, L. C., Hurley, K. M., Katz, J., & Black, R. E. (2015). Nutrition and maternal, neonatal, and child health. *Seminars in Perinatology, 39*(5), 361-372. doi: http://dx.doi.org/10.1053/j.semperi.2015.06.009

Clandinin, M. T., Chappell, J. E., Heim, T., Swyer, P. R., & Chance, G. W. (1981). Fatty acid-utilization in perinatal denovo synthesis of tissues. *Early Human Development, 5*(4), 355-366. doi: 10.1016/0378-3782(81)90016-5

Clandinin, M. T., Chappell, J. E., Leong, S., Heim, T., Swyer, P. R., & Chance, G. W. (1980). Intrauterine fatty-acid accretion rates in human-brain - implications for fatty-acid requirements. *Early Human Development, 4*(2), 121-129. doi: 10.1016/0378-3782(80)90015-8

Clarkson, T. W., Magos, L., & Myers, G. J. (2003). The toxicology of mercury - current exposures and clinical manifestations. *New England Journal of Medicine, 349*(18), 1731-1737. doi: doi:10.1056/NEJMra022471

Colquhoun, D., Ferreira-Jardim, A., Udell, T., & Eden, B. (2008). The nutrition and metabolism committee of the heart foundation. Fish, fish oils, n-3 polyunsaturated fatty acids and cardiovascular disease. Summary of evidence. . Australia.

Connor, W. E., Lowensohn, R., & Hatcher, L. (1996). Increased docosahexaenoic acid levels in human newborn infants by administration of sardines and fish oil during pregnancy. *Lipids, 31*(3 SUPPL.), S-183-S-187.

Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., . . . Brand-Miller, J. (2005). Origins and evolution of the Western diet: health implications for the 21st century. *American Journal of Clinical Nutrition, 81*(2), 341-354.

Correia, H. R., Balseiro, S. C., Correia, E. R., Mota, P. G., & de Areia, M. L. (2004). Why are human newborns so fat? Relationship between fatness and brain size at birth (Retracted Article. See vol 16, pg 111, 2004). *American Journal of Human Biology, 16*(1), 24-30. doi: 10.1002/ajhb.10233

Cosatto, V. F., Else, P. L., & Meyer, B. J. (2010). Do pregnant women and those at risk of developing post-natal depression consume lower amounts of long chain omega-3 polyunsaturated fatty acids? *Nutrients, 2*(2), 198-213. doi: 10.3390/nu2020198

Costa, L. G. (2007). Contaminants in fish: Risk-benefit considerations. *Arhiv za Higijenu Rada i Toksikologiju, 58*(3), 367-374. doi: 10.2478/v10004-007-0025-3

Crawford, M. (2000). Placental delivery of arachidonic and docosahexaenoic acids: implications for the lipid nutrition of preterm infants. *The American Journal of Clinical Nutrition, 71*(1), 275S-284S.

Crozier, S. R., Robinson, S. M., Godfrey, K. M., Cooper, C., & Inskip, H. M. (2009). Women's dietary patterns change little from before to during pregnancy. *Journal of Nutrition, 139*(10), 1956-1963. doi: 10.3945/jn.109.109579

Cunnane, S. C. (2000). The conditional nature of the dietary need for polyunsaturates: a proposal to reclassify 'essential fatty acids' as 'conditionally-indispensable' or conditionally-dispensable' fatty acids. *British Journal of Nutrition, 84*(6), 803-812.

D'Vaz, N. (2012). Effect of postnatal fish oil supplementation in high risk infants on immune function and allergy development: a randomized controlled trial. *Sort, 50*(100), 500.

Daley, C., Patterson, A., Sibbritt, D., & MacDonald-Wicks, L. (2015). Unsaturated fat intakes and mental health outcomes in young women from the Australian Longitudinal Study on Women's Heath. *Public Health Nutr, 18*(3), 546-553. doi: 10.1017/s1368980014000561

Daniels, J. L., Longnecker, M. P., Rowland, A. S., Golding, J., & Team, A. S. (2004). Fish intake during pregnancy and early cognitive development of offspring. *Epidemiology, 15*(4), 394-402.

Darmon, N., & Drewnowski, A. (2008). Does social class predict diet quality? *The American journal of clinical nutrition, 87*(5), 1107-1117.

Davidson, M. H. (2006). Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids. *American Journal of Cardiology, 98*(4A), 27I-33I. doi: 10.1016/j.amjcard.2005.12.024

Davidson, P. W., Cory-Slechta, D. A., Thurston, S. W., Huang, L.-S., Shamlaye, C. F., Gunzler, D., . . . Myers, G. J. (2011). Fish consumption and prenatal methylmercury exposure: Cognitive and behavioral outcomes in the main cohort at 17 years from the Seychelles child development study. *Neurotoxicology, 32*(6), 711-717. doi: 10.1016/j.neuro.2011.08.003

Davidson, P. W., Strain, J. J., Myers, G. J., Thurston, S. W., Bonham, M. P., Shamlaye, C. F., . . . Clarkson, T. W. (2008). Neurodevelopmental effects of maternal nutritional status and exposure to methylmercury from eating fish during pregnancy. *NeuroToxicology, 29*(5), 767-775. doi: http://dx.doi.org/10.1016/j.neuro.2008.06.001

De Vriese, S. R., De Henauw, S., De Backer, G., Dhont, M., & Christophe, A. B. (2001). Estimation of dietary fat intake of Belgian pregnant women - Comparison of two methods. *Annals of Nutrition and Metabolism, 45*(6), 273-278. doi: 10.1159/000046738

De Vriese, S. R., Matthys, C., De Henauw, S., De Backer, G., Dhont, M., & Christophe, A. B. (2002). Maternal and umbilical fatty acid status in relation to maternal diet. *Prostaglandins Leukotrienes and Essential Fatty Acids, 67*(6), 389-396. doi: 10.1054/plef.2002.0446

Deckelbaum, R. J., Worgall, T. S., & Seo, T. (2006). n-3 fatty acids and gene expression. *American Journal of Clinical Nutrition, 83*(6), 1520S-1525S.

Demmelmair, H., von Rosen, J., & Koletzko, B. (2006). Long-term consequences of early nutrition. *Early human development, 82*(8), 567-574.

Denomme, J., Stark, K. D., & Holub, B. J. (2005). Directly quantitated dietary (n-3) fatty acid intakes of pregnant Canadian women are lower than current dietary recommendations. *Journal of Nutrition, 135*(2), 206-211.

Dewailly, É., Blanchet, C., Gingras, S., Lemieux, S., & Holub, B. (2003). Fish consumption and blood lipids in three ethinic groups of Québec (canada). *Lipids, 38*(4), 359-365. doi: 10.1007/s11745-003-1070-4

Dijck-Brouwer, D. A. J., Hadders-Algra, M., Bouwstra, H., Decsi, T., Boehm, G., Martini, I. A., . . . Muskiet, F. A. J. (2005). Lower fetal status of docosahexaenoic acid, arachidonic acid and essential fatty acids is associated with less favorable neonatal neurological

condition. *Prostaglandins Leukotrienes and Essential Fatty Acids, 72*(1), 21-28. doi: 10.1016/j.plefa.2004.08.002

Donahue, S. M. A., Rifas-Shiman, S. L., Olsen, S. F., Gold, D. R., Gillman, M. W., & Oken, E. (2009). Associations of maternal prenatal dietary intake of n-3 and n-6 fatty acids with maternal and umbilical cord blood levels. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 80*(5–6), 289-296.

Droulez, V., Williams, P., Levy, G., Stobaus, T., & Sinclair, A. (2006). Composition of Australian red meat 2002. 2. Fatty acid profile.

Dunstan, J. A., Mori, T. A., Barden, A., Beilin, L. J., Holt, P. G., Calder, P. C., . . . Prescott, S. L. (2004). Effects of n-3 polyunsaturated fatty acid supplementation in pregnancy on maternal and fetal erythrocyte fatty composition. *European Journal of Clinical Nutrition, 58*(3), 429-437. doi: 10.1038/sj.ejcn.1601825

Dunstan, J. A., Mori, T. A., Barden, A., Beilin, L. J., Taylor, A. L., Holt, P. G., & Prescott, S. L. (2003). Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. *Journal of Allergy and Clinical Immunology, 112*(6), 1178-1184.

Dunstan, J. A., Simmer, K., Dixon, G., & Prescott, S. (2008). Cognitive assessment of children at age 2½ years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. *Archives of Disease in Childhood-Fetal and Neonatal Edition, 93*(1), F45-F50.

Dutta-Roy, A. K. (2000). Transport mechanisms for long-chain polyunsaturated fatty acids in the human placenta. *American Journal of Clinical Nutrition, 71*(1), 315S-322S.

Dutta-Roy, A. K. (2009). Transport of fatty acids across the human placenta: A review. *Progress in Lipid Research, 48*(1), 52-61. doi: http://dx.doi.org/10.1016/j.plipres.2008.11.001

Elmadfa, I., & Kornsteiner, M. (2009a). Dietary Fat Intake - A Global Perspective. *Annals of Nutrition and Metabolism, 54*, 8-14. doi: 10.1159/000220822

Elmadfa, I., & Kornsteiner, M. (2009b). Fats and Fatty Acid Requirements for Adults. *Annals of Nutrition and Metabolism, 55*(1-3), 56-75. doi: 10.1159/000228996

Elvevoll, E. O., Barstad, H., Breimo, E. S., Brox, J., Eilertsen, K.-E., Lund, T., . . . Osterud, B. (2006). Enhanced incorporation of n-3 fatty acids from fish compared with fish oils. *Lipids, 41*(12), 1109-1114. doi: 10.1007/s11745-006-5060-3

Emmett, P. (2009). Assessing diet in longitudinal birth cohort studies. *Paediatric and Perinatal Epidemiology, 23*(SUPPL. 1), 154-173. doi: 10.1111/j.1365-3016.2009.01015.x

Emmett, R., Akkersdyk, S., Yeatman, H., & Meyer, B. J. (2013). Expanding awareness of docosahexaenoic acid during pregnancy. *Nutrients, 5*(4), 1098-1109.

Erkkola, M., Karppinen, M., Javanainen, J., Räsänen, L., Knip, M., & Virtanen, S. M. (2001). Validity and reproducibility of a food frequency questionnaire for pregnant finnish women. *American Journal of Epidemiology, 154*(5), 466-476. doi: 10.1093/aje/154.5.466

Ervin, R. B., Wright, J. D., Wang, C. Y., & Kennedy-Stephenson, J. (2004). Dietary intake of fats and fatty acids for the United States population: 1999-2000. *Advance data*(348), 1-6.

Escolano-Margarit, M. V., Ramos, R., Beyer, J., Csábi, G., Parrilla-Roure, M., Cruz, F., . . . Campoy, C. (2011). Prenatal DHA status and neurological outcome in children at age 5.5 years are positively associated. *Journal of Nutrition, 141*(6), 1216-1223. doi: 10.3945/jn.110.129635

European Food Safety Authority. (2009). *Labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids.* (No. EFSA J., 1176, 1-11.). EFSA.

European Food Safety Authority. (2010). *Panel on Dietetic Products Nutrition and Allergies: Scientific Opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol* (No. 1831-4732).

European Food Safety Authority. (2012). *EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA); Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food*. Italy. EFSA J, 10(12).

European Food Safety Authority. (2012). *Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA)*. Italy, EFSA J, 10(7), 2815.

Extier, A., Langelier, B., Perruchot, M.-H., Guesnet, P., van Veldhoven, P. P., Lavialle, M., & Alessandri, J.-M. (2010). Gender affects liver desaturase expression in a rat model of n-3 fatty acid repletion. *Journal of Nutritional Biochemistry, 21*(3), 180-187. doi: 10.1016/j.jnutbio.2008.10.008

Eyres, L. (2000a). *Changes in the lipid composition of New Zealand diets.* Paper presented at the Proceedings of the Nutrition Society of New Zealand, (Vol. 25, pp. 1-9). Nutrition Society of New Zealand (Inc).

Eyres, L. (2000b). Fats, Fatty Acids and Cholesterol. *The New Zealand Food Journal, 29*(4), 143-146.

Faupel-Badger, J. M., Hsieh, C. C., Troisi, R., Lagiou, P., & Potischman, N. (2007). Plasma volume expansion in pregnancy: Implications for biomarkers in population studies. *Cancer Epidemiology Biomarkers and Prevention, 16*(9), 1720-1723. doi: 10.1158/1055-9965.EPI-07-0311

Fawzi, W. W., Rifas-Shiman, S. L., Rich-Edwards, J. W., Willett, W. C., & Gillman, M. W. (2004). Calibration of a semi-quantitative food frequency questionnaire in early pregnancy. *Annals of Epidemiology, 14*(10), 754-762. doi: 10.1016/j.annepidem.2004.03.001

Feskens, E. J., Bowles, C. H., & Kromhout, D. (1991). Inverse association between fish intake and risk of glucose intolerance in normoglycemic elderly men and women. *Diabetes care, 14*(11), 935-941.

Field, A. (2009). *Discovering Statistics Using SPSS* (3rd ed.). London: SAGE.

Fitzsimon, N., Fallon, U., O'Mahony, D., Loftus, B. G., Bury, G., Murphy, A. W., & Kelleher, C. C. (2007). Mothers' dietary patterns during pregnancy and risk of asthma symptoms in children at 3 years. *Irish medical journal, 100*(8), suppl 27-32.

Flachs, P., Rossmeisl, M., & Kopecky, J. (2014). The effect of n-3 fatty acids on glucose homeostasis and insulin sensitivity. *Physiological Research, 63*, S93-S118.

Fleith, M., & Clandinin, M. (2005). Dietary PUFA for preterm and term infants: review of clinical studies. *Critical reviews in food science and nutrition, 45*(3), 205-229.

Flock, M. R., Harris, W. S., & Kris-Etherton, P. M. (2013). Long-chain omega-3 fatty acids: time to establish a dietary reference intake. *Nutrition Reviews, 71*(10), 692-707. doi: 10.1111/nure.12071

Food and Agriculture Organization of the United Nations and World Health Organization. (2010). *Joint Food Agriculture Organization/World Health Organization - Fats and fatty acids in human nutrition: Report of an expert consultation*. Geneva.

Food and Agriculture Organization of the United Nations and World Health Organization. (2011). *Joint Food Agriculture Organization/World*

Health Organization - Expert Consultation on the Risks and Benefits of Fish Consumption (No. 2070-6987). Rome, Italy.

Food Standards Australia and New Zealand. (2011). *Part 1.4 Contaminants and Residues Standard 141 Contaminants and Natuaral Toxicants* (pp. 3). Canberra: FSANZ.

Food Standards Australia and New Zealand (FSANZ). (2011). Mercury in Fish - Advice on Fish Consumption. 2014

Foodworks. (2009). Xyris Software. from High Gate Hill, Qld, Australia.

Fowke, J. H., Schlundt, D., Gong, Y., Jin, F., Shu, X. O., Wen, W., . . . Zheng, W. (2004). Impact of season of food frequency questionnaire administration on dietary reporting. *Annals of Epidemiology, 14*(10), 778-785. doi: 10.1016/j.annepidem.2004.02.002

Fowler, J. K., Evers, S. E., & Campbell, M. K. (2012). Inadequate dietary intakes: Among pregnant women. *Canadian Journal of Dietetic Practice and Research, 73*(2), 72-77. doi: 10.3148/73.2.2012.72

Franke, C., Verwied-Jorky, S., Campoy, C., Trak-Fellermeier, M., Decsi, T., Dolz, V., & Koletzko, B. (2008). Dietary intake of natural sources of docosahexaenoic acid and folate in pregnant women of three european cohorts. *Annals of Nutrition and Metabolism, 53*(3-4), 167-174. doi: 10.1159/000172978

Freemantle, E., Vandal, M., Tremblay-Mercier, J., Tremblay, S., Blachère, J.-C., Bégin, M. E., . . . Cunnane, S. C. (2006). Omega-3 fatty acids, energy substrates, and brain function during aging. *Prostaglandins, leukotrienes and essential fatty acids, 75(3)*, 213-220.

Freund-Levi, Y., Eriksdotter-Jonhagen, M., Cederholm, T., Basun, H., Faxen-Irving, G., Garlind, A., . . . Palmblad, J. (2006). Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study - A randomized double-blind trial. *Archives of Neurology, 63*(10), 1402-+. doi: 10.1001/archneur.63.10.1402

Friesen, R. W., & Innis, S. M. (2009). Dietary arachidonic acid to EPA and DHA balance is increased among Canadian pregnant women with low fish intake. *Journal of Nutrition, 139*(12), 2344-2350. doi: 10.3945/jn.109.112565

Friesen, R. W., & Innis, S. M. (2010). Linoleic acid is associated with lower long-chain n–6 and n–3 fatty acids in red blood cell lipids of Canadian pregnant women. *The American journal of clinical nutrition, 91*(1), 23-31.

Gale, C. R., Robinson, S. M., Godfrey, K. M., Law, C. M., Schlotz, W., & O'Callaghan, F. J. (2008). Oily fish intake during pregnancy -

Association with lower hyperactivity but not with higher full-scale IQ in offspring. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 49*(10), 1061-1068. doi: 10.1111/j.1469-7610.2008.01908.x

Garg, M. L., Wood, L. G., Singh, H., & Moughan, P. J. (2006). Means of delivering recommended levels of long chain n-3 polyunsaturated fatty acids in human diets. *Journal of Food Science, 71*(5), R66-R71. doi: 10.1111/j.1750-3841.2006.00033.x

Gauster, M., Hiden, U., Blaschitz, A., Frank, S., Lang, U., Alvino, G., . . . Wadsack, C. (2007). Dysregulation of placental endothelial lipase and lipoprotein lipase in intrauterine growth-restricted pregnancies. *Journal of Clinical Endocrinology & Metabolism, 92*(6), 2256-2263. doi: 10.1210/jc.2006-2403

Gauster, M., Hiden, U., van Poppel, M., Frank, S., Wadsack, C., Hauguel-de Mouzon, S., & Desoye, G. (2011). Dysregulation of placental endothelial lipase in obese women with gestational diabetes mellitus. *Diabetes, 60*(10), 2457-2464. doi: 10.2337/db10-1434

Gebauer, S. K., Psota, T. L., Harris, W. S., & Kris-Etherton, P. M. (2006). n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *American Journal of Clinical Nutrition, 83*(6), 1526S-1535S.

Genuis, S., & Schwalfenberg, G. (2006). Time for an oil check: the role of essential omega-3 fatty acids in maternal and pediatric health. *Journal of Perinatology, 26*(6), 359-365.

Georgieff, M. K. (2007). Nutrition and the developing brain: nutrient priorities and measurement. *The American journal of clinical nutrition, 85*(2), 614S-620S.

Germann, W. J., & Stanfield, C. L. (2005). *Principles of Human Physiology* (Pearson Education Inc. Ed. Second ed.). San Francisco, CA: Daryl Fox.

Gerrard, J., Popeski, D., Ebbeling, L., Brown, P., & Hornstra, G. (1990). Dietary omega 3 fatty acids and gestational hypertension in the Inuit. *Arctic medical research*, 763-767.

Gibbs, R. A., Rymer, C., & Givens, D. I. (2010). *Intakes in the UK and the potential of a chicken meat prototype to increase them.* Paper presented at the Postgraduate Symposium Long-chain n-3 PUFA, United Kingdon.

Gibson, R. (2005). *Principles of Nutritional Assessment* (2nd ed.). New York: Oxford University Press.

Giltay, E. J., Gooren, L. J. G., Toorians, A., Katan, M. B., & Zock, P. L. (2004). Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. *American Journal of Clinical Nutrition, 80*(5), 1167-1174.

Giuseppe, R. d., Roggi, C., & Cena, H. (2014). n-3 LC-PUFA supplementation: effects on infant and maternal outcomes. *European Journal of Nutrition, 53*(5), 1147-1154.

Givens, D. I., & Gibbs, R. A. (2006). Very long chain n-3 polyunsaturated fatty acids in the food chain in the UK and the potential of animal-derived foods to increase intake. *Nutrition Bulletin, 31*(2), 104-110. doi: 10.1111/j.1467-3010.2006.00554.x

Glatz, J. F. C., Luiken, J. J. F. P., & Bonen, A. (2010). Membrane fatty acid transporters as regulators of lipid metabolism: Implications for metabolic disease. *Physiological Reviews, 90*(1), 367-417. doi: 10.1152/physrev.00003.2009

Golding, J., Steer, C., Emmett, P., Davis, J. M., & Hibbeln, J. R. (2009). High levels of depressive symptoms in pregnancy with low omega-3 fatty acid intake from fish. *Epidemiology, 20*(4), 598-603.

Gould, J. F., Makrides, M., Colombo, J., & Smithers, L. G. (2014). Randomized controlled trial of maternal omega-3 long-chain PUFA supplementation during pregnancy and early childhood development of attention, working memory, and inhibitory control. *American Journal of Clinical Nutrition, 99*(4), 851-859. doi: 10.3945/ajcn.113.069203

Gould, J. F., Smithers, L. G., & Makrides, M. (2013). The effect of maternal omega-3 (n-3) LCPUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials. *American Journal of Clinical Nutrition, 97*(3), 531-544. doi: 10.3945/ajcn.112.045781

Green, J. T., Orr, S. K., & Bazinet, R. P. (2008). The emerging role of group VI calcium-independent phospholipase A(2) in releasing docosahexaenoic acid from brain phospholipids. *Journal of Lipid Research, 49*(5), 939-944. doi: 10.1194/jlr.R700017-JLR200

Grisaru-Granovsky, S., Samueloff, A., & Elstein, D. (2008). The role of leptin in fetal growth: A short review from conception to delivery. *European Journal of Obstetrics & Gynecology and Reproductive Biology, 136*(2), 146-150. doi: http://dx.doi.org/10.1016/j.ejogrb.2007.06.021

Guldner, L., Monfort, C., Rouget, F., Garlantezec, R., & Cordier, S. (2007). Maternal fish and shellfish intake and pregnancy outcomes: a

prospective cohort study in Brittany, France. *Environ Health, 6*(33), 10.1186.

Hadders-Algra, M., Bouwstra, H., Van Goor, S. A., Dijck-Brouwer, D. A. J., & Muskiet, F. A. J. (2007). Prenatal and early postnatal fatty acid status and neurodevelopmental outcome. *Journal of Perinatal Medicine, 35*(SUPPL. 1), S28-S34. doi: 10.1515/JPM.2007.034

Haggarty, P. (2002). Placental regulation of fatty acid delivery and its effect on fetal growth—a review. *Placenta, 23*, S28-S38.

Haggarty, P. (2004). Effect of placental function on fatty acid requirements during pregnancy. *European Journal of Clinical Nutrition, 58*(12), 1559-1570. doi: 10.1038/sj.ejcn.1602016

Haggarty, P., Ashton, J., Joynson, M., Abramovich, D. R., & Page, K. (1999). Effect of maternal polyunsaturated fatty acid concentration on transport by the human placenta. *Biology of the Neonate, 75*(6), 350-359. doi: 10.1159/000014115

Halldorsson, T. I., Meltzer, H. M., Thorsdottir, I., Knudsen, V., & Olsen, S. F. (2007). Is high consumption of fatty fish during pregnancy a risk factor for fetal growth retardation? A study of 44,824 Danish pregnant women. *American Journal of Epidemiology, 166*(6), 687-696. doi: 10.1093/aje/kwm133

Hanebutt, F. L., Demmelmair, H., Schiessl, B., Larqué, E., & Koletzko, B. (2008). Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta. *Clinical Nutrition, 27*(5), 685-693.

Harris, W. S., Varvel, S. A., Pottala, J. V., Warnick, G. R., & McConnell, J. P. (2013). Comparative effects of an acute dose of fish oil on omega-3 fatty acid levels in red blood cells versus plasma: Implications for clinical utility. *Journal of Clinical Lipidology, 7*(5), 433-440. doi: http://dx.doi.org/10.1016/j.jacl.2013.05.001

Hautero, U., Laakso, P., Linderborg, K., Niinivirta, K., Poussa, T., Isolauri, E., & Laitinen, K. (2013). Proportions and concentrations of serum n-3 fatty acids can be increased by dietary counseling during pregnancy. *European Journal of Clinical Nutrition, 67*(11), 1163-1168. doi: 10.1038/ejcn.2013.169

Heird, W. C., & Lapillonne, A. (2005). The role of essential fatty acids in development. *Annu. Rev. Nutr., 25*, 549-571.

Helland, I. B., Saugstad, O. D., Smith, L., Saarem, K., Solvoll, K., Ganes, T., & Drevon, C. A. (2001). Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women. *Pediatrics, 108*(5), e82. doi: 10.1542/peds.108.5.e82

Heppe, D. H. M., Steegers, E. A. P., Timmermans, S., den Breeijen, H., Tiemeier, H., Hofman, A., & Jaddoe, V. W. V. (2011). Maternal fish consumption, fetal growth and the risks of neonatal complications: the Generation R Study. *British Journal of Nutrition, 105*(6), 938-949. doi: 10.1017/s0007114510004460

Herrera, E. (2002). Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal development—a review. *Placenta, 23*, S9-S19.

Herrera, E., & Amusquivar, E. (2012). Polyunsaturated fatty acids and their dietary implications during pregnancy *Fish Oil: Production, Consumption and Health Benefits* (pp. 73-106).

Herrera, E., & Ortega-Senovilla, H. (2014). Lipid metabolism during pregnancy and its implications for fetal growth. *Current Pharmaceutical Biotechnology, 15*(1), 24-31.

Hibbeln, J. R. (2002). Seafood consumption, the DHA content of mothers' milk and prevalence rates of postpartum depression: a cross-national, ecological analysis. *Journal of Affective Disorders, 69*(1-3), 15-29. doi: 10.1016/s0165-0327(01)00374-3

Hibbeln, J. R., Davis, J., Heron, J., Evans, J., Wolke, D., Golding, J., & Team, A. S. (2003). *Low dietary omega-3s and increased depression risk in 14,541 pregnancies.* Paper presented at the American Psychiatric Association Annual Meeting.

Hibbeln, J. R., Davis, J. M., Steer, C., Emmett, P., Rogers, I., Williams, C., & Golding, J. (2007). Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *The Lancet, 369*(9561), 578-585. doi: http://dx.doi.org/10.1016/S0140-6736(07)60277-3

Hibbeln, J. R., Nieminen, L. R., Blasbalg, T. L., Riggs, J. A., & Lands, W. E. (2006). Healthy intakes of n− 3 and n− 6 fatty acids: estimations considering worldwide diversity. *The American journal of clinical nutrition, 83*(6), S1483-1493S.

Högström, M., Nordström, P., & Nordström, A. (2007). n-3 fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: The NO2 study. *American Journal of Clinical Nutrition, 85*(3), 803-807.

Holman, R. (1992). *A long scaly tale: The study of essential fatty acid deficiency at the University of Minnesota.* Paper presented at the Third international congress in essential fatty acids and eicasanoids.

Holman, R. T. (1998). The slow discovery of the importance of ω3 essential fatty acids in human health. *The journal of nutrition, 128*(2), 427S-433S.

Hornstra, G. (2000). Essential fatty acids in mothers and their neonates. *The American journal of clinical nutrition, 71*(5), 1262s-1269s.

Horvath, A., Koletzko, B., & Szajewska, H. (2007). Effect of supplementation of women in high-risk pregnancies with long-chain polyunsaturated fatty acids on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *British Journal of Nutrition, 98*(02), 253-259.

Howe, P., Buckley, J., & Meyer, B. (2007). Long-chain omega-3 fatty acids in red meat. *Nutrition and Dietetics, 64*(SUPPL. 4), S135-S139. doi: 10.1111/j.1747-0080.2007.00201.x

Howe, P., Meyer, B., Record, S., & Baghurst, K. (2006). Dietary intake of long-chain ω-3 polyunsaturated fatty acids: contribution of meat sources. *Nutrition, 22*(1), 47-53. doi: http://dx.doi.org/10.1016/j.nut.2005.05.009

Huiskes, V. J. B., Kuipers, R. S., Velzing-Aarts, F. V., Dijck-Brouwer, D. A. J., van der Meulen, J., & Muskiet, F. A. J. (2009). Higher de novo synthesized fatty acids and lower omega 3-and omega 6-long-chain polyunsaturated fatty acids in umbilical vessels of women with preeclampsia and high fish intakes. *Prostaglandins Leukotrienes and Essential Fatty Acids, 80*(2-3), 101-106. doi: 10.1016/j.plefa.2008.11.003

Hure, A., Young, A., Smith, R., & Collins, C. (2009). Diet and pregnancy status in Australian women. *Public Health Nutrition, 12*(6), 853-861. doi: 10.1017/S1368980008003212

Imhoff-Kunsch, B., Briggs, V., Goldenberg, T., & Ramakrishnan, U. (2012). Effect of n-3 long-chain polyunsaturated fatty acid intake during pregnancy on maternal, infant, and child health outcomes: A systematic review. *Paediatric and perinatal epidemiology, 26*(s1), 91-107.

Ingram, M. A., Stonehouse, W., Russell, K. G., Meyer, B., & Kruger, R. (2012). The new zealand pufa semiquantitative food frequency questionnaire is a valid and reliable tool to assess pufa intakes in healthy new zealand adults. *Journal of Nutrition, 142*(11), 1968-1974. doi: 10.3945/jn.112.162313

Innis, S. M. (2003). Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. *The Journal of Pediatrics, 143*(4, Supplement), 1-8. doi: http://dx.doi.org/10.1067/S0022-3476(03)00396-2

Innis, S. M. (2005). Essential fatty acid transfer and fetal development. *Placenta, 26, Supplement*(0), S70-S75. doi: http://dx.doi.org/10.1016/j.placenta.2005.01.005

Innis, S. M. (2007a). Dietary (n-3) fatty acids and brain development. *Journal of Nutrition, 137*(4), 855-859.

Innis, S. M. (2007b). Fatty acids and early human development. *Early human development, 83*(12), 761-766.

Innis, S. M., & Elias, S. L. (2003). Intakes of essential n-6 and n-3 polyunsaturated fatty acids among pregnant Canadian women. *American Journal of Clinical Nutrition, 77*(2), 473-478.

Innis, S. M., & Friesen, R. W. (2008). Essential n-3 fatty acids in pregnant women and early visual acuity maturation in term infants. *American Journal of Clinical Nutrition, 87*(3), 548-557.

Innis, S. M., & Uauy, R. (2003). Mechanisms of action of LCPUFA effects on infant growth and neurodevelopment: perinatal biochemistry and physiology of LCPUFA discussion. *The Journal of Pediatrics, 143*(4, Supplement), 96-109. doi: http://dx.doi.org/10.1067/S0022-3476(03)00407-4

IOM. (2005). *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids (Macronutrients).* Washington: National Academies Press.

Iso, H., Kobayashi, M., Ishihara, J., Sasaki, S., Okada, K., Kita, Y., . . . Tsugane, S. (2006). Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: The Japan Public Health Center-Based (JPHC) study cohort I. *Circulation, 113*(2), 195-202. doi: 10.1161/CIRCULATIONAHA.105.581355

Jacobson, J. L., Jacobson, S. W., Muckle, G., Kaplan-Estrin, M., Ayotte, P., & Dewailly, E. (2008). Beneficial effects of a polyunsaturated fatty acid on infant development: evidence from the Inuit of Arctic Quebec. *The Journal of pediatrics, 152*(3), 356-364. e351.

Jacques, C., Levy, E., Muckle, G., Jacobson, S. W., Bastien, C., Dewailly, É., . . . Saint-Amour, D. (2011). Long-term effects of prenatal omega-3 fatty acid intake on visual function in school-age children. *The Journal of Pediatrics, 158*(1), 83-90.e81. doi: http://dx.doi.org/10.1016/j.jpeds.2010.06.056

Jans, L. A. W., Giltay, E. J., & Willem Van Der Does, A. J. (2010). The efficacy of n-3 fatty acids DHA and EPA (fish oil) for perinatal depression. *British Journal of Nutrition, 104*(11), 1577-1585.

Janssen, C. I. F., & Kiliaan, A. J. (2014). Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: The influence of LCPUFA on neural development, aging, and neurodegeneration.

Progress in Lipid Research, 53(0), 1-17. doi: http://dx.doi.org/10.1016/j.plipres.2013.10.002

Jensen, C. L. (2006). Effects of n-3 fatty acids during pregnancy and lactation. *American Journal of Clinical Nutrition, 83*(6), 1452S-1457S.

Jia, X., Pakseresht, M., Wattar, N., Wildgrube, J., Sontag, S., Andrews, M., . . . Field, C. J. (2015). Women who take n-3 long-chain polyunsaturated fatty acid supplements during pregnancy and lactation meet the recommended intake. *Applied Physiology, Nutrition, and Metabolism, 40*(999), 1-8.

Joffre, C., Nadjar, A., Lebbadi, M., Calon, F., & Laye, S. (2014). n-3 LCPUFA improves cognition: The young, the old and the sick. *Prostaglandins, leukotrienes, and essential fatty acids, 91*(1-2), 1-20. doi: 10.1016/j.plefa.2014.05.001

Jordan, R. G. (2010). Prenatal Omega-3 Fatty Acids: Review and Recommendations. *Journal of Midwifery & Womens Health, 55*(6), 520-528. doi: 10.1016/j.jmwh.2010.02.018

Judge, M. P., Harel, O., & Lammi-Keefe, C. J. (2007). Maternal consumption of a docosahexaenoic acid-containing functional food during pregnancy: benefit for infant performance on problem-solving but not on recognition memory tasks at age 9 mo. *American Journal of Clinical Nutrition, 85*(6), 1572-1577.

Kaplan, B. J., Giesbrecht, G. F., Leung, B. M. Y., Field, C. J., Dewey, D., Bell, R. C., . . . Martin, J. W. (2014). The Alberta Pregnancy Outcomes and Nutrition (APrON) cohort study: Rationale and methods. *Maternal and Child Nutrition, 10*(1), 44-60. doi: 10.1111/j.1740-8709.2012.00433.x

Karr, J. E., Alexander, J. E., & Winningham, R. G. (2011). Omega-3 polyunsaturated fatty acids and cognition throughout the lifespan: a review. *Nutritional neuroscience, 14*(5), 216-225.

Kartikasari, L., Hughes, R., Geier, M., Makrides, M., & Gibson, R. (2010). *Diets high in linoleic acid reduce omega-3 long chain polyunsaturated fatty acids in chicken tissues.* Paper presented at the 21st Annual Australian Poultry Science Sumposium, Sydney, New South Wales, 2010.

Kaur, G., Cameron-Smith, D., Garg, M., & Sinclair, A. J. (2011). Docosapentaenoic acid (22: 5n-3): a review of its biological effects. *Progress in lipid research, 50*(1), 28-34.

Keelan, J. A., Blumenstein, M., Helliwell, R. J. A., Sato, T. A., Marvin, K. W., & Mitchell, M. D. (2003). Cytokines, prostaglandins and

parturition - A review. *Placenta, 24*, S33-S46. doi: 10.1053/plac.2002.0948

Kidd, P. M. (2007). Omega-3 DHA and EPA for cognition, behavior, and mood: Clinical findings and structural-functional synergies with cell membrane phospholipids. *Alternative Medicine Review, 12*(3), 207-227.

Kind, K. L., Moore, V. M., & Davies, M. J. (2006). Diet around conception and during pregnancy – effects on fetal and neonatal outcomes. *Reproductive BioMedicine Online, 12*(5), 532-541. doi: http://dx.doi.org/10.1016/S1472-6483(10)61178-9

Kirwan, J. P., Hauguel-De Mouzon, S., Lepercq, J., Challier, J.-C., Huston-Presley, L., Friedman, J. E., . . . Catalano, P. M. (2002). Tnf-α is a predictor of insulin resistance in human pregnancy. *Diabetes, 51*(7), 2207-2213. doi: 10.2337/diabetes.51.7.2207

Klemens, C. M., Berman, D. R., & Mozurkewich, E. L. (2011). The effect of perinatal omega-3 fatty acid supplementation on inflammatory markers and allergic diseases: a systematic review. *Bjog-an International Journal of Obstetrics and Gynaecology, 118*(8), 916-925. doi: 10.1111/j.1471-0528.2010.02846.x

Klemens, C. M., Salari, K., & Mozurkewich, E. L. (2012). Assessing omega-3 fatty acid supplementation during pregnancy and lactation to optimize maternal mental health and childhood cognitive development. *Clinical Lipidology, 7*(1), 93-109. doi: 10.2217/clp.12.1

Klingler, M., Demmelmair, H., Larque, E., & Koletzko, B. (2003). Analysis of FA contents in individual lipid fractions from human placental tissue. *Lipids, 38*(5), 561-566. doi: 10.1007/s11745-003-1496-8

Kołakowska, A., Domiszewski, Z., & Bienkiewicz, G. (2006). Effects of biological and technological factors on the utility of fish as a source of n-3 PUFA. *Omega 3 Fatty Acid Research*, 83.

Koletzko, B., Cetin, I., & Brenna, J. T. (2007a). Dietary fat intakes for pregnant and lactating women. *British Journal of Nutrition, 98*(5), 873-877. doi: 10.1017/s0007114507764747

Koletzko, B., Larque, E., & Demmelmair, H. (2007b). Placental transfer of long-chain polyunsaturated fatty acids (LC-PUFA). *Journal of perinatal medicine, 35*(S1), S5-S11.

Koletzko, B., Lien, E., Agostoni, C., Boehles, H., Campoy, C., Cetin, I., . . . Uauy, R. (2008). The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *Journal of Perinatal Medicine, 36*(1), 5-14. doi: 10.1515/jpm.2008.001

Kornsteiner, M., Singer, I., & Elmadfa, I. (2008). Very low n-3 long-chain polyunsaturated fatty acid status in Austrian vegetarians and vegans. *Annals of Nutrition and Metabolism, 52*(1), 37-47. doi: 10.1159/000118629

Krauss-Etschmann, S., Shadid, R., Campoy, C., Hoster, E., Demmelmair, H., Jimenez, M., . . . Nutrition Health Lifestyle Study, G. (2007). Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. *American Journal of Clinical Nutrition, 85*(5), 1392-1400.

Kremmyda, L.-S., Vlachava, M., Noakes, P. S., Diaper, N. D., Miles, E. A., & Calder, P. C. (2011). Atopy risk in infants and children in relation to early exposure to fish, oily fish, or long-chain omega-3 fatty acids: a systematic review. *Clinical reviews in allergy & immunology, 41*(1), 36-66.

Kris-Etherton, P. M., Grieger, J. A., & Etherton, T. D. (2009). Dietary reference intakes for DHA and EPA. *Prostaglandins Leukotrienes and Essential Fatty Acids, 81*(2-3), 99-104. doi: 10.1016/j.plefa.2009.05.011

Kris-Etherton, P. M., Innis, S., & Ammerican, D. A. (2007). Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. *Journal of the American Dietetic Association, 107*(9), 1599-1611.

Kuipers, R. S., Luxwolda, M. F., Offringa, P. J., Rudi Boersma, E., Dijck-Brouwer, D. A. J., & Muskiet, F. A. J. (2012). Fetal intrauterine whole body linoleic, arachidonic and docosahexaenoic acid contents and accretion rates. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 86*(1–2), 13-20. doi: http://dx.doi.org/10.1016/j.plefa.2011.10.012

Kuriki, K., Nagaya, T., Tokudome, Y., Imaeda, N., Fujiwara, N., Sato, J., . . . Tokudome, S. (2003). Plasma concentrations of (n-3) highly unsaturated fatty acids are good biomarkers of relative dietary fatty acid intakes: A cross-sectional study. *Journal of Nutrition, 133*(11), 3643-3650.

Lain, K. Y., & Catalano, P. M. (2007). Metabolic changes in pregnancy. *Clinical Obstetrics and Gynecology, 50*(4), 938-948.

Lakin, V., Haggarty, P., Abramovich, D. R., Ashton, J., Moffat, C. F., McNeill, G., . . . Grubb, D. (1998). Dietary intake and tissue concentration of fatty acids in omnivore, vegetarian and diabetic pregnancy. *Prostaglandins Leukotrienes and Essential Fatty Acids, 59*(3), 209-220. doi: 10.1016/S0952-3278(98)90065-5

Landon, M. B., Mele, L., Spong, C. Y., Carpenter, M. W., Ramin, S. M., Casey, B., . . . Thorp Jr, J. M. (2011). The relationship between maternal glycemia and perinatal outcome. *Obstetrics and gynecology, 117*(2 0 1), 218.

Lappas, M., Hiden, U., Desoye, G., Froehlich, J., Mouzon, S. H.-d., & Jawerbaum, A. (2011). The role of oxidative stress in the pathophysiology of gestational diabetes mellitus. *Antioxidants & redox signaling, 15*(12), 3061-3100.

Lardinois, C. K., & Starich, G. H. (1991). Polyunsaturated fats enhance peripheral glucose utilization in rats. *Journal of the American College of Nutrition, 10*(4), 340-345.

Larqué, E., Gil-Sánchez, A., Prieto-Sánchez, M. T., & Koletzko, B. (2012). Omega 3 fatty acids, gestation and pregnancy outcomes. *British Journal of Nutrition, 107*(S2), S77-S84.

Larsen, M. K., Nielsen, J. H., Butler, G., Leifert, C., Slots, T., Kristiansen, G. H., & Gustafsson, A. H. (2010). Milk quality as affected by feeding regimens in a country with climatic variation. *Journal of Dairy Science, 93*(7), 2863-2873. doi: 10.3168/jds.2009-2953

Lattka, E., Illig, T., Koletzko, B., & Heinrich, J. (2010). Genetic variants of the FADS1 FADS2 gene cluster as related to essential fatty acid metabolism. *Current opinion in lipidology, 21*(1), 64-69.

Lattka, E., Koletzko, B., Zeilinger, S., Hibbeln, J. R., Klopp, N., Ring, S. M., & Steer, C. D. (2013). Umbilical cord PUFA are determined by maternal and child fatty acid desaturase (FADS) genetic variants in the Avon Longitudinal Study of Parents and Children (ALSPAC). *British Journal of Nutrition, 109*(07), 1196-1210.

Lauritzen, L., Hansen, H. S., Jorgensen, M. H., & Michaelsen, K. F. (2001). The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Progress in Lipid Research, 40*(1-2), 1-94. doi: 10.1016/S0163-7827(00)00017-5

Lawrence, J. M., Contreras, R., Chen, W., & Sacks, D. A. (2008). Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005. *Diabetes care, 31*(5), 899-904.

Le, H. D., Meisel, J. A., de Meijer, V. E., Gura, K. M., & Puder, M. (2009). The essentiality of arachidonic acid and docosahexaenoic acid. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 81*(2–3), 165-170. doi: http://dx.doi.org/10.1016/j.plefa.2009.05.020

Leddy, M. A., Power, M. L., & Schulkin, J. (2008). The impact of maternal obesity on maternal and fetal health. *Reviews in Obstetrics and Gynecology, 1*(4), 170.

Lee, & Nieman, D. C. (2010). *Measuring Diet*. In McGraw-Hill (Ed.), *Nutritional Assessment* (5th ed., pp. 68-105). New York.

Levant, B., Ozias, M. K., & Carlson, S. E. (2007). Specific brain regions of female rats are differentially depleted of docosahexaenoic acid by reproductive activity and an (n-3) fatty acid-deficient diet. *The Journal of nutrition, 137*(1), 130-134.

Levant, B., Ozias, M. K., Davis, P. F., Winter, M., Russell, K. L., Carlson, S. E., . . . McCarson, K. E. (2008). Decreased brain docosahexaenoic acid content produces neurobiological effects associated with depression: interactions with reproductive status in female rats. *Psychoneuroendocrinology, 33*(9), 1279-1292.

Lewin, G. A., Schachter, H. M., Yuen, D., Merchant, P., Mamaladze, V., & Tsertsvadze, A. (2005). Effects of omega-3 fatty acids on child and maternal health. *Evidence report/technology assessment (Summary)*(118), 1-11.

Lim, W.-Y., Chong, M., Calder, P. C., Kwek, K., Chong, Y.-S., Gluckman, P. D., . . . Group, G. S. (2015). Relations of plasma polyunsaturated Fatty acids with blood pressures during the 26th and 28th week of gestation in women of Chinese, Malay, and Indian ethnicity. *Medicine, 94*(9), e571.

Lindegaard, M. L. S., Damm, P., Mathiesen, E. R., & Nielsen, L. B. (2006). Placental triglyceride accumulation in maternal type 1 diabetes is associated with increased lipase gene expression. *Journal of Lipid Research, 47*(11), 2581-2588. doi: 10.1194/jlr.M600236-JLR200

Linseisen, J., Schulze, M. B., Saadatian-Elahi, M., Kroke, A., Miller, A. B., & Boeing, H. (2003). Quantity and quality of dietary fat, carbohydrate, and fiber intake in the German EPIC cohorts. *Annals of Nutrition and Metabolism, 47*(1), 37-46. doi: 10.1159/000068911

Liu, J. J., Green, P., John Mann, J., Rapoport, S. I., & Sublette, M. E. (2015). Pathways of polyunsaturated fatty acid utilization: Implications for brain function in neuropsychiatric health and disease. *Brain Research, 1597*(0), 220-246.

Livingstone, M. B. E., & Black, A. E. (2003). Markers of the validity of reported energy intake. *Journal of Nutrition, 133*(3 SUPPL.), 895S-920S.

Loosemore, E. D., Judge, M. P., & Lammi-Keefe, C. J. (2004). Dietary intake of essential and long-chain polyunsaturated fatty acids in pregnancy. *Lipids, 39*(5), 421-424. doi: 10.1007/s11745-004-1246-y

Lopez-Garcia, E., Schulze, M. B., Manson, J. A. E., Meigs, J. B., Albert, C. M., Rifai, N., . . . Hu, F. B. (2004). Consumption of (n-3) fatty acids

is related to plasma biomarkers of inflammation and endothelial activation in women. *Journal of Nutrition, 134*(7), 1806-1811.

Love, J. L., Rush, G. M., & McGrath, H. (2003). Total mercury and methylmercury levels in some New Zealand commercial marine fish species. *Food Additives & Contaminants, 20*(1), 37-43. doi: 10.1080/0265203021000019676

Lukiw, W. J., & Bazan, N. G. (2008). Docosahexaenoic acid and the aging brain. *The Journal of nutrition, 138*(12), 2510-2514. doi: 10.3945/jn.108.096016

Lund, E. K. (2013). Health benefits of seafood: Is it just the fatty acids? *Food Chemistry, 140*(3), 413-420. doi: 10.1016/j.foodchem.2013.01.034

Lyu, L. C., Hsu, Y. N., Chen, H. F., Lo, C. C., & Lin, C. L. (2014). Comparisons of four dietary assessment methods during pregnancy in Taiwanese women. *Taiwanese Journal of Obstetrics and Gynecology, 53*(2), 162-169. doi: 10.1016/j.tjog.2014.04.007

Magnusson, A. L., Waterman, I. J., Wennergren, M., Jansson, T., & Powell, T. L. (2004). Triglyceride hydrolase activities and expression of fatty acid binding proteins in the human placenta in pregnancies complicated by intrauterine growth restriction and diabetes. *Journal of Clinical Endocrinology & Metabolism, 89*(9), 4607-4614. doi: 10.1210/jc.2003-032234

Mahaffey, K. R., Sunderland, E. M., Chan, H. M., Choi, A. L., Grandjean, P., Mariën, K., . . . Yasutake, A. (2011). Balancing the benefits of n-3 polyunsaturated fatty acids and the risks of methylmercury exposure from fish consumption. *Nutrition reviews, 69*(9), 493-508. doi: 10.1111/j.1753-4887.2011.00415.x

Makrides, M. (2008). Outcomes for Mothers and Their Babies: Do n-3 Long-Chain Polyunsaturated Fatty Acids and Seafoods Make a Difference? *Journal of the American Dietetic Association, 108*(10), 1622-1626. doi: http://dx.doi.org/10.1016/j.jada.2008.07.003

Makrides, M. (2009a). Is there a dietary requirement for DHA in pregnancy? *Prostaglandins, Leukotrienes and Essential Fatty Acids, 81*(2–3), 171-174. doi: http://dx.doi.org/10.1016/j.plefa.2009.05.005

Makrides, M. (2009b). *Long chain omega-3 polyunsaturated fatty acids and maternal health (during and after pregnancy), In:*. Paper presented at the Scientific Consensus Workshop, Sydney - Australia. www.omega-3centre.com

Makrides, M., Crowther, C., Gibson, R., Gibson, R., & Skeaff, C. (2002). Docosahexaenoic acid and post-partum depression-is there a link? *Asia Pacific journal of clinical nutrition, 12*, S37-S37.

Makrides, M., Duley, L., & Olsen, S. F. (2006). Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction. *Cochrane database of systematic reviews (Online), 3*.

Makrides, M., & Gibson, R. A. (2000). Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation. *The American journal of clinical nutrition, 71*(1), 307S-311S.

Makrides, M., & Gibson, R. A. (2007). Marine oil supplements for pregnant women good for mum, good for baby? *NeoReviews, 8*(4), e152-e158.

Makrides, M., Gibson, R. A., McPhee, A. J., Yelland, L., Quinlivan, J., Ryan, P., & Team, D. O. I. (2010a). Effect of dha supplementation during pregnancy on maternal depression and neurodevelopment of young children a randomized controlled trial. *Jama-Journal of the American Medical Association, 304*(15), 1675-1683. doi: 10.1001/jama.2010.1507

Makrides, M., Smithers, L. G., & Gibson, R. A. (2010b). Role of long-chain polyunsaturated fatty acids in neurodevelopment and growth. In A. Lucas, M. Makrides, & E. E. Ziegler (Eds.), *Importance of Growth for Health and Development* (Vol. 65, pp. 123-136).

Malcolm, C. A., McCulloch, D. L., Montgomery, C., Shepherd, A., & Weaver, L. T. (2003). Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: A double blind, prospective, randomised trial. *Archives of Disease in Childhood: Fetal and Neonatal Edition, 88*(5), F383-F390.

Malde, M., Alvheim, A., Brunborg, L., & Graff, I. (2012). Maternal seafood concumption in highly educated women is reduced in pregnancy: A pilot study. *International Journal of Nursing and Midwifery, 4*(4).

Malek, L., Umberger, W., Makrides, M., & Zhou, S. J. (2015). Adherence to the Australian dietary guidelines during pregnancy: evidence from a national study. *Public Health Nutrition*. doi: 10.1017/S1368980015002232

Mann, J., & Truswell, S. (2012). *Essentials of human nutrition* (4th ed.): Oxford University Press.

Mann, N. J., O'Connell, S. L., Baldwin, K. M., Singh, I., & Meyer, B. J. (2010). Effects of seal oil and tuna-fish oil on platelet parameters and plasma lipid levels in healthy subjects. *Lipids, 45*(8), 669-681.

Marangoni, F., Colombo, C., De Angelis, L., Gambaro, V., Agostoni, C., Giovannini, M., & Galli, C. (2004). Cigarette smoke negatively and dose-dependently affects the biosynthetic pathway of the n-3 polyunsaturated fatty acid series in human mammary epithelial cells. *Lipids, 39*(7), 633-637. doi: 10.1007/s11745-004-1276-5

Markhus, M. W., Graff, I. E., Dahl, L., Seldal, C. F., Skotheim, S., Braarud, H. C., . . . Malde, M. K. (2013). Establishment of a seafood index to assess the seafood consumption in pregnant women. *Food and Nutrition Research, 57*. doi: 10.3402/fnr.v57i0.19272

Mathers, N., Fox, N., & Hunn, A. (1998). Trent focus for research and development in primary health care: Using interviews in a research project. *Sheffield: Trent Focus Group*.

McCabe-Sellers, B. (2010). Advancing the art and science of dietary assessment through technology. *Journal of the American Dietetic Association, 110*(1), 52-54. doi: 10.1016/j.jada.2009.10.014

McCann, J. C., & Ames, B. N. (2005). Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *American Journal of Clinical Nutrition, 82*(2), 281-295.

McGregor, J. A., Allen, K. G., Harris, M. A., Reece, M., Wheeler, M., French, J. I., & Morrison, J. (2001). The omega-3 story:: Nutritional prevention of preterm birth and other adverse pregnancy outcomes. *Obstetrical & gynecological survey, 56*(5), S1-S13.

McKenzie-Parnell, J. M., Wilson, P. D., Parnell, W. R., Spears, G. F., & Robinson, M. F. (1993). Nutrient intake of Dunedin women during pregnancy. *New Zealand Medical Journal, 106*(959), 273-276.

McMichael, A. J., & Butler, C. D. (2005). Fish, health, and sustainability. *American Journal of Preventive Medicine, 29*(4), 322-323. doi: 10.1016/j.amepre.2005.07.033

McNamara, R. K., & Carlson, S. E. (2006). Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 75*(4), 329-349.

Mehendale, S., Kilari, A., Dangat, K., Taralekar, V., Mahadik, S., & Joshi, S. (2008). Fatty acids, antioxidants, and oxidative stress in pre-eclampsia. *International Journal of Gynecology & Obstetrics, 100*(3), 234-238. doi: 10.1016/j.ijgo.2007.08.011

Melchiorre, K., Sutherland, G. R., Liberati, M., & Thilaganathan, B. (2011). Preeclampsia is associated with persistent postpartum cardiovascular impairment. *Hypertension, 58*(4), 709-715.

Meldrum, S., Dunstan, J., Foster, J., Simmer, K., & Prescott, S. (2015). Maternal fish oil supplementation in pregnancy: A 12 year follow-up of a randomised controlled trial. *Nutrients, 7*(3), 2061-2067.

Mendez, M. A., Plana, E., Guxens, M., Morillo, C. M. F., Albareda, R. M., Garcia-Esteban, R., . . . Sunyer, J. (2010). Seafood consumption in pregnancy and infant size at birth: results from a prospective Spanish cohort. *Journal of epidemiology and community health, 64*(3), 216-222.

Mendez, M. A., Torrent, M., Julvez, J., Ribas-Fito, N., Kogevinas, M., & Sunyer, J. (2009). Maternal fish and other seafood intakes during pregnancy and child neurodevelopment at age 4 years. *Public Health Nutrition, 12*(10), 1702-1710. doi: 10.1017/s1368980008003947

Meyer, B. J. (2011). Are we consuming enough long chain omega-3 polyunsaturated fatty acids for optimal health? *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA), 85*(5), 275-280. doi: http://dx.doi.org/10.1016/j.plefa.2011.04.010

Meyer, B. J., Mann, N. J., Lewis, J. L., Milligan, G. C., Sinclair, A. J., & Howe, P. R. C. (2003). Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. *Lipids, 38*(4), 391-398. doi: 10.1007/s11745-003-1074-0

Meyer, B. J., Swierk, M., & Russell, K. G. (2013). Assessing long-chain omega-3 polyunsaturated fatty acids: A tailored food-frequency questionnaire is better. *Nutrition, 29*(3), 491-496. doi: 10.1016/j.nut.2012.04.002

Micha, R., Khatibzadeh, S., Shi, P., Fahimi, S., Lim, S., Andrews, K. G., . . . Mozaffarian, D. (2014). Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. *BMJ, 348*.

Michaelsen, K. F., Dewey, K. G., Perez-Exposito, A. B., Nurhasan, M., Lauritzen, L., & Roos, N. (2011). Food sources and intake of n-6 and n-3 fatty acids in low-income countries with emphasis on infants, young children (6–24 months), and pregnant and lactating women. *Maternal & child nutrition, 7*(s2), 124-140.

Miller, M. R., Pearce, L., & Bettjeman, B. I. (2014). Detailed distribution of lipids in greenshell (tm) mussel (perna canaliculus). *Nutrients, 6*(4), 1454-1474. doi: 10.3390/nu6041454

Min, Y., Ghebremeskel, K., Lowy, C., Thomas, B., & Crawford, M. A. (2004). Adverse effect of obesity on red cell membrane arachidonic and docosahexaenoic acids in gestational diabetes. *Diabetologia, 47*(1), 75-81. doi: 10.1007/s00125-003-1275-5

Ministry of Health. (1999). *NZ Food: NZ People: Key results of the 1997 National Nutrition Survey* Wellington: Ministry of Health.

Ministry of Health. (2006). *Food and nutrition guidelines for healthy pregnant and breastfeeding women: A background paper*. Wellington: Ministry of Health.

Ministry of Health. (2008a). *Eating for healthy pergnant women*. New Zealand: Ministry of Health.

Ministry of Health. (2008b). *Food and nutrition guidelines for healthy pregnant and breastfeeding women: A background paper*: Wellington: Ministry of Health.

Ministry of Health. (2015). *Report on maternity, 2012*. Wellington: Ministry of Health. Retrieved from http://www.health.govt.nz/publication/report-maternity-2012

Ministry of Primary Industries. (2011). *2009 New Zealand total diet study: Agricultural compound residues, selected contaminant and nutrient elements*. Wellington: MPI. Retrieved from http://foodsafety.govt.nz/elibrary/industry/total-diet-study.pdf

Ministry of Primary Industries. (2013). *Food safety in pregnancy*. Wellington: MPI. Retrieved from http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=3&ved=0CC8QFjAC&url=http%3A%2F%2Fwww.foodsmart.govt.nz%2Felibrary%2Fconsumer%2F2013-mpi-food-safety-in-pregnancy-web.pdf&ei=mjwrVfDIFsTfmgXkxIGIAw&usg=AFQjCNHjNf7uqxpWja86qLWvLO-RAMveXg&bvm=bv.90491159,d.dGY

Mitchell, E. A., Robinson, E., Clark, P. M., Becroft, D. M. O., Glovish, N., Pattison, N. S., . . . Wild, C. J. (2004). Maternal nutritional risk factors for small for gestational age babies in a developed country: A case-control study. *Archives of Disease in Childhood: Fetal and Neonatal Edition, 89*(5), F431-F435. doi: 10.1136/adc.2003.036970

Miyagawa, N., Miura, K., Okuda, N., Kadowaki, T., Takashima, N., Nagasawa, S. Y., . . . Ueshima, H. (2014). Long-chain n-3 polyunsaturated fatty acids intake and cardiovascular disease mortality risk in Japanese: A 24-year follow-up of NIPPON DATA80. *Atherosclerosis, 232*(2), 384-389. doi: 10.1016/j.atherosclerosis.2013.11.073

Miyake, Y., Sasaki, S., Tanaka, K., Ohya, Y., Miyamoto, S., Matsunaga, I., . . . Morimoto, Y. (2007). Fish and fat intake and prevalence of allergic rhinitis in Japanese females: The Osaka maternal and child health study. *Journal of the American College of Nutrition, 26*(3), 279-287.

Montgomery, C., Speake, B. K., Cameron, A., Sattar, N., & Weaver, L. T. (2003). Maternal docosahexaenoic acid supplementation and fetal accretion. *British Journal of Nutrition, 90*(1), 135-145. doi: 10.1079/bjn2003888

Morrison, M. K., Koh, D., Lowe, J. M., Miller, Y. D., Marshall, A. L., Colyvas, K., & Collins, C. E. (2012). Postpartum diet quality in Australian women following a gestational diabetes pregnancy. *European Journal of Clinical Nutrition, 66*(10), 1160-1165. doi: 10.1038/ejcn.2012.84

Morse, N. L. (2012). Benefits of docosahexaenoic acid, folic acid, vitamin D and iodine on foetal and infant brain development and function following maternal supplementation during pregnancy and lactation. *Nutrients, 4*(7), 799-840. doi: 10.3390/nu4070799

Morton, S. M. B., Grant, C. C., Wall, C. R., Carr, P. E. A., Bandara, D. K., Schmidt, J. M., . . . Camargo, C. A. (2014). Adherence to nutritional guidelines in pregnancy: evidence from the Growing Up in New Zealand birth cohort study. *Public health nutrition*, 1-11.

Mossaheb, N., Schloegelhofer, M., Schaefer, M. R., Fusar-Poli, P., Smesny, S., McGorry, P., . . . Amminger, G. P. (2012). Polyunsaturated fatty acids in emerging psychosis. *Current Pharmaceutical Design, 18*(4), 576-591. doi: 10.2174/138161212799316055

Mouratidou, T., Ford, F., & Fraser, R. B. (2006). Validation of a food-frequency questionnaire for use in pregnancy. *Public Health Nutrition, 9*(4), 515-522. doi: 10.1079/PHN2005876

Mozaffarian, D., & Rimm, E. B. (2006). Fish intake, contaminants, and human health - Evaluating the risks and the benefits. *Jama-Journal of the American Medical Association, 296*(15), 1885-1899. doi: 10.1001/jama.296.15.1885

Mozaffarian, D., & Wu, J. H. Y. (2011). Omega-3 fatty acids and cardiovascular disease effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology, 58*(20), 2047-2067. doi: 10.1016/j.jacc.2011.06.063

Mozurkewich, E. L., Clinton, C. M., Chilimigras, J. L., Hamilton, S. E., Allbaugh, L. J., Berman, D. R., . . . Djuric, Z. (2013). The mothers, omega-3, and mental health study: A double-blind, randomized controlled trial. *American Journal of Obstetrics and Gynecology, 208*(4). doi: 10.1016/j.ajog.2013.01.038

Mu, H. L., & Porsgaard, T. (2005). The metabolism of structured triacylglycerols. *Progress in Lipid Research, 44*(6), 430-448. doi: 10.1016/j.plipres.2005.09.002

Muskiet, F. A., van Goor, S. A., Kuipers, R. S., Velzing-Aarts, F. V., Smit, E. N., Bouwstra, H., . . . Hadders-Algra, M. (2006). Long-chain polyunsaturated fatty acids in maternal and infant nutrition. *Prostaglandins, leukotrienes and essential fatty acids, 75*(3), 135-144.

Nelson, M., Black, A. E., Morris, J. A., & Cole, T. J. (1989). Between- and within-subject variation in nutrient intake from infancy to old age: Estimating the number of days required to rank dietary intakes with desired precision. *American Journal of Clinical Nutrition, 50*(1), 155-167.

Nelson, S. M., Matthews, P., & Poston, L. (2010). Maternal metabolism and obesity: modifiable determinants of pregnancy outcome. *Human Reproduction Update, 16*(3), 255-275. doi: 10.1093/humupd/dmp050

NHMRC. (2006). *Nutrient Reference Values for Australia and New Zealand* Canberra: National Health and Medical Research Council, Wellington: Ministry of Health.

Novak, E. M., Dyer, R. A., & Innis, S. M. (2008). High dietary ω-6 fatty acids contribute to reduced docosahexaenoic acid in the developing brain and inhibit secondary neurite growth. *Brain Research, 1237*(0), 136-145. doi: http://dx.doi.org/10.1016/j.brainres.2008.07.107

Oey, N. A., den Boer, M. E. J., Ruiter, J. P. N., Wanders, R. J. A., Duran, M., Waterham, H. R., . . . Wijburg, F. A. (2003). High activity of fatty acid oxidation enzymes in human placenta: Implications for fetal-maternal disease. *Journal of Inherited Metabolic Disease, 26*(4), 385-392. doi: 10.1023/a:1025163204165

Oken, E., Kleinman, K. P., Olsen, S. F., Rich-Edwards, J. W., & Gillman, M. W. (2004). Associations of seafood and elongated n-3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort. *American Journal of Epidemiology, 160*(8), 774-783.

Oken, E., Ning, Y., Rifas-Shiman, S. L., Rich-Edwards, J. W., Olsen, S. F., & Gillman, M. W. (2007). Diet during pregnancy and risk of preeclampsia or gestational hypertension. *Annals of Epidemiology, 17*(9), 663-668. doi: http://dx.doi.org/10.1016/j.annepidem.2007.03.003

Oken, E., Osterdal, M. L., Gillman, M. W., Knudsen, V. K., Halldorsson, T. I., Strom, M., . . . Olsen, S. F. (2008a). Associations of maternal fish intake during pregnancy and breastfeeding duration with attainment of developmental milestones in early childhood: a study from the Danish National Birth Cohort. *American Journal of Clinical Nutrition, 88*(3), 789-796.

Oken, E., Radesky, J. S., Wright, R. O., Bellinger, D. C., Amarasiriwardena, C. J., Kleinman, K. P., . . . Gillman, M. W. (2008b). Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. *American Journal of Epidemiology, 167*(10), 1171-1181. doi: 10.1093/aje/kwn034

Oken, E., Wright, R. O., Kleinman, K. P., Bellinger, D., Amarasiriwardena, C. J., Hu, H., . . . Gillman, M. W. (2005). Maternal fish consumption, hair mercury, and infant cognition in a US cohort. *Environmental Health Perspectives, 113*(10), 1376-1380. doi: 10.1289/ehp.8041

Olafsdottir, A. S., Skuladottir, G. V., Thorsdottir, I., Hauksson, A., Thorgeirsdottir, H., & Steingrimsdottir, L. (2006). Relationship between high consumption of marine fatty acids in early pregnancy and hypertensive disorders in pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology, 113*(3), 301-309.

Olsen, S. F., Mikkelsen, T. B., Knudsen, V. K., Orozova-Bekkevold, I., Halldórsson, T. I., Strøm, M., & Østerdal, M. L. (2007). Data collected on maternal dietary exposures in the Danish National Birth Cohort. *Paediatric and Perinatal Epidemiology, 21*(1), 76-86. doi: 10.1111/j.1365-3016.2007.00777.x

Olsen, S. F., Østerdal, M. L., Salvig, J. D., Kesmodel, U., Henriksen, T. B., Hedegaard, M., & Secher, N. J. (2006). Duration of pregnancy in relation to seafood intake during early and mid pregnancy: prospective cohort. *European journal of epidemiology, 21*(10), 749-758.

Olsen, S. F., Sorensen, J. D., Secher, N., Hedegaard, M., Henriksen, T. B., Hansen, H. S., & Grant, A. (1992). Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. *The Lancet, 339*(8800), 1003-1007.

Olsen, S. F., Sorensen, T. A., Secher, N., Hansen, H., Jensen, B., Sommer, S., & Knudsen, L. (1986). Intake of marine fat, rich in (n-3)-polyunsaturated fatty acids, may increase birthweight by prolonging gestation. *The Lancet, 328*(8503), 367-369.

Olson, D. M. (2003). The role of prostaglandins in the initiation of parturition. *Best Practice & Research in Clinical Obstetrics & Gynaecology, 17*(5), 717-+. doi: 10.1016/s1521-6934(03)00069-5

Otto, S. J., De Groot, R., & Hornstra, G. (2003). Increased risk of postpartum depressive symptoms is associated with slower normalization after pregnancy of the functional docosahexaenoic acid status. *Prostaglandins, leukotrienes and essential fatty acids, 69*(4), 237-243.

Otto, S. J., van Houwelingen, A. C., Badart-Smook, A., & Hornstra, G. (2001a). Changes in the maternal essential fatty acid profile during early pregnancy and the relation of the profile to diet. *American Journal of Clinical Nutrition, 73*(2), 302-307.

Otto, S. J., van Houwelingen, A. C., Badart-Smook, A., & Hornstra, G. (2001b). Comparison of the peripartum and postpartum phospholipid polyunsaturated fatty acid profiles of lactating and nonlactating women. *The American journal of clinical nutrition, 73*(6), 1074-1079.

Øverby, N. C., Serra-Majem, L., & Andersen, L. F. (2009). Dietary assessment methods on n-3 fatty acid intake: a systematic review. *The British journal of nutrition, 102 Suppl 1*, S56-63. doi: 10.1017/S000711450999314X

Pagán, A., Prieto-Sánchez, M. T., Blanco-Carnero, J. E., Gil-Sánchez, A., Parrilla, J. J., Demmelmair, H., . . . Larque, E. (2013). Materno-fetal transfer of docosahexaenoic acid is impaired by gestational diabetes mellitus. *American Journal of Physiology-Endocrinology and Metabolism, 305*(7), E826-E833.

Parker, G., Gibson, N., Brotchie, H., Heruc, G., Rees, A.-M., & Hadzi-Pavlovic, D. (2006). Omega-3 fatty acids and mood disorders. *American Journal of Psychiatry, 163*(6), 969-978.

Patterson, E., Wall, R., Fitzgerald, G. F., Ross, R. P., & Stanton, C. (2012). Health implications of high dietary omega-6 polyunsaturated fatty acids. *Journal of nutrition and metabolism, 2012*.

Patterson, W., & Georgel, P. T. (2014). Breaking the cycle: the role of omega-3 polyunsaturated fatty acids in inflammation-driven cancers. *Biochemistry and Cell Biology-Biochimie Et Biologie Cellulaire, 92*(5), 321-328. doi: 10.1139/bcb-2013-0127

Pauga, M. (2009). *The effect of consuming farmed salmon compared to salmon oil capsules on long chain omega 3 fatty acids and selenium status in humans.* (Masters of Science MSc Thesis,), Massey University,, Auckland, New Zealand,.

Plant and Food Research. (2014). The Concise New Zealand Food Composition. 11th. Version 1

Plourde, M., & Cunnane, S. C. (2007). Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition Et Metabolisme, 32*(4), 619-634. doi: 10.1139/h07-034

Poniedzialek-Czajkowska, E., Mierzynski, R., Kimber-Trojnar, Z., Leszczynska-Gorzelak, B., & Oleszczuk, J. (2014). Polyunsaturated fatty acids in pregnancy and metabolic syndrome: A review. *Current Pharmaceutical Biotechnology, 15*(1), 84-99.

Popeski, D., Ebbeling, L., Brown, P., Hornstra, G., & Gerrard, J. (1991). Blood pressure during pregnancy in Canadian Inuit: community differences related to diet. *CMAJ: Canadian Medical Association Journal, 145*(5), 445.

Potischman, N. (2003). Biologic and methodologic issues for nutritional biomarkers. *Journal of Nutrition, 133*(3 SUPPL.), 875S-880S.

Prescott, S. L., & Clifton, V. (2009). Asthma and pregnancy: Emerging evidence of epigenetic interactions in utero. *Current Opinion in Allergy and Clinical Immunology, 9*(5), 417-426. doi: 10.1097/ACI.0b013e328330634f

Quinn, J. F., Raman, R., Thomas, R. G., Yurko-Mauro, K., Nelson, E. B., Van Dyck, C., . . . Aisen, P. S. (2010). Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: A randomized trial. *JAMA - Journal of the American Medical Association, 304*(17), 1903-1911. doi: 10.1001/jama.2010.1510

Radesky, J. S., Oken, E., Rifas-Shiman, S. L., Kleinman, K. P., Rich-Edwards, J. W., & Gillman, M. W. (2008). Diet during early pregnancy and development of gestational diabetes. *Paediatric and perinatal epidemiology, 22*(1), 47-59.

Rahmawaty, S., Charlton, K., Lyons-Wall, P., & Meyer, B. J. (2013a). Dietary intake and food sources of EPA, DPA and DHA in Australian children. *Lipids, 48*(9), 869-877. doi: 10.1007/s11745-013-3812-4

Rahmawaty, S., Charlton, K., Lyons-Wall, P., & Meyer, B. J. (2013b). Factors that influence consumption of fish and omega-3-enriched foods: A survey of Australian families with young children. *Nutrition & Dietetics, 70*(4), 286-293. doi: 10.1111/1747-0080.12022

Ramakrishnan, U. (2011). Fatty acid status and maternal mental health. *Maternal and Child Nutrition, 7*, 99-111. doi: 10.1111/j.1740-8709.2011.00312.x

Ramakrishnan, U., Imhoff-Kunsch, B., & DiGirolamo, A. M. (2009). Role of docosahexaenoic acid in maternal and child mental health. *The American journal of clinical nutrition, 89*(3), 958S-962S.

Ramel, S. E., & Georgieff, M. K. (2014). Preterm Nutrition and the Brain. In B. Koletzko, B. Poindexter, & R. Uauy (Eds.), *Nutritional care of preterm infants: Scientific basis and practical guidelines* (Vol. 110, pp. 190-200).

Ratnayake, W. M. N., & Galli, C. (2009). Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism: a background review paper. *Annals of Nutrition and Metabolism, 55*(1/3), 8-43. doi: 10.1159/000228994

Ricciotti, E., & FitzGerald, G. A. (2011). Prostaglandins and inflammation. *Arteriosclerosis Thrombosis and Vascular Biology, 31*(5), 986-1000. doi: 10.1161/atvbaha.110.207449

Ristic-Medic, D., & Vucic, V. (2013). Dietary fats and metabolic syndrome. *J Nutrition Health Food Sci, 1*(1), 1-8.

Rodriguez-Bernal, C. L., Ramon, R., Quiles, J., Murcia, M., Navarrete-Munoz, E. M., Vioque, J., . . . Rebagliato, M. (2013). Dietary intake in pregnant women in a Spanish Mediterranean area: as good as it is supposed to be? *Public Health Nutrition, 16*(8), 1379-1389. doi: 10.1017/s1368980012003643

Rolstad, S., Adler, J., & Rydén, A. (2011). Response burden and questionnaire length: Is shorter better? A review and meta-analysis. *Value in Health, 14*(8), 1101-1108. doi: http://dx.doi.org/10.1016/j.jval.2011.06.003

Russell, F., & Bürgin-Maunder, C. (2012). Distinguishing health benefits of eicosapentaenoic and docosahexaenoic acids. *Marine Drugs, 10*(11), 2535-2559.

Russo, G. L. (2009). Dietary n-6 and n-3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention. *Biochemical Pharmacology, 77*(6), 937-946. doi: 10.1016/j.bcp.2008.10.020

Ruxton, C., Reed, S., Simpson, M., & Millington, K. (2007). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J Hum Nutr Diet, 20*(3), 275-285. doi: 10.1111/j.1365-277X.2007.00770.x

Ruyle, M., Connor, W. E., Anderson, G. J., & Lowensohn, R. I. (1990). Placental-transfer of essential fatty-acids in humans - venous arterial difference for docosahexaenoic acid in fetal umbilical erythrocytes. *Proceedings of the National Academy of Sciences of the United States of America, 87*(20), 7902-7906. doi: 10.1073/pnas.87.20.7902

Ryan, A. S., Astwood, J. D., Gautier, S., Kuratko, C. N., Nelson, E. B., & Salem Jr, N. (2010). Effects of long-chain polyunsaturated fatty acid supplementation on neurodevelopment in childhood: A review of human studies. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA), 82*(4–6), 305-314. doi: http://dx.doi.org/10.1016/j.plefa.2010.02.007

Saadeh, D., Salameh, P., Baldi, I., & Raherison, C. (2013). Diet and allergic diseases among population aged 0 to 18 years: Myth or reality? *Nutrients, 5*(9), 3399-3423.

Sagiv, S. K., Thurston, S. W., Bellinger, D. C., Amarasiriwardena, C., & Korrick, S. A. (2012). Prenatal exposure to mercury and fish consumption during pregnancy and attention-deficit/hyperactivity disorder-related behavior in children. *Archives of Pediatrics & Adolescent Medicine, 166*(12), 1123-1131. doi: 10.1001/archpediatrics.2012.1286

Salam, M. T., Li, Y. F., Langholz, B., & Gilliland, F. D. (2005). Maternal fish consumption during pregnancy and risk of early childhood asthma. *Journal of Asthma, 42*(6), 513-518. doi: 10.1081/JAS-67619

Salari, P., Rezaie, A., Larijani, B., & Abdollahi, M. (2008). A systematic review of the impact of n-3 fatty acids in bone health and osteoporosis. *Medical Science Monitor, 14*(3), RA37-RA44.

Saltert, A. M., & Tarling, E. J. (2007). Regulation of gene transcription by fatty acids. *Animal, 1*(9), 1314-1320. doi: 10.1017/s1751731107000675

Salvig, J. D., & Lamont, R. F. (2011). Evidence regarding an effect of marine n-3 fatty acids on preterm birth: a systematic review and meta-analysis. *Acta Obstetricia Et Gynecologica Scandinavica, 90*(8), 825-838. doi: 10.1111/j.1600-0412.2011.01171.x

Sampath, H., & Ntambi, J. M. (2004). Polyunsaturated fatty acid regulation of gene expression. *Nutrition Reviews, 62*(9), 333-339. doi: 10.1301/nr.2004.sept.333-339

Sampath, H., & Ntambi, J. M. (2005). Polyunsaturated fatty acid regulation of genes of lipid metabolism *Annual Review of Nutrition* (Vol. 25, pp. 317-340).

Sanders, T. A. B. (2009a). DHA status of vegetarians. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 81*(2–3), 137-141. doi: http://dx.doi.org/10.1016/j.plefa.2009.05.013

Sanders, T. A. B. (2009b). Fat and fatty acid intake and metabolic effects in the human body. *Annals of Nutrition and Metabolism, 55*(1-3), 162-172.

Sanders, T. A. B. (2014). Protective effects of dietary PUFA against chronic disease: evidence from epidemiological studies and intervention trials. *Proceedings of the Nutrition Society, 73*(01), 73-79. doi: doi:10.1017/S0029665113003789

Saunders, A. V., Davis, B. C., & Garg, M. L. (2012). Omega-3 polyunsaturated fatty acids and vegetarian diets. *Medical Journal of Australia*, 22-26. doi: 10.5694/mjao11.11507

Sausenthaler, S., Koletzko, S., Schaaf, B., Lehmann, I., Borte, M., Herbarth, O., . . . Heinrich, J. (2007). Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 y of age. *American Journal of Clinical Nutrition, 85*(2), 530-537.

Schaeffer, L., Gohlke, H., Müller, M., Heid, I. M., Palmer, L. J., Kompauer, I., . . . Heinrich, J. (2006). Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Human molecular genetics, 15*(11), 1745-1756.

Schaiff, W. T., Bildirici, I., Cheong, M., Chern, P. L., Nelson, D. M., & Sadovsky, Y. (2005). Peroxisome proliferator-activated receptor-gamma and retinoid X receptor signaling regulate fatty acid uptake by primary human placental trophoblasts. *Journal of Clinical Endocrinology & Metabolism, 90*(7), 4267-4275. doi: 10.1210/jc.2004-2265

Schmitz, G., & Ecker, J. (2008). The opposing effects of n-3 and n-6 fatty acids. *Progress in Lipid Research, 47*(2), 147-155. doi: 10.1016/j.plipres.2007.12.004

Schuchardt, J. P., Huss, M., Stauss-Grabo, M., & Hahn, A. (2010). Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. *European journal of pediatrics, 169*(2), 149-164.

Scientific Advisory Committee on Nutrition (SACN). (2004). *Advice on Fish Consumption: Benefits and Risks*. London:TSO. Retrieved from http://www.sacn.gov.uk/pdfs/fics_sacn_advice_fish.pdf

Scorletti, E., & Byrne, C. D. (2013). Omega-3 fatty acids, hepatic lipid metabolism, and nonalcoholic fatty liver disease. *Annual review of nutrition, 33*, 231-248.

Scott, J., Campbell, D., & Davies, M. (2007). *Mothers and Infants*. In M. A. Lawrence, & T. Worsley (Eds.), *Public Health Nutrition: From principals to practice.* (pp. 75). Australia and New Zealand: Open University Press.

Serhan, C. N. (2010). Novel Lipid Mediators and Resolution Mechanisms in Acute Inflammation: To Resolve or Not? *The American Journal of Pathology, 177*(4), 1576-1591. doi: http://dx.doi.org/10.2353/ajpath.2010.100322

Serhan, C. N., Chiang, N., & Van Dyke, T. E. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nature Reviews Immunology, 8*(5), 349-361. doi: 10.1038/nri2294

Serhan, C. N., Dalli, J., Colas, R. A., Winkler, J. W., & Chiang, N. (2015). Protectins and maresins: New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. *Biochim Biophys Acta, 1851*(4), 397-413. doi: 10.1016/j.bbalip.2014.08.006

Shekhawat, P., Bennett, M. J., Sadovsky, Y., Nelson, D. M., Rakheja, D., & Strauss, A. W. (2003). Human placenta metabolizes fatty acids: implications for fetal fatty acid oxidation disorders and maternal liver diseases. *American Journal of Physiology-Endocrinology and Metabolism, 284*(6), E1098-E1105. doi: 10.1152/ajpendo.00481.2002

Sibley, C. P., Turner, M. A., Cetin, I., Ayuk, P., Boyd, C. A. R., D'Souza, S. W., . . . Powell, T. (2005). Placental phenotypes of intrauterine growth. *Pediatric Research, 58*(5), 827-832. doi: 10.1203/01.pdr.0000181381.82856.23

Simmer, k., Gibson, R., D'Vaz, N., Makrides, M., Koletzko, B., Cobiac, L., . . . Sinclair, A. J. (2009). *Omega-3 Fatty Acids for Maternal and Infant Health and Development: Scientific Consensus Workshop*. Australia.

Simopoulos, A. P. (2006). Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomedicine & Pharmacotherapy, 60*(9), 502-507.

Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine, 233*(6), 674-688.

Simopoulos, A. P., Leaf, A., & Salem, N., Jr. (1999). Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids (Bethesda, Maryland; April 1999; National Institute on Alcohol Abuse and Alcoholism-NIH, Office of Dietary Supplements-NIH, Center for Genetics, Nutrition and Health, International Society for the Study of Fatty Acids and Lipids). *Annals of Nutrition and Metabolism, 43*(2), 127-130. doi: 10.1159/000012777

Simopoulos, A. P., Leaf, A., & Salem, N., Jr. (2000). Workshop on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Food Reviews International, 16*(1), 113-117. doi: 10.1081/fri-100100284

Singh, M. (2005). Essential fatty acids, DHA and human brain. *The Indian Journal of Pediatrics, 72*(3), 239-242.

Sinikovic, D. S., Yeatman, H. R., Cameron, D., & Meyer, B. J. (2009). Women's awareness of the importance of long-chain omega-3 polyunsaturated fatty acid consumption during pregnancy: Knowledge of risks, benefits and information accessibility. *Public health nutrition, 12*(04), 562-569.

Sioen, I. A., Devroe, J., Inghels, D., Terwecoren, R., & De Henauw, S. (2010). The influence of n-3 PUFA supplements and n-3 PUFA enriched foods on the n-3 LC PUFA intake of flemish women. *Lipids, 45*(4), 313-320. doi: 10.1007/s11745-010-3403-6

Sioen, I. A., Pynaert, I., Matthys, C., De Backer, G., Van Camp, J., & De Henauw, S. (2006). Dietary intakes and food sources of fatty acids for Belgian women, focused on n-6 and n-3 polyunsaturated fatty acids. *Lipids, 41*(5), 415-422. doi: 10.1007/s11745-006-5115-5

Slater, D. M., Zervou, S., & Thornton, S. (2002). Prostaglandins and prostanoid receptors in human pregnancy and parturition. *Journal of the Society for Gynecologic Investigation, 9*(3), 118-124. doi: 10.1016/s1071-5576(02)00151-x

Smith, K. M., & Sahyoun, N. R. (2005). Fish consumption: Recommendations versus advisories, can they be reconciled? *Nutrition Reviews, 63*(2), 39-46. doi: 10.1301/nr.2005.feb.39-46

Smithers, L. G., Gibson, R. A., & Makrides, M. (2011). Maternal supplementation with docosahexaenoic acid during pregnancy does not affect early visual development in the infant: a randomized controlled trial. *The American Journal of Clinical Nutrition, 93*(6), 1293-1299. doi: 10.3945/ajcn.110.009647

Smuts, C. M., Borod, E., Peeples, J. M., & Carlson, S. E. (2003a). High-DHA eggs: Feasibility as a means to enhance circulating DHA in mother and infant. *Lipids, 38*(4), 407-414. doi: 10.1007/s11745-003-1076-y

Smuts, C. M., Huang, M., Mundy, D., Plasse, T., Major, S., & Carlson, S. E. (2003b). A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy. *Obstetrics & Gynecology, 101*(3), 469-479.

Soltan, S., & Gibson, R. A. (2008). Levels of omega 3 fatty acids in Australian seafood. *Asia Pac. J. Clin. Nutr, 17*(3), 385-390.

Sontrop, J. M., Avison, W. R., Evers, S. E., Speechley, K. N., & Campbell, M. K. (2008). Depressive symptoms during pregnancy in relation to fish consumption and intake of n-3 polyunsaturated fatty acids. *Paediatric and Perinatal Epidemiology, 22*(4), 389-399. doi: 10.1111/j.1365-3016.2008.00941.x

Stanley, J. C., Elsom, R. L., Calder, P. C., Griffin, B. A., Harris, W. S., Jebb, S. A., . . . Sanders, T. A. (2007). UK Food Standards Agency Workshop Report: the effects of the dietary n-6: n-3 fatty acid ratio on cardiovascular health. *British Journal of Nutrition, 98*(06), 1305-1310.

Stark, K. D., Beblo, S., Murthy, M., Buda-Abela, M., Janisse, J., Rockett, H., . . . Salem Jr, N. (2005). Comparison of bloodstream fatty acid composition from African-American women at gestation, delivery, and postpartum. *Journal of Lipid Research, 46*(3), 516-525. doi: 10.1194/jlr.M400394-JLR200

Stewart, D., Conrad, K. P., & Jeyabalan, A. (2013). Prediction and prevention of preeclampsia: Google Patents.

Stewart, F., Rodie, V. A., Ramsay, J. E., Greer, I. A., Freeman, D. J., & Meyer, B. J. (2007). Longitudinal assessment of erythrocyte fatty acid composition throughout pregnancy and post partum. *Lipids, 42*(4), 335-344. doi: 10.1007/s11745-006-3005-5

Stillwell, W., & Wassall, S. R. (2003). Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chemistry and Physics of Lipids, 126*(1), 1-27. doi: 10.1016/s0009-3084(03)00101-4

Stipanuk, M. H., & Caudill, M. A. (2013). *Biochemical, Physiological, and Molecular Aspects of Human Nutrition* (Third ed.). United States of America: Elsevier

Stonehouse, W. (2014). Does consumption of lc omega-3 pufa enhance cognitive performance in healthy school-aged children and throughout adulthood? Evidence from clinical trials. *Nutrients, 6*(7), 2730-2758. doi: 10.3390/nu6072730

Stonehouse, W., Pauga, M. R., Kruger, R., Thomson, C. D., Wong, M., & Kruger, M. C. (2011). Consumption of salmon v. salmon oil capsules: effects on n-3 PUFA and selenium status. *British journal of nutrition, 106*(08), 1231-1239.

Strain, J., Davidson, P. W., Bonham, M. P., Duffy, E. M., Stokes-Riner, A., Thurston, S. W., . . . Georger, L. A. (2008). Associations of maternal long-chain polyunsaturated fatty acids, methyl mercury, and infant development in the Seychelles Child Development Nutrition Study. *Neurotoxicology, 29*(5), 776-782.

Strain, J. J., Davidson, P. W., Thurston, S. W., Harrington, D., Mulhern, M. S., McAfee, A. J., . . . Myers, G. J. (2012). Maternal PUFA status but not prenatal methylmercury exposure is associated with children's language functions at age five years in the Seychelles. *Journal of Nutrition, 142*(11), 1943-1949. doi: 10.3945/jn.112.163493

Stremmel, W., Pohl, J., Ring, A., & Herrmann, T. (2001). A new concept of cellular uptake and intracellular trafficking of long-chain fatty acids. *Lipids, 36*(9), 981-989. doi: 10.1007/s11745-001-0809-2

Su, K.-P., Huang, S.-Y., Chiu, T.-H., Huang, K.-C., Huang, C.-L., Chang, H.-C., & Pariante, C. M. (2008). Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial. *Journal of Clinical Psychiatry, 69*(4), 644.

Subar, A. F., Kirkpatrick, S. I., Mittl, B., Zimmerman, T. P., Thompson, F. E., Bingley, C., . . . Potischman, N. (2012). The automated self-administered 24-hour dietary recall (asa24): A resource for researchers, clinicians, and educators from the national cancer institute. *Journal of the Academy of Nutrition and Dietetics, 112*(8), 1134-1137. doi: 10.1016/j.jand.2012.04.016

Sullivan, B. L., Brown, J., Williams, P. G., & Meyer, B. J. (2008). Dietary validation of a new Australian food-frequency questionnaire that estimates long-chain n-3 polyunsaturated fatty acids. *British Journal of Nutrition, 99*(3), 660-666. doi: 10.1017/s0007114507837408

Sullivan, B. L., Williams, P. G., & Meyer, B. J. (2006). Biomarker validation of a long-chain omega-3 polyunsaturated fatty acid food frequency questionnaire. *Lipids, 41*(9), 845-850. doi: 10.1007/s11745-006-5039-0

Sullivan, E., & Grove, K. (2010). Metabolic imprinting in obesity. *Frontiers in Eating and Weight Regulation, 63*, 186 - 194.

Swierk, M., Williams, P. G., Wilcox, J., Russell, K. G., & Meyer, B. J. (2011). Validation of an Australian electronic food frequency questionnaire to measure polyunsaturated fatty acid intake. *Nutrition (Burbank, Los Angeles County, Calif.), 27*(6), 641-646. doi: 10.1016/j.nut.2010.06.011

Sydenham, E., Dangour, A. D., & Lim, W.-S. (2012). Omega 3 fatty acid for the prevention of cognitive decline and dementia. *Cochrane Database of Systematic Reviews*(6). doi: 10.1002/14651858.CD005379.pub3

Szajewska, H., Horvath, A., & Koletzko, B. (2006). Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *American Journal of Clinical Nutrition, 83*(6), 1337-1344.

Taylor, A. L., Collins, C. E., & Patterson, A. J. (2014). The relationship between potential contaminant exposure from fish and nutrient intakes in Australian women by pregnancy status. *Nutrition & Dietetics, 71*(4), 229-235. doi: 10.1111/1747-0080.12112

Thomas, B., Ghebremeskel, K., Lowy, C., Crawford, M., & Offley-Shore, B. (2006). Nutrient intake of women with and without gestational diabetes with a specific focus on fatty acids. *Nutrition, 22*(3), 230-236. doi: 10.1016/j.nut.2005.07.017

Thompson, F. E., & Subar, A. F. (2013). Chapter 1 - Dietary assessment methodology. In A. M. C. J. B. G. Ferruzzi (Ed.), *Nutrition in the Prevention and Treatment of Disease (Third Edition)* (pp. 5-46): Academic Press.

Thompson, J. M. D., Wall, C., Becroft, D. M. O., Robinson, E., Wild, C. J., & Mitchell, E. A. (2010). Maternal dietary patterns in pregnancy and the association with small-for-gestational-age infants. *British Journal of Nutrition, 103*(11), 1665-1673. doi: 10.1017/S0007114509993606

Troxell, H., Anderson, J., Auld, G., Marx, N., Harris, M., Reece, M., & Allen, K. (2005). Omega-3 for baby and me: Material development for a WIC intervention to increase DHA intake during pregnancy. *Maternal and Child Health Journal, 9*(2), 189-197. doi: 10.1007/s10995-005-4908-0

Uauy, R., & Dangour, A. D. (2006). Nutrition in brain development and aging: role of essential fatty acids. *Nutrition reviews, 64*(s2), S24-S33.

Uauy, R., Hoffman, D. R., Peirano, P., Birch, D. G., & Birch, E. E. (2001). Essential fatty acids in visual and brain development. *Lipids, 36*(9), 885-895. doi: 10.1007/s11745-001-0798-1

United Kingdon Scientific Advisory Committee on Nutrition. (2004). Advice on fish consumption: benefits & risks. Retrieved 20th October, 2014, from https://www.google.co.nz/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CBwQFjAA&url=https%3A%2F%2Fwww.food.gov.uk%2Fsites%2Fdefault%2Ffiles%2Fcot%2Ffishreport2004full.pdf&ei=OISTVdL2KpCD8gWCuYKIBg&usg=AFQjCNGjxdD8Va1-EbRDcbIbcsestSpxvg&bvm=bv.96952980,d.dGc&cad=rja

United States Department of Agriculture (USDA). (2010). *Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans*. USA.

University of Otago. (2011). *Methodology Report for the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health.

University of Otago, & Ministry of Health. (2011). *A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Wellington.

Van Eijsden, M., Hornstra, G., Van Der Wal, M. F., Vrijkotte, T. G., & Bonsel, G. J. (2008). Maternal n− 3, n− 6, and trans fatty acid profile early in pregnancy and term birth weight: a prospective cohort study. *The American journal of clinical nutrition, 87*(4), 887-895.

Vaz, J. d. S., Kac, G., Emmett, P., Davis, J. M., Golding, J., & Hibbeln, J. R. (2013). Dietary patterns, n-3 fatty acids intake from seafood and high levels of anxiety symptoms during pregnancy: Findings from the AVON longitudinal study of parents and children. *Plos One, 8*(7). doi: 10.1371/journal.pone.0067671

Visentin, S., Grumolato, F., Nardelli, G. B., Di Camillo, B., Grisan, E., & Cosmi, E. (2014). Early origins of adult disease: Low birth weight and vascular remodeling. *Atherosclerosis, 237*(2), 391-399. doi: http://dx.doi.org/10.1016/j.atherosclerosis.2014.09.027

Visioli, F., Risé, P., Barassi, M. C., Marangoni, F., & Galli, C. (2003). Dietary intake of fish vs. formulations leads to higher plasma concentrations of n− 3 fatty acids. *Lipids, 38*(4), 415-418.

Wada, T., Hori, S., Sugiyama, M., Fujisawa, E., Nakano, T., Tsuneki, H., . . . Sasaoka, T. (2010). Progesterone inhibits glucose uptake by affecting diverse steps of insulin signaling in 3T3-L1 adipocytes. *American Journal of Physiology-Endocrinology and Metabolism, 298*(4), E881-E888. doi: 10.1152/ajpendo.00649.2009

Wang, W., Zhu, J., Lyu, F., Panigrahy, D., Ferrara, K. W., Hammock, B., & Zhang, G. (2014). Omega-3 polyunsaturated fatty acids-derived lipid metabolites on angiogenesis, inflammation and cancer. *Prostaglandins & Other Lipid Mediators, 113*, 13-20. doi: 10.1016/j.prostaglandins.2014.07.002

Warstedt, K., Furuhjelm, C., Duchen, K., Fälth-Magnusson, K., & Facerás, M. (2009). The effects of omega-3 fatty acid supplementation in pregnancy on maternal eicosanoid, cytokine, and chemokine secretion. *Pediatric Research, 66*(2), 212-217. doi: 10.1203/PDR.0b013e3181aabd1c

Watson, P. E., & McDonald, B. W. (2010). The association of maternal diet and dietary supplement intake in pregnant New Zealand women with infant birthweight. *European Journal of Clinical Nutrition, 64*(2), 184-193. doi: 10.1038/ejcn.2009.134

Watson, P. E., & McDonald, B. W. (2014). Water and nutrient intake in pregnant New Zealand women: association with wheeze in their infants at 18 months. *Asia Pac J Clin Nutr, 23*(4), 660-670. doi: 10.6133/apjcn.2014.23.4.13

Weedon-Fekjaer, M. S., Duttaroy, A. K., & Nebb, H. I. (2005). Liver X receptors mediate inhibition of hCG secretion in a human placental

trophoblast cell line. *Placenta, 26*(10), 721-728. doi: 10.1016/j.placenta.2004.10.005

Whelan, J., Jahns, L., & Kavanagh, K. (2009). Docosahexaenoic acid: Measurements in food and dietary exposure. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 81*(2–3), 133-136. doi: http://dx.doi.org/10.1016/j.plefa.2009.05.008

Wijendran, V., Bendel, R. B., Couch, S. C., Philipson, E. H., Cheruku, S., & Lammi-Keefe, C. J. (2000). Fetal erythrocyte phospholipid polyunsaturated fatty acids are altered in pregnancy complicated with gestational diabetes mellitus. *Lipids, 35*(8), 927-931. doi: 10.1007/s11745-000-0602-2

Wilkinson, S. A., Miller, Y. D., & Watson, B. (2009). Prevalence of health behaviours in pregnancy at service entry in a Queensland health service district. *Australian and New Zealand Journal of Public Health, 33*(3), 228-233. doi: 10.1111/j.1753-6405.2009.00380.x

Williams, C. M., & Burdge, G. (2006). Long-chain n-3 PUFA: plant v. marine sources. *Proceedings of the Nutrition Society, 65*(1), 42-50. doi: 10.1079/pns2005473

Williams, M. A., Frederick, I. O., Qiu, C., Meryman, L. J., King, I. B., Walsh, S. W., & Sorensen, T. K. (2006). Maternal erythrocyte omega-3 and omega-6 fatty acids, and plasma lipid concentrations, are associated with habitual dietary fish consumption in early pregnancy. *Clinical Biochemistry, 39*(11), 1063-1070. doi: 10.1016/j.clinbiochem.2006.09.008

Williams, M. A., Zingheim, R. W., King, I. B., & Zebelman, A. M. (1995). Omega-3 fatty acids in maternal erythrocytes and risk of preeclampsia. *Epidemiology*, 232-237.

Wood, K. E., Mantzioris, E., Gibson, R. A., & Muhlhausler, B. S. (2013). Incorporating macadamia oil and butter to reduce dietary omega-6 polyunsaturated fatty acid intake. *Nutrition & Dietetics, 70*(2), 94-100.

World Health Organization. (2012). *Born Too Soon: The Global Action Report on Preterm Birth.* Geneva: WHO.

Wu, B. T., Dyer, R. A., King, D. J., & Innis, S. M. (2013). Low fish intake is associated with low blood concentrations of vitamin D, choline and n-3 DHA in pregnant women. *British Journal of Nutrition, 109*(5), 936-943. doi: 10.1017/s0007114512002103

Xie, L., & Innis, S. M. (2008). Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during

pregnancy and in breast milk during lactation. *The Journal of nutrition, 138*(11), 2222-2228.

Zamaria, N. (2004). Alteration of polyunsaturated fatty acid status and metabolism in health and disease. *Reproduction Nutrition Development, 44*(3), 273-282. doi: 10.1051/rnd:2004034

Zeijdner, E., Houwelingen, A., Kester, A., & Hornstra, G. (1997). Essential fatty acid status in plasma phospholipids of mother and neonate after multiple pregnancy. *Prostaglandins, leukotrienes and essential fatty acids, 56*(5), 395-401.

Zhou, S. J., Yelland, L., McPhee, A. J., Quinlivan, J., Gibson, R. A., & Makrides, M. (2012). Fish-oil supplementation in pregnancy does not reduce the risk of gestational diabetes or preeclampsia. *The American Journal of Clinical Nutrition, 95*(6), 1378-1384. doi: 10.3945/ajcn.111.033217

Zornoza-Moreno, M., Fuentes-Hernández, S., Carrión, V., Alcántara-López, M. V., Madrid, J. A., López-Soler, C., . . . Larqué, E. (2014). Is low docosahexaenoic acid associated with disturbed rhythms and neurodevelopment in offsprings of diabetic mothers? *European Journal of Clinical Nutrition, 68*(8), 931-937. doi: 10.1038/ejcn.2014.104

APPENDICES

APPENDIX 1 - Ethical approval letter

MASSEY UNIVERSITY
ALBANY

10 July 2014

Michele Eickstaedt
c/- Dr Cath Conlon
Institute of Food Nutrition & Human Health
Massey University
Albany

Dear Michele

HUMAN ETHICS APPROVAL APPLICATION – MUHECN 14/027

Food sources and dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids in pregnant women living in New Zealand

Thank you for your application. It has been fully considered, and approved by the Massey University Human Ethics Committee: Northern.

Approval is for three years. If this project has not been completed within three years from the date of this letter, a reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Dr Lily George
Acting Chair
Human Ethics Committee: Northern

cc Dr Cath Conlon, Dr Kathryn Beck

APPENDIX 2 - Study flyer/poster


Are you pregnant or do you know someone who is?
We are recruiting pregnant women to help us with a research study looking at food sources and dietary intakes of key nutrients during pregnancy.
In return for taking part we will provide you with the latest guidelines on healthy eating during your pregnancy and you will have the chance to WIN one of five parenting books "If Only They'd Told Me".
WHO CAN TAKE PART?
All women in the third trimester of pregnancy, aged 16 and over and who live in New Zealand are invited to participate in our study.
WHAT DOES THE STUDY INVOLVE?
Completion of an anonymous online questionnaire (15 – 20min), available until the 10th February 2015 (hard copy available if requested).
If you are interested in participating in this study, or would like more information, you could:
•Go straight into the survey: www.surveymonkey.com/s/pufa
•Check our website: www.massey.ac.nz/pufa
•Email: pregnancystudynz@gmail.com
•Or Call (09) 4140800 Ext 43658
COMMITTEE APPROVAL STATEMENT: This project has been reviewed and approved by the Massey University Human Ethics Committee: Northern, Application 14/027. If you have any concerns about the conduct of this research, please contact Dr Lily George, Acting Chair, Massey University Human Ethics Committee: Northern, telephone 09 414 0800 x 43279 email humanethicsnorth@massey.ac.nz
MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

APPENDIX 3 - Press advertising for the study

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Mobile: +64 21 660 260
Email: s.yoshioka-braid@massey.ac.nz
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MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

MEDIA RELEASE

Xsday, September X, 2014

Are pregnant women getting enough key nutrients?

Good nutrition during pregnancy is essential for both the mother and the baby, but very little is known about what women are eating at this vital time. A research study at Massey University aims to uncover more about the dietary habits of pregnant women in New Zealand.

It's a topic that Master of human nutrition student Michele Eickstaedt is passionate about. "I have always wanted to study the health of pregnant women, and I have a passion for the important roles of omega-3s for optimal health as well, so this study enables me to combine both interests and hopefully provide some useful information for pregnant women.

"We have such scant knowledge about what pregnant women are eating in New Zealand, and if they are getting enough key nutrients, such as omega-3 and omega-6 polyunsaturated fatty acids in their diets."

Omega-3 and omega-6 polyunsaturated fatty acids are found in the membranes of every cell of the human body. They are found in a range of foods, including meat, poultry, fish, vegetable oils, and some vegetables.

Other studies have reported that modern diets in countries similar to New Zealand do not supply pregnant women with adequate amounts of omega-3 and omega-6 polyunsaturated fatty acids. Information on what pregnant women eat in New Zealand is very limited.

Lecturer in human nutrition, and one of the supervisors of the study Dr Cath Conlon says the limited information about what pregnant women are eating means it is unknown if the daily dietary recommendations are being met.

"The health outcomes are the key. These fatty acids are essential building blocks for almost every cell in the body. They're really important for the baby's brain development and their growth, and they're really important for the Mother's health as well. It's a double whammy – good for baby, good for Mum," she says.

The study is looking for at least 450 women of all ethnic origins from across New Zealand to fill in an anonymous online survey. Participants need to be aged 16 years and over, live in New Zealand, and be in their last trimester of pregnancy.

"If people don't have the facilities to participate online, we can send out hard copies of the survey to them," says Ms Eickstaedt. "The questionnaire takes about 15 to 20 minutes to complete."

All information supplied will be collected anonymously, and none of the study documentation will be able to identify participants.

THE ENGINE OF THE NEW NEW ZEALAND

On completion of the survey, study participants will receive a link to the Ministry of Health's guide *Eating for Healthy Pregnant Women*, and also go into the draw to win one of two subscriptions to *OHBaby!* Magazine for a year.

Ms Eickstaedt says if participants want to receive a summary of the research findings, they can indicate that when they complete the survey. "This is such important information, and we are grateful to the women who give up their time to take part. Hopefully it will help other pregnant women in the future."

The survey is available online until December 2014.

To complete the online survey, go to: https://www.surveymonkey.com/s/pufa

For further information, visit the website: http://www.massey.ac.nz/pufa

The project has been reviewed and approved by the Massey University Human Ethics Committee: Northern, application: 14/027.

Picture caption: Master's student Michele Eickstaedt

To contact Michele Eickstaedt, email : M.Eickstaedt@massey.ac.nz or call: 09 414 0800 ext 43815.

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APPENDIX 4 - Study invitation email (participants and organisations)

Olá, Kia Ora, Talofa lava, Malo e Lelei, Fakalofa lahi atu, Kia Orana, Bula vinaka, Namaste, Nihao and Hello!

Are you pregnant or do you know someone who is?

We are recruiting pregnant women to help us with a research study looking at food sources and dietary intakes of omega-6 and omega-3 fatty acids. These nutrients are important for the baby's growth and brain development and to support a health pregnancy. The study involves an anonymous online questionnaire which will only take about 15 – 20 minutes of your time.

In New Zealand we have very limited information about the diets of pregnant women and it is unknown if dietary recommendations for these key nutrients are being met. In return for taking part we will provide you with the latest guidelines on healthy eating during your pregnancy and you will have the chance to **WIN one of five parenting books "If Only They'd Told Me"**

Information about the study is attached and you can access our study questionnaire on: www.surveymonkey.com/s/pufa.

You can also check the study's press released produced by Massey University through the following link: http://www.massey.ac.nz/massey/about-massey/news/article.cfm?mnarticle_uuid=1573CB41-EA9E-D21A-1D15-37A38CE953F8

This study is part of my Masters in Human Nutrition and has been reviewed and approved by the Massey University Human Ethics Committee: Northern, Application 14/027. If you have any concerns about the conduct of this research, please contact Dr Lily George, Acting Chair, Massey University Human Ethics Committee: Northern, telephone 09 414 0800 x 43279 email humanethicsnorth@massey.ac.nz.

Please share about this study with anyone you know who is pregnant and don't hesitate to contact me for further information.

Thank you for your help

Michele Eickstaedt

MSc Human Nutrition Student

Institute of Food, Nutrition and Human Health, College of Health

Massey University, Albany Campus, Auckland New Zealand

Tel: 09 414 0800 ext 43815 / Mob: 021 123 71 91. Email: m.eickstaedt@massey.ac.nz

APPENDIX 5 - List of organisations that helped promoting the study

Organisation	Contact Person	Email
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Baby Factory communications	Warren Lowe	Warren@babyfactory.co.nz
Health Promotion Coordinator- Heart Foundation	Nicky Williams	nickyw@heartfoundation.org.nz
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Balclutha Parents Centre		balclutha@parentscentre.org.nz
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Birth Balance	Michele Neild	michele@birthbalance.co.nz
Birth West - Midwives centre	Justine Small	justine@birthwest.co.nz
Birth Wise	Sarah	Birth Wise Coordinator - Sarah
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Brain Wave	Shelley Piper	Shelley@brainwave.org.nz
Breastfeeding NZ	Pip	pip.sloan@gslpromotus.co.nz
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East and Bays Parents Centre		east.and.bays@parentscentre.org.nz
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New Plymouth Parents Centre	New Plymouth Parents Centre	parentscentre@hotmail.co.nz
New Zealand College of Midwives Conference 29 to 31 august in Hamilton	Arna Wahl Davies and Nerida Ramsay	nzcom@composition.co.nz
New Zealand Multiple Birth Association		president@multiples.org.nz
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Putaruru Parents Centre		putaruru@parentscentre.org.nz
Rotorua Parents Centre		rotorua@parentscentre.org.nz
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Southland Home Birth Association	Samantha	naturalbirth@mail.com
Stratford Parents Centre		stratford@parentscentre.org.nz
TAHA- Well Pacific Mother and Infant Service	Jacinta Fa'alili-Fidow	j.faalili-fidow@auckland.ac.nz

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Whānau Ora Community Health Worker - Te Korowai Hauora O Hauraki	Nitin Sukumaran	nitin.sukumaran@korowai.co.nz
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Women's Health Action	Isis McKay	info@womens-health.org.nz

APPENDIX 6 - Study information sheet

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids in pregnant women living in New Zealand

Information Sheet

Researchers Introduction

This research is a Master's student project that will be conducted by the student Michele Eickstaedt, and her supervisors Dr Kathryn Beck and Dr Cath Conlon. The Research team is based within the College of Health at Massey University Albany Campus.

Project Description and Invitation

Good nutrition during pregnancy is important for both the mother and the baby. Certain key nutrients, such as omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) are important for the growth and development of the baby as well as to support a healthy pregnancy for the mother.

Omega-6 and omega-3 PUFAs are fatty acids found in the membranes of every cell of the human body. They are found in a range of foods including meat, poultry, fish, vegetable oils and some vegetables.

Several studies report that modern diets in countries such as NZ do not supply adequate amounts of omega-6 and omega-3 PUFAs. There is also limited information about the diets of pregnant women in NZ and it is unknown if dietary recommendations for these key nutrients are being met.

For these reasons, we are inviting you to take part in this study that will investigate food sources and dietary intakes of omega-6 and omega-3 PUFA of pregnant women in NZ, and compare their current intakes to recommended values.

By taking part in this study you will help us to identify what the food sources of the PUFA's are and whether pregnant women are getting enough of these key nutrients. The results from this study will support me in completing my thesis, which is part of obtaining a Master's degree in Human Nutrition at Massey University.

Participants Identification and Recruitment

We are looking for pregnant women in the last trimester of pregnancy, aged 16 years and over, living in NZ and willing to complete an online questionnaire.

This is a nationwide study in which participants are invited to take part until the 10th February 2015.

If you know anyone else who would be interested in taking part in our study please pass on the study link on: https://www.surveymonkey.com/s/pufa.

The research team has consulted with Māori and Pacific representatives whilst planning this study. If you have any concerns please contact a member of our research team (please refer to research team contact details section) who will be happy to discuss your concerns.

Project Procedures

You will be requested to complete an online questionnaire that contains general questions about you, your medical and pregnancy history as well as your dietary intake over the past 6 months. All the information supply will be collected anonymously and none of the study documentation will be able to identify participants.

The questionnaire will take around 15 to 20 minutes to complete. Participation in this research is entirely voluntary and you do not have to answer any questions that you are uncomfortable with.

You are welcome to have a support person, such as a friend or family member, who can assist you in completing the questionnaire.

You can withdraw from this study at any time up until the 10/02/2015.

The questionnaire can be completed online via a secure survey platform link or in hard copy.

If you choose the hard copy, your contact details will be required for postage. You will be posted the questionnaire and a return addressed pre-paid envelope. Your contact details will not be recorded or linked to study documentation.

Upon completion of the questionnaire you will receive the link for *Eating for Healthy Pregnant Women* from the Ministry of Health (a hard copy is available if requested). You will also have the chance to WIN one of five parenting books "If Only They'd Told Me".

If you have any concerns about your health and diet during pregnancy please consult your Midwife, Medical Practitioner or Dietitian.

Data Management

The questionnaire is anonymous and all information gathered will be automatically assigned to a specific study ID number which will allow the researcher to analyse and describe the data while maintaining complete anonymity of participants. Data will be stored securely for five years. Access to the data will be available to the research team only.

Accessing a Summary of the Project Findings

As the data for the study is collected anonymously, we are unable to send out a summary of the findings of the study to participants. However a summary of the findings will be made available on our website around April 2015. Bookmark this page (www.massey.ac.nz/pufa) and visit it for updates on the study.

Alternatively you can inform your email address at the beginning of the questionnaire or contact the research team to request a hard copy of the summary of findings for this research.

Information resulting from this study will also be submitted to a peer-reviewed journal and presented at appropriate research seminars and conferences. If appropriate, the outcomes will also be publicised in the general media.

Participant's Rights

You are under no obligation to take part in this study. If you decide to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study at any time up until the 10/02/2015;
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used;
- be given access to the summary of the project findings when it is concluded.

Human Ethics Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Northern, Application **14/027**. If you have any concerns about the conduct of this research, please contact **Dr Lily George,** Acting Chair, Massey University Human Ethics Committee: Northern, telephone 09 414 0800 x **43279** email humanethicsnorth@massey.ac.nz

Taking Part in the Study

If you are interested in taking part in the study please access the link https://www.surveymonkey.com/s/pufa or contact the research team. Please feel free to tell as many friends, family and colleagues about this study so we can reach a large number of participants.

Project Contacts

MSc Student **Michele Eickstaedt** Institute of Food, Nutrition and Human Health Massey University, Albany Tel: (09) 414 0800 ext 43815 Mobile:021 123 7191 Email: M.Eickstaedt@massey.ac.nz	**Study Supervisor** **Dr Cath Conlon** Institute of Food, Nutrition and Human Health Massey University, Albany Tel: (09) 414 0800 ext 43658 Email:C.Conlon@massey.ac.nz	**Study Co-Supervisor** **Dr Kathryn Beck** Institute of Food, Nutrition and Human Health Massey University, Albany Tel: (09) 414 0800 ext 43662 Email:K.L.Beck@massey.ac.nz

Thank you for considering participating in this study!

APPENDIX 7 - Study questionnaire

Nutrition during pregnancy study

Thank you for your interest in taking part in our study.

This study aims to investigate food sources and dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) of pregnant women in New Zealand.

This is a nationwide study in which participants are invited to take part from July 2014 to December 2014.

We are looking for pregnant women in the last trimester of pregnancy, aged 16 years and over, living in New Zealand and willing to complete our online questionnaire.

This questionnaire aims to collect general information about you, your pregnancy and medical history as well as your diet during pregnancy. All the information supplied will be collected anonymously and none of the study documentation will be able to identify participants.

The questionnaire will take around 15 to 20 minutes to complete. Participation in this research is entirely voluntary and you do not have to answer any questions that you are uncomfortable with. For more information please read the Study Information Sheet or contact the researchers.

Please note that completion of this questionnaire is taken as your consent for the researchers to use the information you have provided for this research.

Just to ensure that you fit the inclusion criteria of the study, we would appreciate it if you could answer the questions below.

If you have any queries or concerns about the questionnaire, please feel free to contact Michele Eickstaedt during working hours on 09 414 0800 Ext 43815 or send an email to m.eickstaedt@massey.ac.nz.

CONSENT

***1. PARTICIPANT CONSENT**

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

◯ Yes ◯ No

***Do you agree to participate in this study under the conditions set out in the Information Sheet?**

◯ Yes ◯ No

2. **Please indicate your email address if you wish to receive the latest "Eating for Healthy Pregnant Women" guidelines and the summary of findings for this research as well as to have the chance to WIN one of five copies of the book If *Only They'd Told Me.***

*Answering these questions is required in order to confirm eligibility and agreement to take part in the study.

ABOUT YOU

***3. Are you currently living in New Zealand, aged over 16 years old and pregnant?**

◯ Yes ◯ No

***4. What is your date of birth?**

DD MM YYYY

Please indicate ☐ / ☐ / ☐☐☐☐

***5. How many weeks pregnant are you?**

◯ Before 28 weeks

◯ Between 28 and 32 weeks

◯ Between 33 and 37 weeks

◯ Between 38 and 40 weeks

◯ 40 weeks and over

*Answering these questions is required in order to confirm illegibility and agreement to take part in the study.

6. What is the highest level of education you have completed?

- ◯ None
- ◯ Primary School
- ◯ Secondary School
- ◯ University Degree
- ◯ Other

Please specify

7. What is your country of birth?

- ◯ New Zealand
- ◯ Other

Please specify

8. How long have you been living in New Zealand?

- ◯ Less than 5 years
- ◯ 5 to 9 years
- ◯ 10 to 19 years
- ◯ 20 years plus

9. Do you identify as:

- ◯ New Zealand European
- ◯ Maori
- ◯ Cook Island Maori
- ◯ Tongan
- ◯ Samoan
- ◯ Fijian
- ◯ Niuean
- ◯ Indian
- ◯ Chinese
- ◯ Other

Please specify (eg. Chilean, Japanese, Malay)

10. How many children do you have?

- ◯ None
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6 or more

11. Including you, how many people live in the same house with you?

- ◯ None
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6 or more

12. What is you annual household income in NZ dollars (before tax)?

- ◯ Under $10,000
- ◯ $10,000 - $19,999
- ◯ $20,000 - $39,999
- ◯ $40,000 - $59,999
- ◯ $60,000 - $79,999
- ◯ $80,000 - $99,999
- ◯ $100,000 plus
- ◯ I prefer not to answer

13. Please indicate your postcode or area that you live in:

14. How did you hear about this study?

- ◯ Midwife or antenatal care centre
- ◯ Email
- ◯ A friend or family member
- ◯ Flyer or poster
- ◯ Newspaper or Magazine
- ◯ Social media (eg. Facebook)
- ◯ Other

Please specify

MEDICAL AND PREGNANCY HISTORY

15. Including this pregnancy, how many times have you been pregnant?

- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6 and over

16. Was your most recent pregnancy planned?

◯ Yes ◯ No

17. Are there any particular foods that you have included in your diet since have you become pregnant?

◯ Yes ◯ No

If yes, please indicate which foods:

18. Are there any particular foods that you have excluded from your diet since you have become pregnant?

◯ Yes ◯ No

If yes, please indicate which foods:

19. Have you made any other changes to your diet since you have become pregnant?

◯ Yes ◯ No

If yes, please indicate which foods:

20. Are you vegetarian?

◯ Yes ◯ No (Go to question 22)

21. If you are vegetarian, please indicate which of the following foods you include in your diet: (You can select more than one option)

◯ Poultry

◯ Eggs

◯ Dairy

◯ Fish and/or seafood

◯ None of those

◯ Other

(please specify)

22. Are you vegan?

◯ Yes ◯ No

23. Have you suffered from morning sickness during your pregnancy?

◯ Yes ◯ No

If yes, please specify how this condition has affected your food intake? For how long and at what stage of pregnancy were you affected by this condition?

24. Have you suffered from hypertension during your pregnancy?

◯ Yes ◯ No

If yes, please specify how this condition has affected your food intake? For how long and at what stage of pregnancy were you affected by this condition?

25. Have you suffered from diabetes during your pregnancy?

◯ Yes ◯ No

If yes, please specify how this condition has affected your food intake? For how long and at what stage of pregnancy were you affected by this condition?

University of Wollongong

MASSEY UNIVERSITY

New Zealand Polyunsaturated Fatty Acid Questionnaire

INSTRUCTIONS: the following 35 questions are about your **usual eating habits over the past 3 months**.

Please fill in the date that you completed this questionnaire: _____

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26. What type of milk do you usually use?

- ○ Anchor Vital Omega 3
- ○ Other Omega-3 enriched milk. Please specify brand: _____
- ○ Whole Milk (Blue Top)
- ○ Skim / Trim / Super Trim / Calci-Trim Milk (Green or Yellow Top)
- ○ Fat Reduced / Lite Milk (Light Blue Top)
- ○ Soy
- ○ Soy Lite
- ○ None of the above

27. How much milk do you usually use per day? (Include milk added to tea, coffee, cereal etc.)

- ○ None
- ○ 1 tablespoon
- ○ 2 tablespoons
- ○ 62.5 ml (1/4 cup)
- ○ 125 ml (1/2 cup)
- ○ 250 ml (1 cup)
- ○ Between 250 and 500 ml (1-2 cups)
- ○ Between 500 and 750 ml (2-3 cups)
- ○ 750 ml (3 cups) or more

28. What type of bread do you usually eat?

- Soy and Linseed Bread
- Vogel's chia & toasted sesame (omega 3)
- Omega-3 Enriched bread. Please specify brand: _____
- Wholemeal/brown bread
- Mixed grain / Multigrain bread
- White bread
- I don't eat bread

29. How many slices of bread do you usually eat per day? (Include fresh or toasted)

- None
- 1/2 slice
- 1 slice
- 2 slices
- 3 slices
- 4 slices
- 5-7 slices
- 8 or more slices

30. What kind of spread do you usually use on bread?

- Butter
- Olive oil spread or blend (e.g. Olivani, Olivio)
- Light olive oil or Canola oil spread or blend (e.g. Olivani Light, Olivio Light, MeadowLea Light)
- Dairy spread or blend (e.g. Mainland Semi-soft, Tararua Supersoft, Anchor Countrysoft)
- Canola spread or blend (e.g. MeadowLea, Gold'n Canola, Flora Buttery Taste, Flora Canola)
- Polyunsaturated spread or blend (e.g. Flora, Rice Bran Oil Spread)
- Light polyunsaturated spread or blend (e.g. Flora Light)
- I don't use any kind of fat spread

31. How much margarine/spread do you usually use per day?

- None
- 1/2 tablespoon
- 1 tablespoon (normal serving size)
- 2 tablespoons
- 3 tablespoons
- 4 tablespoons
- 5-7 tablespoons
- 8 or more tablespoons

32. Which oil/butter/lard do you usually use per day (tick as many as apply)?

- o Butter Fat
- o Canola oil
- o Corn oil +/or cottonseed oil
- o Ghee
- o Grapeseed oil
- o Lard
- o Olive oil
- o Safflower oil
- o Sesame seed oil
- o Soybean oil
- o Sunflower oil
- o Vegetable oil
- o Rice Bran Oil

33. How much oil/butter/lard do you usually use per day?

- o None
- o 1/2 tablespoon
- o 1 tablespoon
- o 2 tablespoons
- o 3 tablespoons
- o 4 tablespoons
- o 5-7 tablespoons
- o 8 or more tablespoons

34. What kind of eggs do you usually eat?

- o Normal chicken eggs (including free range)
- o Duck eggs
- o None of the above

35. On average, how many eggs do you usually eat per week?

- o I don't eat eggs
- o Less than 1 egg per week
- o 1 to 2 eggs per week
- o 3 to 5 eggs per week
- o 6 or more eggs per week

36. On average, how often do you eat breakfast cereals?

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o 2 times per week
- o 3 to 4 times per week
- o Once per day
- o 2 or more times per day

37. When you eat cereal what size bowl would you have?

- Not Applicable
- Small bowl - 28g (½ cup)
- Medium bowl - 45g (1 cup)
- Large bowl - 60g (1½ cup)

38. On average, how often do you eat canned fish? (e.g. tuna, salmon, sardines, etc)

- Never
- Less than once per month
- 1 to 3 times per month
- Once per week
- 2 times per week
- 3 to 4 times per week
- Once per day
- 2 or more times per day

From the list below, please <u>select up to 4 types</u> of canned fish that you regularly eat, and <u>indicate whether it is a small, medium or large can</u> by choosing that option.

(Can sizes may vary, choose the closest option)

○ Tuna in oil	Small 95g	Medium 200g	Large 400g
○ Tuna in oil, drained	Small 95g	Medium 200g	Large 400g
○ Tuna, flavoured (all kinds)	Small 95g		
○ Tuna in brine	Small 95g	Medium 200g	Large 400g
○ Tuna in brine, drained	Small 95g	Medium 200g	Large 400g
○ Tuna in springwater	Small 95g	Medium 200g	Large 400g
○ Tuna, flavoured, Lite (all kinds)	Small 95g		
○ Pink salmon in brine	Small 95g	Medium 200g	Large 400g
○ Pink salmon in brine, drained	Small 95g	Medium 200g	Large 400g
○ Red salmon in brine	Small 95g	Medium 200g	Large 400g
○ Red salmon in brine, drained	Small 95g	Medium 200g	Large 400g
○ Salmon, flavoured (all kinds)	Small 95g		
○ Herring	Small 95g	Medium 200g	Large 400g
○ Sardines	Small 95g	Medium 200g	Large 400g
○ Mackerel	Small 95g	Medium 200g	Large 400g
○ Anchovies	Small 95g	Medium 200g	Large 400g

39. On average, how often do you eat fresh or frozen fish? (Include fish meals, takeaway fish and sushi)

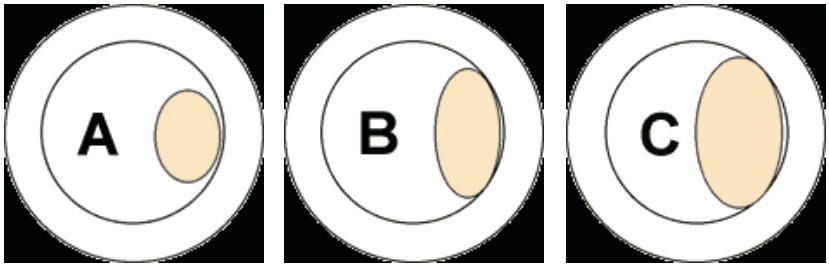
- Never
- Less than once per month
- 1 to 3 times per month
- Once per week
- 2 times per week
- 3 to 4 times per week
- Once per day
- 2 or more times per day

From the list below, please <u>select up to 4 types</u> of fresh or frozen fish that you regularly eat:

- Hoki fish
- Fish Fingers
- Take away (e.g. fish and chips)
- John Dory
- Shark / Lemon Fish
- Salmon
- Sea Perch / White Perch
- Snapper
- Swordfish
- Tuna
- Flounder
- Warehou
- Gurnard
- Terakihi
- Trevally
- Kahawai
- Other type of fish: _____

When you eat fish, <u>what size serving</u> do you usually eat?
Please select from one of the dinner plates which show different sizes of the fish serves.

- Less than A
- A
- Between A and B
- B
- Between B and C
- C
- More than C
- Not Applicable



40. On average, how often do you eat fresh, frozen or canned shellfish?

- ○ Never
- ○ Less than once per month
- ○ 1 to 3 times per month
- ○ Once per week
- ○ 2 times per week
- ○ 3 to 4 times per week
- ○ Once per day
- ○ 2 or more times per day

From the list below, please <u>select up to 4 types</u> of shellfish that you regularly eat and <u>indicate the amount eaten</u> in a single meal (e.g. numbers of pieces)

- ○ Calamari / Squid — Number of pieces eaten: _____
- ○ Crab / Seafood stick / Surimi — Number of pieces eaten: _____
- ○ Lobster / Crayfish — Number of pieces eaten: _____
- ○ Mussels — Number of pieces eaten: _____
- ○ Oysters — Number of pieces eaten: _____
- ○ Prawns / Shrimps — Number of pieces eaten: _____
- ○ Roe / Kina — Number of tablespoons eaten: _____
- ○ Scallops — Number of pieces eaten: _____

41. On average, how often do you eat fish paste?

- ○ Never
- ○ Less than once per month
- ○ 1 to 3 times per month
- ○ Once per week
- ○ 2 times per week
- ○ 3 to 4 times per week
- ○ Once per day
- ○ 2 or more times per day

If you eat fish paste, what serving size would you normally eat?

- ○ 1 teaspoon (approx. 5 g)
- ○ 2 teaspoons (approx. 10 g)
- ○ 1 tablespoon (approx 20 g)
- ○ 2 tablespoons (approx 40 g)
- ○ More than 2 tablespoons

42. Do you take any fish oil capsules?

- o No
- o Yes
- o If yes, do you take them:
- o 2 or more times per day
- o Once per day
- o 3 to 4 times per week
- o 2 times per week
- o Once per week
- o 1 to 3 times per month
- o Less than once per month

When you take them, how many do you consume at a time:

- o 1
- o 2
- o 3
- o 4
- o 5 or more

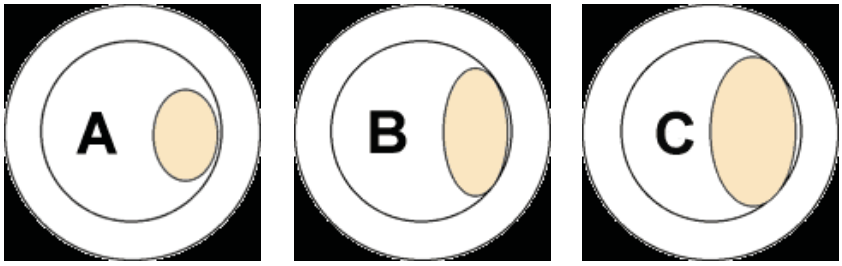
Please specify which brand of fish oil capsules you take from the list below. If your brand is not listed, please select other and specify brand. If you are unsure, please select 'other'.

- o Blackmores Fish Oil 1000mg
- o Blackmores Evening Primrose Oil + Fish Oil
- o Blackmores Omega-3 Daily Concentrated
- o Blackmores Omega Joint
- o Blackmores Omega Heart
- o Blackmores Omega Brain
- o Blackmores Flaxseed Oil
- o Natures Own Omega-3 Fish Oil + Gingko
- o Natures Own Omega-3 Fish Oil + Gingko 300
- o Nature's Own Omega-3 Fish Oil
- o Thompsons Omega Combination
- o Thompsons Flaxseed Oil
- o Thompsons Fish Oil
- o Healtheries Fish Oil 1000
- o Healtheries Flaxseed Oil
- o Fish Oil capsules (Don't know the brand)
- o Other (please specify brand):_____

43. When you eat meat or chicken, what serving size do you usually eat?

Please select from one of the dinner plates which show different sizes of the cuts of meat.

- o Less than A
- o A
- o Between A and B
- o B
- o Between B and C
- o C
- o More than C
- o Not Applicable



On average, how often do you eat the following meats or meat products?

	Not at all	Less than once per month	1-3 times per month	Once per week	2 times per week	3-4 times per week	Once per day	2 or more times per day
Chicken	o	o	o	o	o	o	o	o
Beef	o	o	o	o	o	o	o	o
Lamb	o	o	o	o	o	o	o	o
Veal	o	o	o	o	o	o	o	o
Pork[a]	o	o	o	o	o	o	o	o
Bacon	o	o	o	o	o	o	o	o
Ham	o	o	o	o	o	o	o	o
Deli Meats[b]	o	o	o	o	o	o	o	o
Sausages	o	o	o	o	o	o	o	o

a - Not including bacon or ham

b - Deli meats such as mortadella, pancetta, salami, pepperoni or other luncheon meats

44. If you eat chicken, please specify the type of chicken most often eaten:

- o Chicken breast without skin
- o Chicken thigh without skin
- o Chicken wing without skin
- o Chicken leg without skin
- o Chicken drumstick without skin
- o Other chicken part without skin
- o Chicken breast with skin
- o Chicken thigh with skin
- o Chicken wing with skin
- o Chicken leg with skin
- o Chicken drumstick with skin
- o Other chicken part with skinRoast whole chicken without skin
- o Roast whole chicken with skin

45. If you eat beef, please specify the type of beef most often eaten:

- o Beef (unspecified type)
- o Beef steak
- o Beef blade
- o Beef ribeye/sirloin
- o Beef rump
- o Beef fillet
- o Beef brisket
- o Beef silverside/corned beef
- o Beef mince
- o Beef topside/round
- o Beef round steak
- o Beef roast

46. If you eat lamb, please specify the type of lamb most often eaten:

- o Lamb (unspecified type)
- o Lamb cutlet/chop
- o Lamb forequarter chop
- o Lamb chump chop
- o Lamb leg chop
- o Lamb shank
- o Lamb shoulder
- o Lamb leg roast
- o Lamb mince
- o Lamb steak
- o Lamb roast

47. If you eat veal, please specify the type of veal most often eaten:

- o Veal (unspecified type)
- o Veal steak
- o Veal chop
- o Veal mince
- o Veal stewing cuts
- o Veal leg
- o Veal schnitzel

48. If you eat pork, please specify the type of pork most often eaten:

- o Pork (unspecified type)
- o Pork chop/forequarter
- o Pork steak
- o Pork leg
- o Pork fillet
- o Pork loin
- o Pork shoulder
- o Pork mince
- o Pork spare ribs
- o Pork crackling
- o Pork roast

49. On average, how often do you eat bacon?

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o 2 times per week
- o 3 to 4 times per week
- o Once per day
- o 2 or more times per day

If you eat bacon, how many rashers of bacon do you normally eat on one occasion:_____

(1 rasher = 55g)

50. On average, how often do you eat ham?

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o 2 times per week
- o 3 to 4 times per week
- o Once per day
- o 2 or more times per day

If you eat ham, how many slices of ham do you normally eat on one occasion:_____

(1 slice = 20g)

51. On average, how often do you eat luncheon meats or salami?

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o 2 times per week
- o 3 to 4 times per week
- o Once per day
- o 2 or more times per day

If you eat luncheon meats, please specify the type most often eaten:

- o Luncheon meat
- o Mortadella
- o Pancetta
- o Salami / Pepperoni

How many slices do you normally eat on one occasion:_____

52. On average, how often do you eat sausages?

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o 2 times per week
- o 3 to 4 times per week
- o Once per day
- o 2 or more times per day

If you eat sausages, please specify the type and the number of sausages you would normally eat on any one occasion:

- o Beef sausages Number usually eaten:_____
- o Pork sausages Number usually eaten:_____
- o Lamb sausages Number usually eaten:_____
- o Chicken sausages Number usually eaten:_____
- o Frankfurter / hotdog Number usually eaten:_____
- o Vegetarian sausages Number usually eaten:_____
- o Other sausages Number usually eaten:_____

53. Are there other meats or fish products that you eat regularly? (More than once a month)

	Not at all	Once per month	1-3 times per month	Once per week	2 times per week	3-4 times per week	Once per day	2 or more times per day
Turkey	o	o	o	o	o	o	o	o
Duck	o	o	o	o	o	o	o	o
Venison	o	o	o	o	o	o	o	o
Other	o	o	o	o	o	o	o	o

If 'other' please specify: _____

54. How often do you consume any of the vegetables listed below?

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o 2 times per week
- o 3 to 4 times per week
- o Once per day
- o 2 or more times per day

Please specify up to 4 types of vegetables that you regularly eat and the size of the serve:

o	Broccoli	Small 22g	Medium 44g	Large 66g
o	Brussels sprouts	Small 22g	Medium 44g	Large 66g
o	Avocado	1 slice (15g); 2 slices (30g); quarter (60g); Half (120g); Whole (240g)		
o	Spinach	Small 10g	Medium 30g	Large 50g
o	Tofu / Bean curd	Small 60g	Medium 90g	Large 120g
o	Corn	Small ear (80g or ½ cup)		Large ear (160g or 1 cup)

- o Other (please specify): _____

Small Medium Large

55. Do you consume any peanut butter? If yes, please indicate your typical serving size.

- o None
- o 1 teaspoon
- o 1/2 tablespoon
- o 1 tablespoon (normal serving size)
- o 2 tablespoons
- o 3 tablespoons
- o 4 tablespoons
- o 5-7 tablespoons
- o 8 or more tablespoons

If yes, how often do you consume peanut butter?

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o 2 times per week
- o 3 to 4 times per week
- o Once per day
- o 2 or more times per day

56. What type of nuts and seed products do you usually eat?

Please indicate how often you consume each type of nut, where one handful is equivalent to a small packet or ¼ of a cup.

	Handfuls per month			Handfuls per week				Handfuls per day		
	none	1	2-3	1	2	3-4	5-6	1	2	3 +
Almonds	o	o	o	o	o	o	o	o	o	o
Brazil nuts	o	o	o	o	o	o	o	o	o	o
Cashews	o	o	o	o	o	o	o	o	o	o
Hazelnuts	o	o	o	o	o	o	o	o	o	o
Macadamia	o	o	o	o	o	o	o	o	o	o
Mixed nuts	o	o	o	o	o	o	o	o	o	o
Pecans	o	o	o	o	o	o	o	o	o	o
Peanuts	o	o	o	o	o	o	o	o	o	o
Pine nuts	o	o	o	o	o	o	o	o	o	o
Sesame seeds	o	o	o	o	o	o	o	o	o	o
Sunflower seeds	o	o	o	o	o	o	o	o	o	o
Walnuts	o	o	o	o	o	o	o	o	o	o

57. What type of take-away food do you usually eat (other than fish)?

Please indicate the amount you would normally eat during a single meal (number of slices/pieces) **AND** how often you consume these foods .

	How much of these foods would you normally eat during one meal? Please indicate the number of slices, pieces or serves		**How often do you eat these foods?** Please indicate how many times per month or week or day you eat these foods						
			Times per month:		**Times per week:**			**Times per day:**	
Food types		**Number**	**<1**	**1-3**	**1**	**2**	**3-4**	**1**	**2 +**
Pizza	(1 slice = 79g)	___	o	o	o	o	o	o	o
BBQ chicken	(1 piece = 93g)	___	o	o	o	o	o	o	o
Hamburgers	(1 serve = 303g)	___	o	o	o	o	o	o	o
Hot chips	(1 cup = 113g)	___	o	o	o	o	o	o	o
Meat pies	(1 serve = 250g)	___	o	o	o	o	o	o	o
Sausage rolls	(1 piece = 140g)	___	o	o	o	o	o	o	o
Chiko rolls	(1 piece = 140g)	___	o	o	o	o	o	o	o
Dim sims- steamed	(1 piece = 50g)	___	o	o	o	o	o	o	o
Dim sims- fried	(1 piece = 50g)	___	o	o	o	o	o	o	o
Pasties (Cornish)	(1 piece = 150g)	___	o	o	o	o	o	o	o
Garlic bread	(1 piece = 25g)	___	o	o	o	o	o	o	o
Subway	(1 serve = 230g)	___	o	o	o	o	o	o	o
Kebabs (meat)	(1 piece = 212g)	___	o	o	o	o	o	o	o
Sushi	(1 piece = 26g)	___	o	o	o	o	o	o	o
Thai	(1 serve = 190g)	___	o	o	o	o	o	o	o
Chinese	(1 serve = 190g)	___	o	o	o	o	o	o	o
Spring rolls	(1 piece = 64g)	___	o	o	o	o	o	o	o
Pad thai	(1 serve = 190g)	___	o	o	o	o	o	o	o
Quiche	(1 piece = 100g)	___	o	o	o	o	o	o	o

o Other (please specify): _____

58. How often do you eat pasta?

- o Never
- o Less than once per month
- o 1 to 3 times per month
- o Once per week
- o 2 times per week
- o 3 to 4 times per week
- o Once per day
- o 2 or more times per day

What type of pasta and what sized serve do you usually eat?

o	Cannelloni	Small 90g	Medium 175g	Large 260g
o	Spagetti Bolognese	Small 90g	Medium 175g	Large 260g
o	Lasagne	Small 90g	Medium 175g	Large 260g
o	White dry pasta	Small 90g	Medium 175g	Large 260g
o	Ravioli	Small 90g	Medium 175g	Large 260g
o	Noodles	Small 90g	Medium 175g	Large 260g
o	Other _____	Small 90g	Medium 175g	Large 260g

59. Please specify <u>up to five</u> types of snacks or desserts that you usually eat, indicate the <u>amount</u> you would normally eat on a single occasion (e.g number of pieces of chocolates, number of packet of crisps, etc) and <u>how often</u> you eat them

	How much of these foods would you normally eat during one meal?		**How often do you eat these foods?** Please indicate how many times per month or week or day you eat these foods						
	Please indicate the number of slices, pieces or serves		**Times per month:**		**Times per week:**			**Times per day:**	
Food types		**Number**	**<1**	**1-3**	**1**	**2**	**3-4**	**1**	**2 +**
Biscuits	(1 piece = 22g)	___	o	o	o	o	o	o	o
Cake	(1 slice = 39g)	___	o	o	o	o	o	o	o
Chocolate	(1 piece = 39g)	___	o	o	o	o	o	o	o
Ice cream	(1 scoop = 84g)	___	o	o	o	o	o	o	o
Pastries (ie Danish)	(1 piece = 156g)	___	o	o	o	o	o	o	o
Custard	(1 cup = 120g)	___	o	o	o	o	o	o	o
Potato Crisps	(1 packet = 22g)	___	o	o	o	o	o	o	o
Fruit pies	(1 piece = 100g)	___	o	o	o	o	o	o	o
Cocoa powder	(1 spoon = 25g)	___	o	o	o	o	o	o	o
Cracker/crispbread	(1 piece = 16g)	___	o	o	o	o	o	o	o
Other:_____	(_____g)	___	o	o	o	o	o	o	o

60. Do you consume any other Omega-3 fortified products?

Please provide the details in the space provided (type of food, amount eaten, how often eaten). If not, please leave blank.

Thanks for taking part in the study!

APPENDIX 8 - Email acknowledging participation in the study

Dear Mum,

Thanks for taking part in the study!

If you know anyone else who would be interested in participating, please forward the survey link on: www.surveymonkey.com/s/pufa.

Please get your *Eating for Healthy Pregnant Women* leaflet copy by clicking here. If you have any concerns about health and diet during pregnancy please consult your Midwife, Medical Practitioner or Dietitian.

You have been included in the draw to WIN one of five parenting books "If Only They'd Told Me: Babies, Sex and a Cup of Tea". WE will announce the winners at the start of 2015. We will also send you a copy of the summary of findings once this research is completed.

A summary of the findings will also be available on our website around the end of February 2015. Please feel free to bookmark this page (www.Massey.ac.nz/pufa) and visit it for updates on the study.

Alternatively you can contact our research team to request hard copies of the *Eating for Healthy Pregnant Women* leaflet and summary of findings. Our contacts are:

Research Team Contact		
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We wish you all the best for you and your baby!

Kind Regards,

The Research Team

APPENDIX 9 - Eating for Health Pregnant Women leaflet

NGĀ KAI TOTIKA MĀ TE WAHINE HAPŪ

Eating for Healthy Pregnant Women

Eating well and doing moderate physical activity during pregnancy are important for you and your baby. Nutritional needs are higher when you are pregnant. Meeting these needs helps protect the long-term health of both you and your baby.

Seek antenatal (pre-birth) care as soon as you think that you are pregnant.

Some pregnant women may need special advice from a dietitian about eating. This includes women who:

- are 18 years old or younger
- have a medical condition affecting their eating, such as diabetes
- are having more than one baby (eg, twins or triplets)
- eat very little or have a history of eating problems
- are vegetarian or vegan
- are very obese
- are underweight.

If you think you should see a dietitian, ask your lead maternity carer (LMC, eg, your midwife, doctor or obstetrician) to arrange this for you.

2

Food for a Healthy Mother and Baby

Eat a variety of healthy foods every day from each of the four main food groups below:

1. vegetables and fruit
2. breads and cereals (wholegrain is best)
3. milk and milk products (reduced- or low-fat milk is best)
4. lean meat, chicken, seafood, eggs, legumes, nuts and seeds.

- Limit your intake of foods and drinks that are high in fat (especially saturated fat), salt and/or sugar (see the section on page 9).
- If using salt, choose iodised salt.
- Take care when buying, preparing, cooking and storing food so that the food is as safe as possible to eat. Follow the food safety guidelines in the section Food Safety in Pregnancy on page 11.
- Drink plenty of fluids each day, especially water and reduced - or low-fat milk.
- Avoid alcohol during pregnancy.
- Aim for a healthy weight gain by eating well and being physically active each day (unless advised not to be physically active).

Traditional Māori and Pacific foods can be healthy choices.

Eat a Variety of Foods

You need a variety of healthy foods from the four food groups every day to provide for your growing baby as well as to maintain your own health.

1. Vegetables and Fruit

Vegetables and fruit provide carbohydrates (sugar and starch), fibre, vitamins and minerals and are low in fat.

- Eat plenty of vegetables and fruit.
- Enjoy fresh, well-washed vegetables and fruit or frozen or canned varieties. Steaming or microwaving vegetables is best. Go easy on butter or margarine.
- Include vegetables and fruit in a variety of colours.
- Limit juice and dried fruit intake because these foods have a high sugar content.

*Eat **at least six** servings per day of vegetables and fruit – **at least four** servings of vegetables and **two** servings of fruit. If you do choose juice or dried fruit, have no more than **one** serving per day.*

Serving size examples

Vegetables

- 1 medium piece of potato, kumara, pumpkin, carrot, taro, kamokamo or yam (135 g)
- ½ cup cooked vegetables, eg, puha, watercress, silverbeet, taro leaves, bok choy, Chinese cabbage, broccoli, cabbage, corn or peas (50–80 g)
- ½ cup salad or bean sprouts (60 g)
- 1 tomato (80 g)

Fruit

- 1 apple, pear, banana or orange (130 g)
- 2 small apricots or plums (100 g)
- ½ cup fresh fruit pieces, eg, pineapple or mango (120 g)
- ½ cup stewed fruit (135 g)
- 1 cup fruit juice (250 g)
- 25 g dried fruit, eg, 2 tablespoons of raisins or 3 dates

2. Breads and Cereals

These provide carbohydrates (sugar and starch), fibre, and nutrients such as B vitamins and minerals.

- Eat plenty of breads and cereals, including rice, pasta, breakfast cereals and other grain products.
- Choose wholegrain varieties because they provide extra nutrients and fibre. They also help prevent constipation.
- Choose bread that has had folic acid added to it – this should be written on the label.

*Choose **at least six** servings of breads and cereals each day.*

Serving size examples

- 1 roll (50 g)
- 1 muffin (80 g)
- 1 medium slice rewena bread (30 g)
- 1 medium slice bread (25 g)
- 1 cup cornflakes (30 g)
- ½ cup muesli (55 g)
- ½ cup cooked cereal, eg, porridge (130 g)
- 1 cup cooked pasta (150 g)
- 1 cup cooked rice (150 g)
- 1 cup cassava, sago or tapioca (150 g)
- 2 plain sweet biscuits (14 g)

5

3. Milk and Milk Products

Pregnant women need milk and milk products as sources of protein, vitamins and minerals, especially calcium and iodine.

- Choose reduced - or low-fat milk, yoghurt and hard cheese.
- Milk and milk products provide New Zealanders with most of their calcium. If you do not eat these foods or eat very little of them, ask your LMC about other calcium sources.
- Calcium is also found, in lower amounts, in foods such as wholegrain bread, broccoli, canned salmon, sardines, spinach, baked beans and tofu.
- If you are drinking non-dairy milks, eg, soy or rice milk, choose one that is calcium-fortified (check the label).
- If you follow a vegan diet, you will need to check that your non-dairy milk has vitamin B12 in it.

*Have **at least three** servings each day of milk or milk products, preferably reduced- or low-fat products.*

Serving size examples

- 1 large glass milk (250 mL)
- 1 pottle yoghurt (150 g)
- 2 slices hard cheese (40 g)
- 1 large glass calcium-fortified soy milk (250 mL)

4. Lean Meats, Chicken, Seafood, Eggs, Cooked Dried Beans, Peas and Lentils, and Nuts and Seeds

These foods give you protein, iron, zinc and other nutrients.

- Your body needs more iron and zinc during pregnancy.
- Iron is important for healthy blood and for the development of your baby. During pregnancy, it is important to have a good iron intake to help prevent iron deficiency.
- Iron in lean meats, chicken and seafood is absorbed well by the body. Eggs, cooked dried beans, peas and lentils, and nuts and seeds also contain iron, but the iron is not as easily absorbed.
- Include foods rich in vitamin C with your meals to help absorb iron. Fresh vegetables and fruit, especially taro leaves (cooked), broccoli, tomatoes, oranges, kiwifruit, mangoes and pineapple, are rich sources of vitamin C. This is especially important for vegetarian and vegan women, who may find it hard to get enough iron.
- Liver is a good source of iron, but eat no more than a small piece (100 g) once a week.
- Make sure that vegetables, fruit, meat, chicken and seafood are fresh and that cooked food is cooked well, served hot and eaten immediately after cooking (see the Food Safety in Pregnancy section, page 11).
- Seafood and eggs are also useful sources of iodine (see the Iodine and Iodine Deficiency section on page 17).
- Fish is recommended because it is an important source of long-chain polyunsaturated fatty acids.

Serving size examples

- 2 slices cooked meat (about 100 g), eg, beef, pork or lamb
- ¾ cup mince or casserole (195 g)
- 1 medium steak (120 g)
- 2 drumsticks or 1 chicken leg (110 g)
- 1 medium piece of cooked fish* (100 g), eg, warehou or eel
- 1 medium, freshly cooked pāua (120 g)
- small can of canned fish, eg, skipjack or albacore tuna, sardines, salmon or mackerel (90 g)
- 8 medium, freshly cooked mussels (80 g)
- 1 egg (50 g)
- ¾ cup canned or cooked dried beans, eg, bean salad or lentil dish (135 g)
- ⅓ cup nuts or seeds
- ¾ cup tofu

* See the mercury and fish information below.

*Choose **at least two** servings from this group each day.*

Food safety when choosing fish and seafood

- High intakes of mercury are unsafe for your baby. Some fish have higher levels of mercury, although there is little concern with canned tuna (check that it is skipjack or albacore tuna), canned salmon, mackerel or sardines, farmed salmon, tarakihi, blue cod, hoki, john dory, monkfish, warehou, whitebait and flat fish like flounder.
- Some longer-lived and larger fish (eg, uncanned wild-caught [not farmed] salmon, uncanned albacore tuna or mackerel, as well as kahawai, red cod, orange roughy and ling) can contain more mercury, so consumption of these should be limited to three 150 g servings per week.
- A small number of fish (eg, school shark, southern bluefin tuna, marlin and trout from geothermal regions and Lake Rotomahana) should be eaten only once a fortnight – or not at all if consuming other types of fish or seafood.
- The eating of Bluff and Pacific oysters and queen scallops needs to be limited because of their high cadmium concentrations.
- Mercury levels in fish are actively monitored by the Ministry of Primary Industries (MPI Food Safety). Over time, with new findings, the recommendations regarding mercury may change. For the most up-to-date information, check the MPI Food Safety website at www.foodsmart.govt.nz. Alternatively, contact MPI Food Safety (freephone 0800 693 721) or your health practitioner for more information.

Drink Plenty of Fluids Every Day

Use your thirst as a guide. Aim for nine cups of fluid each day.

Extra fluid may be needed during hot weather, after activity, or if you are vomiting or constipated.

Water or reduced - or low-fat milk are the best choices.

There is evidence that caffeine consumption may affect your baby's growth during pregnancy. Caffeine is naturally occurring in tea, coffee and chocolate and is present in many cola-type drinks. Limit your consumption of caffeinated drinks while pregnant. Have no more than six cups of tea or instant coffee (or three 'single' espresso-type coffees or one 'double' espresso-type coffee) each day.

Be cautious about drinking herbal teas. Discuss this with your LMC.

Avoid drinking tea with meals. The tannins in tea mean you will not absorb the iron in the meal as well as you could.

Limit soft drinks, flavoured waters, fruit drinks, cordials and diet drinks because these are low in nutrients and may be high in sugar. Energy drinks and energy shots are not recommended because they may contain high levels of caffeine and other ingredients not recommended for pregnant women.

Choose and Prepare Foods Low in Fat, Salt and Sugar

The best way to meet your extra needs is to choose foods from the four food groups. These are good sources of fibre, vitamins and minerals.

When shopping, read labels and look for foods that are lower in fat (especially in saturated fat), salt and sugar. If using salt, choose iodised salt.

To cut down on your intake of fat (especially saturated fat), salt and sugar:

- choose polyunsaturated or monounsaturated margarine or lower fat table spreads (fortified with vitamin D) rather than butter or dripping, and spread margarine thinly
- choose foods rich in polyunsaturated fat and omega-3, including green leafy vegetables, nuts and seeds, oily fish (canned tuna, sardines, salmon, mackerel; fresh warehou, eel), and oils (soybean, canola, flaxseed and walnut oils)

- choose lean meats; trim off any fat, remove skin from chicken before or after cooking, skim fat off stews or off the top of boil-ups and eat more grilled, boiled or steamed fish
- reduce intake of sausages or processed meats, which can be high in fat and salt; if eating these foods, grill rather than fry them and always heat until piping hot – then serve them hot to reduce the risk of illness such as listeria (see the Food Safety in Pregnancy section on page 11)
- when cooking, choose to grill, steam, microwave, boil or bake foods, without adding fat
- eat meals without adding extra salt
- choose foods with no added sugar.

Many fast foods, takeaways and processed snacks are high in fat, salt and/or sugar. These include foods such as fish and chips, fried chicken, hamburgers, pies, chocolate bars, muesli bars, chippies, lollies, fruit leathers, cordials and soft/fizzy drinks. Limit intake of these foods and drinks. Only consider eating foods such as fried chicken, hamburgers and pies if they have just been made, are well cooked and are served piping hot (see the Food Safety in Pregnancy section, page 11).

Eat and Keep Active for a Steady Weight Gain

A healthy weight gain during pregnancy is best for you and your baby.

It's normal to gain some weight during pregnancy due to the growth of the baby, placenta and amniotic fluid. However, gaining too much extra weight can increase your chances of:

- high blood pressure in pregnancy (pre-eclampsia)
- diabetes during pregnancy (gestational diabetes)
  needing a caesarean section
- having a large baby. This increases their risk of becoming obese in childhood and early adult life
- difficulty losing weight after your baby is born. This may increase your risk of developing diabetes, heart disease and some cancers later in life.

Not gaining enough weight during pregnancy can increase the changes of having a premature (preterm) birth, or a small for age baby.

Talk to your LMC about what a healthy weight gain during pregnancy is for you.

In the first 12 weeks of pregnancy, you don't need to eat any more food than you would usually eat when not pregnant, but it is important that you eat nutritious food. If you are of normal weight, the total amount of extra food you need each day after the twelfth week of your pregnancy is about the same energy value as a wholegrain cheese and tomato sandwich or a wholegrain peanut butter sandwich and a banana. If you were obese before pregnancy, the extra energy you require is about one slice of wholegrain bread or two apples per day.

Dieting to lose weight during pregnancy is not recommended because it may result in a smaller and less healthy baby and it could also affect your health.

Keeping active is important.

Being physically active each day can help you avoid putting on excess weight, strengthen your heart and lungs and give you the extra energy and strength needed for the birth. Unless your LMC advises otherwise, aim for at least 30 minutes of moderate physical activity on most, if not all, days of the week.

Choose activities you enjoy that match your level of fitness. Suitable activities include brisk walking, swimming, aqua-jogging or any activity that is comfortable for you and leaves you with enough breath to hold a conversation.

Wear suitable clothes when being physically active, for example, a good support bra, loose clothing and supportive footwear. Take breaks for a drink, food or a rest if you need to.

Contact sports and vigorous physical activity are not recommended. Avoid physical activity in extremely hot weather. Don't start a new sport during pregnancy.

You may need more rest. Listen to your body. If you are tired, rest.

Food Safety in Pregnancy

In pregnancy, your immunity is lower, so you and your unborn baby are more susceptible than usual to the kinds of food-borne illnesses that affect everyone. Bacteria such as listeria, salmonella and campylobacter and pathogens such as toxoplasma can cause food-borne illness. When you are pregnant, this can cause infection in you and your baby and miscarriage and stillbirth in extreme cases.

Following some simple food safety steps, including avoiding some foods when you are pregnant, can prevent most food-borne illness and keep you and your baby healthy.

To keep food safe, all foods should be safely handled, stored and protected from cross-contamination. For example, bacteria from raw chicken can contaminate cooked chicken if the same chopping board is used for both.

To keep food safe:

- keep cooked and ready-to-eat foods separate from raw and unprocessed foods so that there is no cross-contamination
- wash your hands, utensils and chopping boards between preparing raw and ready-to-eat foods, to avoid cross-contamination
- cook food thoroughly, especially meat, which should be cooked till the juices run clear
- eat freshly cooked food as soon as possible after cooking or put it in the fridge as soon as it has stopped steaming
- eat canned food immediately after opening the can or transfer the food immediately to a covered, non-metal container and refrigerate
- use cooked, prepared and canned food stored in the fridge **within two days**

- reheat cooked food thoroughly so that it is piping hot, that is, above 70°C, and do not reheat more than once (take special care to heat food thoroughly and evenly when using a microwave oven by stirring frequently).
- wash and dry whole raw fruit, vegetables and herbs thoroughly
- don't eat food that is past its use-by date
- clean the fridge regularly and check that the temperature is between 2 and 4°C
- ideally, consume milk and milk products within two days of opening, particularly cream, ready-made custard and yoghurt. Don't drink or eat raw (unpasturised) milk or cheese.
- you can eat cottage cheese and cream cheese if they are bought in sealed packs and consumed cold or cooked within two days of opening
- avoid prepared ready-to-eat foods such as those bought from a supermarket deli or restaurant buffet unless they are heated until piping hot
- don't eat prepared ready-to-eat foods such as shop-bought sandwiches when you aren't certain of product age, storage conditions or staff food handling.

There are a number of foods that are considered high risk with regard to listeria and other bacterial contamination.

During pregnancy, do not eat any of the following foods:

- processed meats* such as pâté, salami, ham and luncheon
- cold pre-cooked meat* such as chicken (plain or smoked) and corned beef
- raw (unpasteurised) milk and raw milk products
- soft pasteurised cheese* (ie, brie, camembert, feta, blue, mozzarella and ricotta)
- pre-prepared or unrefrigerated salads, including rice or pasta salad, coleslaw, roasted vegetable and green salads
- hummus and other dips containing tahini
- raw, smoked* or pre-cooked fish* or seafood*, including sushi, smoked salmon, marinated mussels or oysters
- foods containing raw egg, eg, smoothies, mayonnaise, hollandaise sauce or desserts such as mousse
- soft-serve ice cream
- cream or custard, especially in pre-made cakes or pastries (unless home-made or pre-packaged and eaten within two days of opening).

* Note that these foods are safe to eat if heated thoroughly until piping hot, that is, above 70°C.

For more information and the most up-to-date list of high-risk foods to avoid, consult the MPI Food Safety resource *Food Safety in Pregnancy*. This can be viewed at www.foodsmart.govt.nz. Alternatively, contact MPI Food Safety freephone 0800 693 721 or your LMC for more information.

Snack Ideas

- **Sandwiches:** Use a variety of fillings such as banana, yeast extract spread, hard cheese, baked beans, jam or peanut butter. Try different bases, for example, wholegrain bread rolls, rewena bread, crackers, rice cakes, crumpets, pita bread, muffins and baked bread fingers.
- **Vegetable sticks:** Keep these in the fridge. Serve with plain unsweetened yoghurt or peanut butter.
- **Fruit:** Try fresh, canned (unsweetened), frozen or dried fruit, served whole, cut up with yoghurt or in an egg-free smoothie.
- **Cereals:** Choose cereals that are low in fat and sugar, for example, porridge, untoasted muesli, cornflakes, bran flakes and wheat biscuits.
- **Popcorn:** Pop using a little oil or margarine or use a microwave. Go easy on the salt.
- **Reduced- or low-fat milk products:** Try yoghurt, cubes of hard cheese, reduced or low-fat milk and milk puddings (eg, creamed rice). Remember to eat pre-packaged items within two days of opening.

Lunch Ideas

Base your lunch on breads or cereals:

- wholegrain bread/toast/roll
- wholegrain toasted sandwich
- pita, focaccia or Turkish bread
- pizza base
- rice or pasta
- rewena bread
- crackers
- panini
- crumpets, muffins or fruit bread

... or try a microwaved baked potato.

Add a filling, topping or spread:

- canned baked beans, corn or spaghetti
- hard cheese
- yeast extract spread, jam, honey or peanut butter
- hard-boiled egg
- banana
- canned fish, such as tuna, sardines, salmon or mackerel (freshly opened)

... or try a pre-prepared frozen meal or pizza, served piping hot.

Add an accompaniment:

- soup, either home-made, canned or made from a mix
- yoghurt
- glass of reduced- or low-fat milk
- freshly made salad or stir-fried vegetables
- vege sticks (eg, baby carrots or tomato)

... or try an egg-free fruit smoothie.

Finish with fruit:

- fresh
- canned
- frozen
- dried.

Buying Your Lunch

When buying your lunch, choose healthy and safe options, such as:

- hot soup and toast
- hot savoury foods, for example, pizza, baked potatoes, rice and pasta dishes. These foods should be heated until they are piping hot (ie, 70°C).
- freshly made, hot toasted sandwiches
- a savoury muffin or scone
- yoghurt
- fruit
- egg-free fruit smoothie, freshly made.

Remember ...

Avoid high-risk foods. Follow the food safety advice provided on pages 11–12.

Eat Well to Cope with Pregnancy Symptoms

Nausea and vomiting are common during early pregnancy, and this is often the first sign of being pregnant. This is referred to as 'morning sickness', but it may occur at any time of the day or night, especially when you are tired or hungry.

Eat as well as you can. Your extra nutrition needs are small during early pregnancy, so nausea and vomiting rarely cause any nutritional problems. However, if your vomiting is severe and you are unable to keep any food or fluids down, seek advice from your LMC.

- Eat regularly, choosing smaller meals or snacks.
- Have fewer high-fat and spicy foods.
- Try a carbohydrate snack (such as a slice of dry toast, a cracker or fruit) before getting out of bed in the morning.

- Drink small sips of flat lemonade or ginger ale.
- Try ginger or foods flavoured with ginger.
- Give yourself extra time in the morning. Rushing can make you feel worse.
- Try to rest more.

Indigestion and Heartburn

These are common towards the end of pregnancy.

- Eat regularly, choosing smaller meals or snacks.
- Have fewer high-fat and spicy foods.
- Avoid drinking fluids with meals.
- If a certain food upsets you, leave it for the time being.
- Avoid lying down straight after a meal.
- Going for a walk may help.
- Raise the head of the bed or use extra pillows.
- Check with your LMC before taking antacids.

Alcohol

Alcohol is not recommended.

Your baby is sensitive to alcohol. The full effects of alcohol on your baby are unknown.

Alcohol, even in small amounts, will enter the baby's bloodstream, so whatever the mother drinks, the baby is having too. Alcohol could affect the development of your baby, especially of his/her brain.

Smoking

Being smokefree is recommended.

Smoking reduces the oxygen and food supplies to the baby and can slow down the baby's growth and development.

Avoid smoky environments. Second-hand smoking (inhaling other people's smoke) has the same effect as smoking.

If you smoke during your pregnancy, your baby is more likely to be born prematurely or be underweight. A small baby does not mean an easier birth.

If you want to quit smoking, seek advice from your LMC.

Medication

Seek advice about taking medication

Use medication only as advised by your LMC because they know which medications are safe for you and your baby.

Taking any other sort of drugs, for example, illicit drugs or party pills, is not recommended because these can affect the baby's growth and development.

Folic Acid

Folic acid is a vitamin that is needed for the formation of blood cells and new tissue. During pregnancy, your need for folic acid is higher. Lack of folic acid has been linked with neural tube birth defects (NTDs) such as spina bifida. The risk of having a child with these birth defects is low and can be reduced by taking a folic acid tablet.

- ***Take a folic acid tablet*** (0.8 mg) daily for four weeks (one month) before you might become pregnant through to 12 weeks (three months) after actually becoming pregnant. If you find out that you are pregnant and you haven't been taking a folic acid tablet, start taking tablets straight away and continue until the 12th week of your pregnancy.
- A higher dose folic acid tablet is also available for women with a higher risk of NTD pregnancy. Talk to your LMC about which folic acid tablet is best for you.
- Choose foods naturally high in folate or fortified with folic acid, such as:
  - well-washed, fresh, raw or lightly cooked vegetables
  - raw fruit, well-washed or peeled (citrus is especially high in folate)
  - cooked dried beans and peas
  - yeast extracts
  - freshly cooked liver and/or kidney (no more than one serving a week)
  - folic acid-fortified wholegrain bread and breakfast cereals.

*Remember: eat **at least six** servings of vegetables and fruit per day, aiming for **10** servings per day.*

Iodine and Iodine Deficiency

Iodine is an essential nutrient required in small amounts to support normal growth and development, including brain development. It is important that unborn babies receive enough iodine. Requirements for iodine increase during pregnancy and breastfeeding. Even with a well-balanced diet, it is difficult to get enough iodine from food alone.

Choose foods that are important sources of iodine and take a daily iodine-only tablet throughout your pregnancy.

Important sources of iodine in foods include well-cooked seafoods, milk, eggs, some cereals, seameal custard and commercially made bread (excluding organic and unleavened bread as they are not required to be made with iodised salt).

If you use salt, choose iodised salt.

- Take one 0.150 milligram (mg)/150 microgram (mcg or µg) iodine-only tablet daily during your pregnancy.

For further information, contact a health practitioner such as your LMC, dietitian, practice nurse or pharmacist.
Supplements containing seaweed, kelp and iodine are not recommended for pregnant women because the iodine content and quality of the supplements is variable.

Supplements

The only supplements recommended for all pregnant women are folic acid-only tablets and iodine-only tablets, which can be purchased from pharmacies at a reduced cost with a prescription from your LMC.

Choosing a variety of foods from the four food groups will meet your other requirements, and supplements will not be necessary.

Using vitamin and mineral supplements will not give you extra energy.

If you are taking any vitamin, mineral or herbal supplements, always let your LMC know. It is best to only take supplements when recommended by your LMC or a dietitian. Make sure they know that you are pregnant.

Vitamin D

Vitamin D is needed for strong bones and joints. While it is found in some foods in the diet, the main source of vitamin D in New Zealand is sunlight. Vitamin D is made in the body through the action of sunlight on the skin. Examples of foods that contain vitamin D are fresh and canned oily fish (tuna, sardines, salmon, herring, mackerel, warehou, eel), eggs and vitamin D-fortified yoghurts, dairy desserts, milk and margarines.

Some sun exposure is recommended so that your body can make vitamin D.

Between September and April sun protection is recommended (shade, clothing coverage, and a hat that shades the face and neck, sunscreen, sunglasses), especially between 10.00 am and 4.00 pm. A daily walk or some other form of outdoor physical activity in the early morning or late afternoon is recommended.

Between May and August some sun exposure is important. A daily walk or another form of outdoor physical activity in the hours around noon, with face, arms, and hands exposed is recommended.

If you have darker skin, completely avoid sun exposure, have liver or kidney disease, or are on certain medications (eg, anticonvulsants), then you are at higher risk of vitamin D deficiency. If you live south of Nelson-Marlborough in winter, you're also more likely to have low vitamin D levels in late winter or early spring.

If you are concerned about not getting enough vitamin D, or are at higher risk of vitamin D deficiency, discuss this with a health practitioner, such as your doctor (GP), dietitian, LMC or Well Child nurse.

Cravings and Aversions

Most women experience strong likes and dislikes (cravings and aversions) for certain foods at some time during pregnancy. If you eat a variety of foods from the four food groups every day, cravings and aversions are unlikely to affect your pregnancy.

If you are experiencing problems with cravings (for example, craving for unhealthy foods), have other eating problems or are unable to eat a variety of foods, ask your LMC to arrange for you to see a dietitian.

Constipation

Constipation can result from the pressure of the growing baby and from hormonal changes that cause your gut muscles to relax.

Choose wholegrain breads and cereals and vegetables and fruit (eg, bran muffins, kiwifruit, figs, corn and peas).

Drink plenty of fluids every day.

Go for a daily walk or be physically active in some other way.

Allergy Prevention

During pregnancy, it is recommended that you eat well from the variety of foods in the four food groups. Avoiding common food allergens during pregnancy is not recommended.

However, if you do choose to avoid common food allergens during pregnancy or breastfeeding, talk to your LMC, doctor or Well Child nurse. They can refer you to a registered dietitian who will make sure that your nutritional needs are being met and help you identify all hidden sources of the food allergen in the diet.

For more information

You are entitled to free care from an LMC during your pregnancy. The booklet ***Your Pregnancy*** (code HE1420) gives you information on choosing an LMC. Once your baby is born, you and your infant are entitled to receive free Well Child care in accordance with the Well Child Tamariki Ora National Programme. This includes advice about and support with your own and your baby's nutrition requirements. This programme is delivered by your LMC from conception until 2–6 weeks after the birth of your baby. From 2–6 weeks onwards, your Well Child provider (Plunket, public health service, Māori or Pacific provider) will provide this care.

Talk to your LMC or Well Child provider about other information you want to know.

Other organisations for information:

Healthline 0800 611 116

New Zealand College of Midwives

La Leche League (for breastfeeding support and information)

Maternity Services Consumer Council

NZ Multiple Birth Association, PO Box 1258, Wellington

Parents Centre New Zealand

Dietitian at local public health unit

Ministry of Primary Industries (for food safety and label reading advice)

For website information

Ministry of Health www.health.govt.nz/your-health/healthy-living/pregnancy

Health Education resources www.healthed.govt.nz

Ministry of Primary Industries www.foodsmart.govt.nz

Raising Children in NZ www.raisingchildren.org.nz

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MINISTRY OF HEALTH
MANATŪ HAUORA

hpa health promotion agency

New Zealand Government

This resource is available from www.healthed.govt.nz or the Authorised Provider at your local DHB.
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APPENDIX 10 – Reject script for participants who have not met the study criteria

Nutrition and Dietetics
College of Health
Massey University
Private Bag 102-904
North Shore Mail Centre
Auckland, New Zealand

Hello!

Thank you for taking the time to access our study page. Our research team appreciates your interest in taking part in our study. However we regret to inform that you do not meet our study criteria (pregnant women*, 3rd trimester of pregnancy, aged 16 years and over, and living in NZ).

> *If you are pregnant, but aren't yet in the third trimester of pregnancy, you can still take part in our study once you reach the third trimester. The study will remain open until the end of December 2014. Your participation is very important for us, so please feel free to bookmark and visit our survey page again (www.surveymonkey.com/s/pufa).

If you know anyone else who would be interested in participating, please forward the survey link on: www.surveymonkey.com/s/pufa.
You can get some tips on Eating for Healthy Pregnant Women by clicking here. If you have any concerns about your health and diet during pregnancy please consult your Midwife, Medical Practitioner or Dietitian.
A summary of findings of this study will be available around February 2015 at www.massey.ac.nz/pufa.

For any questions, suggestions or further information please contact our research team. Our contacts are:

Research Team Contact		
MSc Student	**Study Supervisor**	**Study Co-Supervisor**
Michele Eickstaedt	Dr Cath Conlon	Dr Kathryn Beck
Institute of Food, Nutrition and Human Health	Institute of Food, Nutrition and Human Health	Institute of Food, Nutrition and Human Health
Massey University, Albany	Massey University, Albany	Massey University, Albany
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We wish you all the best for you and your baby.

Kind Regards,

The Research Team