The effect of krill oil supplementation focusing on the incorporation of plasma omega-3 polyunsaturated fatty acids, clinical biomarkers and lipidomic profiles in women

A thesis submitted by

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ABSTRACT

Circulating lipids play an important role in human physiology and pathophysiology. Lipids, as the major components in various cellular membranes, are involved in homeostatic regulation, particularly in relation to immune function and inflammatory mechanisms. With the growing global prevalence of lifestyle-related diseases, including obesity, diabetes, cardiovascular disorders and cancers, dietary lipids have received a great attention. Long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) have been associated with a broad range of health benefits. The three main LC n-3 PUFA are eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and docosapentaenoic acids (DPA, 22:5n-3).

Fish oil and krill oil are currently the most available sources of EPA and DHA as over-the-counter supplements, although other marine sources such as algae oil are also rich in EPA and DHA. Krill oil, derived from Antarctic krill (*Euphausia Superba*), is rich in EPA and DHA found in phospholipids (predominantly phosphatidylcholine) rather than triacylglycerol (TAG), in which EPA and DHA in fish oil are found. Krill oil also contains astaxanthin, a carotenoid contributing to its red colour which may also have beneficial health effects (Barros et al. 2014a, Pashkow et al. 2008). Despite a number of studies examining the effects of krill oil compared with fish oil on the incorporation of LC n-3 PUFA into different tissues, the outcomes have been conflicting, which might be associated with the different study designs using different chemical forms of fish oil and/or different doses of LC n-3 PUFA, and focusing at different target tissues.

The research presented in this thesis consists of nine chapters covering a literature review (*Chapter two*) and two intervention studies in humans (*Chapters four, five, six and seven*) which have examined the effect of krill oil compared with fish oil on the

incorporation of LC n-3 PUFA into plasma lipid fractions. There were a postprandial and a longer-term (30 days) intervention studies, and both clinical studies were randomised crossover designs involved healthy women (n = 10 and n = 11, respectively). All participants were instructed to maintain the habitual dietary intake and habitual physical activity throughout the interventions.

The aim of the postprandial study was to compare the incorporation of LC n-3 PUFA into the plasma and circulating lipids in plasma and chylomicron fractions from five capsules (1 g each) of krill oil compared with five capsules (1g each) of fish oil and 5 g of the olive oil (control) over a 5-hour postprandial period. The second study aimed to investigate the longer-term effect of krill oil supplementation (containing 1,269 mg/d of LC n-3 PUFA including EPA, DHA and DPA) on the plasma LC n-3 PUFA, plasma circulating TAG and inflammatory biomarkers compared with fish oil supplementation (containing the closest possible match to these fatty acids from the capsules, 1,441 mg/d) over a 30-day intervention period. In both studies, lipidomics, was applied to identify the differences in plasma lipid molecular responses between krill oil and fish oil supplementation. Using this technique, a number of plasma lipid classes, and lipid molecular species containing EPA and DHA were identified and quantified.

In the 5-hour postprandial study (*Chapters four and five*), there were no significant differences in the levels of TAG or cholesterol in plasma or chylomicron between the three study oil interventions, although the expected increases in chylomicron TAG were observed in all groups. In comparison to the olive oil, both krill oil (containing 907 mg of LC n-3 PUFA) and fish oil (containing 1,441 mg of LC n-3 PUFA) supplementation significantly increased the level of plasma EPA, which plateaued after three hours; there were no significant differences in the plasma EPA levels

between krill oil and fish oil supplementation groups. There were no significant changes in either DHA or DPA between the three groups. Krill oil, with a lower dose of EPA in this study, showed a similar incorporation outcome of EPA into plasma lipids as fish oil. Given that there were 31% less EPA from krill oil, these results indicate a differential extent of incorporation of EPA between krill oil and fish oil, suggesting that EPA from krill oil may be more efficiently incorporated into the plasma than fish oil.

The advanced technique for lipidomics was performed by high-performance liquid chromatography-mass spectrometer analysis (HPLC MS/MS), which was able to identify and quantify changes in various lipid molecular species containing LC n-3 PUFA in both the postprandial and the longer-term studies. Therefore, the HPLC MS/MS facilitated a comparison between differences in the individual lipid molecular species between krill oil and fish oil supplementation. A more sensitive setting of HPLC MS/MS was applied to the postprandial data than the longer-term data, based on the settings applied by the research laboratory at Baker Heart and Diabetes Institute where these analyses were conducted.

In *Chapter five*, the postprandial plasma lipidomic changes are reported at hours zero (baseline), 3 and 5. A total of 29 lipid classes (≥ 500 pmol/mL) (for example: TAG, diacylglycerol (DAG), phosphatidylcholine (PC), cholesterol esther (CE)) were identified; six of these including O-linked phosphatidylethanolamine classes had significantly greater the incremental area under the curve from baseline (net $iAUC_{0\text{-}5\ h}$) after krill oil supplementation compared with fish oil supplementation. Over the postprandial period, 56 EPA-containing and 76 DHA-containing molecular species (for example 16:0-20:5-PC, 16:0-18:1-20:5-TAG, 16:0-22:6-PC, 16:0-18:1-22:6-TAG) were significantly increased after both krill oil and fish oil supplementation. There were

33 phospholipid molecular species containing EPA, and 16 of these molecular species, including six ether-phospholipid molecular species had significantly greater increased net iAUC $_{0\text{-}5\ h}$ after krill oil than fish oil supplementation. In contrast, for TAG and DAG molecular species containing EPA, seven out of a total of 21 showed significantly increased net iAUC $_{0\text{-}5\ h}$ for fish oil compared with krill oil. Put simply, the EPA from krill oil was associated with increases in phospholipid EPA-molecular species, while the EPA from fish oil was associated with increased TAG and DAG EPA-molecular species.

There were 49 phospholipid molecular species containing DHA, and 11 of these including six ether-phospholipid molecular species, had significantly greater increased net iAUC $_{0\text{-}5\ h}$ after krill oil supplementatin than fish oil supplementation. In a total of 61 AA-containing molecular species (for example 16:0-20:4-PC, 16:1-20:4-DAG) identified, there were 51 phospholipid molecular species containing AA, and seven of these including six ether-phospholipid molecular species, had significantly greater increased net iAUC $_{0\text{-}5\ h}$ after krill oil supplementation than fish oil. A novel finding from this postprandial study was that there was a consistent trend that ether-phospholipid classes (O-linked (containing an alkyl bond) or P-linked (containing an alkenyl bond) phosphatidylcholine and phosphatidylethanolamine) were significantly increased after krill oil supplementation, but decreased after fish oil supplementation. Consistently, it was found that EPA- and DHA-containing ether-phosphatidylethanolamines were significantly increased after the krill oil supplementation, but decreased after the fish oil supplementation. While the significance of this finding is not clear, it is worth noting that plasma levels of O- and P-linked phosphatidylethanolamine have been reported to be decreased in a number of disease states including Alzheimer's disease. Little is known about the origin of these ether-phospholipids in plasma, but the fact that krill oil

increased their post-prandial levels and fish oil decreased them is a clear differentiation between these two omega-3 oils.

In the longer-term study (*Chapters six and seven*), EPA, DHA and DPA were significantly increased after both krill oil and fish oil supplementation over the 30-day period ($p < 0.001$). The main response to the 30-day krill oil supplementation was that the increase of plasma EPA level was significantly greater in the net iAUC $_{0\text{-}30\ d}$ than that of fish oil supplementation ($p < 0.05$). Both krill oil and fish oil significantly reduced plasma TAG over the intervention period ($p < 0.05$ and $p < 0.01$, respectively), but no significant differences were observed between the two groups. Over the 30-day intervention period, some plasma pro-inflammatory cytokines including IL-1β, IL-10, IL-4 and IL-5 ($p \leq 0.05$) were significantly reduced after krill oil supplementation, while no such changes were found after fish oil supplementation.

In *Chapter seven*, the long-term lipidomic changes (≥ 500 pmol/mL), at days zero (baseline), 15 and 30, are reported. Twenty three EPA-containing and 46 DHA-containing molecular species were significantly increased after both krill oil and fish oil supplementation over the 30-day supplementation period. Among EPA-, DHA-, and DPA-containing molecular species, there were 20 cases of net iAUC $_{0\text{-}30\ d}$ significant differences between the two supplementation. Fourteen of these molecular species in phospholipid species, including 12 ether-phospholipid species, had significantly greater increased net iAUC $_{0\text{-}30\ d}$ after krill oil than fish oil ($p \leq 0.05$) supplementation. Consistently, it was found that EPA- and DHA-containing ether-phospholipid species, including six ether-phosphatidylethanolamines, were significantly increased after the krill oil supplementation, and decreased after the fish oil supplementation.

The changes in the ether-phospholipids in the long-term trial were consistent with the changes described in the postprandial trial (*Chapter five*). These results support strongly the differentiation between krill oil and fish oil although there are still many unanswered questions flowing from this novel finding. What is known about plasmalogens is that they play a role in anti-inflammatory response, which might be linked to the significant decrease in pro-inflammatory cytokines observed in the present study.

Overall, both postprandial and longer-term studies demonstrated that EPA from krill oil is efficiently incorporated into plasma, has a similar effect on the plasma TAG-lowering and a greater efficacy on the plasma inflammatory biomarkers when compared with fish oil. No previous studies have investigated plasma lipidomic responses to krill oil and fish oil supplementation in humans. There were significant increases in molecular species containing EPA and DHA following supplementation with krill oil and fish oil over both the postprandial and the longer-term periods. The plasma lipidomic changes of net iAUC over both intervention periods were significantly different between krill oil and fish oil supplementation, particularly for phospholipids (krill oil resulted in a greater increase than fish oil) and TAG (fish oil resulted in a greater increase than krill oil, as described in *Chapter five*). A novel aspect identified in this study was that krill oil increased ether-phospholipids, particularly ether-linked phosphatidylethanolamine, whereas fish oil decreased ether-phospholipids. The biological relevance of this novel lipidomic finding has yet to be fully explored.

Doctor of Philosophy Declaration

"I HYUNSIN (HEDY) SUNG, declare that the PhD thesis entitled "*The effect of dietary krill oil supplementation focusing on plasma biomarkers and lipids, and lipidomic profiles in healthy women*" is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of another degree or diploma. Except where otherwise indicated, this thesis is my own work.

Signature:  Date: 17/ 10/ 2017

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Peer reviewed published manuscripts arising from this thesis

H Sung, A Sinclair, P Lewandowski and X Su 2017, Postprandial long-chain n-3 polyunsaturated fatty acid response to krill oil and fish oil consumption in healthy women: a randomised controlled, single-dose, cross-over study, Asia Pac J Clin Nutr 2018;27 (1):148-157.

Published abstracts

Sung, HH, Sinclair, AJ., Mellett, N., Meikle, PJ., Su, XQ 2017, Postprandial lipidomic profiles after krill oil supplementation compared with fish oil in healthy women, 10th Asia Pacific Conference on Clinical Nutrition incooperating the join annual scientific meeting of Nutrition Society Australia and Nutrition Society New Zealand 2017, vol. 41.

Hyunsin H. Sung, Andrew J. Sinclair, Xiao Q. Su, 2016, The effects of 30-day krill oil supplementation compared with fish oil on inflammatory biomarkers in healthy women, The Victorian Obesity Consortium 2016 Symposium.

HH Sung, AJ Sinclair, PJ Meikle, N Mellett, PA Lewandowski, XQ Su 2016, Lipidomic profiles after a 30-day krill oil supplementation compared with fish oil in healthy women, Nutrition Society Australia, annual scientific meeting 2016, vol. 40, p.46.

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H Sung, J Antonipillai, A Sinclair, and X Su 2014, Krill oil can inhibit platelet adhesion, an ex vivo study, Nutrition Society Australia, Annual scientific meeting 2014, vol. 38, p.94.

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Sung, HH, Sinclair, AJ., Mellett, N., Meikle, PJ., Su, XQ 2017, Postprandial lipidomic profiles after krill oil supplementation compared with fish oil in healthy women, 10th Asia Pacific Conference on Clinical Nutrition incooperating the join annual scientific meeting of Nutrition Society Australia and Nutrition Society New Zealand, 26th - 29th November 2017.

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Society Association Annual scientific meeting, Wellington New Zealand, 1st -4th December 2015.

Sung, H, Sinclair, A, Antonipillai J and Su, XQ 2014, Krill oil can inhibit platelet adhesion, The postgraduate research conference, held at Victoria University, St Albans, Australia in October 2014.

Poster presentation at conferences

The effects of 30-day krill oil supplementation compared with fish oil on inflammatory biomarkers in healthy women, The 3rd Australian Lipid Meeting, 21st - 22nd November 2016.

HS Sung, AJ Sinclair, P Lewandowski and XQ Su 2015, Postprandial lipids response to krill oil supplementation in healthy women, The Australasian section of the American Oil Society (AAOCS) Biennial Conference 9th – 10th September, 2015.

Sung, H, Sinclair, A and Su, XQ 2014, Krill oil can inhibit platelet adhesion, an ex vivo study, Nutrition Society Association Annual scientific meeting, Hobart Australia, 26th – 28th November 2014.

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List of Abbreviations

AA	Arachidonic, 20:4n-6
ADHD	Attention deficit hyperactivity disorder
ALA	α-linolenic acid, 18:3n-3
ANOVA	Analysis of variance
ASD	Autism Spectrum Disorder;
AUC	Area under the curve
AusDiab	Australian Diabetes, Obesity and Lifestyle
ANZCTR	Australian New Zealand Clinical Trials Registry
BGL	Blood glucose level
BHT	Butylated hydroxytoluene
BMI	Body mass index
CE	Cholesterol ester
Cer	Ceramides
CHD	Coronary heart disease
CHO	Carbohydrate
CM	Chylomicrons
COH	Cholesterol
COX	Cyclooxygenase
CRP	C-reactive protein
CVD	Cardiovascular disease
CVS	Cardiovascular system
DAG	Diacylglycerol
DHA	Docosahexaenoic acid, 22:6n-3
dhCer	Dihydroceramide
DPA	Docosapentaenoic acid, 20:5n-3
ELIZA	Enzyme linked immunosorbent assay
EPA	Eicosapentaenoic acid
ESI-MS/MS	Electrospray ionisation-tandem mass spectrometry
FA	Fatty acids
FAME	Fatty acid methyl ester
FFA	Free fatty acids
g	Gram
GC	Gas-liquid chromatography
GM1	GM1 ganglioside
GM3	GM3 ganglioside
HbA1c	glycosylated haemoglobin
HDL-C	High-density lipoprotein cholesterol
Hex1Cer	Monohexosylceramide
Hex2Cer	Dihexosylceramide
Hex3Cer	Trihexosylcermide
HPLC	High performance liquid chromatography

HREC	Human Research Ethics Committee
IL-1	Interleukin-1
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
INF-γ	Interferon-gamma
kg	Kilogram
kg/m^2	Weight (kg) per Height (m) squared
kJ	kilojoule
LA	Linoleic acid, 18:2n-6
LC	Long-chain
LC n-3 PUFA	Long-chain omega-3 polyunsaturated fatty acids
LDL	Low-density lipoprotein cholesterol
LOX	Lipooxygenase
LPC	Lyso-phosphatidylcholine
LPC (O)	Lyso-alkylphosphatidylcholine
LPC (P)	Lyso-alkenylphosphatidylcholine
LPE	Lyso-phosphatidylethanolamine
LPE (P)	Lyso-alkylphosphatidylethanolamine
LPI	Lyso-phosphatidylinositol
MAG	Monoacylglycerol
mg/dL	Milligram per decilitre
mg/L	Milligram per litre
mg/mL	Milligram per millilitre
MI	Myocardial infarction
mL	Millilitre
mmHg	Millimetre of mercury
mmol/L	Millimole per litre
MUFA	Monounsaturated fatty acid
MW	Molecular weight
Net iAUC	Incremental area under the curve from baseline
Net iAUC $_{0\text{-}5\ h}$	Incremental area under the curve from baseline (hours from zero to 5)
Net iAUC $_{0\text{-}30\ d}$	Incremental area under the curve from baseline (days from zero to 30)
ng/L	Nanogram per litre
NHMIC	National Health Medical Research Council
n-3	omega-3
n-6	omega-6
PAF	Platelet-activating factor
PC	Phosphatidylcholine
PC (O)	Alkylphosphatidylcholine
PC (P)	Alkenylphosphatidylcholine
PE	Phosphatidylethanolamine
PE (O)	Alkylphosphatidylethanolamine
PE (P)	Alkenylphosphatidylethanolamine
PG	Prostaglandin

PG	Phosphatidylglycerol
pg/mL	Picogram per litre
PI	Phosphatidylinositol
PL	Phospholipids
PPAR-α	Peroxisome-proliferator-activated receptor-α
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acid
PUFA FFQ	Polyunsaturated fatty acid food frequent questionnaire
RBC	Red blood cells
RCT (s)	Randomised controlled trial(s)
SD	Standard deviation
SDTs	Suggested Dietary Targets
SEM	Standard error mean
SM (D)	Sphingomyelins
Sulfatide	Sulfoglycosphingolipids
TAG	Triaclyglycerol
TLC	Thin layer chromatography
TNF-α,	Tumor necrosis factor-alpha
T2DM	Type 2 Diabetes mellitus
TX	Thromboxane
VLDL	Very low-density lipoprotein cholesterol
wk	Week
yrs	Years

Chapter 1: Introduction

Over the last several decades, dietary long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) have been investigated in relation to human health and the growing prevalence of lifestyle-related diseases worldwide (Diem et al. 2016). A number of observational and experimental studies along with different clinical conditions have shown consistent health benefits of dietary LC n-3 PUFA intake, particularly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), associated with the risks of lifestyle-related diseases and age-related cognitive decline (Alexander et al. 2017, Denis et al. 2015, Lian et al. 2017). The health benefits of EPA and DHA consumption include the reduction in the plasma levels of triglyceride (TAG) (Leslie et al. 2015) and pro-inflammatory mediators in cardiometabolic conditions (Li et al. 2014, Lin et al. 2016), and reduction in the severity of other symptoms such as dry eye disease (Bhargava and Kumar 2015), pain associated with headache (Ramsden et al. 2013) and rheumatoid arthritis (Hill et al. 2016, Senftleber et al. 2017). It has been suggested that EPA and DHA may have different biological functions via cyclooxygenase and lipoxygenase pathways. EPA, but not DHA, directly competes with arachidonic acids (20:4n-6) in relation to anti- and pro-inflammatory lipid mediators (eicosanoids), respectively (Cottin et al. 2011, Mozaffarian and Wu 2012). Eicosanoids derived from EPA are associated with anti-aggregation and vasodilation (Mickleborough et al. 2009). DHA, as an essential constituent in the brain, plays an important role in the cognitive development and the improvements in degenerative and/or behavioural neurological conditions. EPA and DHA need to be obtained from diet since the human body is not able to produce them sufficiently. Based on accumulated evidence in relation to human health and disease, a number of governmental and international health organisations have suggested optimal daily

intakes of LC n-3 PUFA (Mozaffarian and Wu 2012). For instance, the suggested dietary targets of LC n-3 PUFA for adults are 430 mg/day for women and 610 mg/day for men in Australia (National Health Medical Research Council 2006). In relation to the health benefits of EPA and DHA, the demand for LC n-3 PUFA by consumers has rapidly increased over the last decades. The dietary LC n-3 PUFA intake in Australian adults has increased by 62% from 1995 to 2012 (Meyer 2016). However, only one in five Australians meets the suggested dietary targets of LC n-3 PUFA for optimal health (Meyer 2016).

Since there is a high concentration of LC n-3 PUFA in oily fish, marine dietary sources, including fish, algae and krill, have been commonly used to formulate EPA and/or DHA compared with red meat and plant sources (Kwantes and Grundmann 2015). Krill, living in Southern Antarctic oceans, have been recognised as a new source of EPA and DHA in the past decade. In krill, the LC n-3 PUFA are found mainly in phospholipids (predominantly phospholipidcholine) rather than in TAG as is the case of fish oil (Tou et al. 2007). Krill oil and fish oil have been the two major supplemental forms of EPA and DHA available to consumers. This has led to a number of experimental and clinical investigation into whether krill oil has a different bioavailability of LC n-3 PUFA compared with fish oil in humans. However, the findings on the bioavailability of LC n-3 PUFA from these two oils remain insufficient and inconsistent. Some studies have reported a more efficient incorporation of LC n-3 PUFA into the plasma following krill oil supplementation compared with fish oil supplementation, while other studies have shown no differences between the two oil supplementation (Maki et al. 2009, Ramprasath et al. 2013, Ulven and Holven 2015, Ulven et al. 2011). Even though some claims have suggested that krill oil supplementation has resulted in a higher increase of LC n-3 PUFA concentration

compared with fish oil (Ulven and Holven 2015), it is not clear whether these claims refer to the bioavailability of LC n-3 PUFA or other ingredients from krill oil, such as astaxanthin, which is the carotenoid antioxidant contributing to the red colour of krill (Barros et al. 2014a).

The term “bioavailability” has been loosely used in the literature focusing on krill oil and fish oil studies, and in most instances, it has meant the plasma or red blood cell level of LC n-3 PUFA after consuming either krill oil or fish oil for a period of time. In fact, “bioavailability” is not only about the plasma or red blood cell level of LC n-3 PUFA, since it must also take into account the excretion of LC n-3 PUFA together with measurement of peak blood levels over time (Ghasemifard et al. 2014). Hence, the conflicting outcomes based on the currently available literature may be attributed to limitations of study designs in which different amounts of LC n-3 PUFA, chemical forms of LC n-3 PUFA in the oils and/or and target tissues were used in different studies (Ghasemifard et al. 2014, Ulven and Holven 2015). The limitations of those study designs might also include a lack of control in study participants’ variability related to their age, gender, and absorption efficiency (faecal loss) (Cohn, J S et al. 1988; Ghasemifard, Turchini & Sinclair 2014; Lohner et al. 2013). Moreover, the use of conventional methods, using lipid extraction followed by consecutive chromatographic separation, may limit the identification of lipemic responses. The conventional methods provide the ‘mix’ of fatty acids in the TAG via dietary fatty acids pathways where the fatty acids are hydrolysed and mixed together. Due to time-consuming and costly protocols involved, particularly compared with the novel methodology, lipidomics, the conventional methods commonly focus on the total fatty acids and/or specific lipid classes (e.g., TAG, phospholipids) in target tissues. In contrast, the novel lipidomic technology is able to identify comprehensive lipid molecular species and this

may result in a better understanding and the insight into metabolic pathways associated with the discovery of various human diseases at different stages (Ekroos et al. 2010, Zhao et al. 2015).

Therefore, the aims of this PhD project were to investigate the difference in incorporation of LC n-3 PUFA into the plasma, and clinical biomarkers over (i) a postprandial and (ii) a longer-term period between krill oil and fish oil supplementation. The plasma samples, from both studies, were further analysed to examine lipemic changes in plasma molecular lipid species using the novel lipidomic technology over (i) a 5-hour postprandial and (ii) a 30-day period. The specific aims of studies were:

(i) A cross-over randomised postprandial study with supplementation of krill oil, fish oil or olive oil (the control) over a 5-hour period in women (n =10) and,
(ii) A cross-over randomised longer-term study with supplementation of krill oil or fish oil over a 30-day period in women (n = 11).

Specifically, a postprandial intervention was involved in studying the dynamic lipemic changes over the 5-hour postprandial phase. Plasma lipid profiles and the incorporation of LC n-3 PUFA into plasma and chylomicrons were analysed to determine differences between 5 grams of each krill oil and fish oil supplementation in comparison with olive oil (control) in healthy young women.

In a longer-term intervention, the objectives were to verify the 30-day effect of krill oil supplementation (containing 1,269 mg/d of LC n-3 PUFA) on the incorporation of LC n-3 PUFA into plasma, and circulating plasma TAG and inflammatory biomarkers compared with fish oil supplementation (the closest possible match to these fatty acids from the capsules, containing 1,441 mg/d) in healthy young women.

To add to the novel nature of the postprandial and the longer-term studies, the plasma samples from both studies were further analysed to investigate whether plasma lipidomic responses (as various plasma molecular species containing LC n-3 PUFA) to krill oil supplementation is different from those to fish oil supplementation in healthy young women.

The thesis includes the following main chapters: literature review (Chapter 2); general materials and methods (Chapter 3); each of the two studies, over the postprandial and the 30-day periods, divided into two chapters based on the postprandial plasma LC n-3 PUFA and lipid profiles (TAG) in plasma and chylomicrons (Chapter 4); the postprandial lipidomic responses (Chapter 5); the longer-term plasma LC n-3 PUFA profile, and changes in TAG and inflammatory biomarkers (Chapter 6); and the 30-day lipidomic responses (Chapter 7). Following these chapters, there is a general discussion and conclusion in Chapter 8, and finally, future directions are discussed in Chapter 9.

Chapter 2: Review of literature

There have been profound changes in human diet via westernisation and industrialisation over the last 75 years in developed nations as well as rapidly developing nations where one of the crucial nutritional characteristics is the lipid intake and the fatty acid composition of lipids (Cordain et al. 2002, Naughton et al. 2015). The changes in dietary fat intake need to be considered not only for the qualitative but also quantitative perspectives as this has led to the major impact on body composition. Dietary saturated fatty acids and a high-fat have been associated with an increased incidence of inflammatory diseases and neurodegenerative disease such as cardiovascular disease (CVD), obesity, arthritis, cancer and Alzheimer's disease (Corsinovi et al. 2011, Patterson et al. 2012, Simopoulos 2016, Weaver et al. 2009). On the other hand, a growing body of scientific literature has reported that health benefits have been attributed to long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA), particularly eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6 n-3) (Klek 2016, Mateos et al. 2013, Mozaffarian and Wu 2012). Given these health benefits LC n-3 PUFA should be considered for optimal health and even more importantly preventative nutritional therapeutic efficacy for the general population (He 2009, Mozaffarian and Wu 2012).

It is important to know firstly the current dietary lipid intake in Australian adults and recommended guidelines for LC n-3 PUFA followed by available dietary sources; secondly, it is important to know the most common LC PUFA - their structures, metabolic functions and mechanisms of action and differences between LC n-3 and omega-6 (n-6) PUFA; lastly, it is useful to understand the health benefits associated

with n-3 PUFA including any differences between the two common marine oils, fish oil and krill oil.

2.1 DIETARY LIPIDS

2.1.1 The nutrition transition in Australia

The consumption of dietary fatty acids in Australia has changed greatly over the past several decades due to a combination of urbanisation and an increase in multicultural population, followed by food technology advances (Finkelstein 2003, Naughton et al. 2015). According to the recent Australian nutritional transition data between 1961 and 2009, there has been a noticeable increase in the proportion of energy derived from dietary fats from 32.5% to 42.3% (Naughton et al. 2015). The relative change for dietary fats was an increase in polyunsaturated fatty acid (PUFA) from 2.9% in 1961 to 7.7% in 2009 where the proportion of linoleic acids (LA, 18:2) which contributes to PUFA was 75% and 79%, respectively. As a result of the increased consumption of foods high in LA from vegetable cooking oils and processed foods, much higher n-6 PUFA causes an undesirable imbalance with n-3 PUFA (Chilton et al. 2014). Meyer recently reported that, based on 2011–2012 National Nutrition and Physical Activity Survey, Australian adults have increased their dietary LC n-3 PUFA intakes from foods and supplementations by 62% since the last National Nutrition Survey in 1995 (Meyer 2016).

2.1.2 Guidelines for dietary n-3 PUFA intake

In relation to evidence of LC n-3 PUFA intake on human health particularly CVD various governmental, scientific, and international health organisations have suggested optimal daily intake, as shown in Table 2.1 (Mozaffarian and Wu 2012). Most guidelines for general population indicate fish intake per week ranging from one to three times, or daily LC n-3 PUFA consumption (with a combination of EPA and DHA) ranging from 250 mg to 2 g. Many governmental authorities similarly recommended a daily LC n-3 PUFA intake ranging from 1 g up to 4 g for individuals with heart problems or high triglyceride levels (Kris-Etherton et al. 2002, National Heart Foundation of Australia 2009). In Australia, the National Health and Medical Research Council (NHMRC) has recommended the daily total LC n-3 PUFA intake (EPA + DPA + DHA) to reduce chronic diseases. This consists of two sets of recommendation, including an adequate intake (90 mg/d for female adults and 160 mg/d for male adults), and the Suggested Dietary Targets (SDTs) (430 mg/d for female adults and 610 mg/d for male adults) (NHMRC 2006). Additionally, an increase of two to three serves of fish (oily fish) per week was recommended by the Heart Foundation of Australia (Nestel et al. 2015). However, only approx. 20% of Australians meet the SDTs of LC n-3 PUFA for optimal health (Meyer 2016).

2.1.3 Dietary sources of n-3 PUFA

Fish and other seafood are a rich source of LC n-3 PUFA compared with meats and plants sources as shown in Table 2.2. It is important to know that different types of fish contain not only different amounts of LC n-3 PUFA, but also EPA, DPA and DHA (Calder 2012). According to the more recent daily dietary intake in Australian adults,

meat, poultry, and game products (quail and rabbit) were consumed 4.5 to 6 times more than fish/seafood. However, the contribution of LC n-3 PUFA was less than half that of fish/seafood (Meyer 2016).

There is a growing consideration about age and gender-specific disparities in the contribution of LC PUFA composition into different tissues from human clinical trials (Nording et al. 2013, Zulyniak et al. 2016). Compared with men, women tend to have a higher omega-3 index, which represents EPA and DHA (% of total erythrocyte fatty acids) (Howe et al. 2014), and also a significantly higher level of both arachidonic acid (AA, 20:4n-6) and DHA in the total plasma and plasma phospholipids. Moreover, CVD is the major cause of global death in women, although women experience CVD events approximately 10 years later than men. The under-recognition and differences in clinical presentation for CVD may drive women to a lack of self-awareness and identification of CVD risks (Maas et al. 2011). CVD risks in women with a combination of ageing and menopause are more progressed than those of men with ageing alone (Elias-Smale et al. 2015). Additionally, post-menopausal women experience an increased incidence of chronic inflammatory diseases, whereas males experience them earlier than women (Gubbels Bupp 2015). Given these gender-disparities, it may be necessary to consider differential recommendations for dietary intake of LC n-3 PUFA depending on genders. This might contribute to the optimal preventive and therapeutic efficacy.

Table 2.1 National and international guidelines for LC n-3 PUFA intake for adults

Organisation	Target population	Daily intake of LC n-3 PUFA
National Heart Foundation of Australia (2009)	General population	500 mg of EPA + DHA
	Patients with CHD	1 g of EPA + DHA
National Health & Medical Research Council Australia (2006)	Men-SDTs	610 mg of LC n-3 (EPA +DHA + DPA)
	Women-SDTs	430 mg of LC n-3 (EPA +DHA + DPA)
World Health Organization (2003)	General adult population	200 - 500 mg of EPA + DHA
International Society for the Study of Fatty Acids and Lipids (2004)	General adult population	≥ 500 mg of EPA + DHA
European Society of Cardiology and the European Atherosclerosis Society (2011)	Patients with CHD	≥ 1 g n-3 FA
American Heart Association (2002)	Patients with CHD	1 g of EPA + DHA
	Patients with high triglycerides	2-4 g/day EPA + DHA
Ministry of Health, Labour and Welfare in Japan (2015)	Adult men	≥ 2 g of total n-3
	Adult women	≥ 1.6 g of total n-3

Abbreviations: CHD, coronary heart disease; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; g, gram (s); LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; mg, milligram; SDTs Suggested Dietary Targets.

Adapted from (European Society of Cardiology and the European Atherosclerosis Society 2011, International Society for the Study of Fatty Acids and Lipids 2004, Kris-Etherton et al. 2002, National Health Medical Research Council 2006, National Heart Foundation of Australia 2009, National Institute of Health and Nutrition 2015, World Health Organization 2003).

Table 2.2 Dietary sources for long-chain omega-3 PUFA in Australia

Source (mg/ 100 g)	ALA	EPA	DPA	DHA	Total LC n-3 PUFA
Fish and seafood					
Salmon, Pacific king, raw	250	1,106	615	1,700	3,421
Sardine, canned in oil, drained	320	1,127	144	1,257	2,528
Mackerel, baked, roasted, fried, grilled or BBQ'd	50	501	154	1542	2,197
Salmon, Atlantic, raw	470	916	433	821	2,170
Seaweed, nori, dried	0	1,758	21	0	1,779
Mackerel, canned	560	361	111	1,110	1,582
Anchovy, canned	220	178	17	575	770
Sardine, raw	30	168	21	424	613
Oyster, raw	40	229	26	222	477
Tuna, sashimi style, raw	20	71	25	343	439
Squid or calamari, baked, fried, stir-fried, grilled or BBQ'd	0	111	0	306	417
Snapper, baked, roasted, fried, grilled or BBQ'd	0	57	41	265	363
Tuna, baked, roasted, fried, grilled or BBQ'd	20	53	19	261	333
Trevally or kingfish, raw	20	133	42	152	327
Trout, coral, cooked	40	32	12	264	308
Cod or hake, smoked, boiled, steamed or poached	10	75	20	208	303
Barramundi, baked, roasted, grilled, BBQ'd,	310	71	37	140	248
Blue grenadier (hoki), baked, roasted, grilled, BBQ'd	170	38	16	161	215
Octopus, cooked	0	84	7	89	180
Prawn, king or medium, flesh, boiled, steamed or poached	30	83	10	60	153
Meats					0
Beef, chuck steak, untrimmed, boiled, casseroled	110	40	139	20	199
Lamb, loin chop, semi-trimmed, roasted, grilled or BBQ'd	240	67	90	23	180
Beef, silverside steak, separable lean, baked or roasted	50	61	104	16	181
Pork, loin roast, separable fat, baked or roasted	580	0	64	64	128
Chicken, maryland, baked, roasted, fried, grilled or BBQ'd	120	0	21	15	35
Plants					
Oil, linseed or flaxseed	54,590	0	0	0	0
Seed, linseed or flaxseed	23,040	0	0	0	0
Seed, chia, dried	17,900	0	0	0	0
Nut, walnut, raw, unsalted	6,280	0	0	0	0
Lentil, red, dried	180	0	0	0	0
Avocado, raw	170	0	0	0	0
Tahini, sesame seed pulp	120	0	0	0	0

Data are expressed as mg per100 g of dietary sources. Abbreviations: arachidonic acid; ALA, alpha-linolenic acid; BBQ'd, barbecued; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; g, gram(s); LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; mg, milligram; SDTs, Suggested Dietary Targets. Adapted from AUSNUT 2011-2013 food nutrient data (Food Standards Australia New Zealand 2015).

2.2 LIPIDS

2.2.1 Classifications of lipids

Dietary lipids such as fats and oils derived from plant and animal tissues are essential nutrients as a metabolic fuel source and a vital component of cell membranes for human growth and development (Akoh and Min 2008, WHO 2008). The most common food fats (solid) and oils (liquid) are essentially composed of triacylglycerols (TAG), which is the major form of energy storage in the human body and consists of three fatty acids attached to a glycerol backbone. In addition to TAG, minor amounts of phospholipids, monoacylglycerols (MAG), diacylglycerol (DAG), and sterols are present in foods. Lipids are a chemically heterogeneous group of compounds which are insoluble in water but soluble in non-polar solvents such as chloroform, hydrocarbons, benzene, or alcohol (Akoh and Min 2008). Biological lipid constituents are widely distributed to structural and physiological functions in cellular membranes associated with the human health and diseases. The biological lipids can be classified into three subgroups such as 1) derived lipids including fatty acids; 2) simple lipids composed of fatty acids and alcohol including glycerides, sterols, esters and wax, and 3) complex lipids glycerophospholipids (phospholipids) and glycerolipids (TAG, DAG) and sphingolipids (ceramides)) (LIPID MAPS 2016). In this section complex lipids rather than other subgroups of lipids are presented. These data are generated using an advanced lipid analytical technology lipidomics, and focusing on the incorporation of LC n-3 PUFA from krill oil and fish oil into the plasma over both a postprandial and a longer-term period.

2.2.2 Complex lipids

Diverse lipids in the human body physiologically respond to dietary regimen by changing in their levels. Lipids in various lipid classes, as shown in Figure 2.1, can be characterised by variations in fatty acids content, head groups and fatty acid distribution of biological organisms, which can be identified using an advanced analytical methodology, lipidomic profiles (Fahy et al. 2009). It has been well documented that the characteristics of lipids are different throughout the cells and tissues (Fang et al. 2016, McEvoy et al. 2013, Meikle et al. 2014, Meikle et al. 2013, Meikle et al. 2011, Quehenberger et al. 2010). The biosynthesis of lipids initiates in the endoplasmic reticulum in which the lipid compositions of individual membranes can be further stratified to the orientation of the cytosol lipids in the Golgi. The most dominant lipid classes have been revealed through human and animal models, as shown in Table 2.3, although the sterol and glycerolipid species are the most abundant in the plasma (Meikle et al. 2013, Meikle et al. 2011, Quehenberger et al. 2010).

2.2.2.1 Glycerolipids

Glyceroglycolipids (glycolipids) are neutral lipids including DAG and TAG species, and the high proportion of total lipids in human plasma. DAG is a precursor to TAG through reacylation which is also derived from hydrolysis of the head groups in a variety of glycerophospholipid species (LIPID MAPS 2016). Lipids in the human body are predominately TAG as a constituent of lipoproteins, while TAG needs to be hydrolysed into MAG, DAG or free fatty acids (FFA) for absorption in the digestive system (Quehenberger et al. 2010). Dietary intake influences the composition of TAG within the plasma.

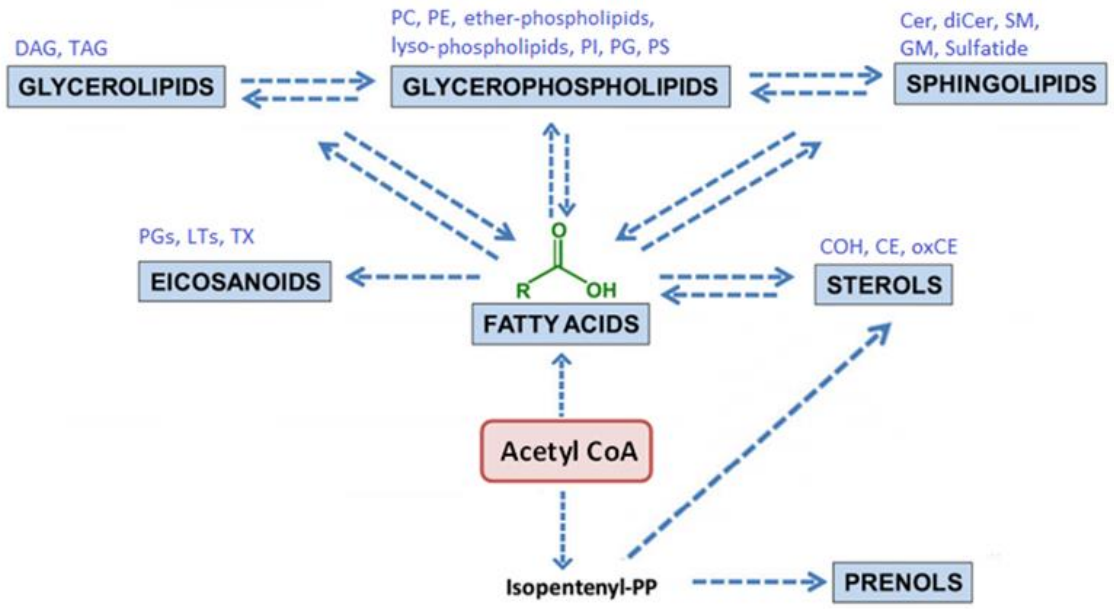


Figure 2.1 Diverse lipids in the human plasma

Ether-phospholipids include alkylphosphatidylcholine (PC (O)), Alkenylphosphatidylcholine (PC (P)), alkylphosphatidylethanolamine (PE (O)) and alkenylphosphatidylethanolamine (PE (P)). Lyso-ether phospholipids include lyso-phosphatidylcholine (LPC), lyso-alkylphosphatidylcholine (LPC (O)) and LPE, lyso-phosphatidylethanolamine. Abbreviations: Cer, ceramide; CE, cholesterol ester; COH, cholesterol; DAG, diacylglycerol; diCer, dihydroceramide; GM, Ganglioside; LTx, leukotrienes; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGs, prostaglandins; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; TAG, triacylglycerol; TX, thromboxane; oxCE, oxidised cholesterol ester. Adapted from (Fahy et al. 2009, Quehenberger et al. 2010).

2.2.2.2 Glycerophospholipids

Glycerophospholipids (phospholipids) molecular species are high in various cellular membranes, particularly in endothelial and vascular smooth muscle cells, cardiac myocytes and neurocytes. Phosphatidic acid is the simplest phospholipids playing a role in a precursor to other phospholipids and to TAG. Phospholipid is an amphipathic lipid containing two fatty acid groups attached to a glycerol-3-phosphate backbone at the sn-1 and sn-2 position. Glycerophospholipids species can be distinguished by their polar head group at the sn-3 position of the glycerol backbone, mainly choline or ethanolamine, and smaller presences of inositol and serine, as shown in Figure 2.2 (Fahy et al. 2009, LIPID MAPS 2016).

In the synthesis of all phospholipids, the head group of phospholipids can be activated with cytidine triphosphate, particularly prior to being attached to the lipid. The polar head part of phospholipids can be used for the synthesis of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), whereas the non-polar tail part of diacylglycerol is used for phosphatidylinositol (PI) and phosphatidylglycerol (PG). Glycerophospholipids species generally undergo fatty acyl hydrolysis through enzymatic activities (transacylase or phospholipases A_2 and/or acyltransferase) with a certain degree of nonselective enzymatic activities. The acyl chain remodelling in the biosynthesis of diacyl- and ether-phospholipids are generally identical, however, the synthesis of ether-phospholipids, is initiated by dihydroxyacetone phosphate in the peroxisome, while in endoplasmic reticulum for diacyl-phospholipids. If alterations of lipids occur, changes in lyso-phospholipids can be correspondent to their diacyl-phospholipid counterpart (LIPID MAPS 2016). Phosphatidylserine (PS) is derived from PC or PE where a head group of PC or PE is exchanged with a calcium-activated transferase in endoplasmic reticulum membranes (Vance and Tasseva 2013).

In the following sections, the subgroups of glycerophospholipids species are further described by their biochemical structure and components, and pathophysiological functions including diacyl-phospholipid, ether-phospholipids, plasmalogens, platelet-activating factors and lyso-phospholipids (Braverman and Moser 2012, Burri et al. 2012). A number of human studies have shown the relationship between glycerophospholipids and human diseases including diabetes, CVD and degenerative cognitive impairment as discussed below (Fahy et al. 2009, Fang et al. 2016, Meikle et al. 2014, Meikle et al. 2013, Meikle et al. 2011). These authors suggested that glycerophospholipid species with a high carbon number and double bond may

represent beneficial effects on human health although the findings are limited to certain diseases at different stages (Ottestad et al. 2012, Rasmiena et al. 2015).

Table 2.3. Top five lipid classes in human and animal models

Lipidomic studies	Identified number of lipids (sub) classes[#], molcular species	Variation factors: diet, tissues	Top five concentration of lipid subclasses[‡] 1	2	3	4	5
30-day cross-over study[1]	30[#], 522	Krill oil	CE	PC	COH	TAG	SM
n = 11 healthy females		Fish oil	CE	PC	COH	TAG	SM
Postprandial study[2]	23[#], 316	Soy meal	CE	PC	COH	TAG	LPC (O)
n = 16 healthy males		Dairy meal	CE	PC	COH	TAG	SM
Pooled human plasma[3]	6[#], 500	--	CE	PC	TAG	Free sterols	PE
n =100 healthy individuals							
6-week animal study[4]	20[#]	Mice Liver	PC	TAG	PE	Cer	PS
n = 6/ diet with krill oil and fish oil		Mice Brain	PC	PC (O)	PS	PE (P)	SM

The superscript ([#]) represents the number of identified lipid classes, and the superscript ([‡]) represents the rank of high concentration of lipid classes. The number of clinical trials represents 1 (a long-term study in this thesis), 2 (Meikle et al. 2015), 3 (Quehenberger et al. 2010) and 4 (Skorve et al. 2015). Abbreviations: CE, cholesterol ester; COH, cholesterol; LPC (O), lyso-alkylphosphatidylcholine; PC, phosphatidylcholine; PC (O), Alkylphosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SM, sphingomyelin; TAG, triacylglycerol.

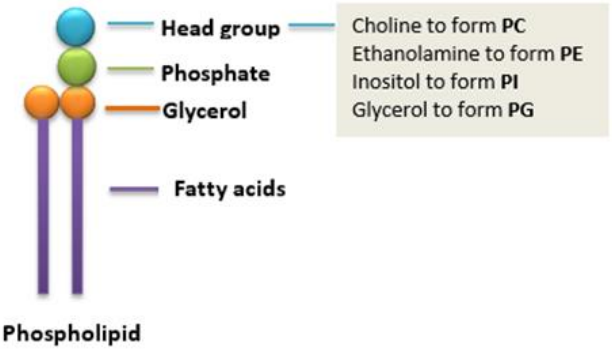


Figure 2.2 Phospholipid structure

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol. Adapted from (Burri et al. 2012).

2.2.2.2.1 Diacyl- and ether-phospholipid species

Diacyl-phospholipids are the most common subgroup of glycerophospholipids in which two acyl chains are linked to the glycerol backbone by an ester bond including PC, known as lecithin, and PE (Fahy et al. 2009). While, ether-phospholipids contain an ether bond (an alkyl or an alkenyl bond) at the sn-1 position of the glycerol backbone rather than an ester bond as in diacyl-phospholipids. Ether-phospholipid species (O-alkyl or P-alkenyl) include alkylphosphatidylcholine, PC (O), alkenylphosphatidylcholine, PC (P), alkylphosphatidylethanolamine, (PE (O), alkenylphosphatidylethanolamine, PE (P) (Braverman and Moser 2012). The most abundant head groups for glycerophospholipids species are choline and ethanolamine, thus this is correspondent to diacyl-phospholipids and ether-phospholipids (Braverman and Moser 2012, Quehenberger et al. 2010).

Accumulated observational and clinical human trials have reported that ether-phospholipids species are negatively associated with diabetes and coronary artery disease as well as rheumatoid arthritis (Fang et al. 2016, 2014, 2013, Meikle et al. 2011). In contrast, the consumption of dietary LC n-3 PUFA in human and animal models has shown health benefits such as the attenuation of atherosclerosis (Ottestad et al. 2012, Rasmiena et al. 2015).

The majority of ether-phospholipid species contain an alkenyl bond rather than an alkyl bond which is known as plasmalogens including PC (P) and PE (P) (Burri et al. 2012, Fahy et al. 2009, LIPID MAPS 2016). While, ether-phospholipid species containing an alkyl bond, particularly PC (O), is known as platelet-activating factor (PAF). Different

pathophysiological roles of plasmalogens and PAF species are discussed in the following sections.

2.2.2.2.2 Plasmalogens

Plasmalogens containing an alkenyl bond (a cis vinyl-ether bond) at the sn-1 position of a glycerol backbone and (Fahy et al. 2005). Plasmalogens are the most abundant form of ether-phospholipids containing either a choline or an ethanolamine moiety in the polar head group of the glycerol backbone although plasmalogen species are minor constituents of cellular membranes (Fahy et al. 2009). The biosynthesis of ether-phospholipids is initiated in peroxisome in which diacyl-phospholipids form alkyl-phospholipids by exchange of the acyl group (fatty acid) for an alkyl group (fatty alcohol). Further metabolism occurs to form alkenyl-phospholipids via enzymatic reactions in the endoplasmic reticulum (Watschinger and Werner 2013). Plasmalogens, PE (P) are synthesised from the corresponding PE (O) by the action of a desaturase, while PC (P) are derived from PE (O) via a combination of enzymatic reactions (Snyder et al. 2002).

The content of plasmalogens is different from organ to organ, and species to species and the level of plasmalogens is correspondent to diverse and complex biological lipids (Brites et al. 2004). For instance, there is a high PE (P) content in the brain myelin, and of high PC (P) in heart/ skeletal muscles and red blood cells (RBC), but a low plasmalogen content in the liver; the liver contains plasmalogen biosynthetic enzymes (Brites et al. 2004, Panganamala et al. 1971, Saitoh et al. 2009). Previous studies have suggested that there is an inverse correlation between PE and PE (P) levels, which could be evidence of compensatory mechanisms for maintaining an

optimal membrane phospholipid composition related to plasmalogen deficiency (Dorninger et al. 2015, Saitoh et al. 2009).

A number of functions of plasmalogen have been suggested. Firstly, plasmalogens are associated with cell membrane fluidity which is regulated by alteration of lipid composition. Experimental animal studies on plasmalogens, PE (P), have shown that plasmalogens form more condensed and thicker bilayers compared with the diacyl-PE (Rog and Koivuniemi 2016, Seelig 1978). This suggests that the presence of the vinyl–ether bond in plasmalogens might be involved in the regulation of membrane fluidity and structural changes in the membrane ER and Golgi compartments, as well as modulation of permeability in the blood-brain barrier by sorting and/ or inserting of protein and lipid molecules (Killian 1998, Rog and Koivuniemi 2016). Secondly, the vinyl-ether bond in plasmalogens plays a role in protecting cells membrane from reactive oxygen species (Gorgas et al. 2006, Lessig and Fuchs 2009). Rasmiena et al (2015) reported that an attenuation of atherosclerosis was attributed to increased level of plasmalogen species in the plasma and the heart of animal models. They proposed that the mechanism might be related to the role of plasmalogens as antioxidants. Lastly, the presence of plasmalogens species was associated with reduced levels of oxidative stress and inflammation (Farooqui and Horrocks 2001, Rasmiena et al. 2015). Plasmalogens, containing AA and DHA at the sn-2 position, may be involved in the metabolic synthesis of eicosanoids and docosanoids. Furthermore, Farooqui and Horrocks suggested that the signal transduction processes associated with cognitive and visual functions can be attributed to plasmalogen species along with plasmalogen-selective PLA_2 and DHA (Farooqui and Horrocks 2001).

2.2.2.2.3 Platelet activating factor

The various pathophysiological functions of platelet-activating factor (PAF), such as PC (P) and PE (P), have been well documented through recent reviews (Liu et al. 2016, Mazereeuw et al. 2015, Reznichenko and Korstanje 2015). PAF, as ether-phospholipids containing an alkyl bond, is considered as a powerful pro-inflammatory mediators associated with inflammatory reactions and allergic responses (Mazereeuw et al. 2013, Reznichenko and Korstanje 2015). PAF is endogenously synthesised by either the PLA_2-dependent remodelling pathway or the de novo pathway (Liu et al. 2016, Snyder 1995). In the remodelling pathway, acetyl hydrolase degrades PAF into inactive form as lyso-PAF by removing the acetate group, whereas, acetyl transferase converts the precursor of PAF (lyso-PAF) into PAF by adding the acetate group (Anand Vijaya Kumar et al. 2017, Snyder 1995). This remodelling pathway, which is regulated by a spontaneous reconversion between PAF and lyso-PAF, primarily occurs in various inflammation and allergic responses by inducing the activation of inflammatory mediators, platelet aggregation, degranulation and endothelial dysfunctions, whereas the de novo pathway is only (Liu et al. 2016, Mazereeuw et al. 2015, Reznichenko and Korstanje 2015). Some other researchers have suggested that PAF is also involved in beneficial pathological roles by reducing of pro-inflammatory mediators and enhancing functional recovery of cerebral infarction when PAF or PAF receptors was blocked (Uchiyama et al. 2006, Wang et al. 2016, Wu et al. 2014).

2.2.2.2.4 Lyso-phospholipids

Lyso-phospholipid species, including lyso-phosphatidylcholine (LPC) and lyso-phosphatidylethanolamine (LPE), are derived from their correspondent diacyl-phospholipids via the PLA_2 hydrolysis. Particularly, LPC species have been associated with inflammatory signalling molecules by enhancing lipid absorption and lipoprotein assembly, as well as lipid clearance (Nakano et al. 2009). Previous studies reported that the level of plasma LPC species positively associated with human and animal models of obesity and type 2 diabetes (Pietiläinen et al. 2007) and LPC species were positively correlated to oxidation of low-density lipoprotein (LDL) (Matsumoto et al. 2007). However, in recent years, observational prospective population-based and cohort studies have reported that the level of plasma LPC species was negatively associated with obesity and/or type 2 diabetes mellitus (T2DM) population when compared with lean individuals (Barber et al. 2012, Lee et al. 2015, Meikle et al. 2011, Stegemann et al. 2014). Barber et al reported via human and animal models that the circulating LPC species were inversely associated with body mass index and the level of plasma insulin, as well as a high-fat diet (Barber et al. 2012). Recently, a 90-day clinical study also reported that most of the LPC species including (16:0) in obese individuals were lower than that of lean individuals (Del Bas et al 2016). The authors suggested that the insensitivity of LPC species in obese individuals following a high-fat diet might be associated with the insulin signal and the regulation of blood glucose levels. Rasmiena et al (2015) reported that an increase in LPC species was associated with the increased tissue plasmalogen level and attenuation of atherosclerosis, there was. They suggested the LPC was derived from the plasmalogen and represented a breakdown product resulting from the oxidation of the plasmalogen.

2.2.2.3 Glycosphingolipids and sterols

Complex sphingolipids containing only single fatty acids esterified to a sphingoid backbone based on variations of unsaturation, hydroxylation and methylation of long-chain sphingolipid base (Fischbeck et al. 2009). High concentration of ceramides including Hex-1, Hex-2, and Hex-3 ceramides are found in cell membranes, which can be further synthesised to sphingomyelin. Ceramide is also metabolised to the complex glycosphingolipids in which ceramide is a hydrophobic sphingolipid back bone (LIPID MAPS 2016). Sterols carrying four fused rings are also found in human plasma which include cholesterol, cholesterol ester and oxidised cholesterol ester, and derivatives (Fahy et al. 2005).

2.2.3 Fat digestion, absorption and transport

The digestion of dietary fats is a complex hydrolytic reaction with enzymes derived from the pancreas and gastrointestinal tract (Gurr et al. 2002). When dietary fats, forming large fat globules in the stomach, are transferred into the small intestine, primarily fat globules are broken down into FFA and MAG from TAG, and into FFA and lyso-phospholipids from phospholipids. Subsequently, fat droplets with bile salts are re-assembled to form micelles for efficient absorption into the intestinal microvillus (Figure 2.3). The lipid contents of micelles such as FFA, 2-MAG, lyso-phospholipids and cholesterol in enterocytes are hydrolysed and re-assembled to form chylomicrons with apolipoprotein B48 (Quehenberger and Dennis 2011). Chylomicrons (CM), TAG-rich lipoproteins, now play a role in transporting fat into the watery bloodstream through the lymphatic system (Gurr et al. 2002, Whitney et al. 2011).

On entering blood circulation, TAG from CM are hydrolysed to FFA and glyceride mainly by lipoprotein lipase via capillary walls and thereafter FFA and glycerol are used as energy or stored in peripheral tissues (muscles) in which adipocytes also store excess FFAs as the form of TAG (Figure 2.4). The remaining smaller particles of CM known as CM remnants are cleared by the liver LDL receptor and LDL receptor proteins. TAG and cholesterol ester in the liver are used in the formation of very-low-density lipoproteins (VLDL) with apolipoprotein B100 to transport lipids via the circulation system; the VLDL are further transformed to intermediate-density lipoprotein (IDL) and LDL by lipoprotein lipase. Through lipase hydrolysis from the liver to peripheral tissues the size of lipoproteins are in the order, VLDL > IDL > LDL > high-density lipoproteins (HDL) where their composition of TAG, cholesterol and phospholipids are changed among lipoproteins as shown Figure 2.4. The cholesterol transport by VLDL, IDL and LDL is from the liver to peripheral tissues, whereas HDL carries cholesterol and phospholipids back from the periphery to the liver which is known as reverse cholesterol transport (Lusis et al. 2004).

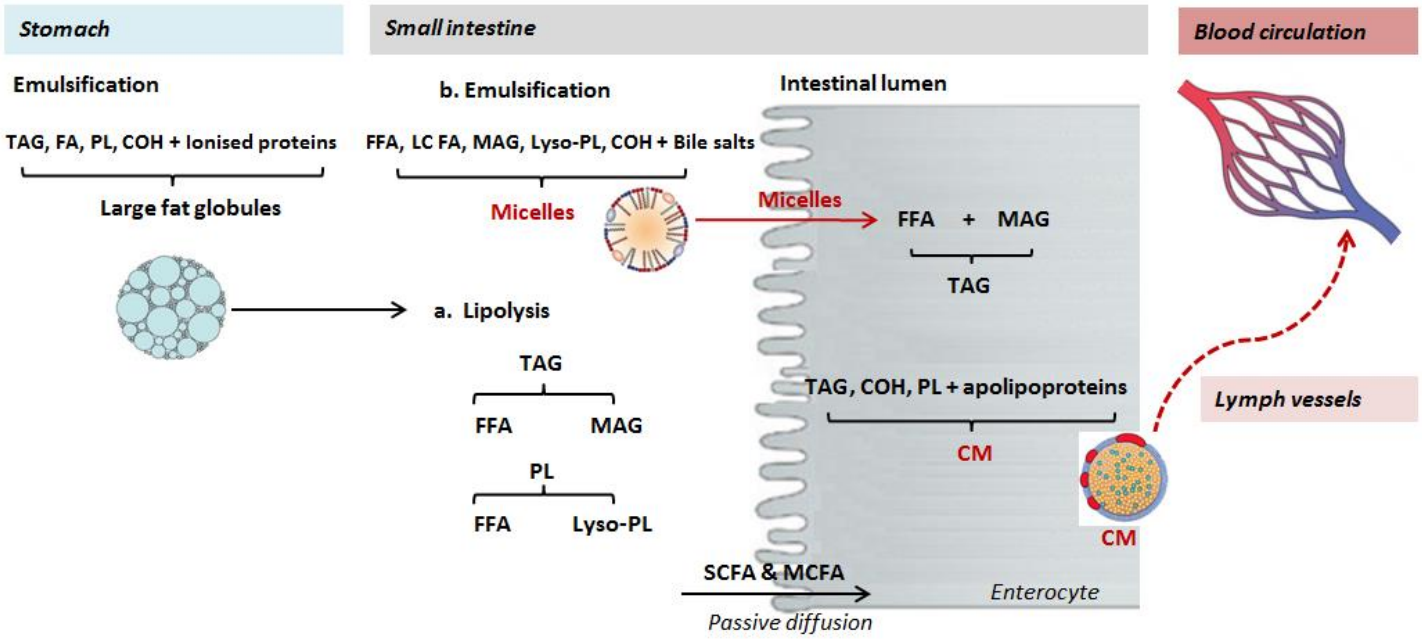


Figure 2.3 Dietary fat digestion, absorption and transport

Abbreviations:, apolipoprotein B48; CM, chylomicron; COH, cholesterol FA, fatty acid; FFA, free fatty acids; LC FA, long-chain fatty acid; Lyso-PL, Lyso-phospholipid; MAG, mono-glyceride; MSFA, medium-chain fatty acid; PL, phospholipids; SCFA, short-chain fatty acid; TAG, triacylglycerol. Adapted from (Lusis et al. 2004, Whitney et al. 2011).

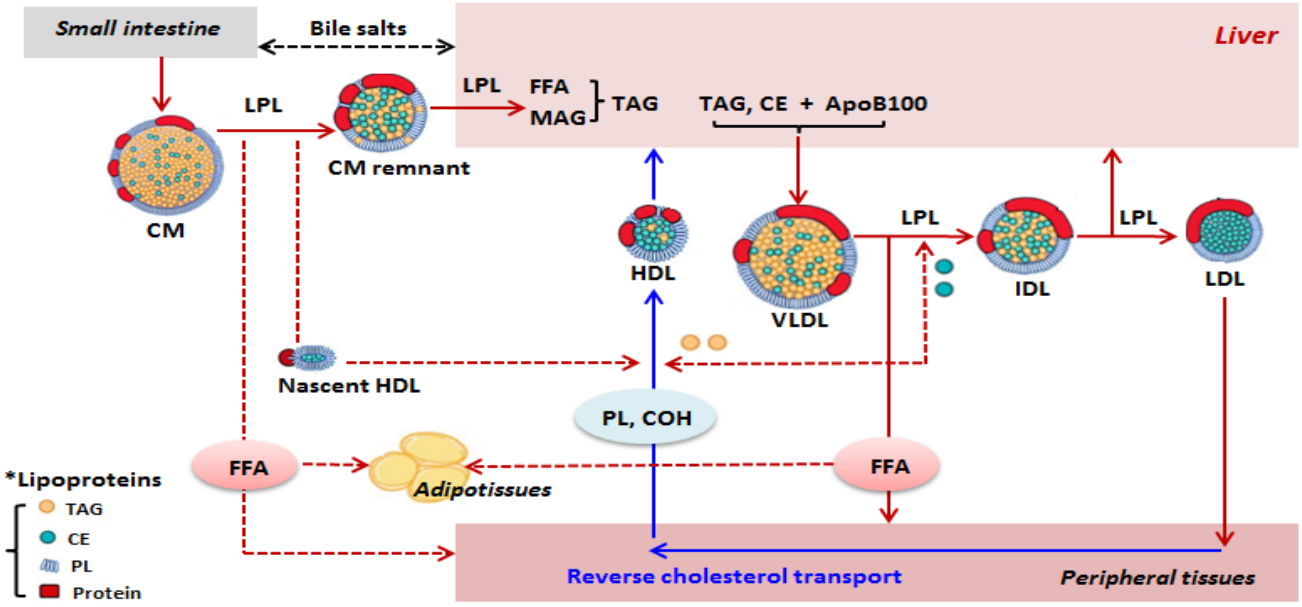


Figure 2.4 Lipoprotein pathways

Abbreviations: apoB100, apolipoprotein B100; CE, cholesterol ester; CM, chylomicron (containing 80% ≤ TAG); COH, cholesterol; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein (containing ≈50% of protein); LDL, low-density lipoprotein (containing ≈50% of cholesterol); LPL, lipoprotein lipase; PL, phospholipid; VLDL, very-low density lipoprotein (containing ≈50% of TAG); TAG, triacylglycerol. Adapted from (Lusis et al. 2004, Whitney et al. 2011).

2.2.4 Fatty acids

Fatty acids are composed of hydrocarbon chains of a carboxyl group (COOH) at one end and a methyl group (CH_3) at the other end (Gurr and James 1971). The biological reactivity of fatty acids depends on the length of the carbon chain and the number and position of any double bonds present, which represents a point of unsaturation between carbon atoms. In relation to degree of saturation in the chemical structure, the common dietary fatty acids have been subdivided into three types, namely saturated fatty acids (SFA) without a double bond, monounsaturated fatty acids with one double bond and polyunsaturated fatty acids (PUFA) with two or more double bonds as shown Figure 2.5.

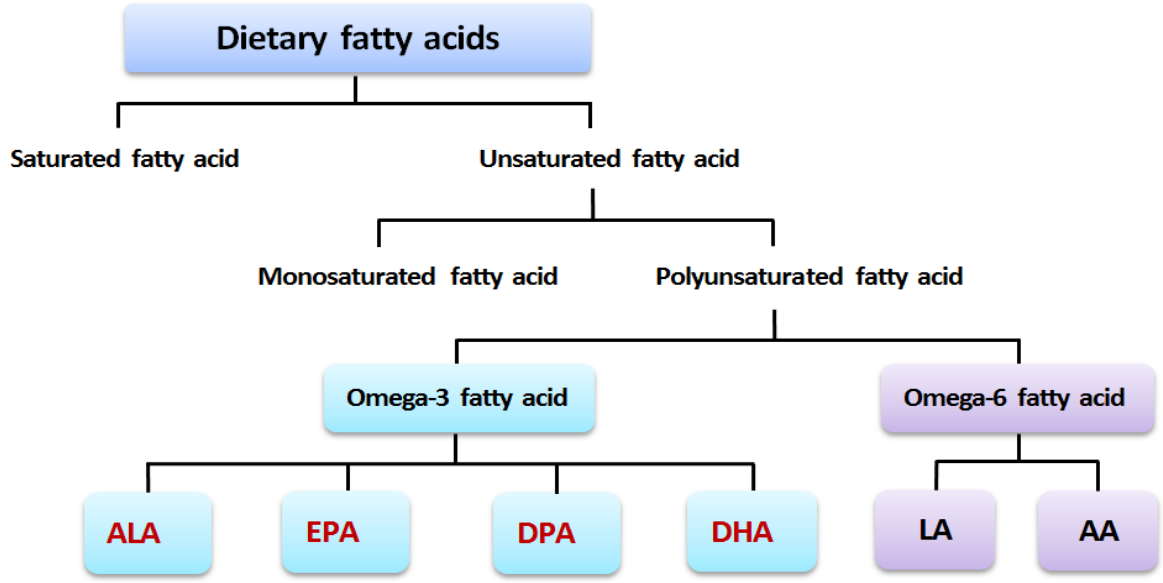


Figure 2.5 Dietary fatty acids

Abbreviations: Arachidonic acid (**AA**, 20:4n-6); Alpha- linolenic acid (**ALA**); Eicosapentaenoic acid (**EPA**, 20:5n-3); Docosahexaenoic acid (**DHA**, 22:6n-3); Docosapentaenoic acid (**DPA**, 22:5n-3); Linoleic acid (**LA**, 18:2n-6). Adapted from (Simopoulos et al. 1986).

Generally, a fatty acid can be described as the number of carbon, the number of double bonds and the location of the first double bond. For instance, a PUFA, alpha-

linolenic acid (ALA) can be described as “18:3n-3”, which indicates ALA is composed of 18 carbons with three double bonds and particularly the first double bond located on the third carbon counting from the methyl end.

2.2.4.1 Saturated fatty acids

Saturated fatty acids carry the maximum possible number of hydrogen atoms in only single carbon bonds. Most common dietary saturated fatty acids are mainly provided from animal fats, as well as palm oil and coconut oil (WHO 2008). Saturated fatty acids can be further classified into four subgroups based on their number carbon chains such as short-chain (containing 3 – 7 carbon atoms), medium-chain (containing 8 -13 carbon atoms), long-chain (containing 14 – 20 carbon atoms), and very long-chain (containing 21 or more carbon atoms) fatty acids. High consumption of saturated fat has been associated with many pathological events in the artery that induce plaque formation and magnify risks for many pro-inflammatory diseases such as diabetes metabolic syndrome (Sudheendran et al. 2010).

2.2.4.2 Unsaturated fatty acids

Unsaturated fatty acids can be divided into two groups such as monounsaturated fatty acids and polyunsaturated fatty acids based on the number of double bonds. The first contains only one double bond, whereas the latter has two or more double bonds that can be found in different length of carbon chain such as short-chain fatty acids (≤ 19 carbon atoms), long-chain fatty acids (20 ≥ carbon atoms) and very-long-chain (25 or more carbon atoms) (WHO 2008).

Polyunsaturated fatty acids (PUFA) have distinct physiochemical structures based on their carbon chain length, the number and the position of the first double bond that results in different physiological functions (He 2009). There are two main types of polyunsaturated fatty acids (PUFA), omega-3 (n-3) and omega-6 (n-6) PUFA (Figure 2.6). The first double bond for n-3 PUFA is located between the third and fourth carbon from the methyl end of the fatty acid chain. Major n-3 PUFA include ALA, EPA, DPA and DHA, whereas major n-6 PUFA include LA and AA (Akoh and Min 2008, Calder 2015).

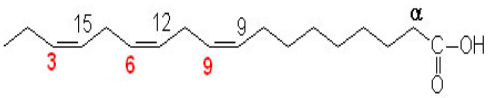
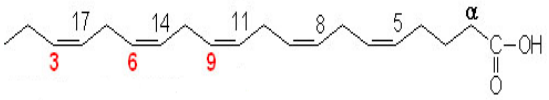
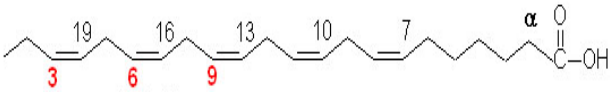
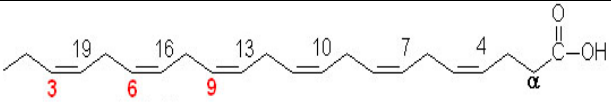
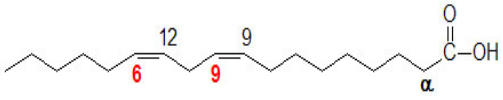
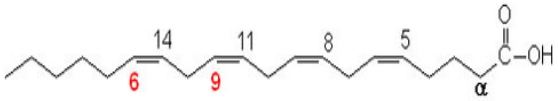
Omega-3 polyunsaturated fatty acids	
α-linolenic acid (ALA, C18:3) Flaxseed, Chia, walnuts, botanical oils	
Eicosapentaenoic acid (EPA, C20:5) Oily fish, shellfish, algae, krill, lean red meats	
Docosapentaenoic acid (DPA, C22:5) Oily fish, shellfish, krill, lean red meats	
Docosahexaenoic acid (DHA, C22:6) Oily fish, shellfish, krill	
Omega-6 polyunsaturated fatty acids	
Linoleic acid (LA, 18:2) Soybean, corn, palm oil and canola oils, basically all vegetable oils	
Arachidonic acid (AA, 20:4) Meat (red & white), eggs, liver, kidney	

Figure 2.6 Structures of polyunsaturated fatty acids

Adapted from (Akoh and Min 2008, Chilton et al. 2014, Mozaffarian and Wu 2012).

2.2.5 Biosynthesis pathways of long-chain polyunsaturated fatty acids

The high number of double bonds in the molecule have an impact on the fluidity even at low temperatures in living organisms (Schuchardt and Hahn 2013). Long-chain polyunsaturated fatty acids (LC PUFA), containing 20 or more carbons, play an important role in lipid metabolism and endothelial functions (Chilton et al. 2014, Mozaffarian and Wu 2012). LC PUFA can be synthesised in the human body from the two main essential dietary fatty acids, ALA (18:3n-3) and LA (18:2n-6), precursors of PUFA n-3 and n-6 PUFA, respectively (Simopoulos 1999). These essential fatty acids need to be obtained from the diet as the body is not able to produce them. ALA and LA increase the chain length (elongation) and degree of unsaturation by adding an extra double bond to the carboxyl group (desaturation) by the same set of enzymes via enzymatic endogenous conversion as shown in Figure 2.7. The endogenous synthesis of PUFA occurs in endoplasmic reticulum where they are translocated to the peroxisome for the final step of DHA synthesis (Dyall 2015). However, the endogenous conversion is limited approximately ranging from 5 to 8% of ALA to EPA, and from 0.07 to 0.3% of ALA to DHA (Mozaffarian and Wu 2011b, Plourde and Cunnane 2007). It is clear that the competition of n-3 and n-6 fatty acids for the same enzymes result in the low rate of endogenous conversion (Plourde and Cunnane 2007). Moreover, Brenna et al (2009) reported that high dietary ratios of LA/ALA and n-6 fatty acid intake inhibit the conversion of ALA to LC PUFA.

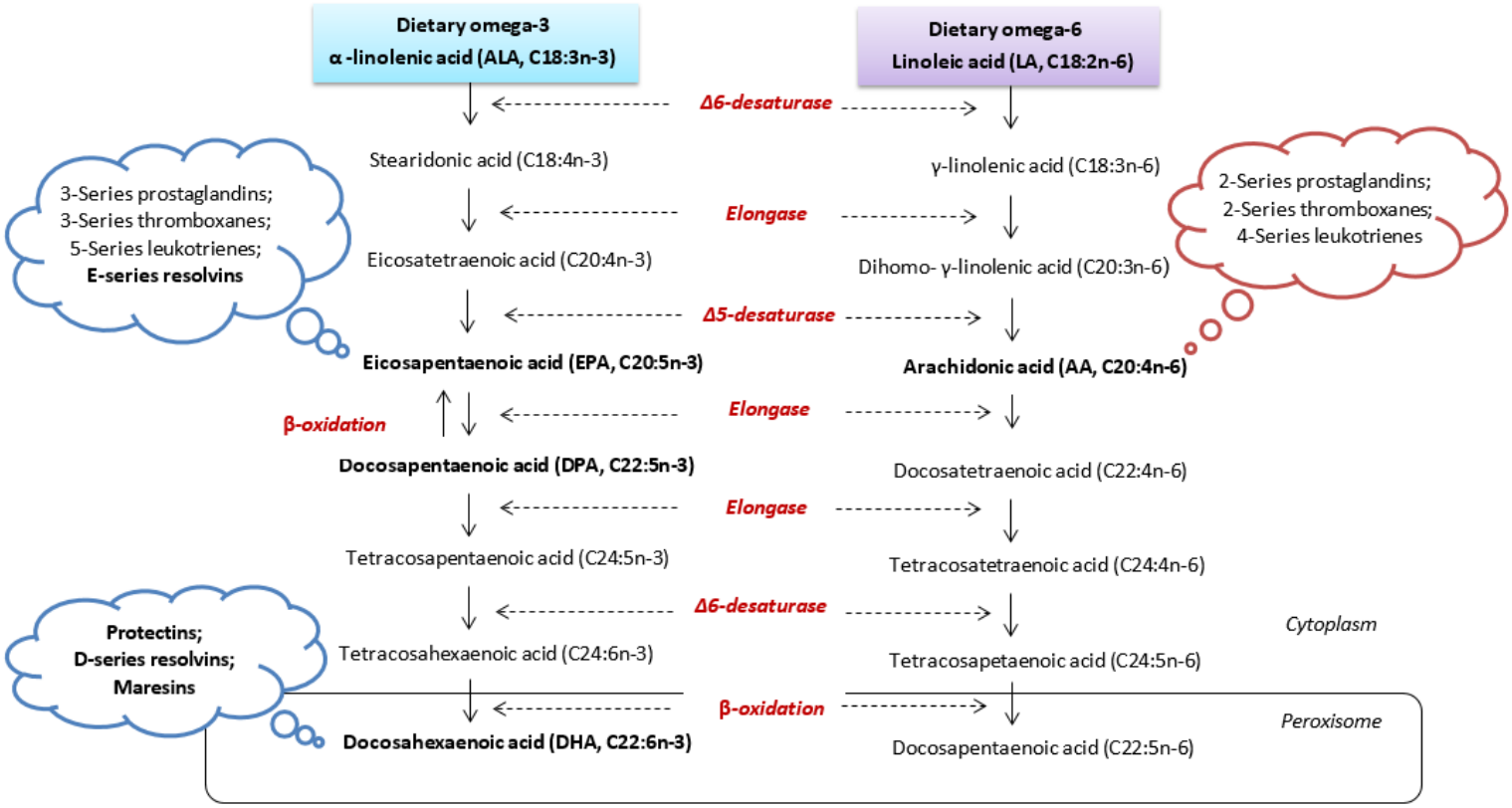


Figure 2.7 The metabolic steps of desaturation and elongation reactions

The enzymatic elongation and desaturation occur in the endoplasmic reticulum, whereas the retro-conversion from C24:6 n-3 to DHA via β-oxidation occurring in peroxisomes. Some people believe a delta 4 desaturase exists instead of the second delta 6 desaturase plus the peroxisomal beta-oxidation (Zhang et al. 2016a). Abbreviation: FADS, fatty acyl desaturases; ELOV, elongation of very long-chain fatty acid. Adapted from (Chilton et al. 2014, Dyall 2015, Kidd 2007, Simopoulos 1991, Williams 2000).

Following the metabolic conversion from LA to LC PUFA, there are two further enzymatic biosynthesis producing two sets of lipid mediators, namely eicosanoid mediators and specialised pro-resolving mediators, from the oxidation of PUFA as shown in Figure 2.8 (Dyall 2015, Miller 2006, Patterson et al. 2012, Serhan and Petasis 2011, Spite et al. 2014). These lipid mediators play a critical role in inflammation which is a physiological local reaction of living tissues to injury or infection for homeostasis (Calder 2009). The two sets of lipid mediators switch over time through inflammation responses from pro-inflammation to pro-resolving (Schwab and Serhan 2006). Eicosanoid mediators including prostaglandins, thromboxane and leukotrienes are

involved in regulating inflammatory responses and levels of the immune cell at the initiation-phase of acute inflammatory responses. Eicosanoid mediators, derived from 20-carbon PUFA, can promote or inhibit inflammation (Calder 2009, Dyall 2015, Mozaffarian and Wu 2012, Tilley et al. 2001). Whereas, specialised pro-resolving lipid mediators including lipoxins, resolvins, protectins and maresins, particularly at the resolution phase of acute inflammation are involved in the clearance and regulation of inflammatory exudates to return to homeostasis (Ishida et al. 2010, Schwab and Serhan 2006, Serhan and Chiang 2013).

Due to a higher proportion of AA than other 20-carbon PUFA, the intensity and duration of inflammatory responses are attributed to AA, as the major substrate for eicosanoid synthesis (Calder 2007). As shown in Figure 2.8, AA in phospholipids of cell membranes can be metabolised by cyclooxygenase and lipoxygenase producing pro-inflammatory eicosanoid mediators including 2-series prostaglandins, thromboxanes A_2 and 4-series leukotrienes (Deutsch 2007, Schuchardt and Hahn 2013, Simopoulos 2002). These pro-inflammatory eicosanoid mediators derive from AA target special organs or cells. Whereas, eicosanoid mediators, derived from LC n-3 PUFA including 3-series prostaglandins (PGE_3), thromboxane A_3 and 5-series leukotrienes, play a critical role in protective inflammation responses and improved blood coagulation leading to chronic heart disease prevention (Calder 2015, Simopoulos 1991, Wijendran and Hayes 2004). In addition to eicosanoid mediators, there are other biosynthetic conversions from both LC n-6 and n-3 PUFA to specialised pro-resolving lipid mediators (SPM) by transcellular metabolism (Norling and Serhan 2010, Spite et al. 2014). These SPM actively stimulate resolution of inflammation to return to the homeostasis. The resolution of inflammation by SPM is followed by enhancing restoration of tissue and macrophage phagocytes, and inhibiting polymorphonuclear

neutrophils infiltration and pro-inflammatory cytokines (Dyall 2015, Norling and Serhan 2010, Spite et al. 2014, Spite and Serhan 2010).

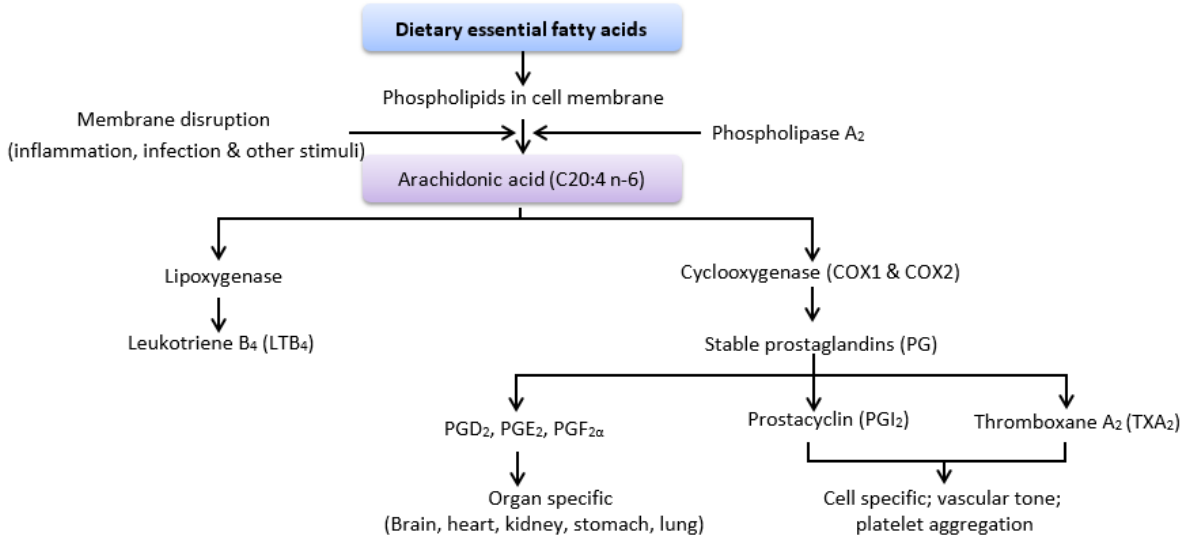


Figure 2.8 Biosynthetic pathways of arachidonic acid (20:4 n-6)

Adapted from (Miller 2006, Patterson et al. 2012).

2.2.6 Balance between LC n-3 and LC n-6 PUFA

The balance between LC n-3 and n-6 PUFA has become important for normal growth and development in humans. However, current diets, particularly Western diets, are much higher in LC n-6 PUFA than LC n-3 PUFA that led to the favourable metabolism of n-6 PUFA over the metabolism of n-3 PUFA. The ratio of n-6/n-3 essential fatty acids in current Western diets has been reported between 15/1 and 17/1, whereas the recommended ratio is 1 to 6/1 (Simopoulos 2008, Wijendran and Hayes 2004). In terms of the importance of balance in n3/n6 PUFA, a randomised 12-week dietary intervention reported that a high n-3 plus low n-6 intervention was significantly effective on chronic headaches (hours/day) compared with a low n-6 intervention. They also found that a high n-3 plus low n-6 intervention significantly increased anti-nociceptive n-3 resolvin pathway precursors from the baseline (Ramsden et al. 2013). One way to assess the status of n-3 PUFA in the body is to measure the sum of two main LC n-3 PUFA (EPA and DHA) in erythrocytes as a percentage of total fatty acids, namely omega 3 index. In order for the longer term status of n-3 PUFA, the omega-3 index has been recognised as the most reliable measure compared with the plasma levels of these fatty acids (Harris and von Schacky 2004, Klem et al. 2012).

2.2.7 Methodologies for assessing n-3 PUFA status

2.2.7.1 Gas chromatography

It has been established that the evaluation of fatty acids status is important, particularly for clinical outcomes. As a result, a number of studies have investigated to determine malnutrition with the essential fatty and the plasma lipids status related to

pathophysiology and pathogenesis of various health conditions including inflammatory responses, cystic fibrosis and CVD (Hodson et al. 2008, Li et al. 2014, Rise et al. 2010). Over the last decades, the increase in the number of individuals with metabolic diseases including T2DM and obesity, which are associated with an elevated risk of CVD, demands more detailed lipid analyses both for diagnostic purposes and for monitoring the efficacy of prescribed therapy. In particular, the status of LC n-3 PUFA, reflecting the dietary intake, has been recognised as critical and essential fatty acids for human health and diseases; growth and development of infants (Harris et al. 2015) and primary and/or secondary preventative treatments of diseases, such as heart diseases (Nestel et al. 2015), depressive disorders, dementia, eye health (Bhargava and Kumar 2015), arthritis (Hill et al. 2016), and cancers (Fu et al. 2015). Therefore, the increasing interest in LC n-3 PUFA has required the development of efficient methodologies for lipid analysis over the time, because the conventional analysis methods for fatty acids have been limited for only target lipid fractions (TAG and/or phospholipids) due to the time-consuming and costly protocols involved. For the conventional methods, the isolation and purification of total lipids from biological samples, which have been introduced by Folch et al (1957), are followed by consecutive chromatographic separations and analyses. There are three conventional chromatographic techniques including gas-liquid chromatography, thin-layer chromatography and high-performance liquid chromatography have been commonly used with the chloroform-methanol extraction of lipids (James 1970, Le Grandois et al. 2009, Volkman and Nichols 1991).

Lepage and Roy (1986) introduced a rapid method to achieve a high-throughput fatty acid analysis, which is known as the direct transesterification method, over the conventional methods requiring a time-consuming process, and significant handling

and costs. This has been further optimised to reduce the time for transesterification setting/steps, labour, equipment and reagents by different researchers, using various biological compartments such as whole blood, total plasma and red blood cells. Each of these approaches has shown the outcomes of the level of n-3 PUFA where various biological compartments and modified methods of the direct transesterification affected the outcomes. Klingler and Koletzko (2012) have reviewed methodologies for the fatty acid quantification using gas chromatography. Based on their systematic review, many approaches which have become greatly simplified methods have been validated in comparison to high-throughput of fatty acids analysis with the reduced duration of the analysis and cost. They, however, suggested that it is necessary to develop a standardised compliance to the analytical procedure associated with different biological compartments, particularly for larger samples sizes of clinical trials (Klingler and Koletzko 2012).

2.2.7.2 Lipidomic techniques

In addition to the total fatty acid analysis, it is quite common to analyse the lipid classes such as CE, TAG, FFA and different phospholipids (PC etc) in plasma, or in the case of red blood cells to analyse the major phospholipid classes (PE, PC, PS, sphingomyelin). The individual lipid species are commonly separated using thin layer chromatography or solid phase extraction with differential solvent elution (Christie 2003). In recent years, an advanced analytical technique, known as lipidomics, has been increasingly used for qualitative and quantitative analysis of lipid components in different biological compartments (refer to the Section 2.2.2 for lipid classifications (LIPID MAPS 2016). The application of lipidomics, providing the characteristics of the molecular species of lipids, is acknowledged as a robust and validated tool to illustrate

biochemical metabolic pathways related to disease diagnosis, prognosis and therapeutic efficacy (Zhao et al. 2015). Lipids from biological specimens are complex and contain diverse molecular species, and can be divided into 8 subcategories; fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides (Fahy et al. 2005). A range of molecular species in each class can be further identified by the position and number of unsaturated double bonds and their carbon length (Wolf and Quinn 2008). Therefore, the identification of diverse lipid molecular species in their structures and functions, particularly for plasma, through lipidomic applications is now considered valuable in understanding homeostatic processes and progression in various diseases (Murphy and Nicolaou 2013).

2.3 LONG-CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS

It has been well documented that dietary LC n-3 PUFA are critical for early human growth and development. The most biologically active LC n-3 PUFA, particularly EPA and DHA play roles in various physiological functions associated with regulating blood lipids, platelet reactivity, blood pressure, insulin sensitivity and inflammation. Hence, the impact of dietary LC n-3 PUFA consumption has been considerably debated for their consistent health benefits as therapeutic agents in a range of human diseases. Therefore, this section reviews the currently available evidence (up to March 2017) on the health effects of dietary LC n−3 PUFA on lifestyle related diseases with specific clinical endpoints related to heart, brain, pregnancy, joints and inflammation. The most recent systematic reviews and/or meta-analyses over the last several years have been mainly adopted to determine the accumulative evidence of the effect of dietary LC n-3 PUFA. The basis of selection of the reviews was as follows: electronic data bases

(PubMed, Google Scholar, and Scopus) were searched using the following search terms in titles and abstracts; “omega-3” or “fatty acid” or “long-chain” or “polyunsaturated fatty acids” or “EPA” or “DHA” or “DPA” or “dietary fat” or “fish oil” or “krill oil”, “systematic” or “meta-analysis” or “randomised” or “trial”. Moreover, the systematic review and/or meta-analysis of all relevant randomised controlled trials covered were in compliance with the NHMRC level. Criteria and outcomes of individual systematic review and/or meta-analysis are summarised within the following section for specific clinical conditions (Table 2.4 - 2.8).

Overall, the evaluation of the efficacy of n-3 PUFA on a range of clinical disorders, including CVD, may need to consider the same type of or at least similar clinical conditions and/or the ethnic background of study individuals within different studies when reviewed. A recent systematic review (Li 2015) reported that there were variations in outcomes between different studies. There are some potential reasons for discrepancies between the currently available reviews on randomised controlled trials (RCTs). There is the lack of control of erythrocyte n-3 PUFA levels at baseline and using a fixed dose of LC n-3 PUFA in intervention groups. Moreover, LC n-3 PUFA, derived from fortified foods and/or habitual dietary intake of fish/ seafood may interfere with dietary intervention with LC n-3 PUFA supplementation (Delgado-Lista et al. 2012, James et al. 2014). James et al (2014) has suggested that future RCTs need to use equivalent dosage between groups and/or adjustment to reach specific targets in order to minimise the current discrepancies.

2.3.1 Cardiovascular diseases

One of the major impacts of dietary LC n-3 PUFA on lifestyle-related diseases is on cardiovascular diseases (CVDs). This has resulted in a number of recent re-evaluations of the effect of LC n-3 PUFA on CVDs. Over the last 15 years, CVDs have been the leading cause of death globally. In 2015, 15 million people died from CVDs such as ischaemic heart disease (8.8 million) and stroke (6.2 million) (World Health Organisation 2017). Australia is no exception to the high death rate from CVDs, particularly ischaemic heart disease as the leading cause of death, even though the rate of CVDs has dropped (Australian Bureau of Statistics 2016).

It has been well documented that the levels of plasma and/or RBC LC n-3 PUFA are inversely associated with risks of CVDs. A recent systematic review and meta-analysis of 18 RCTs and 16 the prospective cohort studies (Alexander et al. 2017) reported that the effect of EPA plus DHA on coronary heart disease were significantly greater in higher risk populations, with high levels of TAG and LDL, than lower risk population. Their re-evaluation also indicated that a higher dose EPA and DHA (> 1 g/d) was associated with a stronger impact on higher risk populations than intervention with low dose of EPA and DHA (< 1 g/d) (Alexander et al. 2017). On the other hand, many other researchers have reported, via systematic review and meta-analysis, that there is insufficient evidence for the beneficial effect of LC n-3 PUFA on CVDs as shown in Table 2.4 (Chowdhury et al. 2014, Enns et al. 2014, Kwak et al. 2012). Those researchers reported that the consumption of LC n-3 PUFA did not show a significant reduction in cardiac death, cardiac events (Rizos et al. 2012), including myocardial infarction and stroke, cerebrovascular risks (Chowdhury et al. 2012) and peripheral arterial disease (Enns et al. 2014) in either primary or secondary CVD preventive trials.

However, a meta-analysis of 12 RCTs (Wen et al. 2014) reported that intake of dietary LC n-3 PUFA was significantly associated with decreased risks for death by all causes including cardiac causes and sudden cardiac death. Another meta-analysis of eight prospective studies (Larsson et al. 2012) revealed that the total stroke risk was significantly reduced following a high intake of LC n-3 PUFA in women although there were insufficient outcomes to determine the relationships between the intake of dietary LC n-3 PUFA and stroke. Moreover, a systematic review of 21 studies (Delgado-Lista et al. 2012) and a meta-analysis of 11 RCTs (Casula et al. 2013) reported that cardiac death and coronary events, particularly in individuals with high cardiovascular risk factors, were decreased by consuming marine n-3 PUFA obtained from food and supplementation. Another meta-analysis of eight prospective studies (Larsson et al. 2012) reported that a high intake of LC n-3 PUFA reduced significantly the total stroke risk, particularly in women. This may indicate variability in health conditions of study participants. Delgado-Lista et al (2012) suggested that 1 gram or more of daily LC n-3 PUFA intake and minimum one year of consumption may be beneficial for CVDs.

In addition, there are a number of observational and clinical studeies of LC n-3 PUFA on platelets and endothelial functions associated with CVDs. Platelet activations via thromboxane biosynthesis are also important in the pathophysiology of cardiac events such as ischemic stroke. A meta-analysis of 15 RCTs reported that the consumption of LC n-3 PUFA significantly reduced platelet aggregation, particularly in individuals with diabetes and/or high risk for CVDs rather than in healthy populations (Gao et al. 2013). This indicates the potential efficacy of LC n-3 PUFA in the secondary prevention rather than in the primary prevention of CVDs. Another meta-analysis of 18 RCTs (Yang et al. 2012) reported that intake of LC n-3 PUFA improved significantly the levels of plasma soluble adhesion molecules, particularly soluble intercellular

adhesion molecule-1, in both healthy individuals and individuals with dyslipidemia. However, there were insufficient evidence to support the effect of LC n-3 PUFA on preventing the development and the progression of atherosclerosis although intake of n-3 PUFA has shown some positive effects (Gao et al. 2013, Yang et al. 2012).

Due to the growing incidence of cardiovascular events, the status of endothelium-dependent vasodilation and flow-mediated dilation have been widely investigated to evaluate the endothelial dysfunction in humans (Abeywardena and Head 2001). A meta-analysis of 16 RCTs reported that there was a significant increase in flow-mediated dilation, particularly in individuals with poor health, but no effect from LC n-3 PUFA was observed in healthy individuals (Wang et al. 2012).

Table 2.4 A summary of the effects of LC n-3 PUFA intake in cardiovascular diseases

Cardiovascular diseases		
Authors	Details of studies	Summary of outcomes and future directions
Alexander et al 2017	SR and MA of 18 RCTs (n = 93,000; EPA+DHA intake ranging from 0.38 to 5.04 g/d; Duration ranging from 0.5 to 7 yrs) and 16 the prospective cohort studies (n = 732,000; dosage NA)	A significant reduction in outcomes of CHD was observed following dietary EPA plus DHA intake in the prospective cohort studies.

Wen et al 2014	MA of 14 RCTs (n = 3,256); subgroup analysis associated with clinical condition of severity; n-3 PUFA intake ≤ 1 g/d vs. >1; Duration < 2 yrs vs. > 2 yrs	The evidence showed that there was no significant protective effect of n-3 PUFA intake on major cardiovascular events, particularly CHD, due to an insufficient sample size of the included trials. Intake of n-3 PUFA may be effective in reducing death from cardiac causes, sudden cardiac death and death from all causes. Further well-designed studies are needed to overcome the limitations of the existing studies with consideration of the optimal dosage of n-3 PUFA, the ratio between EPA and DHA, and the duration of administration. Moreover, it is suggested to exert the protective effect of n-3 PUFA on specific major cardiovascular events.
Enns et al 2014	SR and MA of five randomised trials (n = 396); EPA and/or DHA intake ranging from 0.33 to 4 g/d; Duration ≥ 12 wks	The evidence of the beneficial effect of EPA and/or DHA intake on peripheral arterial disease related to CVD was insufficient.
Chowdhury et al 2014	SR and MA of 32 prospective cohort studies of dietary FA (n = 530,525; dosage NA; mean follow-up ranging from 5 to 23 yrs), 17 observation studies of FA biomarkers (n = 25,721; dosage NA; mean follow-up ranging from 1.3 to 30.7 yrs), and 27 RCTs (n = 103,052; 17/27 LC n-3 PUFA ranging from 0.3 to 0.9 g/d; mean follow-up ranging from 0.1 to 8.0 yrs)	The current cardiovascular guidelines, which recommends a high intake of PUFA and a low saturated fatty acids, was not supported due to insufficient evidence.
Casula et al 2014	MA of 11 RCTs (n = 15,348 with a history of CVD); n-3 intake ranging from 1 to 6 g/d; Duration ranging from 1 to 5 yrs	The efficacy of n-3 PUFA intake on preventing cardiac death, sudden death and myocardial infarction in the second prevention can be enhanced when the dose of LC n-3 PUFA is high (> 1 g/d) and for a minimum one-year administration. More experimental evidence needs to be confirmed for the current suggestion.
Gao et al 2013	MA of 15 RCTs (n = 742); n-3 PUFA intake ranging from 0.6 to 6.8 g/d; Duration ranging from 2 to 15 wks	A significant reduction in platelet aggregation was observed in individuals with poor health status, however, this was not found in healthy individuals or in the primary prevention. The efficacy of dietary n-3 PUFA intake may be greater when individuals are at high-risk for CVD and diabetes. Larger trials are suggested to support the current finding.

Delgado-Lista et al 2012	SR of 21 studies (clinical trials and RCTs); compared with placebo or usual diet ; EPA and/or DHA ranging from 0.32 to 6.9 g/d;≥ 6 mths Duration ranging from 6 to 108 mths; at least with one event of CVD	A significant reduction in cardiovascular event, cardiac death and coronary events was observed after intake of marine LC n-3 PUFA derived from food or supplements when a minimum 6 mths administration and individuals with high CVD risk; Taken together these findings may be considered for the secondary prevention although there was no dose-dependent effect of n-3 PUFA observed.
Rizos et al 2012	SR and MA of 20 RCTs (n = 68,680); n-3 PUFA intake ranging from 0.27 to 6 g/d; Duration ranging from 1 to 6.2 yrs	There was no significant association between n-3 PUFA intake and a lower risk of all-cause mortality, cardiac death, sudden death, myocardial infarction, or stroke.
Chowdhury et al 2012	SR and MA of 26 prospective cohort studies (n = 675,048), (21/26 cohort studies with highest fish intake for up to 4 serves/week; mean follow-up ranging from 4 to 30 yrs), (14/26 of cohort studies with baseline LC n-3 FA intake ranging from 0.1 to 1.6 g/d; mean follow-up ranging from 4 to 29.3 yrs) and 12 RCTs (n = 675,048; EPA and/or DHA and/or DPA intake ranging from 0.4 to 6 g/d; mean follow-up ranging from 4 to 30 yrs) over 15 countries	Intake of LC n-3 PUFA, derived from fish and supplementation, was inversely associated with cerebrovascular risk (not significant); There was insufficient evidence to determine the effect of LC n-3 PUFA on cerebrovascular in primary and secondary prevention trials.
Larsson et al 2012	MA of eight prospective studies in relation to stroke (n = 242, 076); n-3 PUFA intake in the highest (ranging from > 157 to ≥ 600 mg/d) vs. lowest category (ranging from < 39 to < 184 mg/d); follow-up ranging from 4 to 28 yrs	Overall, the current evidence was not supportive the preventive effect of LC n-3 PUFA on stroke although there was a significant reduction in total stroke risk in women following n-3 PUFA intake. This may suggest that higher intake of these PUFA is beneficial for women.
Wang et al 2012	MA of 16 RCTs (n = 901); LC n-3 PUFA intake ranging from 0.5 to 4.5 g/d; Duration ranging from 2 to 52 wks	Intake of LC n-3 PUFA improved significantly the endothelial functions including flow-mediated dilation without affecting endothelium-independent dilation.
Yang et al 2012	MA of 18 RCTs (n = 1,899); LC n-3 PUFA intake ranging from 0.3 to 6.6 g/d; Duration ranging from 4 wks to 3 yrs	Intake of LC n-3 PUFA reduces significantly the levels of plasma sICAM-1 in both healthy individuals and individuals with dyslipidemia. This may suggest that LC n-3 may be considered for preventing the development and the progression of atherosclerosis.

Kwak et al 2012	MA of 14 double-blinded RCTs (n = 20,485); EPA and/or DHA intake ranging from 0.27 to 6 g/d; ≥ 12 mths duration	There was insufficient evidence to support that intake of LC n-3 PUFA is effective on reduction in risks for overall cardiovascular events, all-cause mortality, sudden cardiac death, myocardial infarction, congestive heart failure, or transient ischemic attack and stroke.

n represents the number of participants in a SA and/or MA. Abbreviation: ALA, alpha-linolenic acid; CHD, coronary heart disease; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; MA, meta-analysis; RCTs; mths, months; randomised controlled trials; SR, systematic review; sICAM-1,soluble intercellular adhesion molecule-1; wks, weeks; yrs, years. Adapted from (Alexander et al. 2017, Casula et al. 2013, Chowdhury et al. 2012, Chowdhury et al. 2014, Delgado-Lista et al. 2012, Enns et al. 2014, Gao et al. 2013, Kwak et al. 2012, Larsson et al. 2012, Rizos et al. 2012, Wen et al. 2014, Yang et al. 2012).

2.3.2 Risk factors of cardio-metabolic disease (dyslipidemia, hypertension and hyperinsulinemia)

Metabolic abnormalities including dyslipidemia, hypertension and hyperinsulinemia are coexisting occurrences in CVDs and metabolic syndrome. Dyslipidemia, particularly with elevated levels of circulating TAG, has been shown as an independent risk for developing cardio-metabolic syndrome. There is consistent evidence demonstrating that intake of dietary LC n-3 PUFA reduces significantly the level of circulating TAG as shown in Table 2.5 (Chen et al. 2015a, Leslie et al. 2015, Lopez-Huertas 2012, Ursoniu et al. 2017, Wei and Jacobson 2011). This has also been supported by the latest systematic review and meta-analysis (Ursoniu et al. 2017). The systematic review and meta-analysis of 7 of RCTs (Ursoniu et al. 2017) reported that the dietary intake of EPA plus DHA, particularly derived from krill oil, significantly decreased the level of plasma TAG and LDL although future studies with more participants are needed to confirm the impact of krill oil supplementation on beneficial lipid alterations related to cardio-metabolic risk and on the risk of CVD outcomes. A systematic review of 17 RCTs (Lopez-Huertas 2012) also supported the evidence of TAG-lowering effect of LC n-3 PUFA intake that the TAG-lowering effect can be enhanced when greater than 1 g/d intake of EPA plus DHA and for a minimum of 3

months are applied. However, the levels of total cholesterol, LDL and HDL were controversial (Chen et al. 2015a, Leslie et al. 2015, Wei and Jacobson 2011). A systematic review and meta-analysis of 21 of RCTs (Wei and Jacobson 2011) reported that the EPA and/or DHA intake (ranging from 0.3 to 4 g/d) in the clinical studies may be beneficial to the management of plasma LDL and/or HDL although there were inconsistent outcomes from different clinical studies.

In addition to hypertriglyceridemia, the intake of dietary EPA and DHA has shown to be inversely associated with blood pressure and there is a possible non-pharmacologic adjunct in the treatment of hypertension as reported in several studies (Liu et al. 2011, Miller et al. 2014, Mozaffarian and Wu 2011b, Ueshima et al. 2007). Some recent systematic reviews and/or meta-analysis (Campbell et al. 2013, Miller et al. 2014) have consistently reported that the effect of LC n-3 PUFA intake in individuals with hypertension was greater when compared with normotensive individuals (Table 2.5). Although a systematic analysis of 17 RCTs (Lopez-Huertas 2012) did not find a significant effect of LC n-3 PUFA consumption on hypertension, a systematic review of 17 RCTs (Campbell et al. 2013) reported that EPA plus DHA intake reduced significantly the levels of systolic blood pressure and diastolic blood pressure in individuals with hypertension, and a modest effect of LC n-3 PUFA was also observed in normotensive. Similarly, a meta-analysis of 70 RCTs (Miller et al. 2014) reported that the improvement of blood pressure in hypertensive individuals was greater than controls when 2 g/d or greater LC n-3 PUFA intake for three to four months was applied. Generally, a significant reduction in blood pressure is attributed to DHA rather EPA (Liu et al. 2011, Theobald et al. 2007), however brachial SBP and DBP, and central SBP were significantly lowered following 1.8 g/d intake of EPA for 3 months (Iketani et al. 2013). The efficacy of LC n-3 PUFA intake on blood pressure tends to

be related to significantly high blood pressure rather than modestly elevated levels accompanied by ≥ 3 g/d intake of LC n-3 PUFA (Appel et al. 1993). Further RCTs with intake of dietary n-3 PUFA as anti-hypertensive therapy are required to demonstrate longer-term efficacy as well as the acceptability of lower doses (less than 1 g/d) (Appel et al. 1993).

The prevalence of T2DM has globally increased leading to a number of investigations on the effects of dietary LC n-3 PUFA intake related to risk for T2DM. It has been reported that intake of marine n-3 PUFA improves insulin sensitivity in humans, however, the outcomes are inconsistent as shown in Table 2.5 (Chen et al. 2015a, Wu et al. 2012, Zheng et al. 2012). A systematic review and meta-analysis of 16 prospective studies (Wu et al. 2012) revealed that there were no significant associations between fish/seafood/LC n-3 PUFA supplement and improvement of diabetes risks. Another systematic review and meta-analysis of 31 RCTs (Abbott et al. 2016) reported similar findings that there was insufficient evidence of effect of LC n–3 PUFA intake on the levels of insulin resistance or insulin sensitivity in individuals with/without T2DM. Abbott et al observed that there was a significant improvement to insulin resistance in women only, but not in men when a minimum 6 weeks intervention was applied. However, another systematic review and meta-analysis of 24 cohort and case-control studies (Zheng et al. 2012) reported that intake of marine LC n-3 PUFA was inversely associated with T2DM, particularly in the Asian population although the Western population was positively associated. Zheng et al suggested that their findings may be related to genetics and gene-diet interaction on T2DM within different populations. In contrast, a meta-analysis of 20 RCTs (Chen et al. 2015a) reported that LC n-3 PUFA consumption for less than 12 weeks in Asian population did not improve the levels of plasma glucose or glycosylated haemoglobin (HbA1c). In this meta-

analysis, a high ratio of EPA/DHA was associated with a greater decrease in plasma insulin and HbAc1 although there were no statistical effects observed (Chen et al. 2015a). In relation to insulin resistance, a number of researchers have evaluated effects of LC n-3 PUFA consumption on circulating adiponectin which is a critical biomarker to determine insulin sensitivity as well as the functional adipose tissues (von Frankenberg et al. 2014, Wu et al. 2013). Two recent systematic reviews with meta-analysis reported that there was insufficient evidence to support benefits of LC n-3 PUFA consumption to insulin sensitivity and circulating adiponectin. Intake of LC n-3 PUFA tends to be associated with moderate increases in the levels of adiponectin (von Frankenberg et al. 2014, Wu et al. 2013).

Table 2.5 A summary of the effects of LC n-3 PUFA intake on risks for cardio-metabolic syndrome

Plasma triglycerides		
Authors	Details of studies	Summary of outcomes and future directions
Ursoniu et al 2017	SR and MA of seven RCTs (n = 662); krill oil dosage ranging from 0.5 to 4 g/d; Duration ranging from 4 wks to 3mths	Krill oil supplementation significantly deceased the level of TAG and LDL. Future investigation with more participants could confirm the impact of krill oil supplementation on beneficial lipid modifying related to cardiometabolic risk and on the risk of CVD outcomes.
Leslie et al 2015	14 dietary intervention with n-3 PUFA including ALA derived from food or supplements in normotensive and/or hypertensive individuals; n-3 PUFA intake ranging from 0.19 to 8.42 g/d; D; duration between 2 and 52 wks duration	A high ratio of EPA/DHA contributed to a greater decreasing tendency in plasma insulin, total cholesterol, TAG, and body mass index measures. There is a statistically significant reduction in the circulating TAG, but not other markers observed.

Lopez-Huertas 2012	SR of 17 RCTs including 11 longer-term intervention with EPA plus DHA ranging from 0.2 to 3 g/d (n = 2,427, Duration ranging from 6 wks and to 26 wks), and six postprandial intervention with EPA plus DHA ranging from 1.2 to 3.3 g/d (n = 400, Duration ranging from 4 to 8 hrs)	Intake n-3 PUFA reduced significantly the levels of TAG in individuals with MS when greater than 1 g/d of LC n-3 PUFA intake for at least 3 months were considered. This finding may lead to further benefits by "reducing the % of pro-atherogenic small dense LDL particles (sdLDL) and also perhaps by ameliorating the inflammatory process associated with metabolic syndrome".
Wei and Jacobson 2011	SA and MA of 21 RCTs with EPA and/or DHA supplementation ranging from 0.7 to 4 g/d; Duration ranging from 4 wks to 5 yrs	There was consistent evidence that intake of EPA and/or DHA reduced significantly the levels of plasma TAG. The intake of EPA and/or DHA may be effective on the levels of LDL and/or HDL although there were no consistent outcomes from different RCTs.

Blood pressure

Authors	Details of studies	Summary of outcomes and future directions
Yang et al 2016	MA of 8 prospective cohort studies with LC n-3 PUFA derived from food or supplements; n-3 PUFA intake in the highest (ranging from > 0.2 g/d to ≥ 5-6 serve/week mg/d) vs. lowest category (ranging from < 0.06 to < 1 serve/week); Duration of follow-up ranging from 3 to 20 yrs	There is no association between fish or dietary LC n-3 PUFA consumption and incidence of elevated BP. The circulating LC n-3 PUFA, particularly EPA and DHA was inversely associated with incidence of elevated BP which might be important public health implications for primary prevention of elevated BP.
Miller et al 2014	SR of 70 RCTs with EPA and/or DHA supplementation ranging from 0.2 to 15 g/d; Duration ranging from 3 to 52 wks	Intake of EPA plus DHA may reduce BP, particularly in hypertensive individuals without antihypertensive medication. In addition, a lower dose (between 1 and 2 g/d) may reduce systolic BP, but not diastolic BP. Intake of EPA plus DHA may impact on a clinical and public health perspective by lowering BP.
Campbell et al 2012	SR of 17 of RCTs (n = 1,524 hypertensive and normotensive individuals); EPA and/or DHA supplementation ranging from 0.8 to 13.3 g/d; Duration ranging from 8 to 56 wks	There were significant effects of LC n-3 PUFA intake in hypertensive individuals which may play as important implications for population health and lowering the risk of stroke and ischaemic heart disease. Authors suggested that it is inconclusive to recommend LC n-3 PUFA as an alternative to BP-lowering drugs although there were modest effects of LC n-3 PUFA intake.

Blood glucose, insulin resistance, adiponectin and T2DM		
Authors	Details of studies	Summary of outcomes and future directions
Abbott et al 2016	SR and MA of 31 RCTs (n = 1,848 individuals with/without T2DM) with EPA and/or DHA or ALA supplementation ranging from 0.4 to 11.9 g/d; Duration ranging from 3 to 48 wks	There was no evidence to support that intake of n–3 PUFA is effective on the levels of insulin resistance or insulin sensitivity in individuals with/without T2DM; However, there was a significant improvement to IR in women only indicating a potential a sex-dependent effect with some reductions in women but not in men when a minimum 6 wks intervention were considered.
Chen et al 2015	MA of 20 RCTs (n = 1,209 in 2TDM) with LC n-3 PUFA supplementation ranging from 0.52 to 3.89 g/d of EPA, and ranging from 0 to 3.69 g/d of DHA; a minimum 2 wks of duration	The ratio of EPA/DHA, particularly for individuals with T2DM, and early LC n-3 PUFA supplementation, particularly for healthy individuals may bring out beneficial clinical outcomes.
von Frankenberg et al 2014	SR and MA of 13 RCTs with EPA plus DHA ranging from 0.7 to 4.2 g/d; Duration ranging from 3 to 144 wks	Intake of LC n-3 PUFA increased moderately the levels of adiponectin. Future RCTs are necessary to confirm these findings.
Wu et al 2013	SR and MA of 14 RCTs (n = 1,323); LC n-3 PUFA intake ranging from 0.7 to 4.2 g/d; Duration ranging from 3 to 156 wks	There was no evidence to support possible benefits of LC n-3 PUFA consumption on IS and adipocyte function.
Lopez-Huertas 2012	SR of 17 RCTs including 11 longer-term intervention with EPA plus DHA ranging from 0.2 to 3 g/d (n = 2,427, duration between 6 wks and 26wks), and six postprandial intervention with EPA plus DHA ranging from 1.2 to 3.3 g (n = 400)	There was insufficient evidence to support of effects of LC n-3 PUFA intake on the level of blood glucose in individuals with T2DM.
Zheng et al 2012	SR and MA of 24 prospective cohort and case-control studies (n = 569,784); with fish or marine n-3 PUFA (highest exposure ranging from 0.18 g/d to > 5 serve/week); Duration ranging of follow-up from 4 to 18 yrs	Intake of marine n-3 PUFA was inversely associated with risk for T2DM in Asians, but was positively associated with risk of T2DM in Western populations.

Wu et al 2012	SR and MA of 16 prospective cohort and observational studies (n = 540,154); with EPA and/or DHA or ALA supplementation ranging from 1 to 6 g/d; Duration ranging from 6 to 12 wks	There was insufficient evidence to support of effects of fish/seafood or EPA plus DHA consumption on the risks of T2DM.

n represents the number of study participants in a SA and/or MA. Abbreviation: ALA, alpha-linolenic acid, BP, blood pressure; CHD, coronary heart disease; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HDL, high-density lipoprotein; LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; LDL, low-density lipoprotein; MA, meta-analysis; mths, months; RCTs, randomised controlled trials; SR, systematic review; TAG, triglyceride; T2DM, type 2 diabetes mellitus; wks, weeks; yrs, years. Adapted from (Abbott et al. 2016, Campbell et al. 2013, Chen et al. 2015a, Leslie et al. 2015, Lopez-Huertas 2012, Miller et al. 2014, Ursoniu et al. 2017, von Frankenberg et al. 2014, Wei and Jacobson 2011, Wu et al. 2013, Wu et al. 2012, Yang et al. 2016, Zheng et al. 2012).

2.3.3 Inflammatory responses

Consumption of dietary LC n-3 PUFA has shown beneficial effects on the development and progression of various health conditions associated with inflammatory processes such as rheumatoid arthritis, diabetes, CVDs, cystic fibrosis and cancer. In the homeostasis and regulation of the body functions, inflammatory molecules, including eicosanoids, cytokines, adhesion molecules and lipid mediators, can be evaluated related to inflammatory responses. LC n-3 PUFA, particularly EPA, give rise to eicosanoid mediators related to potent anti-inflammatory action, playing a role in regulating inflammatory pathways (Calder 2014).

A systematic review of RCTs (Rangel-Huerta et al. 2012) revealed that intake of dietary n-3 PUFA was strongly associated with lower levels of inflammation and endothelial regulations in individuals with CVDs and acute and/or chronic other diseases, as shown in Table 2.6. Moreover, a meta-analysis of 68 RCTs (Li et al. 2014) reported that there was a significant reduction in C-reactive protein (CRP), IL-6 and TNF-α after marine LC n-3 PUFA consumptions where the most lowering effect was found in non-obese individuals. Inflammatory responses related to a range of chronic conditions including cancer can be exacerbated, leading to further progression

resulting in cancer. A systematic review and meta-analysis of nine clinical studies on inflammatory biomarkers in individuals with colorectal cancer (Mocellin et al. 2016) showed that the levels of circulating IL-6, albumin and CRP were significantly altered after EPA plus DHA supplementation although TNF-α and lL-1b were not significantly affected. However, a systemic review of 17 RCTs (Lopez-Huertas 2012) indicated insufficient evidence for the effects of EPA plus DHA intake on plasma levels of CRP, IL-6 and TNF-α. A recent systematic review and meta-analysis (Lin et al. 2016) reported finding consistent with previous reviews that the intake of n-3 PUFA decreased significantly the level of CRP in individuals with T2DM. However, the authors suggested that further studies with larger and well-designed RCTs are required to confirm the findings.

Table 2.6 A summary of the effects of LC n-3 PUFA intake on inflammations

Inflammation		
Authors	Details of studies	Summary of outcomes and future directions
Lin et al 2016	SR and MA of eight of RCTs (n = 955) with EPA and/or DHA or ALA supplementation ranging from 1 to 6 g/d; Duration ranging from 6 to 12 wks	Intake of n-3 PUFA decreased significantly the level of CRP in individuals with T2DM although "larger and rigorously designed RCTs are required to confirm this finding and extend it into other inflammatory biomarkers".
Mocellin et al 2016	SR and MA of nine of clinical trials including individuals with colorectal cancer (n = 475) with intake of EPA plus DHA ranging from 0.6 g/d to 0.2 g/kg/day; Duration ranging from 7 postoperative days to 26 wks	Intake of EPA plus DHA showed benefits on some inflammatory mediators in individuals with colorectal cancer. However, future studies are necessary to confirm these findings with specific considerations with certain supplementation protocols involving duration, dose and route of administration as well as the concomitant anti-cancer treatment adapted.
Li et al 2014	MA of 68 RCTs (n = 4,601) with EPA and/or DHA intake derived from food and supplements ranging from 0.1 to 6.6 g/d; Duration ranging from 2 to 52 wks	Intake of marine-derived n-3 PUFA decreased significantly the level of CRP, IL-6 and TNF-α, particularly in non-obese individuals. The lowering effect of LC n-3 PUFA may bring out greater outcomes when a longer-term supplementation is considered.

Rangel-Huerta et al 2012	MA of 26 RCTs and randomised trials (n = 4,601) with EPA and/or DHA intake ranging from 0.3 to 17.2 g/d; Duration ranging from 48 hrs to 8 mths	Dietary n-3 PUFA intake was inversely associated with inflammation and endothelial activation in a range of health conditions such as CVD. There was no effect of LC n-3 PUFA intake on inflammatory biomarkers, particularly in healthy individuals which may be associated with their low levels in serum.
Lopez-Huertas 2012	SR of 17 RCTs including 11 longer-term intervention with EPA plus DHA ranging from 0.2 to 3 g/d (n = 2,427,-Duration ranging from 6 wks and to 26 wks), and six postprandial intervention with EPA plus DHA ranging from 1.2 to 3.3 g/d (n = 400; Duration ranging from 4 to 8 hrs)	There was insufficient evidence to support of effects of LC n-3 PUFA intake on several inflammatory markers including CRP, IL-6, TNF-α, sICAM and adiponectin. More studies are needed to control study designs with a dose of LC n-3 PUFA, a larger number of study individuals to examine the effects of LC n-3 PUFA intake on the inflammation associated with metabolic syndrome.

n represents the number of study participants a SA and/or MA. Abbreviation: CRP, C-reactive protein; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; IL-6, interleukin-6; LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; MA, meta-analysis; mths, months; RCTs, randomised controlled trials; TNF-α, SA, systematic review; sICAM-1,soluble intercellular adhesion molecule-1; tumor necrosis factor-alpha; wks, weeks; yrs, years. Adapted from (Li et al. 2014, Lin et al. 2016, Lopez-Huertas 2012, Mocellin et al. 2016, Rangel-Huerta et al. 2012).

2.3.4. Cognitive impairments including depressive disorder and neurodegenerative decline

The investigation of LC n-3 PUFA related to cognitive impairments has been growing over the last two decades. LC n-3 PUFA is critical for the structure and function of cell membranes in the brain (as a lipid-rich organ). Particularly, DHA is the most abundant PUFA in the brain and retina (Chilton et al. 2014, Sinclair et al. 2007). Currently available reviews on cognitive effect of LC n-3 PUFA intake are summarised in Table 2.7.

A systematic review and meta-analysis of 24 RCTs (Cooper et al. 2015) reported that there was insufficient evidence to support the effects of LC n-3 PUFA intake on the improvement of neurodevelopmental disorders in children and adults with attention deficit hyperactivity disorder (ADHD) or a related-neurodevelopmental disorders.

Another systematic review and meta-analysis of 10 clinical studies reported by the same researchers (Cooper et al. 2016) showed that there was a small effect of LC n-3 PUFA intake on reducing emotional liability and oppositional behaviour, including conduct problems, in subgroups of children with ADHD (Cooper et al. 2016). Similarly, a review of 25 clinical studies (Königs and Kiliaan 2016) reported that the intake of LC n-3 PUFA improved ADHD in children in particularly mild forms of ADHD. However, these three more recent reviews agreed that further clinical studies of LC n-3 PUFA should be conducted with a larger number of study participants, focusing on populations with the LC n-3 PUFA deficiency and significant neuro cognitive deficits (Cooper et al. 2015, Cooper et al. 2016, Königs and Kiliaan 2016).

Other researchers reported inconclusive outcomes of LC n-3 PUFA intake associated with mood disorders in adults (Appleton et al. 2016, Mocking et al. 2016). A comprehensive meta-analysis of 47 RCTs reported that there was a trend of significant clinical outcomes of LC n-3 PUFA intake as an adjuvant rather than a single therapy, particularly in individuals with bipolar disorder. There was, however, insufficient evidence of the effect of LC n-3 PUFA consumption on depressive symptoms, particularly in young and healthy individuals as well as individuals with depression, which is considered as a secondary disease (Grosso et al. 2014). Two recent systematic review and/or meta-analyses suggested that further RCTs with a larger number of individuals are needed to evaluate the role of n-3 PUFA on mood disorders since the current evidence are insufficient. Although one of these reviews reported that the effect of LC n-3 PUFA intake on major depressive disorders can be shown when the doses of EPA were high and in individuals taking antidepressants (Mocking et al. 2016). However, this meta-analysis of 13 RCTs strongly suggested that further

studies are needed to carefully monitor the interactions between EPA and antidepressants.

The investigation of LC n-3 PUFA associated with neurological disorders and cognitive decline has been growing in which marine supplementation containing a high level of LC n-3 PUFA is effective on lowering risks of cognitive impairment (Zhang et al. 2016b). A dose-response meta-analysis of 21 studies (Zhang et al. 2016b) revealed systematic evidence that consumption of marine LC n-3 PUFA from fish or supplementations, particularly with DHA (0.1 g/d), decreased risks of dementia and Alzheimer's diseases. These authors also reported that a high total n-3 PUFA intake (8 g/d) was beneficial to mild cognitive impairment and Parkinson's disease although the total n-3 PUFA intake was not inversely associated with cognitive risks (Zhang et al. 2016b). A systematic review and meta-analysis of 34 RCTs (Jiao et al. 2014) on cognitive function throughout the life span from infancy to old age reported that intake of LC n-3 PUFA improved significantly cognitive development in infants. Moreover, intake of DHA was found to be beneficial to cognitive development during infancy. However, this review of 34 RCTs did not observe that LC n−3 PUFA intake was effective on cognitive functions in children, adults and the elderly (Jiao et al. 2014).

Table 2.7 A summary of the effects of LC n-3 PUFA intake on cognitive impairments

Impaired cognitive functions		
Authors	Details of studies	Summary of outcomes and future directions
Mazahery et al 2017	SR and MA of 15 case-control (n = 1,193); and four RCTs (n = 107; EPA and/or DHA, ranging from 0.70 to 0.84 g/d and ranging from 0.24 to 0.70 g/d, respectively; Duration ranging from 6 to 16 wks)	Individuals with ASD have lower n-3 LC PUFA status. LC n-3 PUFA supplementation may improve some ASD symptoms .although further investigations with large sample size and adequate study duration are required to confirm the efficacy of LC n-3 PUFA.

Mocking et al 2016	MA of 13 RCTs (n = 1,233); details of dosage and duration were not specified	There was evidence to support that intake of n-3 PUFA are beneficial to major depressive disorder, particularly with higher doses of EPA and in individuals taking antidepressants. Future clinical trials are requested to establish whether possible interactions between EPA and antidepressants.
Appleton et al 2016	SA, MA and meta-regression of 20 trials encompassing 26 relevant RCTs with EPA and/or DHA intake ranging from 0.1 to 4 g/d; Duration ranging from 4 to 16 wks	There was insufficient evidence to determine the effects of n-3 PUFA intake on MDD; Significant variability of outcomes heterogeneity between studies; Further research in the form of adequately powered RCTs is needed.
Zhang et al 2016	MA of 21 cohort studies (n = 181,580); details of dosage were not specified; Duration ranging from 2.7 to 21 years	Small effects of n-3 PUFA on reducing EL and oppositional behaviour in subgroups of children with ADHD based on studies excluding the possibility of moderate to large effects; No improvements in EL and RD after n-3 PUFA supplementation via the primary analyses.
Konigs and Kiliaan 2016	Possibly SR of 25 clinical studies including 15 RCTs with LC n-3 PUFA with EPA and/or DHA intake ranging from 0.4 to 1.2 g/d; Duration ranging from 8 to 24 wks	Intake of LC n-3 PUFA treatment resulted in a positive effect on ADHD, particularly for individuals with mild forms of ADHD. Further studies are required to investigate "underlying mechanisms that can lead to a reduction of ADHD symptoms due to LC n-3 PUFA intake" which can be associated with the determination of the optimal concentrations of LC n-3 PUFA, whether used as a single treatment or in combination with other medication.
Cooper et al 2016	SR and MA of 10 clinical studies with n-3 PUFA intake, including EPA, DHA and ALA, in school-aged children and adults; EPA and/or DHA, ranging from 0.08 to 1.11 g/d and ranging from 0.03 to 2.0 g/d, respectively; Duration ranging from 6 to 17 wks	There was insufficient evidence to support that intake of LC n-3 PUFA improve cognitive performance measures including emotional lability (EL) and related domains (e.g. oppositional behaviour, conduct problems). Effect of LC n-3 PUFA intake on reducing EL and oppositional behaviour may be greater in a subgroup of children with ADHD.
Cooper et al 2015	SR and MA of 24 RCTs with n-3 PUFA intake including EPA, DHA and ALA in school-aged children and adults with/without ADHD; EPA and/or DHA, ranging from 0.08 to 1.74 g/d and ranging from 0.008 to 1.8 g/d, respectively; Duration ranging from 4 to 52 wks	There was insufficient evidence to support that intake of LC n-3 PUFA improve cognitive performance in general population including individuals with ADHD and related disorders. Future clinical studies need to employ larger sample sizes and focus on supplementation of individuals with n-3 PUFA deficiency.

Grosso et al 2014	SA and MA of 47 RCTs with EPA and/or DHA and/or ALA intake ranging from 0.2 to 9.6 g/d; Duration ranging from 4 to 160 wks	Significant clinical efficacy was associated with intake of LC n-3 PUFA as adjuvant rather than a single therapy. The effect of LC n-3 PUFA on bipolar disorder, but not LC n-3 PUFA efficacy on depressive symptoms in young or healthy individuals.
Jiao et al 2014	SR and MA of 34 RCTs with EPA and/or DHA intake (n = 12,999 from infant to the elderly); EPA and/or DHA, ranging from 0.06 to 1.67 g/d and ranging from 0.01 to 2.2 g/d, respectively; Duration ranging from 12 wks to 4 yrs	Intake of LC n−3 PUFA improved significantly cognitive development in infants in which particularly DHA intake may be beneficial to cognitive development during infancy. However, there was insufficient evidence to support the effect of LC n-3 PUFA on cognitive function for children, adults, or the elderly.

n represents the number of individuals in a SA and/or MA. **Abbreviation**: ALA, alpha-linolenic acid; ASD, Autism Spectrum Disorder; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; long-chain omega-3 polyunsaturated fatty acids; MA, meta-analysis mths, months; RCTs, randomised controlled trials; SA, systematic review; wks, weeks; yrs, years. Adapted from (Appleton et al. 2016, Cooper et al. 2015, Cooper et al. 2016, Grosso et al. 2014, Jiao et al. 2014, Königs and Kiliaan 2016, Mazahery et al. 2017, Mocking et al. 2016, Zhang et al. 2016b).

2.3.5 Cancers

The consumption of LC n-3 PUFA has been investigated as an adjuvant in some cancer treatments in which different effects of LC n-3 PUFA intake on a different type of cancer (Li 2015). Some meta-analysis of prospective cohort studies have reported that the intake of LC n-3 PUFA is inversely associated with risks of breast cancer and colorectal cancer in men, as shown in Table 2.8 (Chua et al. 2013, Zheng et al. 2013). In contrast, a systematic review of prospective and case-control observational studies (Gerber 2012) reported that there were insufficient evidence to support possible benefits of LC n-3 PUFA consumption to breast, colorectal, and prostate cancers. Gerber suggested that the limited outcomes of review on preventive cancers with LC n-3 PUFA intake might be related to dietary pattern context, the level of intake and genetic polymorphism as the inherent difficulties (Gerber 2012).

A meta-analysis of 21 prospective cohort studies (Zheng et al. 2013) reported that the consumption of dietary marine n-3 PUFA was negatively associated with risk of breast cancer. Similarly, a meta-analysis of 11 cohort studies (Yang et al. 2014) reported that a positive effect of LC n-3 PUFA that higher dietary ratio of n-3/n-6 PUFA was significantly associated with lower risk of breast cancer, recommending an increased marine-derived n-3 PUFA intake to balance with n-6 PUFA. These two reviews suggested that their findings may have public health implications related to preventative dietary and lifestyle interventions for breast cancer. However, these reviews were not able to reach a decisive conclusion due to insufficient evidence of LC n-3 PUFA intake associated with breast cancer. They suggested further investigations with larger study participants (Yang et al. 2014, Zheng et al. 2013).

The outcomes of the effect of LC n-3 PUFA on prostate cancer have been inconsistent, although a systematic review and dose-response meta-analysis (Xu et al. 2015) reported no association between the intake of total fat, saturated fat or unsaturated fat, and risk factors of postprandial cancer. A meta-analysis of nine prospective studies (Chua et al. 2012) reported that the intake of n-3 PUFA or n-6 PUFA was not significantly associated with risk of prostate cancer, and the intake of LC n-3 PUFA was not significantly beneficial to prostate cancer. The intake of n-3 PUFA, particularly ALA (Chua et al. 2012), was negatively associated with risks of prostate cancer, and a similar finding was reported by a systematic review and dose-response meta-analysis (Fu et al. 2015). However, these reviews indicated insufficient evidence particularly with small sample size, short duration, and individual variation. Another meta-analysis of 12 studies (Chua et al. 2013) reported that blood levels of n-3 PUFA, particularly DPA, was inversely associated with prostate cancer While, blood levels of EPA plus DHA may be positively associated with prostate tumour risk. Chua et al

(2013) strongly suggested that their findings need to be interpreted with caution in terms of the etiology of prostate cancer which, as a multifactorial condition, could be complex with the metabolism of LC n-3 PUFA in humans.

Colorectal cancer has been the third most commonly diagnosed cancer in Australia, one of the countries showing the highest rate of bowel cancer in the world (Bowel Cancer Australia 2014). The death rate from colorectal cancer has been decreased due to increased colonoscopy screening rates in Australia and America; while, Austin et al has reported that the incidence of colorectal cancer has been increased in young adults (age < 50 years) in America (Austin et al. 2014, Bowel Cancer Australia 2014, Siegel et al. 2014). A meta-analysis of 14 prospective studies (Chen et al. 2015b) reported that the dietary intake of total n-3 PUFA or marine-derived PUFA including ALA intake was not associated with risk of colorectal cancer, and intake of n-3 PUFA may be beneficial for initiation or early stage of colorectal cancer. This review suggested that the effect of n-3 PUFA on colorectal cancer could be different in a subsite of the colon, and further prospective studies are required to confirm the findings.

Table 2.8 A summary of the effects of LC n-3 PUFA intake on cancers

Breast, prostate and colorectal cancers		
Authors	Details of studies	Summary of outcomes and future directions
Fu et al 2015	MA of 16 prospective studies; details of dosage were not specified Duration of the mean follow-up from 1.9 to 16 yrs	The blood levels of ALA were inversely associated with prostate cancer. In subgroup analyses blood levels of EPA and DHA were positively associated with aggressive prostate cancer risk and nonaggressive, however more prospective studies are required to confirm the findings.
Xu et al 2015	SR and dose-response MA of 14 cohort studies (n = 751,030); details of dosage were not specified; Duration of the mean follow-up from 2 to 17.4 yrs	There was no association between total fat, saturated fat, or unsaturated fat intake and the risk for prostate cancer. Further studies are needed to investigate the association between fat intake and degree of prostate cancer.
Chen et al 2015	MA of 14 prospective studies; n-3 PUFA highest vs. lowest intake (≤ 0.03 -2.13 g/d vs. > 0.21 - 4.48 g/d); Duration ranging from 3 to 22 yrs	Total n-3 or marine PUFAs intake was not associated with risk of CRC. "The benefits of n-3 PUFAs on CRC, particularly at initiation or early stage of CRC, might differ by sub-site within colon cancer. Future prospective studies are required to confirm the current findings.
Yang et al 2014	MA of 11 prospective studies (n = 274,135 adult females); n-3 PUFA highest vs. lowest intake (≥ 0.03 – 14.8 g/d vs. ≤ 0.05 – 7.6 g/d); Duration ranging from 2 to 11 yrs	Intake ratio of n-3/n-6 PUFA was inversely associated with risks of breast cancer in adult women. Increased n-3/n-6 PUFA intake showed a 6% reduction of BC risk among study individuals (USA, Europe and Asia). However, there is still insufficient evidence to support the effect of n-3 PUFA on breast cancer in different populations. Further studies with larger RCTs are required to confirm whether higher intake ratio of n-3/n-6 PUFA can be beneficial for breast cancer risk and/or improve the prognosis of individuals with BC.
Zheng et al 2013	MA of 21 prospective studies (n = 883,585 adult females); details of dosage and duration were not specified for all studies	Evidence from experimental or observational studies suggests that marine n-3 PUFA showed protective effective on BC, however this is still inconclusive due to insufficient evidence.
Gerber 2012	SR of 23 prospective and case-control observational studies including colorectal cancer (9 studies), prostate cancer (6 studies) and breast cancer (9 studies); details of dosage and duration were not specified for all studies	Observational studies on colorectal, prostate and breast cancers only provided limited evidence suggesting a possible role of LC-v3PUFA in cancer prevention due to the heterogeneity of the observations.

Chua et al 2013	MA of 12 studies; details of dosage and duration were not specified for all studies	Serum levels of LC n-3 PUFA, particularly DPA, was inversely associated with total prostate cancer risk, while high blood level of EPA and DHA may be associated with increased high-grade prostate tumour risk.
Chua et al 2012	MA of nine studies; details of dosage were not specified; Duration ranging from 5 to 20 yrs	There was no significant association between intake of n-3 PUFA and n-6 PUFA, and risk of prostate cancer. While, high intake of ALA decreased the risk of prostate cancer although intake of LC n-3 PUFA was not significantly beneficial.

n represents the number of study participants in a SA and/or MA. **Abbreviation**: ALA, alpha-linolenic acid; BC, breast cancer; CRC, colorectal cancer; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; long-chain omega-3 polyunsaturated fatty acids; MA, meta-analysis; mths, months; RCTs, randomised controlled trials; SR, systematic review; wks, weeks; yrs, years. Adapted from (Chen et al. 2015b, Chua et al. 2013, Chua et al. 2012, Fu et al. 2015, Gerber 2012, Xu et al. 2015, Yang et al. 2014, Zheng et al. 2013).

2.3.6 Other health conditions

Evidence for benefits of LC n-3 PUFA consumption in improving the function of a number of other health conditions including dry eye disease, pain, and infant growth and development have been accumulated. In many cases of health conditions, a modifiable diet with the low-cost approach of increasing n–3 LC-PUFA intake could be favourable for improvement these health conditions and health-related quality of life as a primary prevention strategy followed by decreasing the burden from the current condition. However, the current evidence of relationships between LC n-3 PUFA intake and those conditions are still limited and inconsistent in the study participants accounting for the apparent variations in outcomes.

Investigation of LC n-3 PUFA associated with dry eye disease, which is common and complex condition affecting people aged over 65 years (Doughty 2013), has been increased since the prevalence has been growing, particularly associated with increasing impact of digital technologies used causing dry eye disease (Bhargava and Kumar 2015, Bhargava et al. 2015). A meta-analysis of seven RCTS (Liu and Ji 2014)

revealed that the consumption of n-3 PUFA was associated with improvement of dry eye symptom. Three RCTs lasted for 3 to 6 months have also reported that the intake of LC n-3 PUFA alleviated significantly dry eye symptoms and tear stability in individuals with dry eye disease, computer vision syndrome or wearing contact lens (Bhargava and Kumar 2015, Bhargava et al. 2015, Deinema et al. 2016). All those evidence including the meta-analysis require further investigation to confirm the benefits of LC n-3 PUFA administration in dry eye condition (Bhargava and Kumar 2015, Bhargava et al. 2015, Deinema et al. 2016, Liu and Ji 2014).

Chronic pain has been associated with both psychological distress and health-related quality of life leading to an increased investigation of LC n-3 PUFA for improvement of the chronic symptom (Ramsden et al. 2015). A 12-week randomised dietary intervention reported that high n-3 PUFA plus low n-6 PUFA intervention showed significantly greater improvement in the headache impact test score and the number of headache days per month compared with the low n-6 diet (Ramsden et al. 2013). Ramsden et al (2015) also found that high n-3 plus n-6 PUFA improved physical pain, psychological distress, health-related quality of life and physical function in individuals with a chronic headache. A systematic review of 23 RCTs (Miles and Calder 2012) reported that the effect of marine n-3 PUFA intake on rheumatoid arthritis was consistent by a reduction in joint swelling and pain duration. Another meta-analysis of 10 RCTs (Lee et al. 2012) also reported that consumption of LC n-3 PUFA significantly reduced nonsteroidal anti-inflammatory drug consumption in individuals with rheumatoid arthritis although improvement in joint pains and swollen joint pain were not significantly different between LC n-3 PUFA and placebo-treated controls. A recent meta-analysis of 42 randomised trials (Senftleber et al. 2017) observed similar findings that the consumption of marine n-3 PUFA improved significantly pain in individuals

with rheumatoid rather than individuals with osteoarthritis. Those three reviews suggested that further trials need to confirm the effect of LC n-3 PUFA intake as an attractive adjunctive treatment for other pains, particularly associated with rheumatoid arthritis and inflammatory (Lee et al. 2012, Miles and Calder 2012, Senftleber et al. 2017).

The consumption of LC n-3 PUFA has been investigated for maternity and natal health through pregnancy, delivery and infant development. A systematic review of 34 RCTs reported that perinatal death in the singleton gestations was significantly reduced by 73% when n-3 PUFA was consumed before 20 weeks of gestation (Saccone et al. 2016). A recent systematic review and meta-analysis of nine randomised trials (Kar et al. 2016) reported that the consumption of EPA plus DHA reduced significantly rate of the early and any preterm delivery. A total of nine trials showed the clear evidence that the consumption of n-3 PUFA intake reduced the risk of early pre-term and any pre-term deliveries by 58% and 17%, respectively. Moreover, a systematic review and meta-analysis of 13 prospective observational studies and RCTs (Best et al. 2016) reported that increased prenatal intake of LC n–3 PUFA in the maternal diet was positively associated with childhood allergic disease. Other reviews (Gould et al. 2013, Stratakis et al. 2014) were inconclusive to support the effect of LC n-3 PUFA on early childhood cognitive and visual development, and adiposity in childhood due to insufficient evidence. All of these reviews suggested that further investigation with larger RCTs in multiple gestations and longer follow-up are required to confirm due to insufficient current evidence.

2.4 KRILL OIL

2.4.1 Introduction

Krill (*Euphausia superba*) is a relatively new source of LC n-3 PUFA as a marine zooplankton crustacean at the bottom of the food chain. Krill is commercially harvested only in the South Antarctic Ocean (Deutsch 2007). The fatty acid profile of krill is similar to that of other shrimp and crayfish, however krill has a lower proportion of cholesterol than shrimp (Tou et al. 2007, USDA 2017). Krill oil is high in the LC n-3 PUFA found in phospholipids (predominantly phosphatidylcholine) rather than TAG like fish oil (Tou et al. 2007). Limited studies have suggested that krill oil may be a better source of LC n-3 PUFA, EPA and DHA compared with fish oil (TAG or ethyl-esters form) (Schuchardt et al. 2011b) due to their biochemical composition and potential higher bioavailability. Commercially available EPA plus DHA sources such as krill oil and fish oil raise a question about their potential benefits to the human health. Any comparison between krill oil and fish oil needs to be carefully undertaken with specific outcomes such as TAG-lowering or anti-inflammation, and examining similar target tissues.

Figure 2.9 Antarctic krill

The picture was derived from (Brookes 2017).

2.4.2 The term of bioavailability

The term “bioavailability” has often been used in the LC n-3 PUFA studies. In fact, the measurement of bioavailability should be relevant to the rate and extent to which a drug/compound reaches the systemic circulation (Kwan 1997). Moreover, the term “bioavailability”, particularly in a nutrition context, would represent digestibility relevant to the amount of a given nutrient and how much of this is retained in the body. In other words, the measurement of faecal losses can also represent the “bioavailability”, however this estimation has been rarely undertaken in LC n-3 PUFA studies (Ghasemifard et al. 2014). Moreover, gender-specific differences in the contribution of LC n-3 PUFA in different tissues should be considered. It has been reported that women may have a high omega-3 index (Howe et al. 2014) and a significantly high

level of both AA and DHA in the total plasma and plasma phospholipids (Lohner et al. 2013) compared with men.

2.4.3 Comparison of effects of LC n-3 PUFA on blood and tissues between krill oil and fish oil

A number of experimental, observational and clinical studies have investigated the bioavailability of LC n-3 PUFA in krill oil over the last decade. There have been various studies which have examined (i) only krill oil (Ramprasath et al. 2015) without controls (Ulven et al. 2011)/counterparts (Deutsch 2007), or (ii) other forms of krill products such as krill powder (Berge et al. 2013)/ blended krill with salmon oil (Albert et al. 2015). Therefore, this section discusses only studies focusing on the bioavailability of LC n-3 PUFA from krill oil in comparison with fish oil. There are in total 11 randomised human trials, including six RCTs with 1-week to 12-week intervention periods (Tables 2.9) Relevant animal studies are also covered as shown in Table 2.10. In terms of bioavailability, this section, based on the current available literature, also focuses on the effects of LC n-3 PUFA from krill oil on the incorporation of LC n-3 PUFA into the plasma, and briefly covers the clinical benefits of krill oil and fish oil supplementation.

2.4.3.1 Effect of krill oil supplementation on the incorporation of LC n-3 PUFA in human studies

There were three postprandial (72-hour follow-up frame) and five longer-term studies (ranging from 4 to 12 weeks) to investigate the incorporation of LC n-3 PUFA, particularly EPA and DHA, into the plasma (Table 2.9). In the clinical studies, the levels of LC n-3 PUFA were commonly analysed relevant to the total lipids of plasma (or

RBC) or plasma (RBC) lipid fractions (TAG and/or phospholipids), and they were represented as concentration or percentage relative to total fatty acids. In these studies, different dose ranges of LC n-3 PUFA supplementation were used between the postprandial and the longer-term interventions, ranging from 1,296 mg to 1,680 mg, and from 240 mg/d to 1,100 mg/d, respectively. It is important to note that seven of 11 clinical trials used different doses of EPA and/or DHA between krill oil and fish oil although three of 11 studies (Banni et al. 2011, Schuchardt et al. 2011b, Yurko-Mauro et al. 2015) used similar doses and the data of supplementation were insufficient to clarify in a 12-week RCT (Bunea et al. 2004).

All study participants in the postprandial and the longer-term interventions were healthy individuals, except for two longer-term interventions in individuals with elevated plasma lipid levels including TAG (Cicero et al. 2015, Ulven et al. 2011). The participants in the postprandial and the longer-term interventions were commonly mixed gender cohorts although a postprandial (Schuchardt et al. 2011b) and a 12-week intervention were in male participants (Konagai et al. 2013).

In terms of the incorporation of LC n-3 PUFA into the plasma, there were no consistent findings from the three postprandial studies (Table 2.9). Only a 72-hour postprandial study (Kohler et al. 2015) reported that the incorporation of EPA and DHA into the plasma phospholipids following the krill oil supplementation was significantly higher than the fish oil Supplementation. The finding of the efficacy of LC n-3 PUFA incorporation into phospholipids rather than TAG by Kohler et al (2015) might be related to the biochemical form of LC n-3 PUFA in krill oil, particularly phosphatidylcholine in krill oil (Tou et al. 2007). EPA from krill oil is mainly found in the phospholipids (particularly, phosphatidylcholine and phosphatidylethanolamine) and

FFA compared with fish oil where the EPA is found in TAG (Kutzner et al. 2016). It has been known that the pathways of the digestion and absorption of fatty acids from phospholipids and TAG are different. The dietary phospholipids following digestion and absorption might be transported into the bloodstream in chylomicrons, HDL or as lyso-phospholipids. Whereas, dietary TAG fatty acids are mostly transported via chylomicrons which can be followed by uptake in the liver and/or incorporation into different lipids of exported lipoproteins (e.g., VLDL) (Küllenberg et al. 2012a, Lusis et al. 2004, Zierenberg and Grundy 1982). Some researchers (Kwantes and Grundmann 2015, Sampalis et al. 2003) proposed that phospholipids, due to their structural polarity, are able to be digested without bile salts leading to an efficient absorption, while TAG need to be emulsified by bile salts before the formation of micelles, as described in the section 2.2.3 with Figure 2.3. In contrast, other two postprandial studies did not observe either a significant changes from the baseline (as the area of under curve of plasma EPA and/or DHA) or differences between the krill oil and fish oil groups over the postprandial period (Schuchardt et al. 2011b, Yurko-Mauro et al. 2015). It was suggested that the limited capacity to detect significant differences between the study oils might be attributed to a substantial variability between participants.

In the longer-term clinical studies, there was always a substantial rise in the level of LC n-3 PUFA compared with the control group following supplementation of krill oil or fish oil. Five out of eight longer-term studies reported the changes in the levels of LC n-3 PUFA over the intervention period. In all five of these studies, there was a significant increase in plasma and/or RBC EPA, DHA and total n-3 PUFA, particularly compared with the control during a period of 4 – 12-week intervention (Konagai et al. 2013, Laidlaw et al. 2014, Maki et al. 2009, Ramprasath et al. 2013, Ulven et al. 2011).

However, only the 8-week study (Ramprasath et al. 2013) reported that the incorporation of EPA from krill oil was significantly higher than fish oil. Therefore, the information available from the literature is not consistent with regards to the bioavailability of EPA (and/or DHA) from krill oil in comparison with fish oil.

2.4.3.2 Effect of krill oil supplementation on the incorporation of LC n-3 PUFA in animal studies

In a total of 11 longer-term experimental studies (ranging from 4 to 12 weeks), seven of them investigated the incorporation of EPA and DHA into different tissues including the plasma, liver, brain, and adipose tissues. Four of seven studies fed different amount of LC n-3 PUFA in the diet, while the rest of studies used similar amount of LC n-3 PUFA. Hence, comparison regarding uptake are difficult to make, and a summary of these studies are presented in Table 2.10.

2.4.4 Comparison of potential health benefits of LC n-3 PUFA between krill oil and fish oil supplementation

2.4.4.1 Effect of krill oil on clinical biomarkers in human studies

There is insufficient data to compare clinical biomarkers such as the plasma TAG and inflammatory markers between krill oil and fish oil (Table 2.9). In a total of eight longer-term human trials, five of them investigated the effect of TAG-lowering. Only two of them (Bunea et al. 2004, Laidlaw et al. 2014) reported a significant improvement on the levels of TAG, however the alteration was inconsistent between krill oil and fish oil.

Dietary LC n-3 PUFA have shown the modulation of hepatic lipid metabolism, particularly for a reduction in the plasmatic level of TAG, which has been consistent in clinical and experimental studies. The recent reviews on the effect of LC n-3 PUFA on TAG-lowering effect in humans have agreed that the consumption of dietary EPA and DHA has shown a significant reduction in plasma TAG although the outcomes of other plasma lipids, such as cholesterol, LDL and HDL, have been inconsistent from different RCTs as described in the section 2.3.2 and presented in Table 2.5 (Alexander et al. 2017, Lopez-Huertas 2012). This turns in positively associated with the prevention of CVD and metabolic syndromes in which the evidence has been reported that the effect of EPA and DHA on TAG-lowering, particularly more pronounced in individuals with elevated TAG (Harris 2007). The possible mechanisms of TAG-lowering by LC n-3 PUFA have been proposed including a reduced production of VLDL followed by an increased VLDL clearance in the liver and a reduced rate of delivery of circulating non-esterified fatty acids (NEFA) to the liver (Shearer et al. 2012).

There was also limited evidence to show the health effect of LC n-3 PUFA from krill oil compared with fish oil as shown in Table 2.9. Only three longer-term studies (Maki et al. 2009, Ulven et al. 2011) measured inflammatory markers. These interventions resulted in a non-significant differences between krill oil and fish oil although a 4-week krill oil supplementation (Cicero et al. 2015) resulted in a significant improvement in hs-CRP compared with fish oil.

Development and progression of lifestyle-related diseases, including T2DM, CVDs and cancers, can be determined by pro-inflammatory responses. Intake of LC n-3 PUFA has been reported to be associated with the modulation of inflammatory responses, as described in section 2.2.5. Several systematic reviews and meta-

analyses reported the consistent effect of LC n-3 PUFA on inflammatory biomarkers such as CRP in individuals with diabetes (Lin et al. 2016). Moreover, a meta-analysis of 68 RCTs reported by Li et al. (2014) that marine LC n-3 PUFA consumption resulted in a significant reduction in CRP, IL-6 and TNF-α, particularly in non-obese individuals. It has been suggested that a decrease in pro-inflammatory eicosanoids, such as PGE_2 derived from AA, may be attributed to the intake of LC n-3 PUFA (Calder 2015). Moreover, there was evidence that an increased cellular uptake of n-3 PUFA resulted in improved fluidity of fatty acid molecules through the intestinal wall (Calder 2015). This consequently initiates alterations of derived eicosanoids production and leads to balanced lipid-based pro-inflammatory and anti-inflammatory mediators (Schuchardt et al. 2011b). It has also been suggested that the presence of high level of LC n-3 PUFA may ameliorate the expression of genes involved in inflammatory processes (Arita et al. 2005).

2.4.4.2 Effect of krill oil on clinical biomarkers in animal studies

Four of 11 experimental studies investigated the inflammatory responses. In addition to different amount of LC n-3 PUFA in the diet, different target tissues may be also related to the conflicting outcomes. A summary of these studies are presented for the further details (Table 2.10).

2.4.5 A summary of the krill oil supplementation studies in humans

Overall, current evidence on the bioavailability of krill oil versus fish oil in human and animal studies in this section are conflicting (Tables 2.9 and 2.10). These inconsistent findings of incorporation of LC n-3 PUFA might be related to a large inter-individual

variability in responses (Russell and Burgin-Maunder 2012, Schuchardt et al. 2011a, Yurko-Mauro et al. 2015) and different dosage of EPA plus DHA within clinical trials and between interventions (Cicero et al. 2015, Konagai et al. 2013, Maki et al. 2009, Ramprasath et al. 2013, Ulven et al. 2011) which may also affect inconsistent outcomes of other clinical biomarkers such as the level of plasma lipids and inflammatory responses. Moreover, different animals species (rats, mice or rabbits) with different health conditions (obesity or arthritis or inflammation) through different lengths of dietary treatment (ranging from 4-week to 12-week) might be related to the variation of the current outcomes. Therefore, future studies with a well-designed study including a larger number of participants and equivalent doses of EPA and DHA are needed to confirm the health benefits of krill oil supplementation in comparison with fish oil.

Table 2.9 Evidence of effects of LC n-3 PUFA in krill oil compared with fish oil from human clinical studies

Study design	Number of individuals	Dietary n-3 (mg or mg/d) consumption	Fatty acid composition/ changes	Lipids (plasma TAG) / Inflammation (CRP)	Limitations / future directions suggested by authors
Postprandial randomised double-blind crossover trial (72 hrs); 2 wks wash-out between treatments	n = 12 healthy male individuals (aged 31 ± 5 yrs; BMI 24.6 ± 2.2 kg/m^2)	Three groups-High Fat (29.6 g) breakfast containing EPA plus DHA (1,680 mg). G1: KO (mainly PL) (1,050 mg EPA and 630 mg DHA); G2: FO r-esterified TAG (1,008 mg EPA and 672 mg DHA); G3: FO ethyl-esters (1,008 mg EPA and 672 mg DHA)	The highest incorporation of EPA plus DHA (AUC % * hour) in KO group was in the plasma PL although there was no significant differences between the groups.	NA	High standard deviation values leading to no significant difference in DHA or the sum of EPA and DHA; The total fat (7 g/d) consumed in krill oil group was two-fold higher than other groups; Further studies with a larger sample size and a longer period are required (Schuchardt et al 2011).
Postprandial randomised single-blind crossover trial (72 hrs); 13-29 days between treatments	n = 15 healthy individuals (F:6, M:7; aged 58 ± 11.1 yrs; BMI 24.9 ± 2.4 kg/m^2)	Three groups-High fat (29 g) breakfast containing EPA plus DHA (ranging from 1,102 to 1,455 mg). G1: KO (896 mg EPA and 504 mg DHA); G2: krill meal (692 mg EPA and 410 mg DHA); G3: FO (875 mg EPA and 580 mg DHA)	A higher incorporation of plasma PL EPA plus DHA (iAUC) in KO administration compared with krill meal or FO administration.	NA	A large inter-individual variability in responses was observed; The dose of EPA and DHA were different between groups; Further longer-term studies are required to investigate a parameter reflecting tissue fatty acid composition (n-3 index) (Kohler et al 2015).

Postprandial and 4-week randomised SB parallel trial	n = 66 healthy individuals (F:48, M:18; n = 22/group; (aged 35 ± 1.4 yrs; BMI 25.3 ± 0.3 kg/m^2)	Three groups-High Fat (not specified) breakfast containing EPA and plus DHA (ranging from 1,296 to 1,380 mg). G1: KO (810 mg EPA and 486 mg DHA; astaxanthin); G2: FO TAG (840 mg EPA and 540 mg DHA; tocopherol); G3: FO ethyl-esters (840 mg EPA and 540 mg DHA; tocopherol)	No significant difference in the concentration of total plasma EPA plus DHA, and omega-3 index between groups in postprandial state (< 48 hrs) and week 4.	NA	Inter-individual variability in responses and the smaller samples size may be related to variability and confidence intervals (Yurko-Mauro et al 2015).
12-week randomised double-blind multi-centre prospective RCT	n = 120 individuals with hyperlipidemia (4 groups, 30 individuals/group - BMI dependant; aged 51 ± 9.5 yrs)	Four groups (1-3 g/d oils). G1: KO (2 g/d for BMI < 30 kg/m2; 3 g/d for BMI > 30 kg/m2); G2: KO (1 g/d for BMI < 30 kg/m2; 1.5 g/d for BMI > 30 kg/m2); G3: FO (3 g/d, 540 mg EPA and 360 mg DHA); G4: control (3 g/d, microcrystalline cellulose).	NA	1-3 g/d KO consumption were significantly effective on the levels of plasma glucose, TAG and LDL; A significant increase in HDL after KO and FO consumption, but not the control; 1 and 1.5 g/d KO was significantly effective when compared with 3 g/d FO; 2 and 3 g/d KO showed a significant reduction in glucose, TAG and LDL compared with 3 g/d FO consumption.	Contents of EPA and DHA in KO, standard deviation and p values for groups comparison were not specified; KO was used which was provided by Neptune Technologies & Bioresources (Bunea et al 2004).

4-week double-blind Parallel RCT	n= 76 overweight or obese individuals (3 groups, n = 25 KO, n = 26 MO, n = 25 control; Among groups, mean age (47 - 50 yrs), mean BMI (32 - 33 kg/m^2)	Three groups (2 g/d oils). G1: KO (216 mg/d EPA and 90 mg/d DHA); G2: Menhaden oil (MO, 212 mg/d EPA and 178 mg/d DHA); G3: Olive oil control	Significant increases in the plasma levels EPA and DHA after KO and MO compared with the control; No significant difference between KO and MO.	No significant differences in the levels of plasma glucose homeostasis, lipids (TAG, HDL) and hs-CRP between groups.	The dose of DHA was different between groups. Future studies with a longer intervention are required to achieve steady-state plasma levels of EPA and DHA (Maki et al 2009).
4-week double-blind crossover RCT; 8 wks wash-out between treatments	n = 24 healthy individuals (F:14, M:20; aged 28 ± 5.4 yrs; BMI 23.8 ± 3 kg/m^2)	Three groups (3 g/d oils) G1: KO (371 mg/d EPA and 214 mg/d DHA); G2: FO (332 mg/d EPA and 215 mg/d DHA) G3: 3 g Corn oil control	Both KO and FO supplementation significantly increased the levels of plasma EPA, DPA and DHA compared with control. A greater incorporation of plasma EPA, RBC EPA and DHA after KO supplementation compared with FO supplementation (p < 0.005).	No significant alteration in the levels of lipids (TAG, LDL and HDL); The levels of plasma TAG and HDL were not changed across all groups, while the levels of LDL was significantly increased by KO and FO consumption compared with control.	Doses of EPA and DHA were different between KO and FO groups. There was an insufficient control of n-3 PUFA intake from individuals' diet over the study period. Further studies with longer intervention are required to compare the effect of KO and FO over different time points (Ramprasath et al 2013).
4-week double-blind parallel RCT	n = 63 healthy individuals (F:53, M:10; n = 21 KO, n = 23 MO, n = 19 control; Among groups, mean age (47 - 50 yrs), mean BMI (31 - 32 kg/m^2)	Three groups (2 g/d oils). G1: KO (216 mg/d EPA and 90 mg/d DHA); G2: MO (216 mg/d EPA and 178 mg/d DHA); G3: Olive oil control	NA	NA	Data for changes in the levels of plasma LC PUFA were not sufficiently provided to compare between groups; Further studies with a larger sample size, a longer period and higher doses of KO are required to investigate the effects on the metabolic syndrome (Banni et al 2011).

12-week randomised double-blind multi-centre prospective trial	n = 45 healthy Japanese elderly men (n = 15/group; aged 67 ± 3.4 yrs; Among groups, mean BMI (23 - 24 kg/m^2)	Three groups (2 g/d oils). G1:KO (193 mg/d EPA, 5 mg/d DPA and 92 mg/d DHA); G2:Sardine oil (SO, 491 mg/d EPA, 46 mg/d DPA and 54 mg/d DHA); G3: medium-chain TAG control	A lesser amount of EPA and DHA in KO showed equivalent to or better effects on cognitive function along with an increased level of oxyhemoglobin compared with SO.	NA	Doses of EPA and DHA were different between KO and SO groups; Further studies with a larger sample size are required to confirm the findings (Konagai et al 2013).
7-week parallel, open single-centre RCT	n = 113 healthy individuals with normal or elevated TAG and/or total cholesterol levels (n = 36 KO, n = 40 FO, n = 42 control)	Three groups (3 g/d oils). G1: KO (348 mg/d EPA and 195 mg/d DHA); G2: FO (450 mg/d EPA and 195 mg/d DHA); G3: Corn oil control; Vitamins A, E and D were added	A significant increase in plasma levels of EPA, DPA and DHA in KO and FO groups, but not in control group although there were no significant differences between KO and FO groups. The levels of AA were increased in KO group, while decreased in FO leading a significant difference between KO and FO groups.	There were no significant differences in the plasma lipids between groups; LDL was significantly increased only in FO group and the HDL/TAG ratio in KO group was significantly increased.	Doses of EPA and DHA were different between KO and SO groups (Ulven et al. 2011).
4-week double-blind cross-over RCT; 4 wks wash-out between treatments	n = 25 individuals with mild hypertension (n = 13 KO, n = 12 FO); Among groups, mean age (50 - 51 yrs), mean BMI (27.6 - 27.9 kg/m^2)	Two groups (1 -2 g/d oils). G1: 1 g/d KO (150 mg/d EPA and 90 mg/d DHA); G2: 2 g/d esterified n-3 (approximately 1,700 mg/d EPA plus DAH)	NA	A significant improvement in the plasma levels of TAG and hs-CPR in both group in which esterified n-3 group was more effective than KO group ($p < 0.05$); A significant increase in the plasma levels of HDL following only KO supplementation.	Doses of EPA and DHA were different between groups (Cicero et al 2015).

28-day randomised double-blind crossover trial; 4 wks wash-out between treatments	n = 35 healthy individuals (F:17, M:18; age 35 ± 14 yrs; BMI 25.8 ± 6.1 kg/m^2)	Three groups. G1: KO PL (150 mg/d EPA and 90 mg/d DHA); G2: concentrated rTAG FO (650 mg/d EPA and 450 mg/d DHA); G3: EE (756 mg/d EPA and 228 mg/d DHA), G4: TAG salmon oil (180 mg/d EPA and 220 mg/d DHA); All samples had tocopherols added, and PL and TAG supplements contained the naturally occurring astaxanthin	The changes in the whole blood EPA following rTAG consumption were significantly greater than other groups.	NA	Total weights of oil consumptions in all groups were not specified; Doses of EPA and DHA were very different between groups (Laidlaw et al 2014).

Abbreviation: ALA, alpha-linolenic acid, AUC, area under the curve; BMI, body mass index; CRP, C-reactive protein; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; EE FO, ethyl ester fish oil; F, females; FO, fish oil; HDL, high-density lipoprotein; G, group; hs-CRP, high-sensitivity C-reactive protein; iAUC, incremental area under the curve from the baseline; LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; LDL, low-density lipoprotein; KO, krill oil; n, the number of study participants; M, males; MO, Menhaden oil; mths, months; NA, not available; PL, phospholipids; RCT, randomised controlled trials; SO, sardine oil; TAG, triglyceride; wks, weeks; yrs, years. Adapted from (Banni et al. 2011, Bunea et al. 2004, Cicero et al. 2015, Kohler et al. 2015, Konagai et al. 2013, Laidlaw et al. 2014, Maki et al. 2009, Ramprasath et al. 2013, Schuchardt et al. 2011a, Ulven et al. 2011, Yurko-Mauro et al. 2015).

Table 2.10 Evidence of effects of LC n-3 PUFA in krill oil compared with fish oil from animal studies

Details of study design	Dietary n-3 (mg)/d consumption	Fatty acid composition/ changes	Lipids (plasma TAG) / Inflammation (CRP)	Limitations suggested by authors
4-week administration; n = 18 male Zucker rats (obese model; 4 wks old)	Three groups (0.8% of energy in the rat diet); G_1: KO (300 mg EPA and 140 mg DHA)/100 g of diet; G_2: FO (290 mg EPA and 180 mg DHA)/100 g of diet; G_3: control with soybean oil plus blended oils	In comparison to control, KO and FO significantly increased the levels of plasma EPA and DHA although there was no significant difference between KO and FO; LC n-3 PUFA administration with KO and FO significantly decreased the levels of plasma AA, in which the changes of the levels of AA in KO was greater than that of FO.	KO significantly decreased the levels of TAG in liver and heart compared with FO and control in which KO for heart TAG was significantly effective compared with FO; The levels of plasma LDL in KO and FO were significantly low compared with the control; No significant differences in the levels of HDL, IL-1β, IL-6, TNF-α, IL-10 and CRP between groups.	Further studies are needed to measure the same parameters in same target tissues to confirm the current findings (Batetta et al 2009).
6-week administration; n = 26 Male transgenic mice expressing human TNF (n = 8 in each KO and FO; n = 10 control; 6-8 wks old)	Three groups fed HF diet (23.6% w/w lipids consisting lard and soy oil); G_1: KO (5.39 wt% EPA and 2.36 wt% DHA)/day; G_2: FO (5.23 wt% EPA and 2.82 wt% DHA)/day; G_3: Control (0.03 wt% EPA and 0.05 wt% DHA)/day	In comparison to control, KO and FO significantly increased the plasma levels of LC n-3 PUFA and decreased levels of AA although there was no significant differences between KO and FO; A similar rise in plasma DHA in KO and FO although there was a significant low dose of DHA in KO compared with FO.	KO and FO significantly decreased the plasma levels of total cholesterol, cholesterol ester and HDL compared with control although there was no significant differences between KO and FO; A significant decrease in plasma TAG by KO, and plasma LDL by FO compared with control; A significant increase in hepatic pro-inflammatory IL-17 after only FO compared with control; No differences in other cytokines in liver and mesenteric adipose tissue between groups.	(Vigerust et al 2013).

25-day administration; DBA/1 mice (arthritis model); n = 42 (n = 14 in each group; 3.5 wks old collagen-induced arthritis)	Three groups fed a similar total fatty acids with soybean oil and a blended oils; G_1:KO (300 mg EPA and 140 mg DHA)/100 g diet; G_2: FO (290 mg EPA and 180 mg DHA)/100 g diet; G_3: Control (rapeseed oil, 2,500 mg/100 g of diet)	NA	The levels of infiltration in inflammatory cells after KO was significantly lower than control and FO; KO and FO significantly reduced the levels of hyperplasia and total histology score compared with control; There were no significant changes in inflammatory cytokines except for IL-1α and IL-13 which were increased after FO.	(Ierna et al 2010).
8-week intervention; n = 60 female Sprague-Dawley rats (n = 10/each group; 28 days old)	Six groups fed a HF diet (12% wt) consisting different n-3 PUFA sources; G_1: KO (20.3 mg EPA and 5.9 mg DHA)/g diet; G_2: Salmon oil (SO, 14.4 mg EPA and 3.5 mg DHA)/g diet; G_3: Tuna oil (TO, 8.6 mg EPA and 5.9 mg DHA)/g diet; G_4: Menhaden oil (MO, 3.9 mg EPA and 3.3 mg DHA)/g diet; G_5: Flaxseed (FxO, not detected); G_6: Control corn oil (CO)	MO, TO and SO diets showed significantly higher levels of renal EPA and DHA.	A significant decrease in urinary PGE_2 after MO, TO and SO diets; A significant decrease in NFkB and TNF-α following TO and SO diets.	Different doses of EPA and DHA were fed between groups; "Further studies with awareness of a potential risk of increasing phospholipids consumption on renal health are required to confirm the findings"(Gigliotti et al 2013).
2-month administration; n = 24 New Zealand white male rabbits (between 3 and 3.5 mths old); Intravenous glucose tolerance test (IVGTT)	Four groups fed isocaloric diet (approximately 450 kcal/d); G_1: KO (600 mg/d n-3); G_2: Castrated FO (600 mg/d n-3); G_3: Castrated control; G_4: Non-castrated control, Castrated	NA	A significant reduction in fasting blood glucose after KO and FO administrations.	Details of composition of n-3 PUFA and comparison *p* values were not specified between KO and FO; More studies are required to measure the same parameters related to gene expressions to confirm the current findings (Ivanova et al 2014).

4-week administration; n = 18 male Zucker rats (obesity model; 4 wks old)	Three groups fed similar total fatty acids diet; G_1: KO (298 mg EPA and 140 mg DHA)/ 100 g of diet; G_2: FO (292 mg EPA and 179 mg DHA)/ 100 g of diet; G_3: Control (rapeseed oil with blended oils)	The levels of EPA and DHA in brain PL after KO were significantly higher than control and FO; No significant differences in brain PL AA between groups.	NA	(Di Marzo et al 2010).
6-week administration; n = 20 male C57BL/6J mice (n = 6 KO; n = 5 FO; n = 9 control)	Two groups fed a HF (24% fat wt/wt) diet containing n-3 PUFA; G_1: 5.7%E KO (5.23% EPA and 2.28% DHA of total fatty acids); G_2: 5.8%E FO (8.97% EPA and 6.4% DHA of total fatty acids); G_3: HF control (0.03% EPA and 0.05% DHA of total fatty acids)	The levels of EPA and DHA in plasma and liver PL were significantly increased by KO and FO compared with control although the dose s of EPA and DHA in KO were lower than that of FO; The levels of AA in the plasma and liver were significantly decreased by KO compared with control in which no significant difference between KO and FO.	The levels of plasma cholesterol, TAG and PL were significantly decreased by FO compared with control in which no significant difference between KO and FO; The levels of VLDL in cholesterol and TAG were significantly decreased by FO compared with control, and VLDL in cholesterol in FO was also significantly reduced compared with KO.	Different doses of EPA and DHA were fed between groups (Tillander et al 2014).
8-week administration; n = 10 female Sprague-Dawley rats (age 28d) each group	Six groups fed a HF diet (12% wt) supplemented with different oils; G_1: KO (13.2 mg EPA and 4.6 mg DHA)/g diet; G_2: Menhaden oil (MO, 5.5 mg EPA and 2.0 mg DHA)/g diet; G_3: Salmon oil (SO, 10.0 mg EPA and 1.9 mg DHA)/g diet; G_4: Tuna oil (TO, 2.6 mg EPA and 2.9 mg DHA)/g diet; G_5: Flaxseed oil (FxO, not detected); G_6: Control corn oil	SO and TO groups resulted in the highest levels of n-3 PUFA in brain and liver compared with other marine diets, FxO and control; The lowest total lipid digestibility was resulted from KO group; The highest deposition of EPA and DHA in adipose tissue was found in FxO group.	NA	Further studies are required to clarify the relationship between different type of PL and fatty acid digestibility (Tou et al 2011).

1-8 week administration; male Wistar rats	Three groups fed different oils (2.5% each oil); G_1: KO (0.30 g EPA and 0.17 g DHA)/100g diet; G_2: FO (0.2 g EPA and 0.29 DHA)/100g diet; G_3: Control olive oil	NA	The levels of hepatic TAG and total cholesterol were significantly decreased in KO and FO in which KO showed more pronounced effect.	(Ferramosca et al 2012).
12-week administration; Male CBA/J mice (6 weeks old)	Three groups fed different oils (2.5% each oil); G_1: KO (0.19 g EPA and 0.11 g DHA)/100g diet; G_2: FO (0.17 g EPA and 0.11 g DHA)/100g diet; G_3: Control soybean oil	NA	There were no significant differences in plasma levels of TAG, total cholesterol, free fatty acids, PL or insulin between groups.	Further studies are needed to confirm the current findings of gene expression associated with metabolic diseases (Burri et al 2011).
6-week administration; n = 21 male C57BL/6 J mice (n = 6 each KO and FO group; n = 9 control; 9 - 10 wks old)	Three groups fed a HF (245 wt/wt) diet containing different oils; G_1: KO (5.2% EPA and 2.3% DHA in KO diet); G_2: FO (9.0% EPA and 6.4% DHA in FO diet); G_3: Control soy oil	Both krill oil and fish oil significantly increased the levels of liver EPA and DHA, and significantly decreased the levels of liver AA although there was no significant difference between two groups.	NA	Further studies are required to confirm the findings with same markers related to anti-inflammatory reaction (Skorve et al 2015).

Abbreviation: ALA, alpha-linolenic acid, AUC, area under the curve; BMI, body mass index; CRP, C-reactive protein; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; EE FO, ethyl ester fish oil; F, females; FO, fish oil; HDL, high-density lipoprotein; HF, high-fat; G, group; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; IL-1β, interleukin 1 beta; LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; LDL, low-density lipoprotein; KO, krill oil; kcal, kilocalories; n, the number of study participants; M, males; MO, Menhaden oil; mths, months; NA, not available; NFkB, nuclear factor kappa B activity; PGE2, pro-inflammatory 2- series prostaglandins; PL, phospholipids; RCT, randomised controlled trials; TAG, triglyceride; TNF-α, tumor necrosis factor alpha; VLDL, very low-density lipoprotein; wks, weeks; yrs, years; %E, % of total energy. Adapted from (Batetta et al. 2009, Burri et al. 2011, Di Marzo et al. 2010, Ferramosca et al. 2012, Gigliotti et al. 2013, Ierna et al. 2010, Ivanova et al. 2015, Skorve et al. 2015, Tillander et al. 2014, Tou et al. 2011, Vigerust et al. 2013).

2.5 STUDY AIMS AND HYPOTHESES

Overall, the aim of this thesis, with two crossover studies in healthy women, was to invetigate the effect of krill oil supplementation on the incorporation of LC n-3 PUFA (EPA, DPA and DHA) into the plasma, circulating plasma lipids (particularly TAG) and their impacts on the inflammatory biomarkers compared with the fish oil supplementation. Moreover, the plasma fatty acids from both studies are further analysed to determine the responses of lipid molecular species, particularly those containing LC n-3 PUFA, using the novel lipidomic technology, for both postprandial and long-term interventions.

2.5.1 A postprandial study and a longer-term study

The first aim of this thesis was to compare the incorporation of LC n-3 PUFA (EPA, DPA, DHA) into the plasma and circulating plasma lipids over a 5-hour postprandial period between a single dose of krill oil supplementation compared with fish oil and the control (olive oil).

The specific hypotheses tested were that:

1. Five grams of krill oil supplementation (with a smaller amount of LC n-3 PUFA, 907 mg) would result in a similar post-prandial incorporation of plasma LC n-3 PUFA compared with five grams of fish oil supplementation (with 1,441 mg of LC n-3 PUFA). The equal number of oil capsules was provided with the intention of having participants blinded for practical reasons.

2. Five grams of krill oil (with a smaller amount of LC n-3 PUFA) supplementation would result in similar post-prandial responses in circulating plasma lipids/biomarkers of CVD (TAG, total cholesterol, low-density lipoproteins, high-density lipoprotein) in both plasma and chylomicron over the five hours of postprandial period.

The second study aimed to determine the differences in the incorporation of plasma LC n-3 PUFA, plasma circulating TAG and inflammatory biomarkers to investigate how krill oil supplementation affects the plasma LC n-3 PUFA levels in comparison with fish oil supplementation in a longer-term (30 days); and whether a 30-day krill oil supplementation has different impacts on lipid metabolism and inflammatory responses compared with the fish oil supplementation.

The specific hypotheses tested were that:

1. A 30-day krill oil supplementation (providing 1,269 mg/d of LC n-3 PUFA) would increase the plasma LC n-3 PUFA levels greater than the fish oil supplementation (providing 1,441 mg/d of LC n-3 PUFA).

2. 30-day supplementation of krill oil would be more effective on the plasma TAG and inflammatory biomarkers compared with the fish oil supplementation.

2.5.2 Lipidomic study

The third aim of the thesis was to investigate the plasma lipidomic profiles as a novel measurement in order to determine whether the krill oil supplementation would result in similar changes in lipid molecular species compared with fish oil supplementation over both postprandial and longer-term periods. This would provide a better insight into the lipid mechanisms at molecular levels, and help us to understand better the

variation of impact between the krill oil and fish oil consumption in both the postprandial phase and the longer-term period.

The specific hypotheses tested were that:

1. Lipidomic profiles would provide more specific and comprehensive data on the plasma lipid molecular species to enable the comparison of differences between the impact of krill oil and fish oil supplementation.

2. EPA and DHA from krill oil and fish oil would be differentially incorporated into the plasma EPA and/or DHA-molecular species in different lipid classes over both the postprandial phase and the long-term intervention period.

Chapter 3: General Materials and Methods

3.1 CLINICAL HUMAN TRIALS

Both a postprandial and a longer-term dietary interventions, with krill oil supplementation compared with fish oil supplementation, were randomised and crossover studies. The postprandial study included also a control (olive oil) treatment.

3.1.1 Human ethics and trial registration

Human ethics approval was required to ensure research projects involving humans adhere to the value and principles of ethical conduct prior to the conducting of each dietary intervention. Ethics approval for the postprandial study and the longer-term study were obtained from the Victoria University Human Ethics research (VUHREC). The titles of the ethics submissions were *"The effect of dietary krill oil supplementation on cardiovascular risk in healthy females"* (HRE14-040) and '*The effect 30-day of dietary krill oil supplementation focusing on plasma biomarkers and lipids and lipidomic profiles in healthy women'* (HRE15-031), respectively.

Both dietary interventions were also registered to Australian New Zealand Clinical Trial Registry (ACTRN) in 2014 and 2015, and were allocated the ACTRN (ACTRN12615000620527 and ACTRN 12615000472572, respectively).

3.1.2 Recruitment of study participants

Recruitment for study participants was undertaken by emails to all Victoria University staff and students, and flyer advertisements on the Victoria University at five

campuses (City, Footscray Park, Footscray Nicholson, Werribee and St. Albans), the general public, community centres, and medical practices.

Participants were screened for their suitability for the postprandial and the longer-term studies using a medical questionnaire and anthropometric/physiological measurements at the initial meeting. All necessary information was conveyed to the participants in the *Information to Participants Involved in Research*. This explanatory statement enabled participants to read through and to be aware of the procedures involved in the studies. The potential participants were given an opportunity to ask questions during the initial meeting and written consent was obtained from all participants prior to enrolling into the study.

Inclusion criteria for the postprandial study and the longer-term study were similar: healthy women, who have not experienced menopause, were aged between 18 and 45 years within BMI 20-30 (kg/m^2), and aged between 18 and 50 years within BMI 20-35 (kg/m^2), respectively. Additionally, exclusion criteria were cigarette smokers, pregnant or lactating, or had heart, liver, kidney or inflammatory bowel disease, diabetes, or medications interfering with lipid metabolism or lowering blood lipids, allergy to fish or seafood or intake of oily fish more than twice a week or supplement including omega-3 fatty acids in the past four weeks prior to the dietary interventions.

Ten participants took part in the postprandial study and there were no dropouts during that study. On the other hand, 13 participants originally started the longer-term study, of which two dropped out after washout period due to relocating overseas and health issues, thus data presented were derived from 11 participants.

All participants in the postprandial study were given a lunch voucher and a sustainable drink (Sanitarium, UP & GO liquid breakfast, Australia) after the last postprandial blood sample at 5 hours was taken. This helped participants to restore energy intake on the study day. Participants who completed the longer-term study were given $50 voucher and a thank-you card for their time and efforts to the study over three months.

3.2 STUDY DESIGN

All participants in both studies were instructed to maintain their habitual food intake and exercise regime, and limit to consumption of fish/seafood or omega-3 fortified foods no more than once a week during the study period including washout period.

3.2.1 Postprandial study

3.2.1.1 Study protocol

The postprandial crossover study was conducted with test meals containing three study oils which were consumed in a randomised order. The participants were required to attend three times the Exercise and Metabolism Unit, at Victoria University, St. Albans Campus at intervals of a minimum one week washout period.

Prior to each study day, participants were required to consume a standardised dinner, as one of most common low-fat dishes in their diet, and to avoid drinking alcohol and strenuous physical activity, and fast approximately 10 hours overnight. On each study day, participants arrived at the clinic between 7 am and 9 am, and a fasting blood sample (baseline, 10 mL) was collected. A single test meal was provided with 250 mL of water. All participants finished the test meal within 15 minutes. Participants were

only allowed to drink water during the 5-hour postprandial intervention period. Postprandial blood samples (10 mL) were collected using intravenous cannulation by a qualified practitioner, the student researcher after a test meal consumption over 5 hours. Strategies that were used for difficulty drawing blood samples due to the cold weather included cover the arm with a towel and hot water bottle, and offering a heater or warm water to drink during the postprandial intervention.

3.2.1.2 Test meal with study oils

Three test meals were provided with krill oil, fish oil or olive oil. The test meal consisted of 150 g of fresh mashed potato with 20 g of olive oil (control, 20 g of olive oil; krill oil, 15 g of olive oil + 5 g of krill oil; fish oil, 15 g of olive oil + 5 g of fish oil). Peeled potatoes with one tablespoon of water were cooked using a microwave oven at the Exercise and Metabolism Unit, at Victoria University, St. Albans Campus before participants arrived. 150 g of cooked potatoes were mixed with 15 g of olive oil (Coles™, olive oil, Australia) and mashed after adding a 5 g of salt. The test meal was served with 150 g of mashed potatoes, 5 g of krill oil capsules (containing 542 mg of EPA and 298 mg of DHA) or 5 g of fish oil capsules (containing 786 mg EPA and 473 mg DHA), and 250 mL of water. Whereas, test meal with olive oil was prepared that 20 g of olive instead of 15 g were added to the mashed potato.

The study oils, including krill oil (Swisse Wellness Pty Ltd., high Strength deep sea krill oil, Victoria, Australia) and fish oil (Swisse Wellness Pty Ltd., Odourless wild fish oil, Victoria, Australia) purchased from the local pharmacy, were analysed for the fatty acid profiles prior to the commencement of intervention, as shown in Table 3.1. The single capsule fill weight was 1,054 mg for krill oil and 1,063 mg for fish oil, which was used to calculate the EPA, DPA and DHA contents. In brief, five randomly chosen oil

capsules were mixed together and analysed for six times using gas chromatography (GC) which was performed for each study oils.

3.2.2 Longer-term study

3.2.2.1 Study protocol

The longer-term crossover study was conducted with krill oil and fish oil supplementation for 30 days each, and a minimum 30-day washout period between. During the 30-day intervention period, all participants were asked to ingest omega-3 supplement such as seven capsules (1 g each) of krill oil and five capsules (1 g each) of fish oil on a daily basis. The participants were required to attend five times the Exercise and Metabolism Unit, at Victoria University, St. Albans Campus for each supplementation period. Based on the fatty acids analysis, the amount of long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) from the two omega-3 oils was matched, seven capsules (1g each) of krill oil or five capsules (1g each) of fish oil were determined to provide not only an appropriate amount of LC n-3 PUFA, but also the closest possible match to these fatty acids (FA) from the two omega-3 oils.

All protocols were identical for each intervention. Anthropometric and physiological parameters were measured on days zero and 30. All participants were requested to complete a *24-hour dietary recall* at days 5 and 10, a *3-day food diary* at days zero, 15 & 30, and the *Internal Physical Activity Questionnaire* at days zero and 30. A blood sample after overnight fasting (approximately 10 hours) was collected using venepuncture by the student researcher at five-time points (days zero, 5, 10, 15 and 30).

Table 3.1 The analysis of fatty acids in study oils

Fatty acids	mg/ g			% of total fatty acids		
	Olive oil	Fish oil	Krill oil	Olive oil	Fish oil	Krill oil
14:0	0.0	64.4 (1.0)	66.3 (0.8)	0.0	6.7	9.0
14:1n-5	0.0	0.0	1.5 (0.1)	0.0	0.0	0.2
15:0	0.0	4.5 (0.1)	2.8 (0.0)	0.0	0.5	0.4
16:0	96.9 (12.9)	142.2 (2.2)	141.7 (1.6)	10.7	14.9	19.3
16:1n-7	6.2 (0.8)	75.2 (1.1)	33.0 (0.4)	0.7	7.9	4.5
17:0	0.0	5.8 (0.1)	0.3 (0.2)	0.0	0.6	0.0
16:2n-4	0.0	10.0 (0.2)	3.1 (0.2)	0.0	1.0	0.4
16:3n-4	0.0	12.0 (0.2)	0.6 (0.3)	0.0	1.3	0.1
18:0	31.3 (4.2)	27.7 (0.5)	8.4 (0.1)	3.5	2.9	1.1
18:1n-9 t	1.7 (0.2)	1.9 (0.4)	0.2 (0.2)	0.2	0.2	0.0
18:1n-9	691.0 (92.6)	73.5 (0.9)	70.1 (0.8)	76.6	7.7	9.5
18:1n-7	18.1 (2.4)	26.2 (0.5)	45.4 (0.5)	2.0	2.7	6.2
18:2n-6 t	0.0	0.5 (0.3)	1.6 (0.2)	0.0	0.1	0.2
18:2n-6	41.5 (5.5)	11.9 (0.2)	13.3 (0.2)	4.6	1.2	1.8
18:3n-6	0.0	2.2 (0.5)	0.6 (0.3)	0.0	0.2	0.1
18:3n-4	0.0	0.3 (0.2)	0.5 (0.2)	0.0	0.0	0.1
18:3n-3	4.8 (0.6)	6.6 (0.1)	7.2 (0.2)	0.5	0.7	1.0
20:0	4.0 (0.5)	4.4 (0.2)	0.3 (0.2)	0.4	0.5	0.0
20:1n-11	0.0	28.6 (0.4)	30.7 (3.4)	0.0	3.0	4.2
20:1n-9	7.2 (0.9)	16.5 (0.8)	9.7 (1.0)	0.8	1.7	1.3
21:0	0.0	8.7 (1.3)	18.0 (4.6)	0.0	0.9	2.4
20:2n-6	0.0	11.5 (1.5)	10.4 (2.4)	0.0	1.2	1.4
20:3n-6	0.0	23.4 (5.5)	19.8 (2.3)	0.0	2.4	2.7
20:4n-6	0.0	14.7 (0.8)	6.6 (0.5)	0.0	1.5	0.9
20:3n-3	0.0	17.5 (2.3)	13.3 (1.5)	0.0	1.8	1.8
20:4n-3	0.0	2.9 (0.9)	6.8 (1.2)	0.0	0.3	0.9
22:1n-11	0.0	17.4 (1.5)	7.1 (1.2)	0.0	1.8	1.0
22:1n-9	0.0	17.3 (2.3)	7.8 (0.5)	0.0	1.8	1.1
20:5n-3	0.0	157.3 (2.6)	108.4 (1.4)	0.0	16.4	14.7
24:0	0.0	6.8 (0.2)	4.1 (0.1)	0.0	0.7	0.6
22:4n-6	0.0	8.8 (2.0)	8.0 (0.7)	0.0	0.9	1.1
22:5n-6	0.0	14.6 (1.9)	7.9 (1.1)	0.0	1.5	1.1
24:1n-9	0.0	10.7 (1.4)	7.7 (2.1)	0.0	1.1	1.0
22:5n-3	0.0	36.3 (5.6)	13.4 (1.0)	0.0	3.8	1.8
22:6n-3	0.0	94.6 (2.7)	59.5 (0.8)	0.0	9.9	8.1
n-3 PUFA	4.8 (0.0)	315.2 (1.7)	208.6 (0.6)	0.5	32.9	28.4
n-6 PUFA	41.5 (0.0)	87.6 (0.9)	68.1 (0.4)	4.6	9.2	9.3
Total LC n-3 PUFA	0.0	288.2 (1.0)	181.3 (1.7)	0.0	30.1	24.6

Values are expressed as mean (SEM) of five randomly chosen oil capsules mixed together and analysed for six times using gas chromatography. The total LC n-3 PUFA represents EPA, DHA and DPA. Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; mg/g, milligram per gram.

Table 3.2 The analysis of lipid classes in study oils

Lipid class	% of lipid class		
	Krill oil	Fish oil	Olive oil
Wax esters	1.0	0.5	0.3
Triacyglycerols	**23.8**	**97.7**	**98.0**
Free fatty acids	12.9	0.5	0.4
Sterols	1.0	0.7	0.5
Phospholipids	**61.4**	**0.6**	**0.7**

The study oils, five randomly chosen oil capsules mixed together and analysed for three times, were quantified for individual lipid classes using an Iatroscan thin-layer chromatography-flame ionization detector analyser (TLC-FID). The separation of lipids classes using an Iatroscan TLC-FID was supported by Mina Brock at CSIRO Tasmania, Australia.

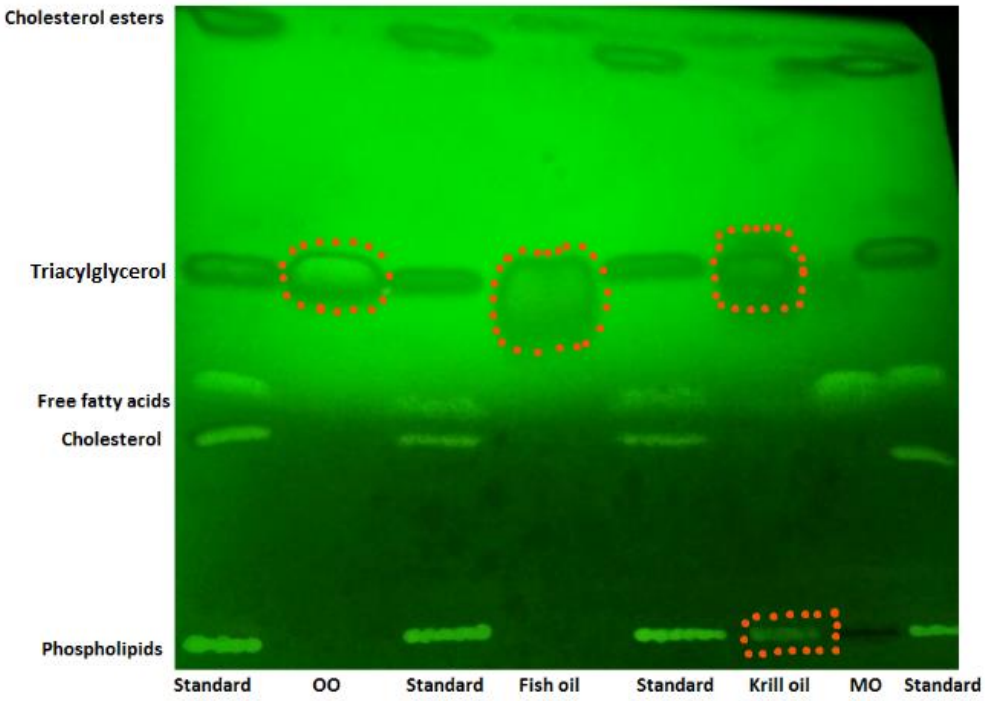


Figure 3.1 Visualised lipid fractions of the study oils

The study oils, five randomly chosen oil capsules mixed together, were quantified for individual lipid classes using a thin layer chromatography (TLC). The separation of lipids classes using TLC supported by Professor Andrew Sinclair at the Metabolic Research Unit, Deakin University, Geelong Waurn Ponds Campus, Australia.

3.2.2.2 Supplementation with krill oil and fish oil

All participants were instructed to ingest the supplement capsules daily with a meal having the highest fat content of the day, which was generally dinner. Only one participant ingested the supplement with her lunch as it was her highest fat meal of the day.

3.3 CLINICAL ASSESSMENTS

3.3.1 Anthropometric and physiological measurements

All measurements were performed using standard technique and equipment (Centers for Disease Control and Prevention 2016). All measurements were taken in duplicate and the mean of the measurements was used as the final result.

Participants were asked to wear light clothing and were barefooted while anthropometric measurements, such as body weight and waist circumference, were conducted. Participants were asked to stand with their backs to the stadiometer while height was measured (to the nearest 0.5 cm) using a standardised stadiometer (0-2000 mm) (Mentone Educational Australia 2011).

Body weight was determined to the nearest 0.1 kg using an electronic digital scale after removal of heavy clothing and shoes which was placed on a level surface at the Exercise and Metabolism Laboratory at Victoria University, St Albans Campus. Body mass index (BMI) was calculated using the following formula: BMI = body weight (kg)/ height (m^2) (WHO 2000).

Waist circumference and hip circumference were measured using an inelastic measurement tape. Waist circumference was measured to the nearest 0.1 cm at the midway between the lowest point of the rib cage and the most lateral points of the iliac crest in the narrowest site. Hip circumference was taken at the level of the greatest protuberance of the buttocks. The ratio of waist circumference to hip circumference (WHR) was calculated using the following formula: WHR = waist circumference (cm)/hip circumference (cm) (Australian Institute of Health and Welfare 2011).

Blood pressure for systolic and diabolic blood pressure, and the heart rate were measured after the participant was seated and relaxed for at least 10 minutes using an automated digital blood pressure monitor (Omron Healthcare, Kyoto, Japan).

3.3.2 Health, dietary and physical activity assessment

3.3.2.1 Medical questionnaire

A medical questionnaire was used to screen participants' eligibility for the postprandial study and the longer-term study. Potential participants provided their age, fish/seafood consumption, general health condition and history including food allergies using the medical questionnaire, which included a question about menstrual cycle regularity.

3.3.2.2 Food and physical activity records

It is important to monitor food consumption and physical activity of participants over the dietary intervention periods as participants may have potential variations in their food intake and physical activities on a daily basis. All participants were provided detailed instructions on how to measure and record their food consumption including beverage, and they were advised to record a detailed description of food consumption

such as type, portion size, brand, cooking methods and ingredients in recipes. All participants completed food records including physical activity using a *24-hour dietary recall* and a *3-day food diary* prior to each clinic visit day. Participants also recorded their general physical activities on a daily basis using the dietary forms in which the type of exercise, duration and intensity were determined as leisure-time physical activities (walking, jogging, swimming). The research student clarified the dietary records for any unclear records with the individual participant when required. A *24-hour dietary recall* was used at each study day during the postprandial study, and at days 5 and 10 for each supplementation period in the longer-term study. A *3-day food diary* (two week days and one day of the weekend) including physical activity was used at days zero, 15 and 30 for each supplementation period in the longer-term study.

International Physical Activity Questionnaire (IPAQ) was used to monitor participants' habitual physical activity during the longer-term study period. The previously validated *IPAQ* consists of 27 questions to evaluate the total weekly physical activity undertaken across a comprehensive set of four domains including work-related, transportation, housework and leisure time physical activity (Craig et al. 2003). The self-administered *IPAQ* also includes questions on time spent sitting to evaluate sedentary behaviour.

3.3.2.2.1 Analysis of food intake

Food records were analysed using *FoodWorks* Professional (version 8, Xyris Software 2015) with NUTTAB 2010 and AUSNUT 2013 based on Australian Food Composition Database. *FoodWorks* provides three categories of data including macro-nutrients, vitamins and minerals. The results of macro-nutrients were given as total energy intake (kJ), carbohydrates (g), proteins (g), total fats (g), fibres (g) and alcohol (g), and the total fats (g) includes saturated fats, monosaturated fats and polyunsaturated fats.

3.3.2.2.2 Polyunsaturated fatty acids frequency questionnaire (PUFA FFQ)

In addition to food diary analysis, dietary fat intakes were analysed particularly for LC n-3 PUFA intake using an electronic polyunsaturated fatty acids frequency questionnaire (PUFA FFQ, PUFA v1, The University of Wollongong) in the longer-term study. The same questionnaire was also used to screen participants for n-3 PUFA intake based on the data from previous 3 months, and assess the habitual food intake at days zero (baseline), 5, 10, 15 and 30 for each treatment period. The PUFA FFQ consisted of 39 questions; frequency of consumption of common sources of omega-3 fatty acids such as meats, fresh fish and shellfish, canned fish, and vegetables throughout various common main dishes, snack foods and desserts. The frequency of consumption categories was once per day, 2-3 times per week, once per month or once per a couple of months. The PUFA FFQ analysis determines an average of total n-3 intake per day and average LC n-3 intake per day (Sullivan et al. 2008, Sullivan et al. 2006, Swierk et al. 2011).

3.3.3 Blood sampling and preparation

Blood samples were collected at the Exercise and Metabolism Unit, at Victoria University, St. Albans Campus for the analysis of plasma fatty acids, lipids (total cholesterol, triglyceride and high-density lipoprotein), glucose and other biomarkers in the postprandial study and the longer-term study. Participants in both studies were asked to refrain from exercise and alcohol consumption, and fast for approximately 10 hours (overnight) prior to the blood collection.

Approximately 10 mL of venous blood at each time was drawn from the cubital vein using a winged needle and ethylene diamine tetraacetic acid (EDTA) vacutainer

(Beckton Dickinson, UK). Blood samples were gently mixed by inverting about several times immediately after collection. Blood samples then were immediately spun for 10 minutes at 3,500 g at 4°C to obtain plasma. The plasma was aliquoted into cryotubes and was stored at -80°C until further analysis.

3.3.3.1 Postprandial study

Venous blood samples were collected at the fasting state and thereafter an hourly basis for five hours after consumption of a test meal.

3.3.3.1.1 Chylomicron isolation

Postprandial plasma at each time point in the acute study was used for further chylomicron (CM) separation. Chylomicron fractions were isolated using ultracentrifugation.

Density check for a salt solution was undertaken prior to CM isolation. For instance, 1 mL of salt solution should weigh 1.006 g. If the density was high, then water was added to lower the density whereas salt was used to increase the density.

Postprandial plasma (2.7 mL) was transferred into three tubes (2 mL) in which 900 µL plasma without foam or a bubble on the top was transferred into each tube. 800 µL of 1.14% NaCl salt solution (density = 1.006 kg/ L) on top of 900 µL of postprandial plasma was laid and followed by ultracentrifugation at 36, 000 g at 4 °C for 30 min in a Sigma (3-30K) with 12154-H rotor. Chylomicrons can be seen as a fluffy white layer on the very top in which 0.5 mL of CM fraction from the very top was removed and aliquoted into cryotubes, and stored at -80°C until further analysis. In the fasted sample, the top layer contained little or nothing visible, however 0.5mL of the very top

layer of each sample was removed as CM fraction. The rest of plasma after CM fraction removal was aliquoted into cryotubes and labelled as non-chylomicron plasma. The detail of this protocol was obtained from Dr Maxine Bonham (2013).

3.3.3.2 Longer-term study

20 mL of fasting blood sample at baseline (day 0), days 15 and 30, and 10 mL of fasting blood sample at days 5 and 10 were collected. Blood samples were centrifuged to obtain plasma and then aliquoted into cryotubes and stored at -80 °C until further analysis.

3.3.3.2.1 Full blood count and fasting glucose analysis

Two EDTA and a citrated vacutainers in order were used for blood collection at days zero, 15 and 30. 1 mL of whole blood sample for full blood count (FBC) was taken from the second EDTA vacutainer to avoid potentially increased white blood cell (WBC) and/or plasma due to a needle puncture. A whole blood sample was aliquoted into a sterilised eppendorf tube and immediately kept on ice until FBC analysis. FBC were analysed within 2 hours after the blood collection using an automated haematology analyser (Sysmex, KX-21N, USA) at Institute of Sport, Exercise & Active Living (ISEAL), at Victoria University, Footscray Park Campus.

A drop of whole blood sample was transferred from the second EDTA vacutainer onto a strip and glucose was analysed using an automatic glucometre (Precision Xtra system, Abbott Diabetes Care Inc., Australia).

3.4 FATTY ACIDS ANALYSIS

3.4.1 Lipid class separation

Lipid class separation using thin layer chromatography (TLC) was performed for dietary study oils including krill oil, fish oil and olive oil prior to the postprandial study, and some plasma samples from the postprandial study. Lipid class separation using TLC was used to investigate differences of study oils within distinctive lipid classes including phospholipid (PL), cholesterol (COH), free fatty acid (FFA), triacylglycerol (TAG) and cholesterol ester (CE), and furthermore differences of LC n-3 PUFA, particularly in TAG and PL fractions rather than total plasma lipids, between krill oil and fish oil. For the separation of a mixture lipid components using TLC technique, the student researcher was trained and supported by Professor Andrew Sinclair at the Metabolic Research Unit, Deakin University, Geelong Waurn Ponds Campus.

3.4.1.1 Thin layer chromatography

TLC plates were prepared by mixing a slurry of 7 g of Silica Gel 60 G powder (Merck Millipore, Darmstadt, Germany) with 17 ml distilled water in a 50 mL falcon tube, then the slurry was spread evenly on a 20 x 20 cm^2 ethanol cleaned glass TLC plate. Prepared plates were conditioned in an oven at 100°C for 1 hour after being air-dried. TLC plates were removed from the oven and placed in a desiccator for approximately 1 hour prior to sample application which allowed the silica gel to cool down.

Standard mixtures of fatty acid methylation

Saturated fatty acid, C17:0 (Heptadecanoic (Margaric), MW: 270.48 g/mol)) (Nu-Chek Prep Inc., Elysian, MN).

Triglyceride fatty acid, C17:0 (Triheptadecanoin, MW: 849.42 g/mol) (Nu-Chek Prep, Inc., Elysian, MN).

Phosphatidylcholine (PC), C17:0 (PC, 1 2-diheptadecanoyl-sn-glycero-3-phosphocholine, MW (762.15 g/mol) (Avanti Polar Lipids, Alabaster, USA).

To make the Internal Standards

250 mL of dichloromethane-methanol (DCM) (1:1) + 5 mg of BHT = 5 mg (5000 µg)/ 250 mL ⇨ 20 µg/ mL

Weighed out 26.9 mg TAG and 26.3 mg PC into two separate 25 mL volume flask (2 flasks)

Take 3 mL of each standard TAG/PC and dilute to 250 mL in DCM; that is, 3 mL x 26.9 (TAG) mg /25 ⇨ 250 mL DCM; 3mL x 26.3 mg (PL)/25⇨250 mL DCM

Internal standard triacylglycerol (TAG) – details of calculations:

26.9 mg/ TAG MW (849.42 g/mol) x 3 x FA MW (270.48g/mol) = 25.69 mg C17:0 from TAG in 25 mL.

25.6 g/ 25 mL x 3/250 mL (DCM) x 7 mL (Std. extraction) = 0.0887 mg (= 88.7 µg) = amount of TAG IS per sample of plasma extracted in presence of the IS.

Therefore concentration of TAG-FA is

= (Area FA/ area C17:0) x 88.7 µg of C 17:0 x (1mL / 0.7mL plasma)

Internal standard phospholipids (PL) – details of calculations:

26.3 mg / PL MW (762.15 g/mol) x 2 x FA MW (270.48 g/mol)

= 18.667 = 18.67 mg C17:0 from PL in 25 mL.

18.67 mg/ 25 mL x 3/250 mL (DCM) x 7 mL (Std. extraction) = 0.0627 mg (=62.7 µg)

= amount of PL IS per sample of plasma extracted in the presence of the IS.

Therefore concentration of PL-FA is

= (Area FA/ area C17:0) x 62.7 µg of C 17:0 x (1mL / 0.7mL plasma)

TLC of the study oils

Standards (C18:5-A Nu-Chek Prep Inc., C18-5A).

25 mg/ 2.5mL= 10 mg / mL: CE, TAG, FFA, COH, PL

Mussel oil (MO, 200 mg / 10mL = 20 mg / mL): CE, TAG, FFA

Prior to use, all glassware equipment including extraction tubes and a 100 µL glass syringe were cleaned with DCM/ MeOH (2:1, v/v) to avoid potential contamination from the previous use. For instance, the glass syringe was cleaned for four times with DCM/MeOH (2:1, v/v) before transferring each sample including dietary study oil and the standard. The standard and mussel oil as references were used to identify each individual lipid class. Each dietary oil sample was streaked separately onto the plate where one sample per lane next to the standard (C18:5-A) was spotted with a 100 µL glass syringe (e.g. standard/ olive oil/ standard/ fish oil/ standard/ krill oil/ mussel oil/ standard. The TLC plate was then placed into a TLC developing tank containing a solvent system of petroleum ether: di-ethyl ether: acetic acid (85:15:2 v/v/v). Lipid

fractions were allowed to separate in the TLC tank for approximately 1 hour or at which point the solvent front had reached 1-2 cm away from the top of the TLC plate. The TLC plate was removed from the TLC tank and allowed to air-dry for 30 minutes in a fume cupboard. Dichlorofluoroscein (DCF) solution (0.05 g/L of methanol) was sprayed onto the TLC plate followed by being air-dried on a flat surface. The DCF solution was used to visualise the bands of lipid under a UV light (Spectroline®CM UV-viewing cabinet, Sigma-Aldrich Co., Australia). The visualised lipid fractions on each study oil sample are presented in Figure 3.1.

3.4.1.2 Iatroscan thin-layer chromatography-flame ionization detector analyser

In addition to the method of TLC, the study oils including krill oil, fish oil and olive oil were quantified for individual lipid classes using an Iatroscan MK V TH10 thin-layer chromatography-flame ionization detector (TLC-FID) analyser (Iatron Laboratories, Tokyo, Japan). Firstly, an aliquot of each oil was dissolved in chloroform and analysed (Ackman 1981, Volkman & Nichols 1991) and secondly samples were applied to silica gel SIII chromarods (5 µm particle size) using 1 µl micropipettes. Thirdly, chromarods were developed in a glass tank lined with pre-extracted filter paper. The primary solvent system used for the lipid separation was hexane-diethyl ether-acetic acid (70:10:0.1, v:v:v), a mobile phase resolving non-polar compounds such as WE, TAG, FFA and sterols. Fourthly, the chromarods were analysed immediately to minimize the absorption of atmospheric contaminants when oven dried. The calibration of FID was performed for each compound class (phosphatidylcholine, cholesterol, cholesteryl oleate, oleic acid, squalene, TAG (derived from fish oil), wax ester (derived from orange roughy, Hoplostethus atlanticus, oil) and DAGE for diacylglycerol ethers (derived from deep-sea shark liver oil; 0.1-10 µg range). Peaks were quantified on an IBM compatible computer using DAPA Scientific software (Kalamunda, Western

Australia, Australia). TLC-FID results are generally reproducible to ±10% of individual lipid class abundances (Volkman and Nichols 1991).

3.4.1.3 Determination of lipids classes of postprandial plasma samples using TLC

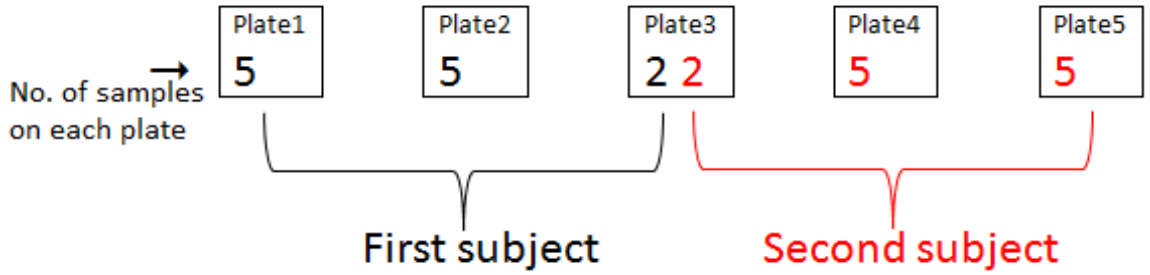
Postprandial plasma samples were randomly chosen from four participants from each treatment: krill oil and fish oil at hours zero, 2, 3, 4 and 5, and olive oil at time points at hours zero and 5 resulting in 12 plasma samples for each participant (Figure 3.2).

Firstly, 24 extraction tubes were rinsed with 0.5 mL DCM/MeOH (1:1) to avoid potential contamination from the previous use. The correct plasma samples were selected from minus 80°C freezer and placed in the fume hood until defrosted. Appropriate labelling on the extraction tubes for 24 samples and rinsing the tubes with 0.5 µL DCM/MeOH (1:1) were undertaken. When plasma samples thawed, the samples were inverted to mix thoroughly and then 0.7 mL was dispensed into the correctly labelled extraction tubes. A volume of 7 mL standard extraction solution was added into each extraction tube, from an automatic dispenser, followed by vortexing to mix well. The samples were left for several hours, at 4°C temperature before the next step.

Secondly, the plasma samples with the standard extraction solution in extraction tube were filtered into labelled glass centrifuge tubes through filter paper. 3.5 mL DCM/ MeOH (2:1) was added into the extract tube to rinse and filtered this into the labelled tube. Once all filtered, the filter funnel and filter paper were removed. 3.5 mL DCM and 2.8 mL 0.9% saline were added into each labelled tube followed by centrifuge (Speed (1000), for 10 min at 20°C).

Thirdly, the upper phase was removed by suction (discard) and the lower phase was transferred into a round bottom flask prior to using a rotary evaporator (Buchi Rotavapor R-114, Radiometer Pacific Pty. Ltd., Victoria, Australia). In order for the efficient evaporation, Milli-Q water was filled in the water bath (Buchi water bath B-480, Radiometer Pacific Pty. Ltd., Victoria, Australia), whereas ice was filled in the water chiller to maintain 0°C (Hetofrig water bath, Gemini BV, Netherlands). Once evaporate was not milky, lipids were transferred into a methylation tube in 2 mL $CHCl_3$ and then stored at 4°C.

Fourthly, TLC plates in a desiccator with activated silica gel and TLC tanks containing the solvent system of petroleum ether: di-ethyl ether: acetic acid (85:15:2 v/v/v) were prepared beforehand. Plasma extracts in methylation tubes were evaporated under a stream of nitrogen in a warm H_2O bath and then 150 µL of $CHCl_3$ was added to plasma extracts in methylation tubes. The plasma lipid extraction for 24 samples from two participants was carefully designed to work efficiently and accurately. For instance, 4 - 5 spots were designed on a paper with same constant 2 cm to put a gap from the edge on the bottom and the left which was used for each sample spot on a TLC plate. Between different plasma samples, the syringe and the needle were cleaned thoroughly by rinsing five times with DCM: methanol (2:1, v/v). 40 µL of standard or 80 µL of each plasma sample on a spot with 5 - 6 dots overlapped for approximately 1.5 cm followed by a gap before the next plasma sample.



Test meal	Time point	Number of plasma samples per participant
Krill oil	0, 2, 3, 4 and 5	5
Fish oil	0, 2, 3, 4 and 5	5
Olive oil	0 and 5	2
Total		12

Figure 3.2 A set of plasma lipid extraction using TLC

Then, the TLC plate was placed into a TLC developing tank containing a solvent system of petroleum ether: di-ethyl ether: acetic acid (85:15:2 v/v/v). Lipid fractions were allowed to separate in the TLC tank. The TLC plate was removed from TLC tank when the front solvent reached 1-2 cm away from the top of the TLC plate and allowed to air-dry for 30 minutes in a fume cupboard. Dichlorofluoroscein (DCF) solution (0.05 g/L of methanol) was sprayed onto the TLC plate followed by being air-dried on a flat surface. The bands of TAG and PL fractions for each plasma sample on a TLC plate were marked under a UV light (Spectroline®CM UV-viewing cabinet, Sigma-Aldrich Co., Australia) and scraped off plates and carefully transferred into the corresponding labelled methylation tubes. and then 2.8 mL of H_2SO_4 in methanol was added into each tube to form methylation which was placed in the oven at 100°C for 2 hours in which constant shaking sample tubes was undertaken every 20 minutes. After the incubation in the oven, the samples were cooled on ice and 2 mL Mill-Q water and 3 mL petroleum ether were added, shaken, and then centrifuged.

Lastly, the upper phase was transferred into a clean labelled tube and the samples were stored at minus 20°C until injection into the GC (samples were stored for no longer 10 days).

3.4.2 Fatty acid methylation

3.4.2.1 Preparation for fatty acid methylation

The extraction of fatty acids as methyl esters (FAME) was performed using human plasmas from the postprandial study and the longer-term study. Total plasma lipids were extracted using methanol: toluene (4:1 v/v), based on the Folch method (Folch et al. 1957), with modifications by Ghasemi Fard et al and Lepage and Roy (Christie 2003, Ghasemi Fard et al. 2014, Lepage and Roy 1986).

All glassware such as tubes (12 mL), Schott bottles, volumetric flasks, funnels and cylinders were rinsed with methanol or petroleum and dried in a fume hood prior to use. Fatty acids were isolated from plasma sample via FAME and thereafter injected to gas chromatography (GC) at Victoria University, Werribee Campus to identify individual fatty acids based on a standard mixture and an internal standard.

3.4.2.2 Reagents/Chemicals Required

Standard mixtures of fatty acid methylation

A standard mixture of fatty acids, GLC reference standard 403 (Nu-Chek Prep, Inc., Elysian, MN) was used as an external standard.

Internal standard solution

To make 100 mL of Methanol/ Toluene (4:1, v:v)

To make 400 mL of internal standard (Heneicosanoic acid, C21:0) solution

The desired concentration of the internal standard solution was 90 µg of C21:0 in one mL of methanol: toluene 4:1(v/v)). Firstly, 36 mg of the Heneicosanoic acid, C21:0 (Nu-Chek Prep, Inc., Elysian, MN) were weighed and carefully placed in a volumetric flask using a funnel which was followed by washing the funnel with methanol/ toluene (4:1) to make sure all Heneicosanoic acid (C21:0) dissolved and transferred to the volumetric flask. Approximately 150 mL of the methanol/ toluene (4:1) and a magnetic flea were added into the volumetric flask and placed on a magnetic stirrer to stir until all the Heneicosanoic acid (C21:0) was dissolved. Secondly, the magnetic flea can be removed using another magnetic flea to draw it out of the flask when Heneicosanoic acid was completely dissolved, and then 50 mL of the methanol/ toluene (4:1) was added to make 200 mL of the solution. Thirdly, the solution was carefully tipped into a 500 mL Schott bottle, and another 200 mL of methanol/ toluene (4:1) were added into the 500 mL Schott bottle making 400 mL of internal standard solution. Lastly, the internal standard solution was aliquoted into 100 mL Schott bottles covered with aluminium foil and stored in a refrigerator at 4°C.

3.4.2.3 Fatty acid methylation (FAME) using direct transesterification method

To adhere identical and accurate FAME work for all samples, 18 plasma samples from the postprandial study or 20 plasma samples from the longer-term study were extracted at once. The correct plasma samples were selected from minus 80°C freezer and placed under the fume hood until being defrosted. The internal standard (IS)

solution needs to be warmed up in warm water (< 30°C) and well mixed before use. Due to optimizing methods for the two studies, different internal standards, heneicosanoic acid (C21:0) and tricosanoic acid (C23:0) were used for the postprandial study and the longer-term study, respectively.

Firstly, 2 mL of internal standard (tricosanoic acid, C23:0, 90 µg/mL methanol: toluene 4:1(v/v)) was added into methylation glass tube (12 mL), and then 200 µL plasma sample (or in the case of oils, 25 µL oil sample) was added under the fume hood.

Secondly, 200 µL of acetyl chloride (C_2H_3ClO) was slowly added into tubes over a period of 1 minute after vortex the plasma sample with IS. This step needed to be cautious as C_2H_3ClO reacts very vigorously with water. Then methylation glasses tubes of plasma samples were tightly closed and then placed in the oven at 100°C for methanolysis for 1 hour.

Thirdly, plasma samples in methylation glasses tubes were placed in a cold water bath to cool down 10 minutes when the oven incubation was completed. 5 mL of 6% potassium carbonate (K_2CO_3) was slowly added to stop the reaction and neutralize the mixture. Methylation glasses tubes of plasma samples were tightly closed and mixed thoroughly for 1 minute using a shaker vortex before centrifuge.

Fourthly, the plasma samples were centrifuged at 3000 x *g* for 10 minutes and the top layer (toluene upper phase) of plasma sample was carefully transferred using a glass Pasteur pipette into a glass tube (4 mL).

Fifthly, the plasma samples were placed in warm water bath (40°C) in which toluene was evaporated using nitrogen gas through an evaporator (Organomation Associates, Inc.,N.EVAP™, USA).

Lastly, 200 µL of petroleum ether sprit was added into the plasma sample when all dried, and the plasma samples were transfer into gas chromatography (GC) vial (~1.5 mL). Samples were stored at minus 20°C until injection into the GC (samples can be stored for about 10 days).

3.4.2.4 Gas chromatography

Gas Chromatography (GC) at Victoria University, Werribee Campus was used to isolate and determine fatty acid methyl esters (Ackman 2002). The GC, Varian Star 3400Cx (Agilent Technologies, CA, USA) was equipped with an SGE BPX 70 capillary column (60 m × 0.25 mm internal diameter, 0.25 µm film thickness, SGE Analytical Science, Melbourne, Australia) with a flame ionization detector (FID) and an auto-sample (Varian Star8200 Cx) injecting 2µL of samples set to splitless mode (1: 10) using an SGE micro-syringe into the injector. The initial oven temperature was set at 160 °C for 1min, then from 160 °C to 225 °C increasing by 2.5 °C/min and held at 225°C for 12 min. The injector and detector temperature were set at 290 °C and 300 °C, respectively. The resulting peaks for individual fatty acids were identified by comparison of retention times with those of standard mixtures of FAME, GLC reference standard 403 (Nu-Chek Prep, Inc., Elysian, MN). Individual fatty acids were quantified with its peak area relative to the total area and expressed as concentration (mg/g) and/ or composition (the percentage of total fatty acids).

3.4 LIPIDOMIC PROFILES

3.5.1 Extraction procedure

Plasma lipids were isolated using a single phase chloroform: methanol ($CHCl_3$:MeOH) extraction as previously described by Weir et al (2013). Briefly, randomised plasma samples (10 µL) were extracted in a single-phase extraction with 20 volumes of $CHCl_3$: MeOH (2:1) and 10 µL of an internal standard mix (in $CHCl_3$: MeOH (1:1) containing between 50 and 1000 pmol each of 23 non-physiological or stable isotope-labelled lipid standards (Table 3.3).

3.5.1.1 Lipid analysis

Lipid analysis for both the 5-hour postprandial study and the 30-day supplementation study was performed by high-performance liquid chromatography electrospray ionisation-tandem mass spectrometry (HPLC ESI-MS/MS) using an Agilent 1290 HPLC coupled to an Agilent 6490 triple quadrupole mass spectrometer. The setting for the two studies were identical, however the sensitivity was optimised to detect TAG species for the postprandial study. The settings for HPLC ESI-MS/MS as follows: gas temperature 150°C, gas flow 17 L/min, nozzle pressure 20 psi, sheath gas temperature 200°C, sheath gas flow 10 L/min, capillary voltage 3500V and nozzle voltage 1000V. Liquid chromatography was performed on a Zorbax Eclipse Plus C18, 1.8 µm, 50 x 2.1 mm column (Agilent Technologies) using solvents A and B consisting of water: acetonitrile: isopropanol, 50:30:20 and 1:9:90 respectively, both containing 10mM ammonium formate. The column was heated to 60°C and the auto sampler regulated to 25°C. Lipid extracts (1 µL) were injected and separated under gradient conditions with a flow rate of 400 µL/min: 10% B to 45% B over 2.7 minutes, increase

to 53% B over 0.1 minutes, increase to 89% B over 6.2 minutes, increase to 93% B over 1.9 minutes, increase to 100% over 0.1 minutes and hold at 100% B for 0.8 minutes. The solvent was then decreased to 10% B over 0.1, held at 10% B for 0.9 minutes, increased flow rate to 600µl/min over 0.1 minutes, held at 600 µl/min 10%B over 1 minute, reduce flow to 400 µl/min over 0.1 minutes and remained at 400 µl/min, 10% B until next injection at 15.4 minutes. The first minute and final 2.3 minutes of each analytical run were diverted to waste.

Table 3.3 Concentrations of corresponding internal standards

All powerful standard Mix – 10µl per sample	added per sample (pmol)	tR	5 µL inj height (CPS)	5 µL inj area	1 µL inj height (CPS)	1µL inj area
Bis(monoacylglycero) phosphate (BMP) 14:0/14:(	100	4.8	4.00E+04	3.00E+05		
Ceramide 17:0	100	7	1.00E+05	6.00E+05		
Galactosylceramide 15:0	100					
Ceramide-1-P 12:0	100					
CE 18:0 (d6)	1000	9.4	2.00E+05	1.16E+06		
Dihydroceramide 8:0	50	5.6	2.00E+04	1.44E+05		
Lysophosphatidylcholine 13:0	100	1	2.00E+05	2.55E+06		
Phosphatidic Acid 17:0/17:0	100					
Phosphatidylcholine 13:0/13:0	100	5.3	4.00E+05	3.82E+06		
Phosphatidylethanolamine 17:0/17:0	100	6.8	5.50E+05	6.20E+05		
Phosphatidylglycerol 17:0/17:0	100	5.8	4.00E+04	2.23E+05		
Phosphatidylserine 17:0/17:0	100	6.8	2.00E+04	3.26E+05		
Sphingomyelin C12:0	200	5	5.50E+05	4.64E+06		
Sphingosine (17:1 base)	100	3.7				
Sphingosine-1-phosphate (17:1 base)	100					
Sphinganine (17:0 base)	100	3.9				
Sphinganine-1-Phosphate (17:0 base)	100					
Glyceryl triheptadecanoate 17:0 17:0 17:0	100	5.1			1.00E+05	1.45E+06
Lyso PA 17:0	100					
DAG 15:0/15:0	200	2			3.00E+04	3.49E+05
Cholesterol (D7)	10000	6.6	3.00E+04	2.79E+05		
1-O-acylceramide (17:0 18:1)	100	8.9	4.00E+05	2.50E+06		
Monohexosylceramide 16:0 d3	50	6.2	3.00E+04	1.87E+05		
Dihexosylceramide 16:0 d3	50	5.9	3.00E+04	1.99E+05		
Trihexosylcermide 17:0	50	5.9	2.00E+03	1.88E+05		

Abbreviation: CPS, counts per second for intensity; tR, retention time.

3.5.1.2 Identification of plasma lipid species

The lipidomic technology is described as 'semi-quantitative' since the response factors are not known for all species (personal communication, Peter Meikle, Baker Heart and Diabetes Institute, Melbourne, Australia). For the postprandial lipidomic profiles, the instrument was specifically optimised for detecting TAG species, particularly those containing LC n-3 PUFA, although the TAG responses are generally thought to be only about 33% of the true response (personal communication, Peter Meikle, Baker IDI, Melbourne, Australia).

A total of 522 lipid species were measured using dynamic multiple reaction monitoring (dMRM) where data was collected for a retention time window specific to each lipid species. Results from the chromatographic data were analysed using Mass Hunter Quant where relative lipid abundances were calculated by relating each area under the chromatogram for each lipid species to the corresponding internal standard. Correction factors were applied to adjust for different response factors, where these were known.

3.6 BIOCHEMICAL ANALYSIS

3.6.1 Plasma lipid profiles and glucose

The plasma levels of total cholesterol (TC), TAG and high-density lipoprotein (HDL), low-density lipoprotein (LDL) and glucose were quantified using an auto-biochemistry analyser (Thermo Fisher Scientific Inc., Indiko™ Clinical and Specialty Chemistry System, USA) at Monash University using commercially available enzymatic

colorimetric kit methods (Cholesterol, Triglyceride, LDL A & B, HDL A & B and Glucose HK).

Reagents

Multi-analyte controls (Thermo Fisher Scientific Inc., USA)

Nortrol, abtrol, lipitrol, chem 1 and chem 3.

Multi-calibrators (Thermo Fisher Scientific Inc., USA)

Scal and LDL/HDL were used.

Plasma lipid profiles and glucose analysis

All procedures were performed in accordance with the manufacturer's instructions. In brief, quality controls and calibration with reagents for lipid profiles including total cholesterol, TAG, LDL and HDL, and glucose were undertaken prior to running plasma samples on a daily basis. Approximately 200 µL of plasma sample for all markers were transferred to a sample cup after being defrosted on ice followed by gentle inverting a couple of times. The sample cups were loaded into the auto-biochemistry analyser and analysed. Reading the concentration of all markers was duplicated for the first 50 samples and thereafter every 10^{th}.

3.6.2 Inflammatory biomarkers

Several cytokines were analysed using Bioplex cytokine immunoassay (171A1001P, Bio-Rad Laboratories, Munich, Germany). All procedures were conducted according

to the manufacturer's instructions. The cytokines analysed from this immunoassay are highlighted in Table 3.4.

Table 3.4 Inflammatory biomarkers

Analyte	Role in human physiology	Findings on cytokines related dietary LC PUFA
Pro-inflammatory cytokines		
Interleukin 1β (IL-1β)	Released by macrophage; cause a rise in body temperature	LC n-3 PUFA were effective on the reduction of neurogenesis caused by IL-1β (Borsini et al. 2017).
Interleukin 2 (IL-2)	Secreted by T help cells; Involved in T cell and B cell proliferation	IL-2 secretion in a vivo experiment decreased following purified DHA feeding (Fan et al. 2004).
Interleukin 5 (IL-5)	Released by some T cells and mast cells; a stimulatory effect on immunoglobulin A secretion	A high level of n-3 PUFA significantly reduced the production of IL-5 in the dermatitis models (Park et al. 2013).
Interleukin 6 (IL-6)	Secreted predominately from adipose tissue/ the skeletal muscle; a stimulatory effect on platelet formation	Marine-derived n-3 PUFAs supplementation had a significant lowering effect on IL-6 based on 68 randomised clinical trials (Li et al. 2014).
Interleukin-12p70 (IL-12p70)	Released by dendritic cells and macrophages; stimulate cytotoxic T cells and natural killer cells	The production of IL-12p70 was inhibited by DHA in vivo expression (Kong et al. 2010).
Tumour Necrosis Factor α (TNFα)	Released by monocytes and macrophages; promote the release of inflammatory cytokines including IL-1β and IL-6	Refer to a review on randomised clinical trials, dietary n-3 PUFAs reduced significantly TNF-α level (Li et al. 2014).

Anti-inflammatory cytokines		
Interleukin 4 (IL-4)	Secreted by T help cells; Involved in T cell differentiation and B cell activation	Fish oil supplementation decreases the production of several pro-inflammatory cytokines, however there was no change in IL-4 secretion (Wallace et al. 2003).
Interleukin 10 (IL-10)	Released by macrophages; suppresses macrophage and dendritic cells function and inhibits the production of pro-inflammatory cytokines	A high EPA diet was associated with a greater reduction in the systemic macrophage inflammatory response with increased IL-10 production compared with a high DHA diet (Sierra et al. 2008); “Fish oil might down-regulate the mRNA expression of IL-1β, XOR and TNF-α through enhancing the expression of IL-10 and PPAR-γ” (Lin et al. 2013).
Interleukin 13 (IL-13)	Suppresses the production of several cytokine (IL-1, IL-8 and TNF)	A significant inverse relationship between levels of n-3 PUFA in neonatal cell membranes and plasma IL-13. The level of plasma IL-13 in neonates, whose mothers consumed fish oil, was significantly lower than the control group (Dunstan et al. 2003); fish oil increased the levels of IL-13, whereas krill oil did not modulate the levels of cytokines in experimental rheumatoid arthritis models (Ierna et al. 2010).

Adapted from (Marieb and Hoehn 2014, Tortora)

Prior to preparing plasma samples for analysis, buffers and diluents were removed from the fridge to room temperature. Calibration and validation kits were placed on bench top shaker to bring them to room temperature.

Firstly, one vial of lyophilised standard in 500 µL of serum based standard diluent was reconstituted followed by vortex for 30 seconds and incubation on ice for 30 minutes (add serum to samples 60 µL).

Secondly, a 96-well plate was washed twice using the magnetic plate washer (Run 4: Meg 2) and coupled beads were vortexed. 200 µL of magnetic beads was diluted in 5,080 µL of assay buffer (DO NOT DISCARD REMAINING BEADS). Magnetic beads I assay buffer was placed in multichannel pipette reservoir and 50 µL of this was added to each well of a 96 well magnetic plate (BLACK). This step needed to be protected from light with aluminium foil as beads are light sensitive.

Thirdly, eight different concentrations of standard dilutions were prepared. Eight eppendorf tubes (1.5 mL) with S1-S8 and one tube with B for the blank were labelled. 374.4 µL of standard diluent in S1, 300 µL in S2-S8, and 120 µL in B were transferred. Once incubated 25.6 µL of the reconstituted standard was added to S1 followed by vortex and transferring 100 µL to S2, repeat process until S8. Each dilution was a four-fold dilution of the previous standard, the S1 values were recorded on the product insert. Reconstituted standards on the ice were kept until required.

Fourthly, 40 µL of plasma sample was reconstituted in 80 µL of serum based diluent then 50 µL each of standards, the blank and plasma samples after vortex were added into the 96-well magnetic plate in duplicate.

Fifthly, the plasma samples in the 96-well magnetic plate were incubated at room temperature for 60 minutes on the bench top shaker at approximately 200 RPM after being covered and sealed with tape and foil. With 10 minutes remaining 300 µL of detection antibody after vortex was added to 2,700 µL antibody diluent using 15 mL tube wrapped with foil. At end of 60 minutes, the plate was washed three times (Run5: Meg3) and 25 µL of antibody was added to each well.

The plasma samples in the 96-well magnetic plate were covered and protected from light and returned to bench top shaker at RT for 30 minutes incubation. With 10 minutes left 60 µL of SV-PE (strreptavidi-PE) vortexed was reconstituted in 5,940 µL of assay buffer. At end of 30 minutes, the plate was removed and washed three times. 50 µL of diluted SV-PE was added to each well using a multichannel pipette.

The plasma samples in the 96-well magnetic plate were then covered/ protected from light prior to incubation at room temperature on bench top shaker for 10 minutes. When the incubation completed, the plate was washed three times using the wash station. 125 µL of assay buffer was added to each well using the multichannel pipette and sealed/ covered with foil prior to incubation for 30 seconds on bench top shaker.

During incubation of samples, the calibration and validation process can be undertaken. Additionally, the plate on the BioPlex manager software and the analytes (check bead region specified) can be set up. Plate as duplicate and standard values (S1) for all analytes by calculation can be selected and entered on the BioPlex manager software.

Lastly, the plate was loaded into the Bioplex machine after removal of tape and foil, and run by BioPlex 200 in which high PMT setting, plate set to 25 bead map region in

advanced settings and 100 beads per region when specifying data acquisition needed to be selected.

3.7 STATISTICAL ANALYSIS

3.7.1 Postprandial study

The minimum number of study participants was determined to allow an 80% power to detect a difference in plasma EPA based on a postprandial study (Kagan et al. 2013). Data were expressed as means ± standard error mean except for participant characteristics (mean ± standard deviation) and they were analysed using SPSS version 22 (SPSS, Chicago, IL, USA) and GraphPad Prism version 7 for Windows (GraphPad Software, San Diego, CA, USA). Distributions of data were tested for normality prior to data analysis. Postprandial plasma fatty acids were analysed to determine significant effects on changes from baseline values by time, meal, and interaction between time and meal using two-way ANOVA for repeated measures and post-hoc analysis was undertaken using the Tukey's test for the plasma levels of lipids, glucose and fatty acids, and using the Benjamini-Hochberg false discovery rate for lipidomic profiles. The incremental area under the curve from baseline (net $iAUC_{0-5\ h}$) over the 5-hour postprandial period was calculated using the trapezoid rule. The values of $AUC_{0-5\ h}$ were compared between the treated groups using a paired *t*-test. $P \leq 0.05$ was considered significant.

3.7.2 Longer-term study

The sample size was determined by statistical power analyses (two-tailed *t*-test at the 5% significance level for the power of 90%) to detect a difference in plasma total n-3 PUFA composition based on a previous 4-week study by Ramprasath et al (2013); the minimum required number of participants was eight in order to obtain a significant difference in outcome measures such as plasma EPA and total LC n-3 PUFA. Data, including plasma cytokines, TAG and fatty acids, were expressed as means ± standard error mean (SEM) and they were analysed using GraphPad Prism version 7.01. The normality of data distribution was checked using D'Agostino & Pearson normality test. Two-way analysis of variance (ANOVA) for repeated measurements was performed to assess supplementation effect over time (interaction time x supplementation), the difference between time point within supplementation, and the same time point between the treated groups. All *p* values were corrected for multiple comparisons using the Holm-Sidak method for plasma fatty acids, TAG and inflammatory markers, using the Benjamini-Hochberg false discovery rate for lipidomic profiles. The incremental area under the curve from baseline (net iAUC $_{0\text{-}30\ d}$) for the 30-day intervention period was calculated using the trapezoid rule. The values of AUC $_{0\text{-}30\ d}$ were compared between the treated groups using a paired *t*-test. $P < 0.05$ was considered significant.

Chapter 4: Postprandial long-chain n-3 polyunsaturated fatty acid response to krill oil and fish oil consumption in healthy women: a randomised controlled, single-dose, crossover study

The following is a direct excerpt from the published manuscript, H Sung, A Sinclair, P Lewandowski and X Su 2017, Postprandial long-chain n-3 polyunsaturated fatty acid response to krill oil and fish oil consumption in healthy women: a randomised controlled, single-dose, crossover study, Asia Pac J Clin Nutr 2018;27 (1), In press. It has only been edited for formatting to be consistent with this thesis. In addition to this manuscript please refer to Appendix One.

Postprandial long-chain n-3 polyunsaturated fatty acid response to krill oil and fish oil consumption in healthy women: a randomised controlled, single-dose, crossover study by H Sung, A Sinclair, P Lewandowski and X Su was published in the peer review journal, Asia Pacific Journal of Clinical Nutrition, 27(1), pp. 148-157, 2018
DOI: https://doi.org/10.6133/apjcn.092017.03

Chapter 5: The comparative effects of krill oil and fish oil supplementation on postprandial plasma lipidomic profiles in healthy women: a randomised, crossover study

5.1 ABSTRACT

Background: Krill oil has been suggested to have a higher bioavailability than fish oil due to its characterised chemical composition and thus potentially better health outcomes compared with fish oil. This research examined the plasma lipidomic responses to the krill oil supplementation compared with the fish oil supplementation in a 5-hour postprandial study, in an attempt to differentiate the bioavailability of omega-3 polyunsaturated fatty acids between the two omega-3 oils.

Methods: In a randomised crossover postprandial study, 10 healthy women (aged 18 – 45 years) consumed five capsules (1 g each) of krill oil or fish oil that provided 907 mg and 1, 441 mg of long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA), respectively. There was at least one week washout period between the interventions in which the 5-hour postprandial lipidomic responses were investigated. Plasma samples were analysed using a liquid chromatography electrospray ionisation-tandem mass spectrometry. Concentrations of lipid molecular species in plasma (pmol/mL) at zero (baseline), 3 and 5 hours following each supplementation were analysed using a two-way ANOVA for repeated measures. The differences between the two supplementation groups over the 5-hour postprandial period were analysed using the paired *t*-test for net incremental area under the curve from baseline (net iAUC $_{0\text{-}5\ h}$, pmol/mL *hour).

Results: Twenty nine total lipid classes (≥ 500 pmol/mL) were identified in the plasma. The krill oil supplementation significantly increased 12/29 lipid classes, whereas the fish oil supplementation significantly increased only 7/29 and decreased 1/29 class over the 5-hour postprandial period. For 6/29 total lipid classes, the net iAUC $_{0\text{-}5\ h}$ following the krill oil supplementation was significantly greater than the fish oil supplementation. Following the supplementation of both omega-3 oils, a total of 56 eicosapentaenoic acid (EPA, 20:5n-3) - and 76 docosahexaenoic acid (DHA, 22:6n-3)-containing molecular species, in TAG, DAG, sterol and phospholipid classes, were significantly increased over the 5-hour postprandial period. For phospholipid molecular species containing EPA, the net iAUC $_{0\text{-}5\ h}$ following the krill oil supplementation were significantly greater than the fish oil supplementation in 16 out of a total 33 molecular species. In contrast, for TAG and DAG molecular species containing EPA, the iAUC $_{0\text{-}5\ h}$ following the fish oil supplementation were significantly greater than the krill oil supplementation in 7 out of 21 molecular species. The krill oil and fish oil supplementation increased significantly a similar number of DHA-containing molecular species (21/25 for krill oil and 23/25 for fish oil) for triacylglycerol (TAG) and (DAG) molecular species over the postprandial period. For 11/49 phospholipid molecular species containing DHA, the net iAUC $_{0\text{-}5\ h}$ for the krill oil supplementation were significantly greater than the fish oil supplementation ($p \leq 0.05$). There was a consistent trend that molecular species containing LC n-3 PUFA in ether-phospholipid species were significantly increased after the krill oil supplementation, while these molecular species were decreased after the fish oil supplementation.

Conclusions: No previous studies have investigated plasma lipidomic responses to the krill oil and fish oil supplementation in humans. There were significant postprandial increases in molecular species containing EPA and DHA following the

supplementation with both krill oil and fish oil. A novel finding was that the plasma lipidomic changes were different between the two supplementation groups, particularly in phospholipid species (krill oil greatly increased several species compared with fish oil), and TAG species (fish oil greatly increased several species compared with krill oil). A second finding identified in this study was that the krill oil supplementation increased the concentration of plasma ether-phospholipid molecular species, particularly ether-phosphatidylethanolamine, whereas the fish oil supplementation decreased the level of plasma ether-phospholipid molecular species. The biological relevance of this finding is under further investigation.

5.2 INTRODUCTION

In spite of a number of studies investigating whether krill oil has different bioavailability of LC n-3 PUFA compared with fish oil in humans and animals, findings on the bioavailability of LC n-3 PUFA in these two oils remain insufficient. This is related to the apparent limitations of the study designs including different doses, chemical forms of LC n-3 PUFA in the oils, and target tissues used in those studies (Ghasemifard et al. 2014, Ulven and Holven 2015). The commonly used method for fatty acid analysis represent only the total lipids or specific fractions, which may comprise a complex of phospholipids, free fatty acids (FFA), TAG and cholesterol esters (CE). The tissues used are normally plasma, red blood cells and/or organ cells (heart and liver). Moreover, the conventional analysis, focusing on the total fatty acids or specific lipid classes (TAG, phospholipids) in target tissues, is not able to see the associations between different fatty acids in a lipid class (e.g. a TAG containing 3 different fatty acids – 16:0 in position 1, 18:1 in position 2 and 20:5 in position 3) since all the fatty acids are hydrolysed and mixed together, and then analysed as the ‘mix’ of fatty acids

in the TAG, by a technique such as gas chromatography. This process of conventional lipid analysis is very time consuming and therefore expensive in labour costs, and is not very sensitive when compared with lipidomics.

A novel methodology, lipidomics, has been introduced in 2003 with the aim to quantify and identify all lipids (lipidomics) of a target cell or tissue. The lipidomics approach uses liquid chromatography to which is attached a highly sensitive and highly specific mass-spectrometer, and is more cost effective and less time consuming than the conventional lipid analysis. As a tool for the study of lipid/lipoprotein metabolism, lipidomics reveals the characteristics of a large amount (hundreds) of lipid molecular species in biological samples. These detailed data can illustrate the roles of individual lipid molecules and biochemical metabolism. Individual lipid molecules can be identified in various lipid classes including CE, TAG, diacyl-phospholipids and ether-phospholipids (Zhao et al. 2015). This technique identifies the structures and possible functions of diverse lipids. In addition, lipidomics may provide an insight on signalling lipid molecules for regulation of multiple cellular functions resulting in a better understanding of homeostatic processes and progression in various health conditions (Mundra et al. 2016, Murphy and Nicolaou 2013, Zhao et al. 2015).

Krill oil, extracted from krill (*Euphausia superba*), has been used as one of main sources of LC n-3 PUFA over recent decades because of its higher contents of EPA and DHA (Tou et al. 2007). Krill oil is different from fish oil in that LC n-3 PUFA (mainly EPA and DHA) are mainly found in the phospholipid and FFA forms, whereas in fish oil they are commonly found in the TAG form (Tou et al. 2007, Winther et al. 2011). The phospholipids in krill oil are mostly diacyl-phospholipids such as phosphatidylcholine, but they also contain a small proportion of ether-phospholipids

and lyso-phospholipids (Winther et al. 2011). Several human and animal studies have suggested that LC n-3 PUFA from phospholipids may lead to a more efficient incorporation of omega-3 fatty acids into plasma and erythrocytes compared with those from the TAG (Küllenberg et al. 2012a, Schuchardt et al. 2011b, Skorve et al. 2015).

Several reports from human trials suggested that krill oil may have a higher bioavailability than fish oil (Ramprasath et al. 2013, Schuchardt et al. 2011b). However, Ghasemifard et al pointed out that there are a number of factors that could influence the apparent bioavailability of LC n-3 PUFA. These include the matrix of the food, the fat content of the test meal and dose rate consumed by study participants, as well as individual's variability related to their age, gender, and absorption efficiency (faecal loss) (Cohn et al. 1988, Ghasemifard et al. 2014, Lohner et al. 2013). Typically, in studies comparing the bioavailability of krill oil and fish oil, these factors are not controlled sufficiently, and presumably have contributed to the inter-individual variability. The application of novel lipidomic approach has been extended in recent years to detect and quantify lipid molecules in various target tissues. This method can help to gain pathological insights on lipid metabolism and regulation, particularly in given health conditions including cardio-metabolic disorders or degenerative cognitive function (Boon et al. 2013, Meikle et al. 2014, Mundra et al. 2016).

Currently, there are only a limited number of studies reporting the plasma lipidomic profiles following the LC n-3 PUFA supplementation in humans (Block et al. 2010, Ottestad et al. 2012). The characteristics of lipidomic changes in the plasma have not yet been investigated with regards to krill oil and fish oil supplementation. To the best of our knowledge, this is the first postprandial study to reveal the plasma lipidomic

profiles with the krill oil supplementation compared with the fish oil supplementation in humans. To minimise gender and inter-individual variabilities, healthy young women were recruited for this study. We investigated the effect of the krill oil supplementation in comparison with the fish oil supplementation on multiple lipid classes and lipid molecular species, particularly those containing EPA, DHA and DPA. It was hypothesised that a single dose of 5 g of krill supplementation would result in a different incorporation of n-3 PUFA into plasma lipid molecular species compared with same amount of fish oil supplementation (5 g).

5.3 MATERIAL AND METHODS

5.3.1 Study design

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and the protocol was approved by the Ethics Committee of Victoria University Human Research (HRE 14-040), and informed consent was obtained from all participants prior to the study. This trial was registered with the Australian and New Zealand Clinical Trial Registry (ACTRN 12615000620527). The study was a randomised crossover design with three test meals containing different dietary oils consumed in a randomised order with seven days washout period between the test meals as described in *Chapter four* (Figure 4.1). During the study period, all participants were instructed to maintain their habitual diet and not to consume fish/seafood or omega-3 fortified foods more than twice a week.

Prior to the study day, the participants were required to consume the most common low-fat dish in their diet, such as pasta, and avoid drinking alcohol and strenuous

physical activity, and fast for approximately 10-hour overnight. On the study day, standardised procedures were performed where the participants arrived at the clinic between 7 a.m. and 9 a.m. and a fasting blood sample (baseline, 10 mL) was collected via intravenous cannulation by a qualified practitioner. The participants then consumed a single test meal (breakfast), which consisted of 150 g of fresh mashed potato mixed with 15 g of olive oil, together with five capsules of krill oil or fish oil with 250 mL of water. Krill oil and fish oil are very different in colour, smell, and taste so for this trial both omega-3 oils were provided as capsules rather than as oils in order to provide a degree of blinding to the trial. For the control group, the participants consumed 5 g of olive oil added into the mashed potato in place of the five capsules (1 g each) of krill oil or fish oil. It was recognized that the 5 g of each krill oil and fish oil do not contain identical amounts of LC n-3 PUFA, but it was deemed that was preferable to maintain the test oils as blinded a fashion as possible, and to minimise the participants withdrawing from the trial owing to smell and taste considerations. All participants finished the test meal, including the capsules, within 15 min and they were only allowed to drink water during the 5-hour intervention period. After the test meal consumption, postprandial blood samples (10 mL) were collected at every hour for 5 hours, thus making a total of six blood samples, including the sample at baseline, per participant per test meal.

5.3.2 Study participants

Ten healthy women aged between 18–45 years within BMI 20–30 (kg/m^2), who had not experienced menopause, were recruited by emails to all Victoria University staff and students, and flyer advertisements via the Victoria University Nutritional Therapy Teaching Clinic, the general public, community centres, and local medical practices.

Participants were screened for their suitability for the postprandial study using a medical questionnaire and anthropometric measurements prior to enrolling into the study. Participants were excluded if they were cigarette smokers; pregnant or lactating; or had heart, liver, kidney or inflammatory bowel disease; diabetes; or medications interfering with lipid metabolism or lowering blood lipids; allergy to fish or seafood; or had consumed oily fish more than twice a week or supplements including omega-3 fatty acids in the past four weeks prior to the study.

5.3.3 Lipid extraction of human plasma

Plasma lipids were isolated using a single phase chloroform: methanol ($CHCl_3$: MeOH) extraction as previously described (Ghasemi Fard et al. 2014, Weir et al. 2013). Briefly, randomised plasma samples (10 µL) were extracted in a single-phase extraction with 20 volumes of CHCl3: MeOH (2:1) and 10 µL of an internal standard mix (in $CHCl_3$: MeOH (1:1)) containing between 50 and 1000 pmol each of 23 non-physiological or stable isotope-labelled lipid standards.

5.3.4 Lipidomics analysis

Lipid analysis was performed by high-performance liquid chromatography, electrospray ionisation-tandem mass spectrometry (HPLC ESI-MS/MS), using an Agilent 1290 HPLC coupled to an Agilent 6490 triple quadrupole mass spectrometer. The details of settings of LC ESI-MS/MS were described in *Chapter three* and detailed in section 3.5.1.1.

5.3.5 Identification of plasma lipid molecular species

A total of 724 lipid species was measured and analysed using dynamic multiple reaction monitoring (dMRM) where data were collected for a retention time window specific to each lipid species. Results from the chromatographic data were analysed using Mass Hunter Quant where relative lipid abundances were calculated by relating each area under the chromatogram for each lipid species to the corresponding internal standard. Correction factors were applied to adjust for different response factors, where these were known.

5.4 STATISTICAL ANALYSIS

The power calculation to determine the minimum number of participants to detect a difference in plasma LC n-3 PUFA, plasma TAG based on a previous postprandial study (Kagan et al. 2013) was six, using a two tailed *t*-test at the 5% significance level for a power of 90%. Statistical analyses were performed to compare the significant differences in plasma lipidomic responses between krill oil and fish oil consumption over the 5-hour postprandial period. Values are expressed as mean of concentration ± standard error mean (SEM) for 10 participants. The normality of data distribution was checked using D'Agostino & Pearson normality test. Log-transformation of data was carried out where appropriate. Two-way analysis of variance (ANOVA) for repeated measurements was performed to analyse the supplementation effect over time (interaction time x supplementation), differences between time point within the supplementation, and the same time point between the supplementation groups. All *p* values were corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR). The incremental area under the curve from baseline (net iAUC

$_{0\text{-}5\ h}$) of plasma lipid molecular species for 10 participants over the 5-hour postprandial period was calculated using the trapezoid rule. The net iAUC $_{0\text{-}5\ h}$ was compared between krill oil and fish oil supplement using paired *t*-test. $P < 0.05$ was considered significant. The data were analysed using GraphPad Prism version 7.01.

5.5 RESULTS

A total of 37 lipid classes and 724 molecular lipid species were identified and quantified in the plasma samples collected at hours zero, 3 and 5 after the krill oil or fish oil supplementation over the 5-hour postprandial period. Of these lipid molecular species, glycerophospholipids species were predominant among the six different lipid classes accounting for 51% of the total lipid molecular species. This was followed by sphingolipids accounting for 22% of the total molecular species as shown in Table 5.1.

However, there was an arbitrary decision not to show the plasma total lipid classes under 500 pmol/mL. Even though, all individual molecular species containing LC n-3 PUFA (the main focus on this study) are discussed to investigate a trend of postprandial changes in these fatty acids following the supplementation of krill oil compared with the fish oil supplementation. For some molecular species, the concentrations at baseline were low, however they following the krill oil supplementation were significantly changed, particularly in phospholipid species, compared with the fish oil supplementation over the postprandial period. Therefore, a total identified of 57 EPA (20:5n-3) molecular species, 76 DHA (22:6n-3) molecular species, 29 DPA (22:5n-3) molecular species, 61 arachidonic acid (AA, 20:4n-6) molecular species and 101 LA (18:2n-6) molecular species are herein discussed.

5.5.1 Distribution of total plasma lipid classes

As mentioned above, there were a total of 37 lipid classes detected. However, only 29 of them are described in details based on the arbitrary decision that consideration was given to those showing a concentration over 500 pmol/mL. A total of 29 plasma lipid classes showed significant changes following the krill oil and fish oil supplementation over the 5-hour postprandial period, as presented in Supplementary Table 5.1. The concentrations of total lipid classes ranged from 530.7 to 2,444,439.7 pmol/mL. Cholesterol ether (CE) was the highest in concentration followed by cholesterol (COH), phosphatidylcholine (PC), TAG, sphingomyelin (SM) and lyso-phosphatidylcholine (LPC). The identified molecular species are summarised in Supplementary Table 5.1. The krill oil supplementation increased 12/29 lipid classes ($p < 0.05$), whereas the fish oil supplementation increased only 7/29 lipid classes ($p < 0.05$) and decreased 1/29 lipid class, TAG, ($p = 0.032$), over the 5-hour postprandial period as shown in Figure 5.1.

Figure 5.2 shows that six of 29 lipid classes in the net iAUC $_{0\text{-}5\ h}$ were significantly different following the two omega-3 oil supplementation. All cases of the krill oil supplementation showed significantly greater the net iAUC $_{0\text{-}5\ h}$ than the fish oil supplementation over the 5-hour postprandial period ($p < 0.05$).

Table 5.1 Identified lipid classes and molecular species in the plasma over the 5-hour postprandial period after krill oil or fish oil supplementation

Lipid classes (n = 37)	Abbreviation; Description	Number of identified molecular species (n =724)
Glycerolipid		**137**
Diacylglycerol	DG	40
Ether TAG	TAG (O)	3
Triacylglycerol	TAG	94
Glycerophospholipids		**369**
Phosphatidylcholine	PC	70
Alkylphosphatidylcholine	PC (O)	22
Alkenylphosphatidylcholine	PC (P), plasmalogen	26
Lysophosphatidylcholine	LPC	61
Lysoalkylphosphatidylcholine	LPC (O), platelet activating factor	10
Lysoalkenylphosphatidylcholine	LPC (P), plasmalogen	6
Phosphatidylethanolamine	PE	37
Alkylphosphatidylethanolamine	PE (O)	14
Alkenylphosphatidylethanolamine	PE (P), plasmalogen	55
Lysophosphatidylethanolamine	LPE	14
Lysoalkenylphosphatidylethanolamine	LPE (P), plasmalogen	4
Phosphatidylinositol	PI	27
Lysophosphatidylinositol	LPI	8
Phosphatidylglycerol	PG	4
Phosphatidylserine	PS	7
Lysoalkenylphosphatidylserine	LPS	4
Sphingolipids		**157**
Sphingomyelin	SM, SM (d)	44
Dihydroceramide	dhCer	6
Ceramide	Cer	54
Monohexosylceramide	HexCer	14
Dihexosylceramide	Hex2Cer	10
Trihexosylcermide	Hex3Cer	6
GM1 ganglioside	GM1	1
GM3 ganglioside	GM3	7
Sulfoglycosphingolipid	Sulfatide	6
Sphingosine-1-Phosphate	S1P	5
Sphingosine	Sph	3
Ceramide 1-phosphate	Cer1P	1
Sterols		**46**
Cholesterol	COH	1
Cholesterol ester	CE	27
Oxidised cholesterol ester (fatty acid)	OxCE	2
CE others (Hydroxylcholesterol/ Desmosterol)	OH cholesterol (9)/ Demosterol (6)	16
Prenol lipids		**1**
Ubiquinone	Ubiquinone	1
Fatty acyls		**14**
Acylcarnitine	Acylcarnitine	14

The lipids classes (n = 30) are presented for the all identified molecular species (n = 522) in the current study although only 24/30 of lipid classes (> 500 pmol/mL) are discussed in this Chapter.

5.5.2 Distribution of plasma long-chain n-3 PUFA

There were three main LC n-3 PUFA including EPA, DPA and DHA which were identified in different lipid molecular species. Multiple molecular species showed significant changes following krill oil and fish oil supplementation over the 5-hour postprandial period. The notation used in Supplementary Tables 5.1 – 5.3 shows the lipid classes such as CE, PC, alkylphosphatidylcholine (PC (O)), alkenylphosphatidylcholine, (PC (P)), phosphatidylethanolamine, (PE), alkylphosphatidylethanolamine, (PE (O)), and alkenylphosphatidylethanolamine, (PE (P)) followed by the molecular species (such as PE (O)-18:1-22:6)).

Lipidomic profiles of plasma lipid species were presented in various lipid classes followed by one or greater than one of fatty acids, in which the structures of the esterified fatty acids were presented with various degree of carbon chain lengths and saturation. The designation PC (XX/XX) or PE (XX/XX) refers to diacyl-lipid species, while PC (P-XX/XX) or PC (O-XX/XX) or PE (P-XX/XX) or PE (O-XX/XX) refers to ether-lipid species (containing an alkyl bond or an alkenyl bond, presented O- and P-, respectively) in which alkenyl-linked lipid species is referred to as plasmalogens. Any designation followed using (a) or (b) refers to molecular species for which the exact assignment of the fatty acid and fatty alkyl /alkenyl moieties has not been determined at this point of time. It is possible some of the molecular species may be mixtures of two distinct molecular species.

Sometimes, with the HPLC-MS/MS it was possible to assign specific fatty acid e.g., species PC (16:0/20:5) for EPA molecular specie. While, in other cases only the

carbon number and number of double bonds could be assigned e.g., species PC (38:5). It was assumed that a molecular species with this designation PC (38:5) was a molecular species containing both 18:0 and 20:5, if its concentration increased over the 3 or 5 hours it was assumed to contain 20:5, since both krill oil and fish oil contain EPA (20:5). It is possible that such a species could be composed of 18:1/20:4 (38:5), however this is possible, it would unlikely if the concentration of this species increased in both the krill oil and fish oil supplementation periods.

5.5.2.1 Plasma eicosapentaenoic acid (20:5n-3)

A total of 56 EPA-containing molecular species were detected, as shown in Supplementary Table 5.2. The concentrations of plasma EPA (20:5n-3) molecular species ranged from 0.7 to 75,384.2 pmol/mL. The CE species was the highest in concentration with the remaining species detected including PC, PC (O), PC (P), desmosterol, PE (P), PE, and diacylglycerol (DAG) species.

As shown in Supplementary Table 5.2, both krill oil and fish oil supplementation significantly increased 37/56 EPA-containing molecular species in which the krill oil supplementation was more effective on EPA-phospholipid molecular species, whereas the fish oil supplementation was more effective on EPA-TAG and EPA-DAG molecular species.

The net iAUC $_{0\text{-}5\ h}$ for 23/56 EPA-containing molecular species were significantly different between the two omega-3 oil supplementation ($p < 0.05$). 16 cases in 33 EPA-phospholipid species, including six of ether-phospholipid species, following the krill oil supplementation were significantly greater than the fish oil supplementation ($p \leq 0.05$), as shown in Figure 5.3. Whereas, for the other case of the net iAUC $_{0\text{-}5\ h}$, seven of 21

EPA-containing TAG and DAG molecular species, following the fish oil supplementation were significantly greater than the krill oil supplementation. There was a trend in EPA-containing ether-phospholipid molecular species in which six of 33 EPA-containing ether-phospholipid molecular species were significantly different between the two omega-3 supplementation in which all six cases in the net $iAUC_{0\text{-}5\ h}$ were significantly increased following the krill oil supplementation. In contrast, the fish oil supplementation increased only one of these EPA-containing ether-phospholipid molecular species, PE (O-36:5), and decreased five of them ($p < 0.05$).

5.5.2.2 Plasma docosahexaenoic acid (22:6n-3)

A total of 76 DHA-containing molecular species were detected, as shown in Supplementary Table 5.4. The concentrations of plasma DHA (22:6n-3) molecular species ranged from 5.0 to 41,934.1 pmol/mL. The CE species was the highest in concentration with the remaining species detected including PC, PE (P), TAG, DAG and PE species.

As shown in Supplementary Table 5.4, both krill oil and fish oil increased significantly multiple molecular species. The krill oil supplementation increased significantly 40/76 DHA-containing molecular species, whereas the fish oil supplementation increased significantly 41/76 DHA-containing molecular species over the 5-hour postprandial period. Particularly, for phospholipid molecular species, the krill oil supplementation significantly increased 19/49 DHA-phospholipid molecular species. Whereas, the fish oil supplementation significantly increased 12/49 DHA-phospholipid molecular species and decreased 5/49 DHA-phospholipid molecular species over the postprandial period ($p \leq 0.05$).

The net iAUC $_{0\text{-}5\ h}$ for 11 of 76 DHA-containing molecular species were significantly different following the two omega-3 oil supplementation periods. The concentrations of all 11 cases in 49 DHA-phospholipid species, including six of ether-phospholipid species, following the krill oil supplementation were significantly greater than the fish oil supplementation ($p \leq 0.05$), as shown in Figure 5.4. There was a similar trend to EPA molecular species, particularly in ether-phospholipid species. All six DHA-containing ether-phospholipid molecular species were significantly increased following the krill oil supplementation. While, the fish oil supplementation increased significantly only 1/11 DHA-containing ether-phospholipid molecular species, PE (O-16:0/22:6), and decreased five of them.

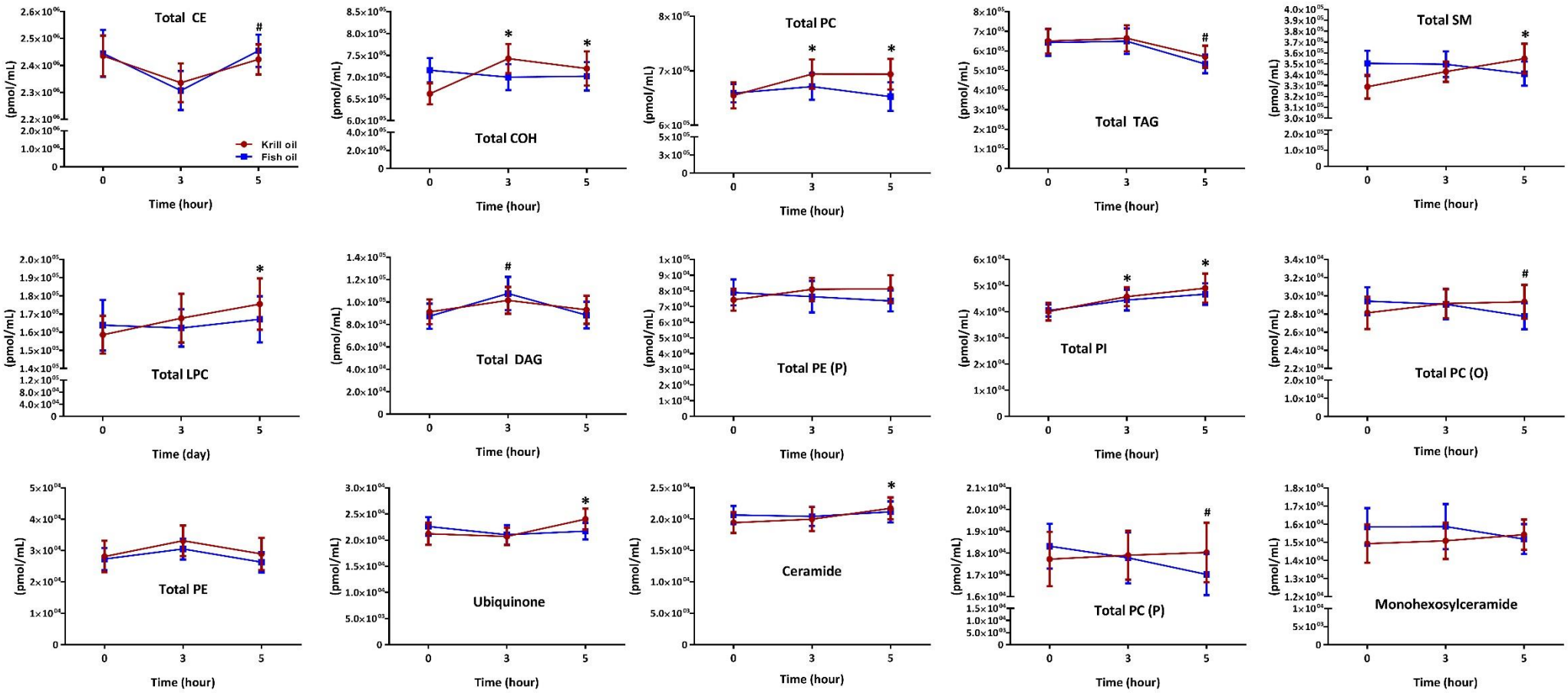


Figure 5.1 (A.1) Postprandial changes in a total of 29 lipid classes in the plasma after the krill oil or fish oil supplementation

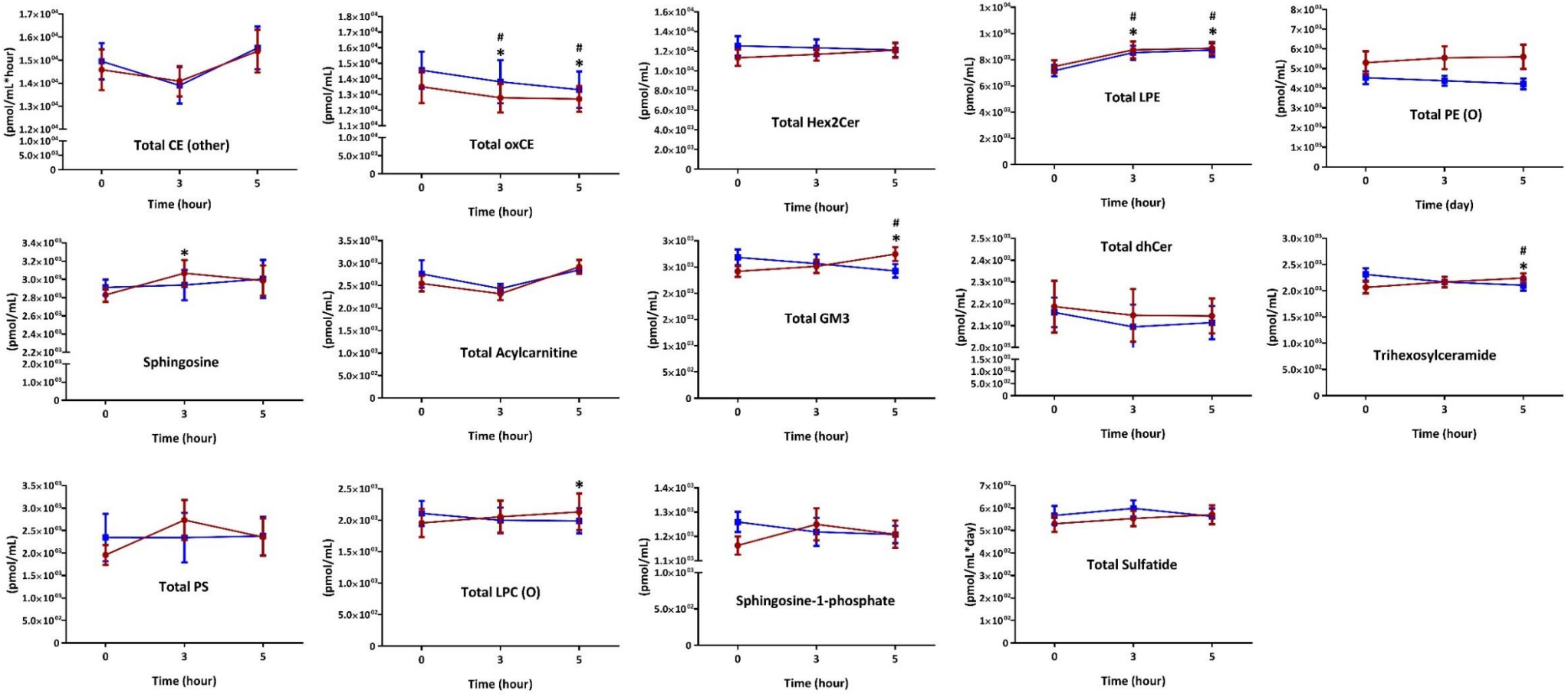


Figure 5.1 (A.2) Postprandial changes in a total of 29 lipid classes in the plasma after the krill oil or fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) for 10 women. Two-way ANOVA for repeated measurements was applied to assess a significant difference at each time point between the two supplementation. All *p* values were corrected for multiple comparisons using the Benjamini-Hochberg FDR. * and # in graphs indicate significant differences ($p < 0.05$) compared with the baseline for krill oil (*) or fish oil (#). Abbreviation: CE, cholesterol ester; CE (others): Desmosterol; COH, cholesterol; DAG, diacylglycerol; dhCer,

dihydroceramide; GM3, GM3 ganglioside; Hex1Cer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lyso-phosphatidylcholine; LPC (O), lyso-alkylphosphatidylcholine; LPE, lyso-phosphatidylethanolamine; net iAUC, net incremental area under the curve from baseline (hours from zero to five); oxCE, oxidised cholesterol ester (fatty acid); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; SM, sphingomyelin; Sph: Sphingosine; S1P, Sphingosine-1-Phosphate; TAG, triacylglycerol.

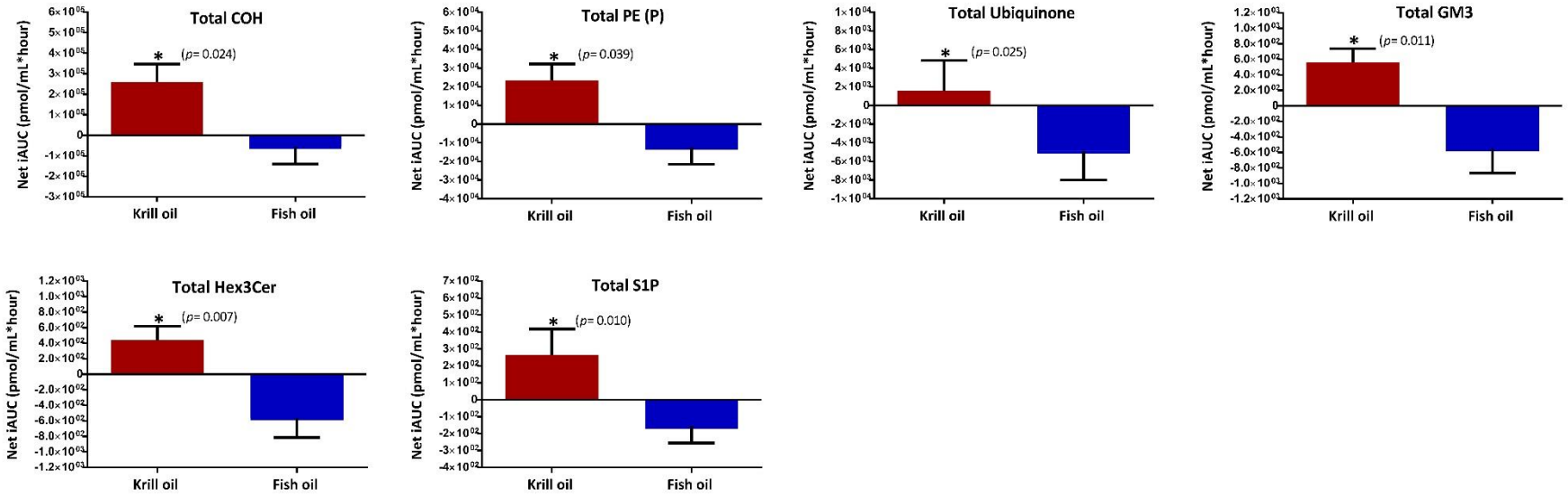


Figure 5.2 (A) Significant differences in net iAUC of total plasma lipid classes over the 5-hour postprandial changes between the krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) for 10 women. The net iAUC from baseline (hours from zero to five) of plasma lipid classes was calculated using the trapezoid rule and compared between the two supplementation groups using paired t-test. * in graphs indicates significant differences ($p < 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: COH, cholesterol; GM3, GM3 ganglioside; Hex3Cer, trihexosylceramide; net iAUC, net incremental area under the curve from baseline (hours from zero to five); PE (P), alkenylphosphatidylethanolamine; S1P, Sphingosine-1-Phosphate.

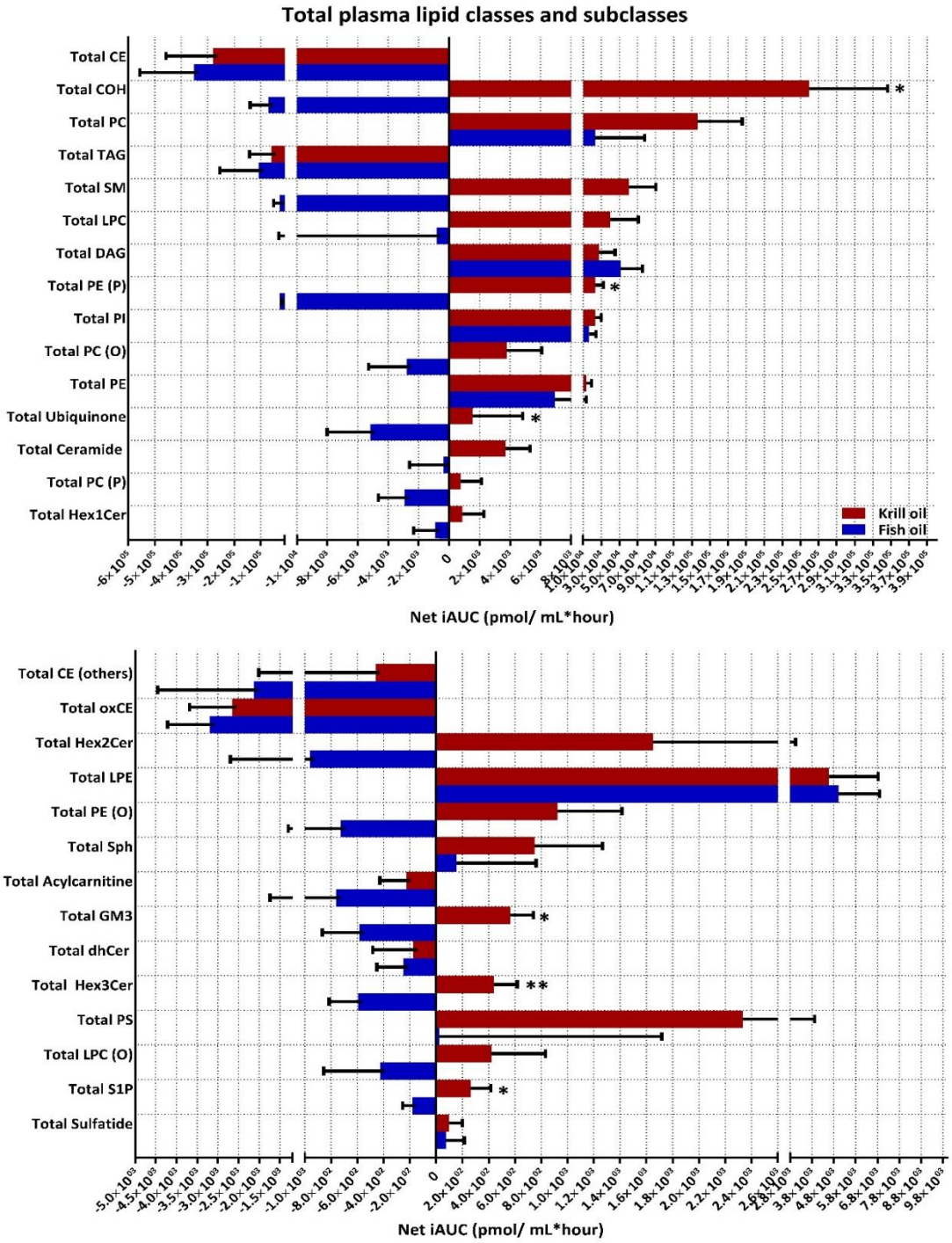


Figure 5.2 (B) The differences in the net iAUC of a total of 29 plasma lipid classes over the 5-hour period between krill oil or fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) for 10 women. The net iAUC from baseline (hours from zero to five) of plasma lipid classes was calculated using the trapezoid rule and compared between the two supplementation groups using paired *t*-test. ***** in graphs indicates significant differences ($p < 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: CE, cholesterol ester; CE (others): Desmosterol; COH, cholesterol; DAG, diacylglycerol; dhCer, dihydroceramide; GM3, GM3 ganglioside; Hex1Cer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lyso-phosphatidylcholine; LPC (O), lyso-alkylphosphatidylcholine; LPE, lyso-phosphatidylethanolamine; net iAUC, net incremental area under the curve from baseline (hours from zero to five); oxCE, oxidised cholesterol ester (fatty acid); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE,

phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; SM, sphingomyeline; Sph, Sphingosine; S1P, Sphingosine-1-Phosphate; TAG, triacylglycerol.

5.5.2.3 Plasma docosapentaenoic acid (22:5n-3)

A total of 29 DPA-containing molecular species were detected, as shown in Supplementary Table 5.4. The concentrations of plasma DPA (22:5n-3) molecular species ranged from 20.1 to 2,703.2 pmol/mL. The PE species was the highest in concentration with the remaining species detected including PC, CE and DAG species. As shown in Supplementary Table 5.4, both krill oil and fish oil increased significantly more than half of the total 29 DPA-containing molecular species. The krill oil supplementation significantly increased 15/29 DPA molecular species, whereas the fish oil supplementation significantly increased 18/29 DPA-containing molecular species over the 5-hour postprandial period. For the significant changes in DPA-phospholipids molecular species, 4/12 molecular species were increased following the krill oil supplementation, while 4/12 molecular species were increased following the fish oil supplementation over the 5-hour postprandial period.

Three out of 29 DPA-containing molecular species in the net iAUC $_{0\text{-}5\ h}$ were significantly different between the krill oil and fish oil supplementation. The net iAUC $_{0\text{-}5\ h}$ for all three species in CE and phospholipid species, including two ether-phospholipid species, following the krill oil supplementation was significantly greater than the fish oil supplementation ($p \leq 0.05$), as shown in Figure 5.5.

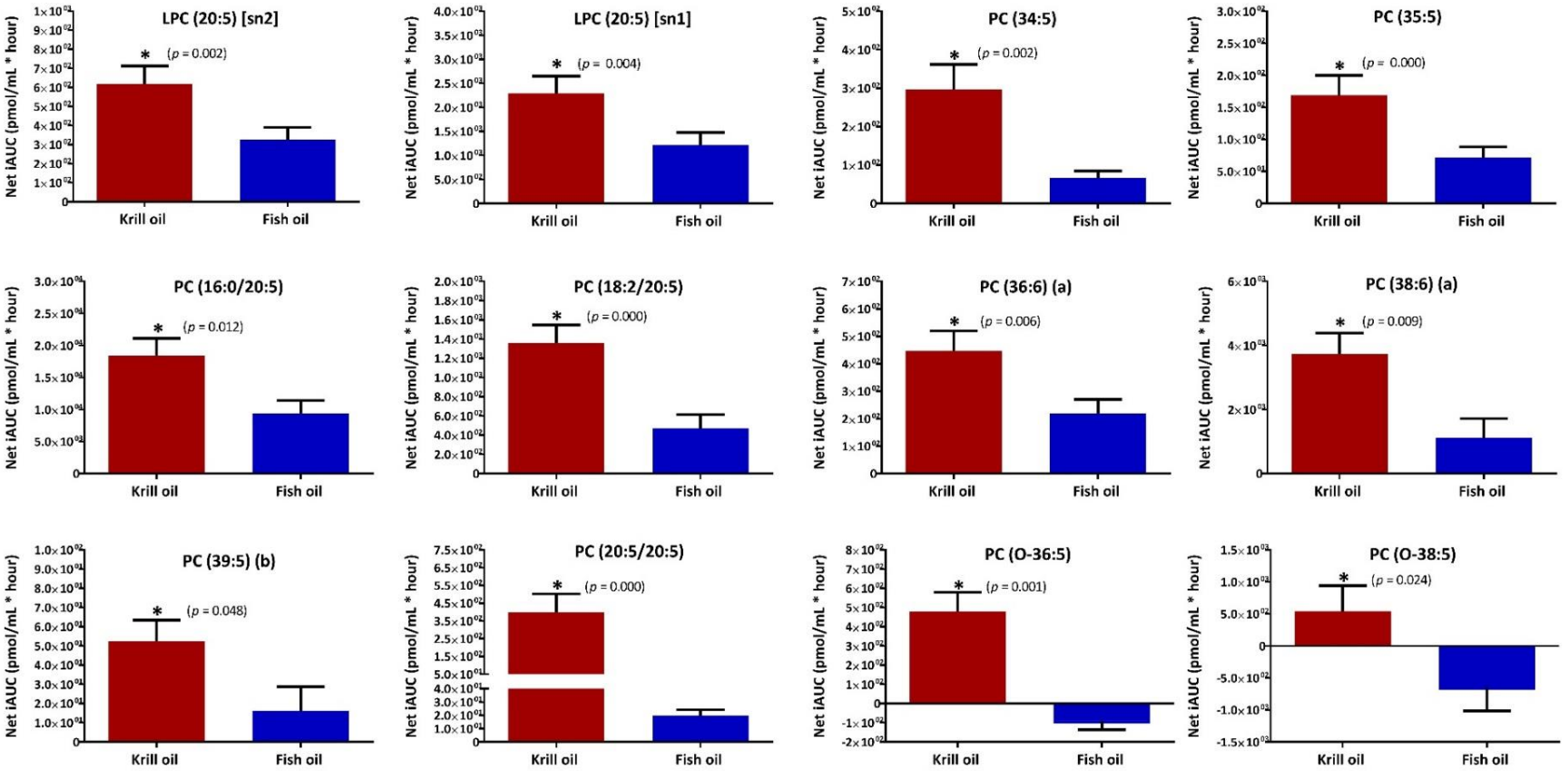


Figure 5.3 (A) Significant differences in the net iAUC of plasma EPA (20:5) molecular species over the 5-hour postprandial period between the krill oil and fish oil supplementation

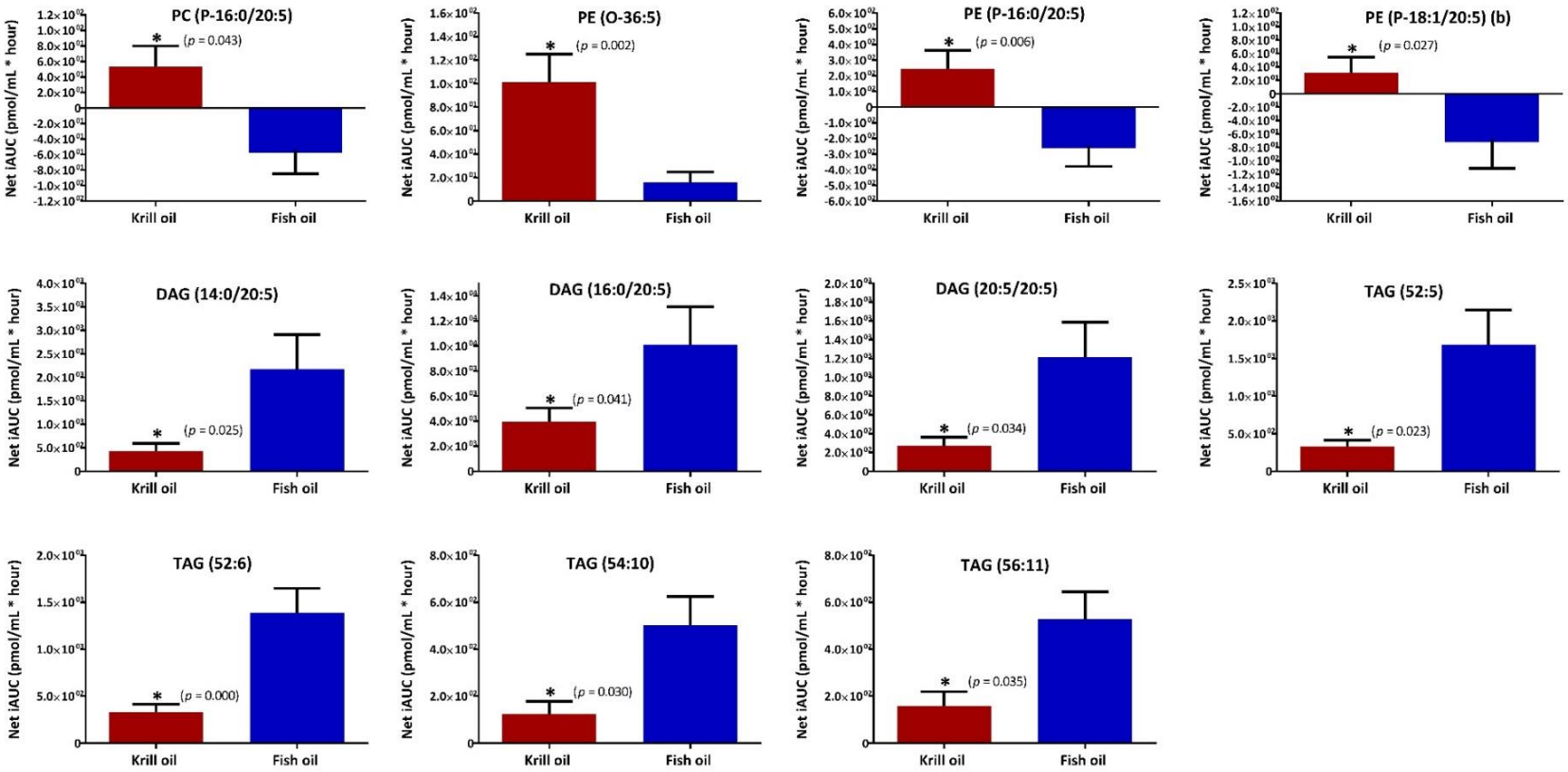


Figure 5.3 (B) Significant differences in net iAUC of plasma EPA (20:5) molecular species over the 5-hour postprandial period between the krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) for 10 women. The net iAUC from baseline (hours from zero to five) of plasma EPA molecular species was calculated using the trapezoid rule and compared between the two supplementation groups using paired t-test. ***** in graphs indicates significant differences ($p < 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: DAG, diacylglycerol; LPC, lyso-phosphatidylcholine; net iAUC, net incremental area under the curve (hours from zero to five); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; pmol/mL, picomole per millilitre; TAG, triacylglycerol.

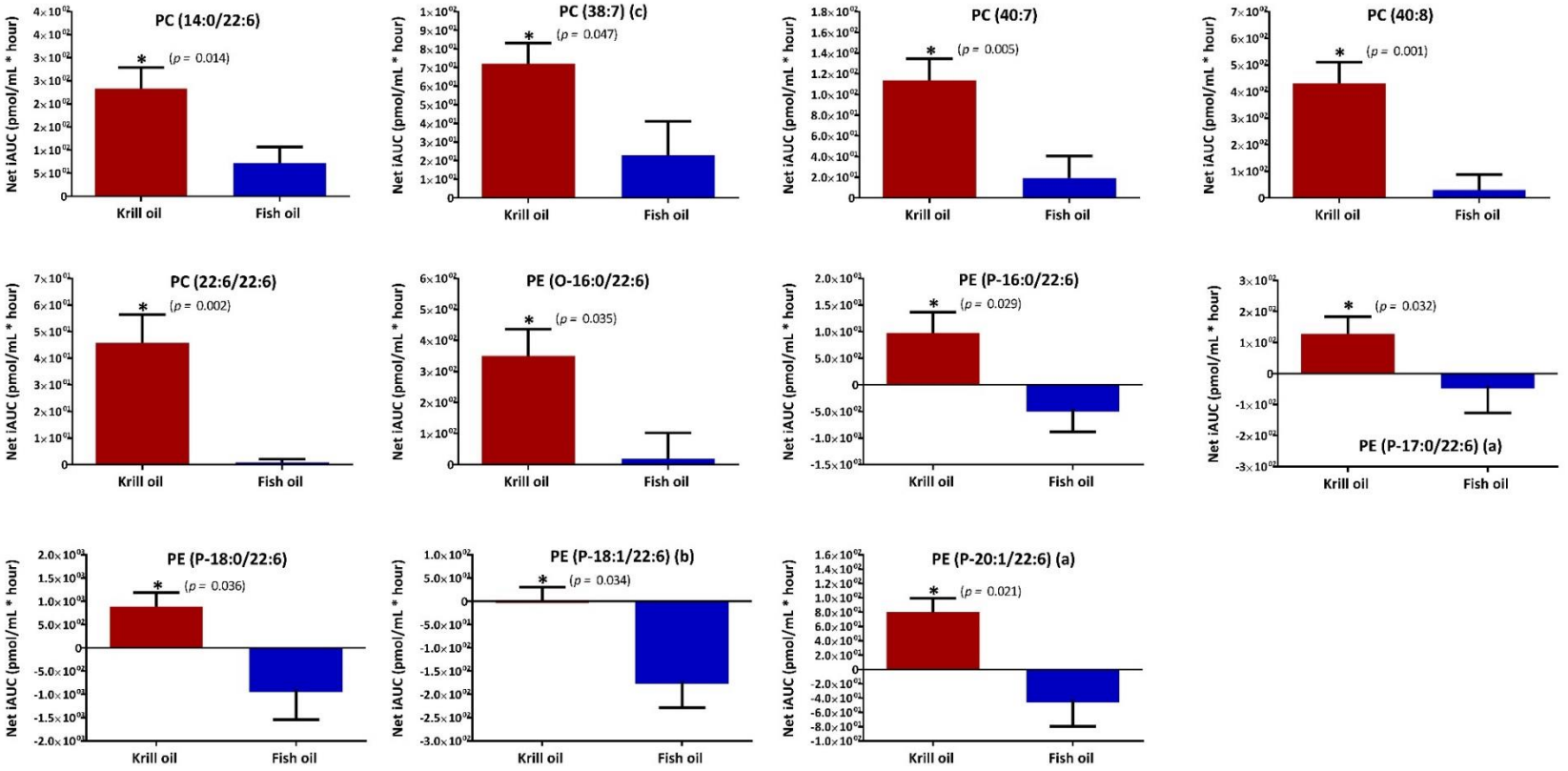


Figure 5.4 Significant differences in net iAUC of plasma DHA (22:6) molecular species over the 5-hour postprandial period between krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) for 10 women. The net iAUC from baseline (hours from zero to five) of plasma DHA molecular species was calculated using the trapezoid rule and compared between the two supplementation groups using paired *t*-test. * in graphs indicates significant differences ($p < 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: DAG, diacylglycerol; LPC, lyso-phosphatidylcholine; net iAUC, net incremental area under the curve (hours from zero to five); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; ; PC (P), alkenylphosphatidylcholine;PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; pmol/mL, picomole per millilitre; TAG, triacylglycerol.

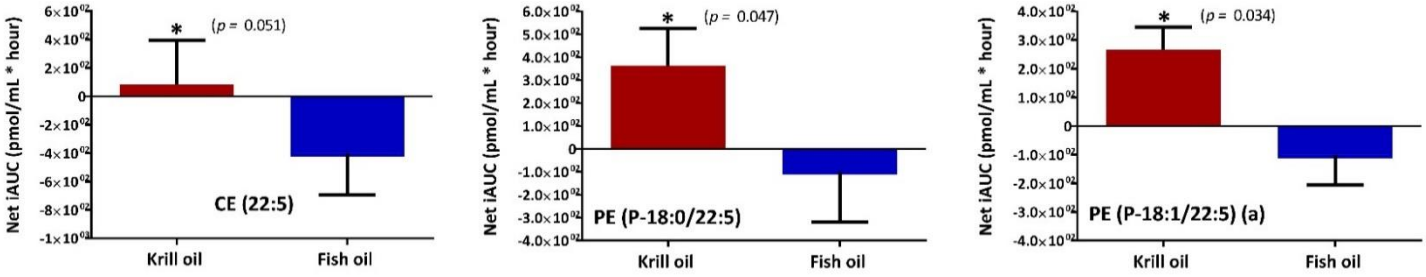


Figure 5.5 Significant differences in the net iAUC of plasma DPA (22:5) molecular species over the 5-hour postprandial period between the krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) for 10 women. The net iAUC from baseline (hours from zero to five) of plasma DPA molecular species was calculated using the trapezoid rule and compared between the two supplementation groups using paired *t*-test. * in graphs indicates significant differences ($p \leq 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: CE, cholesterol ester; net iAUC, net incremental area under the curve (hours from zero to five); PE (P), alkenylphosphatidylethanolamine; pmol/mL, picomole per millilitre.

5.5.3 Distribution of plasma n-6 PUFA

There were two n-6 PUFA observed including arachidonic acid (20:4n-6) and linoleic acid (LA, 18:2n-6), which were identified in various lipid classes and a number of molecular species.

5.5.3.1 Plasma arachidonic acid (20:4n-6)

A total of 61 AA-containing molecular species were detected, as shown in Supplementary Table 5.5. The concentrations of plasma AA (20:4n-6) molecular species ranged from 11.2 to 307,907.3 pmol/mL. The PC species was the highest in concentration with the remaining species detected including PI, PE (P), PC (O), PE, PC, LPC, TAG, DAG, desmosterol and PE (O) species.

As shown in Supplementary Table 5.5, there were differences in postprandial changes between the krill oil and fish oil supplementation groups over the 5-hour postprandial

period. The krill oil supplementation significantly increased 28/61 AA molecular species. Whereas, the fish oil supplementation significantly increased 8/61 species, and decreased 6/61 ($p \leq 0.05$). For the AA-phospholipid molecular species, the krill oil supplementation significantly increased 23/51 molecular species, while the fish oil supplementation increased 5/51 and decreased 6/51 molecular species over the 5-hour postprandial period.

Seven out of 61 AA-containing molecular species in the net iAUC $_{0\text{-}5\ h}$ were significantly different between the krill oil and fish oil supplementation. For phospholipid species, including six ether-phospholipid species, the net iAUC $_{0\text{-}5\ h}$ following the krill oil supplementation was significantly greater than the fish oil supplementation ($p < 0.05$), as shown in Figure 5.6.

5.5.3.2 Plasma linoleic acid (18:2n-6)

A total of 101 linoleic acid (LA) containing molecular species were detected, as shown in Supplementary Table 5.6. The concentrations of plasma LA (18:2n-6) molecular species ranged from 4.2 to 1,169,479.0 pmol/mL. The CE species was the highest in concentration with the remaining species detected including PC, TAG, LPC, DAG and SM species.

As shown in Supplementary Table 5.6, there were differences in postprandial changes between the krill oil and fish oil supplementation over the 5-hour postprandial period. The krill oil supplementation resulted in significant changes in 34/101 LA-containing molecular species including an increase in 16/31 LA-sphingolipid species and 7/41 LA-phospholipid species. Whereas, the fish oil supplementation resulted in significant changes in 27/101 LA-containing species including an increase in 1/31 LA-

sphingolipid species and 3/41 LA-phospholipid species. Over the 5-hour postprandial period, both the krill oil and fish oil supplementation significantly decreased LA-TAG and LA-DAG molecular species. Six of 19 LA-TAG and LA-DAG molecular species were significantly decreased following the krill oil supplementation, whereas the fish oil supplementation significantly decreased 11/19 LA-TAG and LA-DAG molecular species.

As shown in Figure 5.7, ten of 101 LA-containing molecular species in ceramide, GM_3 ganglioside (GM_3), SM, Sphingosine (Sph) and phospholipid species, including four LA-containing ether-phospholipid species were significantly different in net $iAUC_{0\text{-}5\ h}$ between krill oil and fish oil supplementation. The net $iAUC_{0\text{-}5\ h}$ for nine of ten cases was greatly increased by the krill oil than the fish oil, while one of ten, phosphatidylglycerol (PG 36:2) was greatly increased by the fish oil than the krill oil. For ether-phospholipid species, the pattern of postprandial changes was in line with other PUFA including EPA and DHA. The net $iAUC_{0\text{-}5\ h}$ for four out of 41 LA-phospholipid species following the krill oil supplementation was significantly increased, whereas these LA-containing ether-phospholipid species were significantly decreased following the fish oil supplementation.

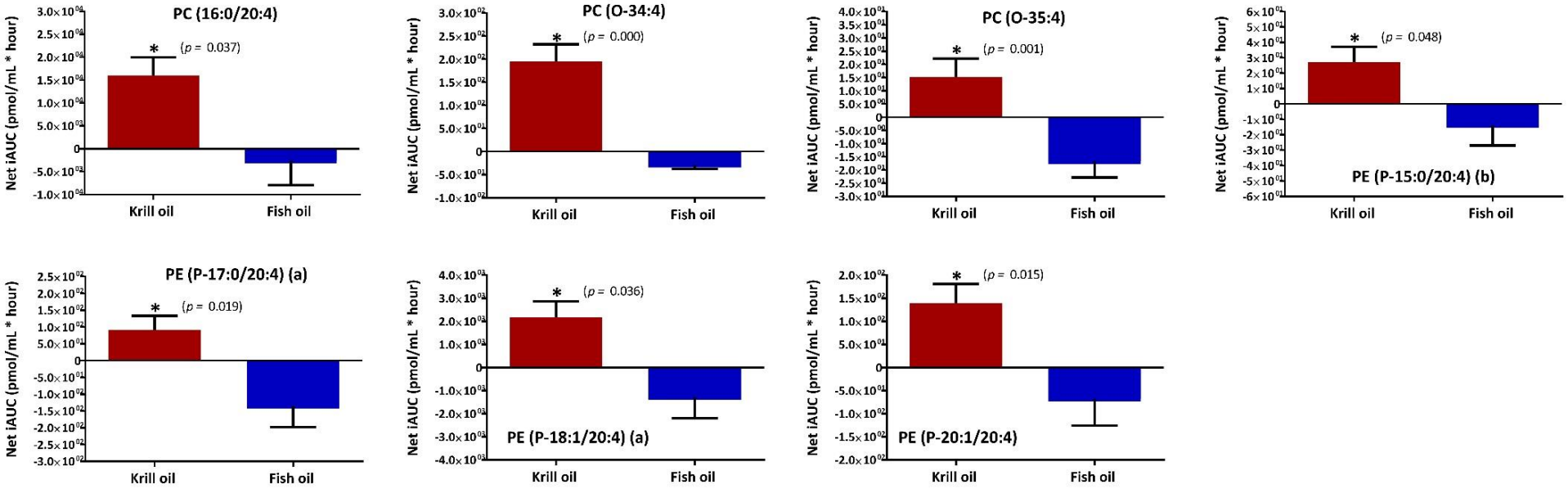


Figure 5.6 Significant differences in the net iAUC of plasma AA (20:4) molecular species over the 5-hour postprandial period between the krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) for 10 women. The net iAUC from baseline (hours from zero to five) of plasma AA molecular species was calculated using the trapezoid rule and compared between the two supplementation groups using paired *t*-test. * in graphs indicates significant differences ($p < 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: net iAUC, net incremental area under the curve (hours from zero to five); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PE (P), alkenylphosphatidylethanolamine; pmol/mL, picomole per millilitre.

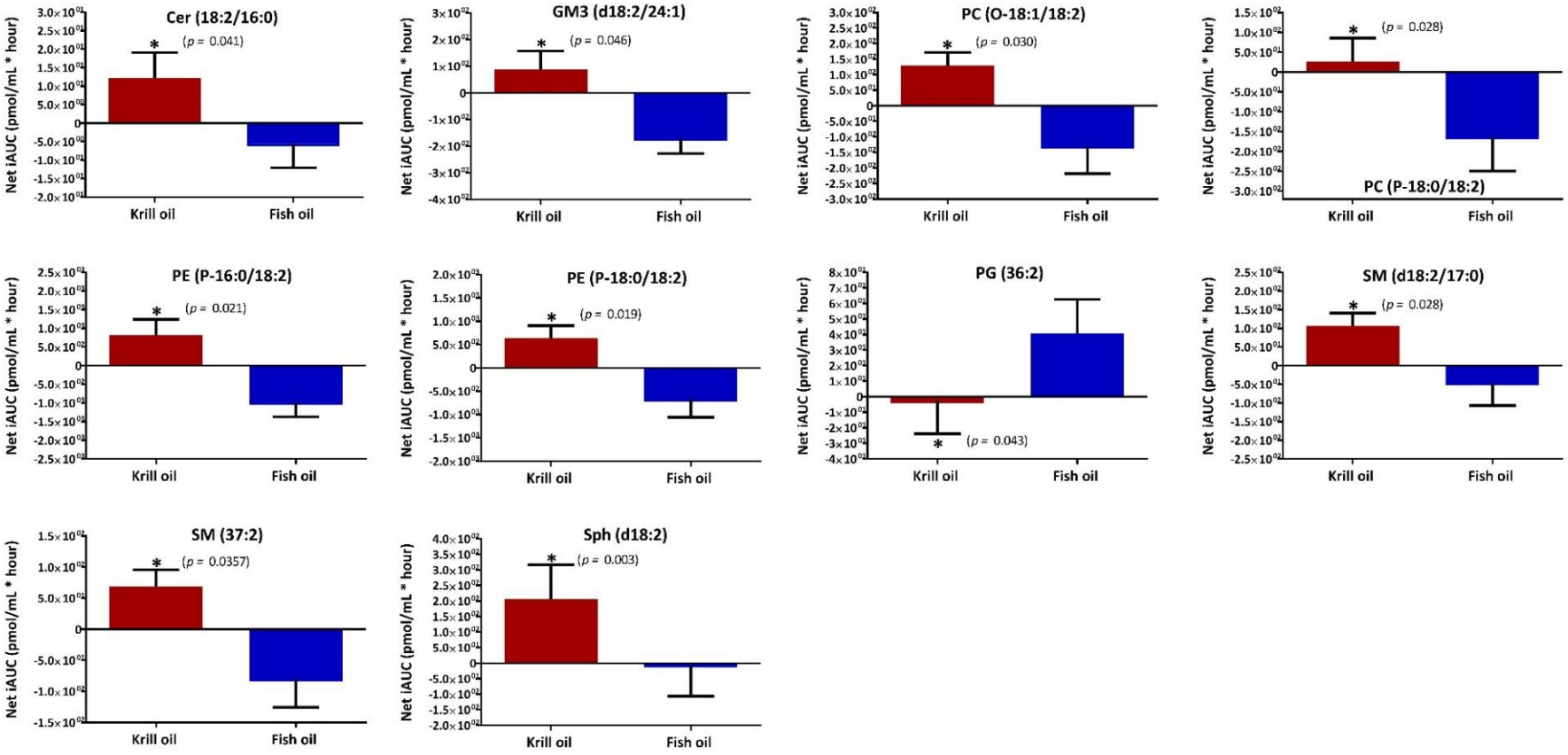


Figure 5.7 Significant differences in the net iAUC of plasma LA (18:2) molecular species over the 5-hour postprandial period between krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) for 10 women. The net iAUC from baseline (hours from zero to five) of plasma AA molecular species was calculated using the trapezoid rule and compared between the two supplementation groups using paired *t*-test. * in graphs indicates significant differences ($p < 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: Cer, ceramide; GM3, GM3 ganglioside; PC (O), alkylphosphatidylcholine; PC (P), PE (P), alkenylphosphatidylethanolamine; PG, phosphatidylglycerol; pmol/mL, picomole per millilitre; SM, sphingomyelin; Sph, Sphingosine.

5.6 DISCUSSION

This randomised crossover study investigated the differentiation of lipidomic responses between krill oil and fish oil supplementation in healthy women over a 5-hour postprandial period. For the purpose of comparing changes in lipid molecular species between the two omega-3 supplementation groups, a summary table is presented below showing the differences in the incremental area under the curve from baseline (net iAUC $_{0\text{-}5\ h}$) over the 5-hour postprandial period (Table 5.2).

A total of 29 lipid classes were detected as shown in Supplementary Table 5.1. The most abundant fatty acid-containing lipid classes detected in the plasma were CE, TAG and PC, which is consistent with a previous human study by Quehenberger et al (2010). Interestingly, as shown in Table 5.8, among the fatty acid-containing lipid classes, only the PE (P) class showed a significant difference in the net iAUC $_{0\text{-}5\ h}$ between krill oil and fish oil supplementation. The krill oil showed a significant increase in the net iAUC $_{0\text{-}5\ h}$ of PE, while the fish oil resulted in a significant decrease in the net iAUC $_{0\text{-}5\ h}$ of this class. These differences will be discussed later.

Previously, it was shown in *Chapter four* that plasma EPA levels from total plasma lipids were significantly increased from 3 to 5 hours after the start of supplementation with krill oil and fish oil compared with the olive oil (control). In this lipidomic study, it can be seen from Table 5.2 that there were significant increases in many molecular species containing EPA, DHA and DPA in the CE, TAG and phospholipids (the main one being PC) following supplementation with the n-3 oils over the postprandial period. Of the 36 molecular species containing LC n-3 PUFA, which showed significant

differences in the net iAUC $_{0\text{-}5\ h}$, the majority were for phospholipids and only eight were for CE, DAG and TAG. This has not been reported previously since the most authors have focused on changes in the n-3 PUFA in TAG or total fatty acids following ingestion of omega-3 oils in postprandial studies, which was the protocol in *Chapter four* where total plasma fatty acids were analysed.

This novel data highlights the importance of being able to study changes in many different lipids classes and molecular species in a postprandial study, and to date the only technique, which can do this, is lipidomics. The older methods such as thin-layer chromatography separation of lipids are not sufficiently sensitive and do not yield any data on molecular species.

Three things stand out from the data from this postprandial study. Firstly, the principal phospholipids containing LC n-3 PUFA, which circulate in plasma (diacyl-phospholipid species, in particular PC), were increased significantly more by the krill oil supplementation than by the fish oil supplementation, when expressed as the net iAUC $_{0\text{-}5\ h}$ (Figures 5.3 and 5.4, Table 5.2). Secondly, in contrast, the main plasma neutral lipids (CE, TAG and DAG) containing LC n-3 PUFA showed a significantly greater net iAUC $_{0\text{-}5\ h}$ following the fish oil compared with the krill oil supplementation (Figure 5.3, Table 5.2). Lastly, There was a consistent trend that ether-phospholipid molecular species, containing LC n-3 PUFA, were significantly increased after the krill oil supplementation, but decreased after the fish oil supplementation, expressed as the net iAUC $_{0\text{-}5\ h}$, (Figures 5.3 - 5.5, Table 5.2) ($p \leq 0.05$).

These findings that diacyl- and ether-phospholipids showed different responses between krill oil and fish oil are novel, as is the distinct difference between the krill oil

and fish in terms of the neutral lipids. It is of interest that the results from *Chapter four*, using thin layer chromatography, were suggestive of different responses between krill oil versus fish oil for phospholipids and TAG. This is the first time that it has been possible to differentiate between krill oil and fish oil in terms of LC n-3 fatty acids and it is most likely that such findings were possible because of the sensitivity and specificity of lipidomics.

There are no obvious explanations for these novel findings, which have shown significantly different lipid responses in the postprandial period between krill oil and fish oil supplementation. However, first, let's examine the TAG content of krill oil and fish oil:

[i] Krill oil has a lower proportion of TAG (24% of total lipid classes) compared with fish oil which contains 98% TAG. Also in this study, there was large difference in total LC n-3 PUFA content between the ingested krill oil compared with fish oil (907 mg and 1,441 mg/dose, respectively). The reason for this has been explained previously in *Chapter four*, but the oils used in the postprandial study were normalised to a 1 gram capsule of each oil. Therefore, based on the well-known lipid digestion and absorption of fatty acids from TAG, it should be expected that there would be a lower net iAUC for LC n-3 in krill oil than that in fish oil.

[ii] Krill oil contains 61% phospholipids (of the total lipid classes) compared with fish oil which contains 1% phospholipids. In this study, the total amount of phospholipids ingested in the krill oil arm was 553 mg/dose (calculated by 61% x 907 mg) compared with 14 mg/dose (calculated by 1% x 1,441 mg) for fish oil. Therefore, it should be expected that there would be a difference in the phospholipid digestion and absorption

in the postprandial period. But, the pathways of digestion and absorption of phospholipids, both for diacyl and ether-phospholipids, are poorly understood (see Figure 5.8).

The literature suggests that phospholipids in the intestine (from food or bile) are digested by pancreatic phospholipase A_2 (Küllenberg et al. 2012a, Lusis et al. 2004) into FFA and lyso-phospholipids (Figure 5.8). The lyso-phospholipids and FFA are then absorbed and re-assembled into phospholipids in the mucosal cells and exported into the blood circulation via chylomicrons (CM). Some of the FFA from the dietary/bile phospholipids are believed to be incorporated into the TAG, and are also exported in the CM (Cohn et al. 2010). Other reports suggest that some intestinal phospholipids may be absorbed passively without hydrolysis, and then preferentially incorporated into high-density lipoproteins (HDL) (Cohn et al. 2010). A third possibility which has not been extensively studied is that lyso-phospholipids may be transferred directly from the intestinal mucosa to the portal vein for direct transport to the liver (Zierenberg and Grundy 1982). Since lyso-phospholipids bind strongly to serum albumin, it is likely that this is how the absorbed lyso-phospholipids from the digestion of dietary phospholipids are transported in the blood circulation. Phospholipids from HDL, via the action of lecithin-cholesterol acyl transferase, can be transferred to the plasma membranes of various cells and tissues (Glomset 1968). It has been suggested that lipoprotein phospholipid fatty acids, derived from dietary phospholipids, can be incorporated into cellular membranes resulting in alteration of the cellular membrane composition, although the mechanism is complex and unclear (Küllenberg et al. 2012b, Taylor et al. 2010). While, there is considerable uncertainty about the digestion and absorption of diacyl-phosphatidylcholine species as described above, almost nothing is known about such processes for ether-phospholipid species. Perhaps,

these are metabolized in the same way as the diacyl phospholipid species, but this is uncertain.

Therefore, in summary, dietary phospholipids might be transported into the bloodstream following digestion and absorption in CM, HDL or as lyso-phospholipids bound to albumin. In contrast, dietary TAG are mostly transported via CM, followed by uptake in the liver and redistribution of the TAG fatty acids into different lipids of exported lipoproteins (very low-density lipoproteins). The above suggestions about possible metabolism of dietary PL reveal that fatty acids from these phospholipids could be incorporated into plasma phospholipids (actually lipoproteins) in the postprandial phase. It is relevant to consider the concentration of the LC n-3 in neutral lipids (CE, TAG and DAG) compared with phospholipids at 5 hours to give an estimate of the relative importance of the incorporation of LC n-3 into these two broad lipid class groups. In the krill oil supplementation, 25.1% of the LC n-3 PUFA were found in the phospholipids compared with 74.9% in the neutral lipids. In contrast, in the fish oil supplementation, 11.3% of the LC n-3 PUFA were found in the phospholipids compared with 88.7% in the neutral lipids (data calculated from Supplementary Tables 5.3 – 5.5).

The important issues arising from these novel postprandial data are that there are clear differences between krill oil and fish oil supplementation and that differences are most noticeable in the changes in ether-phospholipids in the postprandial period. It is not known whether the changes observed in the postprandial translate to changes in these phospholipid species in a longer-term. The biological relevance of changes in ether-phospholipids will be discussed in *Chapter seven*.

Table 5.2 Summary of significant differences in the net iAUC between krill oil and fish oil supplementation over the 5-hour postprandial period

Total lipid classes (n = 6)	*P* values	Comparison of the net iAUC $_{0\text{-}5\ h}$ for krill oil versus fish oil
Total COH	0.024	krill oil > fish oil
Total PE (P)	0.039	krill oil > fish oil
Total Ubiquinone	0.039	krill oil > fish oil
Total GM3	0.011	krill oil > fish oil
Total Hex3Cer	0.007	krill oil > fish oil
Total S1P	0.010	krill oil > fish oil
EPA (20:5n-3) molecular species (n = 23)	***P* values**	**Comparison of the net iAUC $_{0\text{-}5\ h}$ for krill oil versus fish oil**
PC (16:0/20:5)	0.012	krill oil > fish oil
PC (O-38:5)	0.024	krill oil > fish oil
PC (38:6) (a)	0.009	krill oil > fish oil
PE (P-16:0/20:5)	0.006	krill oil > fish oil
PC (O-36:5)	0.001	krill oil > fish oil
LPC (20:5) [sn1]	0.004	krill oil > fish oil
DAG (16:0/20:5)	**0.041**	**krill oil < fish oil**
PC (P-16:0/20:5)	0.043	krill oil > fish oil
PC (18:2/20:5)	0.000	krill oil > fish oil
TAG (52:5)	**0.023**	**krill oil < fish oil**
TAG (52:6)	**0.000**	**krill oil < fish oil**
PE(P-18:1/20:5) (b)	0.027	krill oil > fish oil
LPC (20:5) [sn2]	0.002	krill oil > fish oil
PC (39:5) (b)	0.048	krill oil > fish oil
PE (O-36:5)	0.002	krill oil > fish oil
PC (36:6) (a)	0.006	krill oil > fish oil
PC (34:5)	0.002	krill oil > fish oil
DAG (14:0/20:5)	**0.025**	**krill oil < fish oil**
PC (35:5)	0.000	krill oil > fish oil
DAG (20:5/20:5)	**0.034**	**krill oil < fish oil**
PC (20:5/20:5)	0.001	krill oil > fish oil
TAG (56:11)	**0.035**	**krill oil < fish oil**
TAG (54:10)	**0.030**	**krill oil < fish oil**

DHA (22:6n-3) molecular species (n = 11)	*P* values	Comparison of the net iAUC $_{0\text{-}5\ h}$ for krill oil versus fish oil
PE (P-16:0/22:6)	0.029	krill oil > fish oil
PE (P-18:0/22:6)	0.036	krill oil > fish oil
PE (O-16:0/22:6)	0.035	krill oil > fish oil
PC (40:8)	0.001	krill oil > fish oil
PE (P-17:0/22:6) (a)	0.032	krill oil > fish oil
PE (P-18:1/22:6) (b)	0.034	krill oil > fish oil
PC (14:0/22:6)	0.014	krill oil > fish oil
PC (40:7) (a)	0.005	krill oil > fish oil
PE (P-20:1/22:6) (a)	0.021	krill oil > fish oil
PC (38:7) (c)	0.047	krill oil > fish oil
PC (22:6/22:6)	0.002	krill oil > fish oil
DPA (22:5n-3) molecular species (n = 3)	***P* values**	**Comparison of the net iAUC $_{0\text{-}5\ h}$ for krill oil versus fish oil**
CE (22:5)	0.051	krill oil > fish oil
PE (P-18:0/22:5)	0.047	krill oil > fish oil
PE (P-18:1/22:5) (a)	0.034	krill oil > fish oil

Values are expressed as mean ± SEM (pmol/mL) (n =10). Two-way ANOVA for repeated measurements was applied to assess a significant difference at each time point between the two supplementation. All p values were corrected for multiple comparisons using the Benjamini-Hochberg FDR. Abbreviation: CE, cholesterol ester; CE (others): Desmosterol; COH, cholesterol; DAG, diacylglycerol; GM3, GM3 ganglioside; Hex3Cer, trihexosylceramide; LPC, lyso-phosphatidylcholine; net iAUC, incremental area under the curve from baseline (hours from zero to five); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PG, phosphatidylglycerol; ; SM, sphingomyelin; Sph: Sphingosine; S1P, Sphingosine-1-Phosphate; TAG, triacylglycerol.

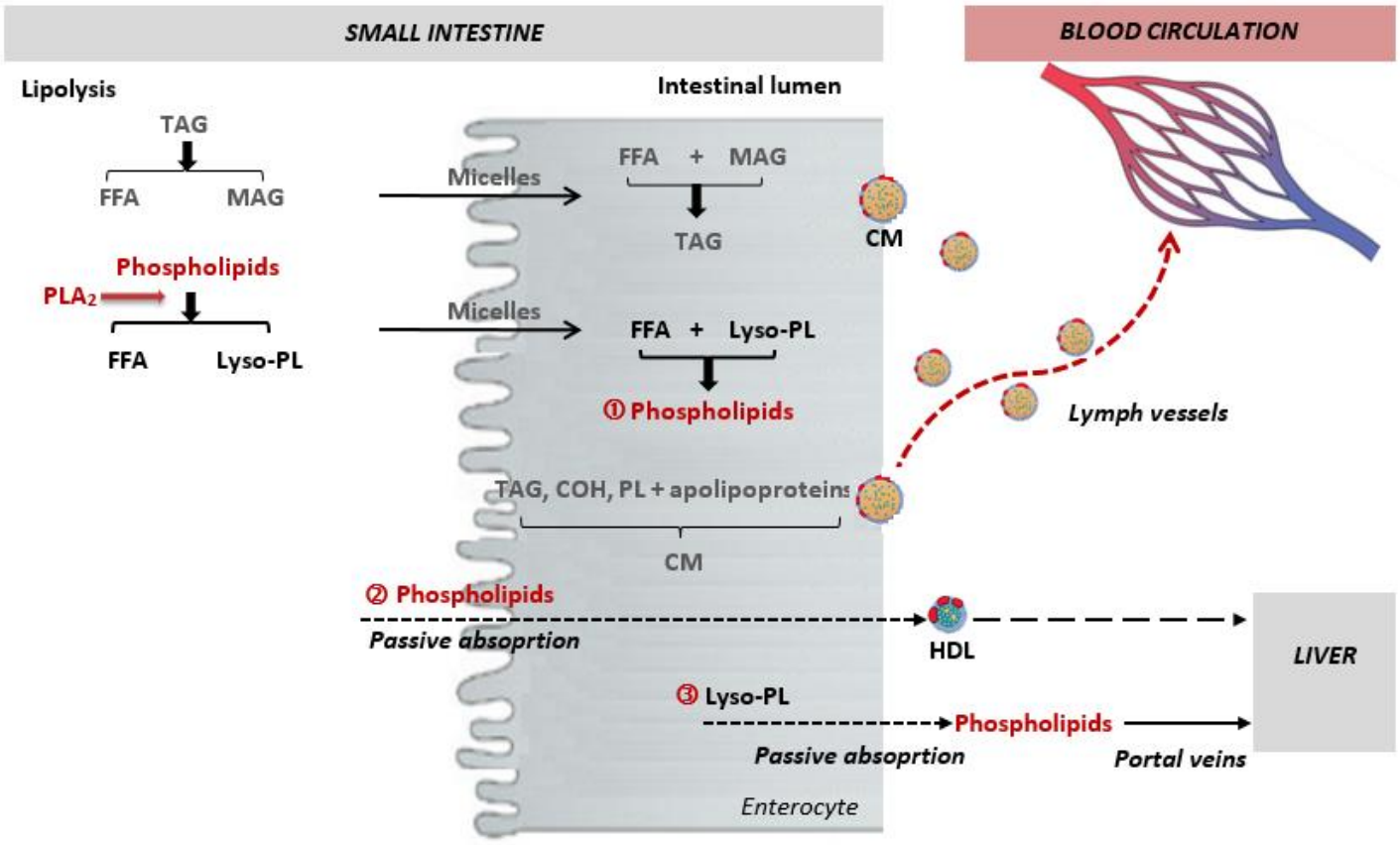


Figure 5.8 Dietary phospholipids digestion, absorption and transport

Abbreviations: CM, chylomicron; COH, cholesterol; FFA, free fatty acids; HDL, high-density lipoproteins; LC FA, long-chain fatty acid; Lyso-PL, Lyso-phospholipid; MAG, mono-glycerol; PL, phospholipids; TAG, triacylglycerol. Adapted from (Cohn et al. 2010, Küllenberg et al. 2012a, Lusis et al. 2004, Zierenberg and Grundy 1982).

5.7 CONCLUSION

Overall, the current study demonstrated that the postprandial lipidomic responses were significantly different between the krill oil and fish oil supplementation groups, especially in terms of the partitioning of the LC n-3 FA. The use of lipidomics to analyse the plasma samples enabled a novel differentiation between these two omega-3 oils that has not been previously reported. It is clear that there needs to be more studies on the digestion and absorption of dietary phospholipids including both diacyl-and ether-phospholipids.

5.8 STUDY SPECIFIC ACKNOWLEDGMENTS

I would like to thank Mina Brock at CSIRO Tasmania, for her assistance with the Iatroscan thin-layer chromatography-flame ionization analysis of the study oils. I am grateful to A/Prof. Peter Meikle, Baker and Heart and Diabetes Institute, Melbourne, Australia for his support and advice on lipidomic analysis. I also thank Natalie Mellett at Baker Heart and Diabetes Institute for her technical assistance with lipidomic analysis using HPLC ESI-MS/MS).Finally, the study participants are thanked for their genuine cooperation and support throughout the study.

Supplementary Table 5.1 Postprandial changes in a total of 29 plasma lipid classes (> 500 pmol/mL) over the 5-hour period after krill oil or fish oil supplementation

Total lipid classes	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5				
Total CE	2435181.3	2335145.2	2422155.1	2444439.7	2306417.6	2453960.8	0.078	0.810	0.121	**0.019**	0.861	**0.013**	0.865	0.598	0.560	0.709	0.112	0.960	0.725
Total COH	661758.8	742398.6	719703.1	715655.9	699814.8	701779.7	**0.007**	**0.041**	0.401	0.555	0.605	0.941	0.056	0.124	0.505	**0.024**	0.332	0.916	**0.048**
Total PC	654852.9	693898.7	693804.5	659032.1	671062.7	652551.2	**0.009**	**0.009**	0.994	0.376	0.631	0.180	0.756	0.102	**0.006**	0.101	0.178	0.135	0.077
Total TAG	650237.1	664843.4	570268.8	642697.2	649851.1	533948.3	0.737	0.139	0.101	0.869	**0.032**	**0.032**	0.981	0.981	0.966	0.733	0.887	**0.002**	0.807
Total SM	329010.1	342892.3	354836.2	350503.6	349663.1	341008.1	0.180	**0.018**	0.246	0.934	0.353	0.396	**0.045**	0.505	0.182	0.209	0.575	0.637	0.066
Total LPC	158578.1	167718.0	175489.8	163844.0	162328.2	167099.7	0.222	**0.031**	0.296	0.836	0.657	0.517	0.475	0.465	0.260	0.354	0.308	0.717	0.391
Total DAG	91323.0	101541.3	93143.8	87383.7	107649.4	88401.4	0.136	0.784	0.216	**0.006**	0.878	**0.009**	0.555	0.363	0.478	0.396	0.078	0.937	0.443
Total PE (P)	74379.1	81002.4	81372.0	79071.7	76380.6	73600.5	0.095	0.079	0.923	0.483	0.162	0.468	0.227	0.234	**0.053**	**0.039**	0.626	0.716	0.076
Total PI	40134.6	45822.0	49099.7	40518.9	44549.7	46796.2	**0.033**	**0.002**	0.223	0.158	**0.021**	0.306	0.866	0.578	0.318	0.567	**0.000**	1.000	0.737
Total PC (O)	28141.3	29167.3	29348.8	29416.1	29075.9	27750.7	0.175	0.114	0.806	0.645	**0.034**	0.085	0.096	0.902	**0.041**	0.085	0.698	0.883	**0.039**
Total PE	28111.4	33131.3	28933.1	27311.3	30503.0	26294.6	0.512	0.103	0.307	0.512	0.639	0.267	0.144	0.851	0.778	0.373	0.398	0.364	0.583
Total Ubiquinone	21207.1	20713.3	24000.7	22620.0	21030.3	21719.8	0.625	**0.017**	**0.007**	0.322	0.610	0.610	0.833	0.904	0.770	**0.025**	**0.038**	**0.028**	0.942
Total Ceramide	19428.2	19996.1	21714.7	20658.1	20427.2	21146.3	0.294	**0.000**	**0.004**	0.665	0.365	0.188	**0.031**	0.423	0.294	0.207	0.066	0.690	0.079
Total PC (P)	17721.7	17905.0	18028.4	18318.7	17781.5	17016.4	0.525	0.419	0.859	0.399	**0.025**	0.131	0.200	0.859	0.068	0.166	0.698	0.883	**0.039**
Total Hex1Cer	14934.5	15088.6	15429.0	15862.0	15876.1	15185.4	0.783	0.748	0.783	0.979	0.510	0.510	0.889	0.889	0.889	0.328	0.263	0.901	0.723
Total CE (others)	14580.4	14085.3	15389.6	14947.0	13903.2	15529.4	0.527	0.515	0.274	0.338	0.457	0.123	0.986	0.986	0.986	0.295	0.881	**0.038**	0.915
Total oxCE	13501.9	12796.5	12716.3	14560.4	13822.9	13316.0	**0.034**	**0.020**	0.797	**0.027**	**0.001**	0.116	0.877	0.877	0.877	0.658	**0.009**	0.507	0.512
Total Hex2Cer	11337.1	11687.4	12113.3	12552.1	12355.6	12119.9	0.542	0.212	0.542	0.827	0.681	0.827	0.640	0.805	0.995	0.174	0.147	0.849	0.566
Total LPE	7480.0	8753.5	8870.4	7165.8	8542.1	8733.5	**0.003**	**0.002**	0.759	**0.002**	**0.001**	0.617	0.414	0.581	0.720	0.831	**0.012**	0.702	0.946
Total PE (O)	5303.6	5553.0	5605.1	4533.7	4382.0	4223.4	0.217	0.139	0.792	0.446	0.129	0.427	**0.001**	**0.000**	**0.000**	0.119	0.859	**0.051**	0.107
Total Sph	2830.3	3067.4	2986.9	2913.0	2937.8	3004.2	**0.038**	0.349	0.216	0.752	0.530	0.349	0.349	0.127	0.530	0.184	0.744	1.000	0.171
Total Acylcarnitine	2547.0	2320.2	2919.2	2760.3	2433.7	2852.3	0.229	0.056	**0.004**	0.090	0.620	**0.034**	0.257	0.542	0.718	0.631	**0.007**	0.628	0.556
Total GM3	2418.0	2512.6	2747.1	2683.6	2567.0	2427.0	0.239	**0.001**	**0.007**	0.151	**0.004**	0.088	0.324	0.781	0.284	**0.011**	0.723	0.999	**0.000**
Total dhCer	2187.0	2147.7	2144.6	2161.1	2095.0	2113.9	0.644	0.619	0.971	0.439	0.580	0.822	0.760	0.535	0.718	0.876	0.610	0.560	0.971
Total Hex3Cer	2066.2	2170.0	2244.7	2310.6	2168.4	2105.8	0.254	**0.038**	0.278	0.086	**0.014**	0.362	0.282	0.993	0.579	**0.007**	**0.001**	0.920	0.803
Total PS	1958.6	2733.8	2356.5	2345.9	2343.1	2377.9	0.116	0.408	0.432	0.995	0.946	0.942	0.420	0.416	0.964	0.353	0.462	0.982	0.516
Total LPC (O)	1958.0	2056.1	2132.6	2108.5	1998.0	1991.7	0.247	**0.048**	0.365	0.195	0.172	0.939	0.083	0.488	0.103	0.090	0.933	0.887	0.058
Total S1P	1162.9	1250.2	1209.0	1259.9	1218.7	1208.5	0.188	0.543	0.543	0.618	0.618	0.825	0.448	0.886	0.993	**0.010**	0.137	0.691	0.724
Total Sulfatide	530.7	553.8	570.6	567.7	598.5	563.0	0.599	0.365	0.698	0.479	0.925	0.424	0.406	0.313	0.864	0.914	0.568	0.426	0.660

Values are expressed as mean of concentration (pmol/mL) of total lipid molecular classes for 10 women. Two-way ANOVA for repeated measurements were performed to analyse supplementation effect over time, supplement and interaction (time x supplement), difference between time point within supplementation (superscript with a) and each time point between the two supplementation (superscript with b). All *p* values (< 0.05) were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (hours from zero to five) of plasma lipid molecular species for 10 women was calculated using the trapezoid rule and compared between krill oil and fish oil supplementation using paired *t*-test. Abbreviation: CE, cholesterol ester; CE (others), desmosterol and hydroxylcholesterol; COH, cholesterol; DAG, diacylglycerol; dhCer, dihydroceramide; GM3, GM3 ganglioside; Hex1Cer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lyso-phosphatidylcholine; LPC (O), lyso-alkylphosphatidylcholine; LPE, lyso-phosphatidylethanolamine; net iAUC, net incremental area under the curve from baseline (hours from zero to five);oxCE, oxidised cholesterol ester (fatty acid); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; SM, sphingomyelin; Sph, sphingosine; S1P, sphingosine-1-phosphate; TAG, triacylglycerol; T0, baseline; T3, 3 hours; T5, 5 hours.

Supplementary Table 5.2 (A) Postprandial changes in a total 23/56 plasma EPA (20:5) TAG, DAG and sterol molecular species over the 5-hour period after krill oil or fish oil supplementation

EPA (20:5) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5				
TAG (54:6)	801.2	3164.6	2486.6	806.0	3290.8	3011.2	**0.000**	**0.002**	0.163	**0.000**	**0.000**	0.557	0.992	0.790	0.276	0.733	**0.000**	0.661	0.717
TAG (56:7)	408.6	3055.6	2241.4	420.0	3298.4	2916.5	**0.000**	**0.004**	0.165	**0.000**	**0.000**	0.506	0.984	0.671	0.246	0.680	**0.000**	0.550	0.704
TAG (54:7)	364.6	1159.0	1038.3	399.0	1453.1	1338.4	**0.000**	**0.000**	0.508	**0.000**	**0.000**	0.325	0.253	0.325	**0.015**	0.818	**0.000**	0.367	0.439
TAG (56:8)	208.8	757.7	681.4	232.4	794.1	843.0	**0.000**	**0.000**	0.484	**0.000**	**0.000**	0.652	0.827	0.737	0.147	0.779	**0.000**	0.562	0.609
TAG (52:5)	167.4	510.3	416.7	201.4	744.0	527.4	**0.008**	**0.044**	0.427	**0.000**	**0.011**	0.077	0.771	0.058	0.350	**0.023**	**0.000**	0.275	0.480
TAG (52:6)	158.1	618.3	397.7	164.0	995.8	607.9	**0.009**	0.145	0.178	**0.000**	**0.011**	**0.024**	0.970	**0.028**	0.198	**0.000**	**0.000**	0.174	0.273
TAG (56:6)	136.8	478.9	392.0	134.5	542.3	469.0	**0.000**	**0.003**	0.264	**0.000**	**0.000**	0.345	0.976	0.413	0.321	0.528	**0.000**	0.512	0.734
TAG (54:8)	54.7	175.2	145.6	58.9	266.1	224.9	**0.000**	**0.000**	0.343	**0.000**	**0.000**	0.848	0.525	0.076	**0.007**	0.252	**0.000**	0.217	0.263
TAG (50:5)	51.7	172.8	82.1	60.4	369.8	159.2	0.062	0.623	0.154	**0.000**	0.123	**0.003**	0.888	**0.005**	0.222	0.092	**0.000**	0.062	0.115
TAG (52:7)	34.2	131.3	83.4	35.7	215.4	157.0	**0.008**	0.148	0.157	**0.000**	**0.002**	0.089	0.964	**0.019**	**0.036**	0.133	**0.000**	0.093	0.176
TAG (60:12)	31.8	170.2	141.5	27.8	243.2	258.5	**0.000**	**0.000**	0.357	**0.000**	**0.000**	0.340	0.307	0.493	**0.017**	0.882	**0.000**	0.084	0.369
TAG (56:10)	7.4	127.3	82.6	8.2	296.7	261.7	**0.032**	0.161	0.396	**0.000**	**0.000**	0.504	0.989	**0.004**	**0.003**	0.054	**0.000**	**0.024**	**0.042**
TAG (56:11)	1.3	55.3	25.2	1.5	159.5	134.4	0.068	0.402	0.292	**0.000**	**0.000**	0.378	0.994	**0.002**	**0.001**	**0.035**	**0.000**	**0.015**	**0.020**
TAG (54:10)	0.7	43.9	16.2	0.8	154.9	118.6	0.137	0.583	0.331	**0.000**	**0.001**	0.208	0.997	**0.001**	**0.002**	**0.030**	**0.000**	**0.012**	**0.019**
DAG (18:1/20:5)	1122.6	9361.8	7735.2	1247.3	12390.5	13418.0	**0.000**	**0.003**	0.400	**0.000**	**0.000**	0.593	0.948	0.126	**0.008**	0.265	**0.000**	0.156	0.143
DAG (18:2/20:5)	535.4	1799.5	2150.4	609.9	2148.9	2858.1	**0.000**	**0.000**	0.070	**0.000**	**0.000**	**0.001**	0.119	0.452	**0.008**	0.847	**0.000**	0.276	0.312
DAG (16:0/20:5)	384.3	1570.6	1385.3	419.5	3500.1	2842.8	**0.000**	**0.000**	1.000	**0.000**	**0.000**	0.744	0.704	**0.002**	**0.001**	**0.041**	**0.000**	**0.022**	**0.035**
DAG (16:1/20:5)	127.4	519.8	428.4	148.1	1255.2	1012.8	**0.000**	**0.000**	0.814	**0.000**	**0.000**	0.707	0.329	**0.002**	**0.000**	0.100	**0.000**	**0.009**	0.071
DAG (18:0/20:5)	53.6	141.4	134.4	55.2	237.8	203.9	**0.018**	**0.028**	0.837	**0.000**	**0.000**	0.329	0.963	**0.011**	0.054	0.145	**0.000**	0.142	0.153
DAG (14:0/20:5)	52.7	201.1	106.0	47.4	758.6	443.0	**0.001**	**0.021**	0.135	**0.000**	**0.000**	0.853	0.559	**0.003**	**0.000**	**0.025**	**0.000**	**0.003**	**0.004**
DAG (20:5/20:5)	4.1	91.3	54.4	4.4	383.8	269.4	0.238	0.490	0.612	**0.000**	**0.002**	0.127	0.996	**0.001**	**0.008**	**0.034**	**0.001**	**0.018**	**0.026**
CE (20:5)	67270.4	67242.5	84277.4	75384.2	64598.3	81639.8	0.995	**0.001**	**0.001**	**0.026**	0.176	**0.001**	0.085	0.559	0.560	0.559	**0.025**	0.910	0.171
Desmosterol (20:5)	1520.0	1530.3	1779.6	1547.6	1481.2	1666.5	0.979	**0.001**	**0.001**	0.319	0.068	**0.008**	0.873	0.385	0.107	0.173	**0.031**	0.713	0.436

Supplementary Table 5.2 (B.1) Postprandial changes in a total 33/56 plasma EPA (20:5) phospholipid molecular species over the 5-hour period after krill oil or fish oil supplementation

EPA (20:5) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5				
PC (38:5) (a)	9676.9	10399.1	10559.9	9284.0	9401.3	9699.4	**0.028**	**0.009**	0.601	0.703	0.187	0.338	0.211	**0.004**	**0.011**	0.173	0.066	**0.049**	0.355
PC (16:0/20:5)	7430.5	11969.6	14462.5	7525.9	9461.5	12074.8	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**	0.861	**0.000**	**0.000**	**0.012**	**0.000**	0.093	**0.004**
PC (38:5) (b)	5335.2	6404.9	7152.7	5218.6	5988.1	6606.1	**0.000**	**0.000**	**0.002**	**0.002**	**0.000**	**0.008**	0.578	0.058	**0.016**	0.294	**0.000**	0.482	0.340
PC (O-38:5)	4082.4	4258.6	4184.7	4189.0	4035.7	3927.8	0.141	0.384	0.527	0.198	**0.035**	0.359	0.364	0.068	**0.038**	**0.024**	0.594	0.256	0.071
PC (38:6) (a)	4063.0	4996.8	5463.4	4056.9	4301.9	4566.7	**0.000**	**0.000**	**0.012**	0.162	**0.007**	0.132	0.972	**0.001**	**0.000**	**0.009**	**0.000**	**0.004**	**0.004**
PC (P-38:5) (a)	2025.5	2055.6	2056.0	2075.8	2022.2	1958.3	0.577	0.573	0.995	0.326	**0.040**	0.245	0.356	0.537	0.083	0.173	0.688	0.775	0.171
PE(P-18:0/20:5)	1403.8	1492.3	1574.9	1588.3	1518.8	1462.9	0.272	**0.042**	0.304	0.385	0.126	0.484	**0.030**	0.739	0.169	0.063	0.834	0.899	**0.048**
PE (38:5) (a)	1125.2	1312.1	1215.3	1036.4	1128.0	1043.5	**0.019**	0.370	0.115	0.159	1.000	0.159	0.285	**0.041**	0.058	0.482	0.109	0.314	0.710
PE (O-38:5) (a)	920.8	956.2	961.9	765.1	744.5	685.0	0.301	0.233	0.867	0.544	**0.027**	0.091	**0.000**	**0.000**	**0.000**	0.124	0.408	0.084	0.059
PE (P-16:0/20:5)	875.6	938.7	964.9	887.8	818.2	816.7	0.189	0.069	0.577	0.149	0.141	0.975	0.795	**0.018**	**0.005**	**0.006**	0.933	0.660	0.054
PE(P-18:1/20:5) (a)	764.3	877.2	912.8	790.8	796.8	772.4	0.088	**0.029**	0.576	0.924	0.772	0.701	0.678	0.216	**0.038**	0.082	0.059	0.641	0.190
PI (38:5) (a)	667.8	788.8	825.6	654.9	689.9	737.4	**0.013**	**0.002**	0.411	0.435	0.076	0.292	0.773	**0.036**	0.059	0.193	**0.001**	0.156	0.339
PE (38:5) (b)	627.1	762.0	724.3	573.7	659.8	684.5	**0.001**	**0.009**	0.246	**0.009**	**0.003**	0.614	0.318	**0.039**	0.614	0.581	**0.000**	0.495	0.477
PC (O-40:5)	491.5	503.3	512.5	517.2	507.1	493.9	0.494	0.231	0.593	0.560	0.186	0.445	0.146	0.824	0.287	0.269	0.989	0.774	0.209
PI (38:5) (b)	469.6	576.5	636.8	514.7	558.8	617.7	**0.000**	**0.000**	**0.019**	0.076	**0.000**	**0.022**	0.070	0.462	0.427	0.084	**0.000**	0.947	0.115
PC (O-36:5)	455.7	547.4	705.1	544.5	523.1	508.7	**0.001**	**0.000**	**0.000**	0.363	0.136	0.539	**0.001**	0.303	**0.000**	**0.001**	**0.000**	0.556	**0.000**

Supplementary Table 5.2 (B.2) Postprandial changes in a total 33/56 plasma EPA (20:5) phospholipid molecular over the 5-hour period after krill oil or fish oil supplementation

EPA (20:5) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5				
LPC (20:5) [sn1]	398.6	978.2	1247.4	405.6	648.2	1020.4	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.894	**0.000**	**0.000**	**0.004**	**0.000**	**0.004**	**0.001**
PC (P-38:5) (b)	334.1	334.2	331.4	342.8	334.5	321.7	1.000	1.000	1.000	0.281	0.156	0.716	0.716	0.156	0.081	0.333	0.697	0.721	0.563
PC (P-16:0/20:5)	265.9	279.4	285.3	287.3	276.7	259.9	0.198	0.072	0.572	0.308	**0.015**	0.116	**0.049**	0.789	**0.022**	**0.043**	0.810	0.962	**0.015**
PC (18:2/20:5)	232.6	594.8	686.1	243.2	346.2	455.1	**0.000**	**0.000**	**0.004**	**0.002**	**0.000**	**0.001**	0.706	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**
PE (O-38:5) (b)	142.3	147.9	154.7	130.0	125.7	126.9	0.472	0.123	0.388	0.580	0.690	0.877	0.122	**0.009**	**0.002**	0.237	0.640	0.272	0.369
PE (16:0/20:5)	129.2	219.8	224.6	121.7	193.6	223.1	**0.000**	**0.000**	0.792	**0.001**	**0.000**	0.123	0.686	0.169	0.934	0.633	**0.000**	0.620	0.618
PE(P-18:1/20:5) (b)	113.1	120.7	125.1	127.9	106.8	112.5	0.197	**0.046**	0.432	0.158	0.792	0.243	0.895	**0.015**	**0.035**	**0.027**	0.419	0.620	0.127
PS (38:5)	111.5	127.2	116.8	117.0	119.0	121.8	0.136	0.603	0.315	0.846	0.636	0.779	0.593	0.423	0.625	0.507	0.363	0.881	0.559
LPC (20:5) [sn2]	110.4	265.4	342.6	114.0	179.9	274.5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.804	**0.000**	**0.000**	**0.002**	**0.000**	**0.003**	**0.001**
PC (39:5) (b)	105.5	118.1	126.5	108.5	112.6	114.2	**0.001**	**0.000**	**0.011**	0.171	0.064	0.594	0.354	0.073	**0.001**	**0.048**	**0.000**	0.490	**0.007**
PE (O-36:5)	83.5	106.2	128.0	73.7	77.9	78.9	**0.001**	**0.000**	**0.002**	0.491	0.394	0.867	0.117	**0.000**	**0.000**	**0.002**	**0.000**	**0.024**	**0.001**
PC (36:6) (a)	66.2	184.3	218.8	63.8	115.1	155.6	**0.000**	**0.000**	**0.009**	**0.000**	**0.000**	**0.003**	0.849	**0.000**	**0.000**	**0.006**	**0.000**	**0.013**	**0.001**
PC (34:5)	62.7	144.0	156.1	61.3	77.9	85.9	**0.000**	**0.000**	0.208	0.087	**0.015**	0.389	0.889	**0.000**	**0.000**	**0.002**	**0.000**	**0.020**	**0.000**
PS (40:5)	60.9	79.1	65.2	68.8	71.1	69.9	0.404	0.825	0.295	0.627	0.627	1.000	0.825	0.566	0.627	0.970	0.530	0.826	0.743
PC (39:5) (a)	59.8	63.2	63.2	61.3	66.9	63.4	0.330	0.147	0.612	0.155	0.458	0.476	0.424	0.743	0.132	0.932	0.367	0.758	0.681
PC (35:5)	47.8	90.2	111.1	53.3	68.6	86.8	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.233	**0.000**
PC (20:5/20:5)	4.0	119.8	112.4	4.4	9.2	12.2	**0.000**	**0.000**	0.659	**0.000**	**0.000**	0.103	0.572	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**

Values are expressed as mean of plasma EPA (20:5) molecular species concentration for 10 women. Two-way analysis of variance for repeated measurements was performed to analyse supplementation effect over time, supplement and interaction (time x supplement), difference between time point within supplementation (superscript with a) and each time point between the two supplementation (superscript with b). All *p* values (< 0.05) were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (hours from zero to five) of plasma lipid molecular species for 10 women was calculated using the trapezoid rule and compared between krill oil and fish oil supplementation using paired *t*-test. Abbreviation: CE, cholesterol ester; Desmosterol; DAG, diacylglycerol; LPC, lyso-phosphatidylcholine; net iAUC, net incremental area under the curve from baseline (hours from zero to five); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; T0, baseline; T3, 3 hours; T5, 5 hours; TAG, triacylglycerol.

Supplementary Table 5.3 (A) Postprandial changes in a total 27/76 plasma DHA (22:6) TAG, DAG and sterol molecular species over the 5-hour period after krill oil or fish oil supplementation

DHA (22:6) Molecular species	Concentration (pmol/mL)												*P* value						
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5				
TAG (56:7)	3763.2	8377.9	8565.8	3486.9	7477.0	9079.2	**0.000**	**0.000**	0.832	**0.000**	**0.000**	0.082	0.755	0.315	0.563	0.854	**0.000**	0.886	0.528
TAG (18:1/18:1/22:6)	1565.0	5513.6	4854.9	1738.1	5350.2	5868.9	**0.000**	**0.000**	0.309	**0.000**	**0.000**	0.421	0.786	0.798	0.125	1.000	**0.000**	0.646	0.413
TAG (56:8)	1524.8	3310.3	3352.8	1543.5	3086.9	3878.0	**0.000**	**0.000**	1.000	**0.000**	**0.000**	**0.006**	0.112	0.906	**0.008**	0.550	**0.000**	0.559	0.116
TAG (54:7)	650.5	1456.5	1157.0	603.7	1495.7	1347.5	**0.000**	**0.003**	0.057	**0.000**	**0.000**	0.328	0.755	0.794	0.213	0.615	**0.000**	0.828	0.527
TAG (54:6)	648.0	1224.9	1169.2	619.4	1249.7	1345.5	**0.001**	**0.002**	0.694	**0.000**	**0.000**	0.500	0.839	0.860	0.221	0.656	**0.000**	0.827	0.568
TAG (58:9)	452.4	1040.6	1186.6	515.8	1001.5	1401.0	**0.001**	**0.000**	0.325	**0.003**	**0.000**	**0.013**	0.666	0.790	0.155	0.863	**0.000**	0.708	0.473
TAG (52:6)	209.0	373.6	229.6	191.2	439.3	289.0	**0.005**	0.693	**0.012**	**0.000**	0.072	**0.009**	0.733	0.216	0.261	0.319	**0.001**	0.645	0.454
TAG (56:9)	191.8	408.0	386.1	205.7	439.8	489.6	**0.000**	**0.000**	0.730	**0.000**	**0.000**	0.132	0.097	0.492	**0.017**	0.744	**0.000**	0.423	0.412
TAG (56:10)	158.5	354.2	358.7	184.2	382.6	461.2	**0.000**	**0.000**	0.843	**0.000**	**0.000**	**0.027**	**0.033**	0.491	**0.009**	0.523	**0.000**	0.364	0.295
TAG (56:6)	128.9	203.0	177.6	116.0	254.4	197.8	**0.022**	0.117	0.403	**0.000**	**0.013**	0.072	0.670	0.100	0.505	0.211	**0.000**	0.691	0.332
TAG (58:7)	127.7	330.2	260.5	117.2	350.8	306.8	**0.000**	**0.011**	0.153	**0.000**	**0.001**	0.358	0.824	0.663	0.334	0.586	**0.000**	0.755	0.694
TAG (54:8)	124.4	281.7	203.5	126.1	291.1	265.7	**0.000**	**0.000**	**0.041**	**0.000**	**0.000**	0.862	0.439	0.546	**0.017**	0.798	**0.000**	0.480	0.309
TAG (52:7)	87.8	165.2	97.0	81.1	190.9	126.3	**0.002**	0.660	**0.004**	**0.000**	**0.042**	**0.006**	0.750	0.229	0.173	0.293	**0.000**	0.606	0.416
TAG (56:11)	33.1	179.0	141.9	37.9	285.6	232.7	**0.000**	**0.000**	0.397	**0.000**	**0.000**	0.740	0.119	0.210	**0.022**	0.996	**0.000**	0.132	0.685
TAG (60:12)	31.8	170.2	141.5	27.8	243.2	258.5	**0.000**	**0.000**	0.448	**0.000**	**0.000**	0.127	0.322	0.536	**0.008**	0.831	**0.000**	0.202	0.224
TAG (60:13)	7.9	43.1	43.5	8.1	53.5	68.9	**0.002**	**0.002**	0.971	**0.000**	**0.000**	0.121	0.988	0.291	**0.015**	0.350	**0.000**	0.275	0.195
DAG (18:0/22:6)	3727.7	14564.2	11386.3	6517.4	12216.8	5617.3	0.176	**0.034**	0.386	0.904	0.695	0.609	0.159	0.856	0.236	0.126	0.329	0.822	0.191
DAG (18:1/22:6)	1753.6	4619.7	5029.7	1717.2	4409.2	5946.5	**0.000**	**0.000**	0.468	**0.000**	**0.000**	**0.012**	0.948	0.708	0.115	0.848	**0.000**	0.780	0.324
DAG (18:2/22:6)	1143.2	2344.7	2843.3	1222.3	2251.8	3598.1	**0.000**	**0.000**	0.087	**0.000**	**0.000**	**0.000**	**0.027**	0.554	**0.004**	0.253	**0.000**	0.367	0.177
DAG (16:0/22:6)	674.6	1319.1	1519.7	710.8	1601.1	1860.1	**0.002**	**0.000**	0.260	**0.000**	**0.000**	0.150	0.836	0.119	0.064	0.375	**0.000**	0.481	0.433
DAG (16:1/22:6)	425.5	849.1	956.5	412.4	1067.2	1268.5	**0.000**	**0.000**	0.701	**0.000**	**0.000**	0.077	0.445	0.155	**0.008**	0.506	**0.000**	0.296	0.311
DAG (16:0/22:6)	352.7	631.5	706.6	372.7	746.7	885.0	**0.004**	**0.001**	0.393	**0.000**	**0.000**	0.124	0.818	0.196	**0.052**	0.441	**0.000**	0.493	0.438
DAG (14:0/22:6)	166.9	340.0	255.4	160.2	652.8	454.8	0.079	0.353	0.375	**0.000**	**0.005**	**0.047**	0.944	**0.003**	**0.046**	0.069	**0.002**	0.075	0.073
DAG (22:6/22:6)	25.7	89.4	111.3	24.4	156.2	204.1	**0.000**	**0.000**	0.133	**0.000**	**0.000**	**0.018**	0.065	**0.036**	**0.004**	0.696	**0.000**	0.101	0.622
DAG (20:5/22:6)	22.6	252.3	150.3	19.9	897.3	654.2	**0.000**	**0.000**	0.087	**0.000**	**0.000**	**0.000**	**0.027**	0.554	**0.004**	0.253	**0.000**	0.367	0.177
CE (22:6)	41882.8	39451.3	44718.5	41934.1	36040.1	45064.9	0.226	0.161	**0.014**	**0.007**	0.124	**0.000**	0.979	0.096	0.860	0.352	**0.024**	0.815	0.335
Desmosterol (22:6)	525.5	457.7	542.1	469.6	440.5	538.5	**0.024**	0.554	**0.007**	0.304	**0.022**	**0.002**	0.056	0.540	0.896	0.085	0.102	0.550	0.394

Supplementary Table 5.3 (B.1) Postprandial changes in a total 49/76 plasma DHA (22:6) phospholipid molecular species over the 5-hour period after krill oil or fish oil supplementation

DHA (22:6) Molecular species	Concentration (pmol/mL)												*P* value						
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5				
PC (16:0/22:6)	24462.0	25499.4	26456.5	24896.3	25290.2	25403.1	0.276	**0.044**	0.313	0.674	0.590	0.904	0.643	0.823	0.268	0.499	0.265	0.851	0.532
PC (18:0/22:6)	6656.8	6978.4	7081.6	6737.1	7026.4	6933.1	0.140	0.057	0.626	0.182	0.360	0.660	0.704	0.820	0.485	0.773	0.164	0.987	0.707
PE (P-16:0/22:6)	3983.6	4244.4	4308.2	4256.5	4167.6	4025.8	0.137	0.068	0.707	0.602	0.185	0.408	0.120	0.652	0.109	**0.029**	0.661	0.848	0.086
PE (P-18:0/22:6)	3873.8	4107.3	4180.1	4333.2	4118.3	3981.1	0.162	0.162	1.000	0.227	0.113	0.682	**0.052**	0.540	0.311	**0.036**	0.935	0.898	0.082
PE (16:0/22:6)	3314.7	3465.2	3411.2	3303.6	3308.0	2999.4	0.300	0.530	0.675	0.907	**0.037**	**0.047**	0.815	0.172	**0.006**	0.192	0.413	0.661	0.150
PE (P-18:1/22:6) (a)	2043.2	2118.0	2235.5	2193.1	2083.0	2055.0	0.516	0.106	0.312	0.343	0.237	0.807	0.201	0.760	0.127	0.165	0.710	0.795	0.145
PE (18:0/22:6)	1541.6	1707.3	1514.1	1531.8	1608.8	1432.4	**0.025**	0.689	**0.011**	0.270	0.159	**0.018**	0.886	0.163	0.243	0.315	0.127	0.790	0.624
PC (18:1/22:6) (a)	1415.6	1523.2	1520.2	1372.8	1414.9	1455.0	**0.034**	**0.038**	0.951	0.380	0.096	0.403	0.372	**0.033**	0.180	0.350	0.117	0.543	0.612
PC (O-16:0/22:6)	1256.2	1307.5	1344.6	1373.5	1367.2	1307.9	0.248	0.054	0.399	0.885	0.144	0.184	**0.014**	0.181	0.404	0.167	0.832	0.337	0.061
PI (18:0/22:6)	857.2	989.6	1022.2	891.3	975.0	1073.0	**0.030**	**0.009**	0.569	0.154	**0.005**	0.099	0.553	0.797	0.379	0.719	**0.000**	0.655	0.700
LPC (22:6) [sn1]	748.3	856.4	1016.2	753.5	806.8	969.6	**0.040**	**0.000**	**0.004**	0.290	**0.000**	**0.004**	0.917	0.324	0.353	0.521	**0.001**	0.510	0.678
PE (O-16:0/22:6)	713.4	800.2	846.7	684.6	694.9	677.7	**0.019**	**0.001**	0.185	0.763	0.842	0.617	0.404	**0.006**	**0.000**	**0.035**	**0.016**	**0.043**	**0.029**
PC (18:1/22:6) (b)	614.5	642.3	642.8	635.4	630.0	633.8	0.200	0.193	0.982	0.800	0.941	0.858	0.332	0.564	0.673	0.313	0.750	0.998	0.481
PC (P-16:0/22:6)	586.1	592.5	582.1	605.4	590.9	578.2	0.748	0.839	0.601	0.468	0.182	0.527	0.337	0.936	0.848	0.437	0.573	0.885	0.660
PC (O-18:0/22:6)	574.6	595.7	601.4	625.1	619.0	594.6	0.270	0.165	0.762	0.746	0.117	0.204	**0.014**	0.224	0.718	0.223	0.843	0.264	0.120
PC (O-40:7) (a)	499.0	505.1	528.3	521.5	506.0	478.5	0.685	0.063	0.134	0.307	**0.009**	0.080	0.145	0.953	**0.003**	0.129	0.920	0.481	**0.008**
PC (40:8)	476.1	597.3	604.6	493.5	503.4	498.9	**0.000**	**0.000**	0.634	0.515	0.722	0.766	0.259	**0.000**	**0.000**	**0.001**	**0.001**	**0.009**	**0.000**
LPE (22:6) [sn1]	452.4	469.1	575.3	425.6	514.8	603.3	0.580	**0.001**	**0.002**	**0.007**	**0.000**	**0.008**	0.374	0.139	0.354	0.164	**0.000**	0.685	0.220
PI (38:6)	429.6	511.6	521.0	438.6	496.3	525.8	**0.014**	**0.007**	0.756	0.071	**0.009**	0.339	0.768	0.617	0.876	0.713	**0.000**	0.990	0.832
PE (18:1/22:6) (a)	392.3	564.2	525.5	387.5	604.1	590.9	**0.000**	**0.000**	0.201	**0.000**	**0.000**	0.543	0.674	0.907	**0.054**	0.360	**0.000**	0.804	0.208
PE (P-17:0/22:6) (a)	385.5	422.8	419.9	435.5	428.9	414.4	**0.053**	0.114	0.683	0.417	0.541	0.838	**0.015**	0.838	0.683	**0.032**	0.732	0.516	0.125
PE (O-18:0/22:6)	344.3	352.6	350.7	307.0	288.4	292.3	0.592	0.679	0.902	0.235	0.345	0.798	**0.024**	**0.001**	**0.001**	0.332	0.772	0.196	0.435
PC (16:1/22:6)	305.2	371.8	400.6	300.8	338.0	360.8	**0.000**	**0.000**	0.074	**0.025**	**0.001**	0.151	0.774	**0.039**	**0.017**	0.102	**0.000**	0.375	0.239
PE (P-18:1/22:6) (b)	303.7	300.7	313.9	326.2	275.7	281.4	0.871	0.580	0.476	**0.012**	**0.024**	0.753	0.231	0.186	0.091	**0.034**	**0.022**	0.680	0.095
PE (O-18:1/22:6)	287.3	303.3	309.5	252.5	242.3	246.9	0.374	0.224	0.731	0.571	0.752	0.800	0.065	**0.003**	**0.002**	0.294	0.631	**0.022**	0.470

Supplementary Table 5.3 (B.2) Postprandial changes in a total 49/76 plasma DHA (22:6) phospholipid molecular species over the 5-hour period after krill oil or fish oil supplementation

DHA (22:6) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-	Inter-
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5		Time	ment	action
LPC (22:6) [sn2]	279.1	307.2	361.0	269.7	297.5	342.7	0.060	**0.000**	**0.001**	0.062	**0.000**	**0.005**	0.508	0.496	0.208	0.907	**0.001**	0.437	0.880
PC (17:0/22:6)	273.6	283.7	289.0	278.5	285.8	283.2	0.342	0.155	0.616	0.491	0.658	0.803	0.640	0.841	0.583	0.755	0.512	0.980	0.753
LPE (22:6) [sn2]	260.4	272.1	327.4	251.4	286.6	333.2	0.489	**0.001**	**0.004**	**0.048**	**0.000**	**0.012**	0.592	0.395	0.730	0.469	**0.000**	0.846	0.607
PC (14:0/22:6)	248.8	308.4	333.4	240.3	258.9	265.7	**0.000**	**0.000**	**0.014**	0.058	**0.013**	0.471	0.368	**0.000**	**0.000**	**0.014**	**0.000**	0.222	**0.001**
PC (15:0/22:6)	243.8	266.1	288.2	250.4	262.2	269.1	**0.017**	**0.000**	**0.018**	0.179	**0.040**	0.427	0.443	0.652	**0.037**	0.319	**0.001**	0.778	0.127
PC (15-MHDA/22:6)	241.5	265.5	274.4	247.0	262.5	266.6	**0.043**	**0.008**	0.431	0.178	0.094	0.718	0.623	0.790	0.487	0.569	**0.025**	0.935	0.692
PC (O-40:7) (b)	210.1	217.0	213.5	216.3	212.2	204.6	0.325	0.621	0.617	0.560	0.104	0.279	0.378	0.491	0.207	0.193	0.599	0.725	0.295
PE (P-17:0/22:6) (b)	203.0	208.5	207.7	232.0	224.5	225.7	0.644	0.539	0.877	0.758	0.758	1.000	0.076	0.287	0.359	0.549	0.977	0.066	0.779
PE (P-20:0/22:6)	201.5	223.3	209.2	221.1	216.7	214.0	0.064	0.493	0.219	0.696	0.531	0.811	0.094	0.555	0.673	0.138	0.219	0.416	0.269
PC (40:7) (a)	180.8	213.8	211.9	170.2	175.1	177.2	**0.001**	**0.001**	0.811	0.550	0.394	0.795	0.205	**0.000**	**0.000**	**0.005**	**0.004**	**0.002**	**0.050**
PC (P-18:1/22:6)	170.8	168.3	173.1	176.4	172.8	166.8	1.000	0.565	0.565	0.624	0.069	0.168	0.160	0.346	0.305	0.467	0.679	0.653	0.197
PC (P-18:0/22:6)	158.7	159.1	161.0	171.0	168.9	158.8	0.559	0.603	0.948	0.696	**0.028**	0.063	**0.018**	0.122	0.769	0.278	0.296	0.462	0.141
PE (P-20:1/22:6) (a)	131.1	154.2	153.2	170.0	155.4	164.3	**0.032**	**0.039**	0.919	0.159	0.573	0.382	**0.001**	0.903	0.276	**0.021**	0.545	0.271	**0.040**
PC (38:7) (c)	117.1	135.2	143.9	118.8	124.4	127.9	**0.000**	**0.000**	**0.042**	0.180	**0.034**	0.383	0.667	**0.013**	**0.001**	**0.047**	**0.000**	0.196	**0.015**
PE (18:1/22:6) (b)	87.7	84.3	76.4	80.5	79.8	75.5	0.635	0.125	0.275	0.926	0.479	0.538	0.318	0.531	0.889	0.644	0.538	0.630	0.823
PE (P-15:0/22:6) (b)	83.0	89.8	93.1	89.3	104.2	98.3	0.359	0.180	0.655	**0.054**	0.231	0.421	0.394	0.061	0.482	0.598	0.177	0.238	0.623
PS (40:6)	80.7	101.9	89.8	98.0	92.3	86.4	0.211	0.586	0.467	0.731	0.488	0.724	0.305	0.562	0.839	0.327	0.618	0.870	0.490
PE (P-15:0/22:6) (a)	62.8	58.4	59.7	70.7	69.5	69.0	0.286	0.447	0.752	0.765	0.668	0.896	0.063	**0.012**	**0.032**	0.554	0.745	0.296	0.854
PE (17:0/22:6)	37.8	40.7	38.0	36.3	43.5	36.9	0.329	0.969	0.348	**0.021**	0.832	**0.033**	0.596	0.339	0.720	0.384	0.184	0.989	0.513
PE (15-MHDA/22:6)	29.1	33.4	31.3	28.4	32.9	28.9	0.117	0.411	0.431	0.105	0.839	0.150	0.792	0.839	0.379	0.911	0.257	0.771	0.863
PI (37:6)	19.6	20.3	19.2	21.5	20.4	17.6	0.554	0.679	0.320	0.357	**0.003**	**0.025**	0.121	0.938	0.197	0.144	0.146	0.877	0.140
PE (P-20:1/22:6) (b)	17.8	20.9	18.8	18.1	18.3	17.5	0.164	0.656	0.331	0.917	0.799	0.719	0.891	0.243	0.573	0.258	0.482	0.368	0.643
PI (39:6)	14.2	15.4	15.7	13.3	15.0	15.7	0.269	0.171	0.780	0.128	**0.034**	0.494	0.382	0.663	0.978	0.676	0.185	0.554	0.830
PC (22:6/22:6)	5.0	17.0	20.8	6.1	6.3	6.3	**0.000**	**0.000**	**0.040**	0.879	0.901	0.978	0.549	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**	**0.000**

Values are expressed as mean of plasma DHA (22:6) molecular species concentration for 10 women. Two-way analysis of variance for repeated measurements was performed to analyse supplementation effect over time, supplement and interaction (time x supplement), difference between time point within supplementation (superscript with a) and each time point between the two supplementation (superscript with b). All *p* values (≤ 0.05) were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (hours from zero to five) of plasma lipid molecular species for 10 women was calculated using the trapezoid rule and compared between krill oil and fish oil supplementation using paired *t*-test. Abbreviation: CE, cholesterol ester; Desmosterol; DAG, diacylglycerol; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; net iAUC, net incremental area under the curve from baseline (hours from zero to five); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; T0, baseline; T3, 3 hours; T5, 5 hours; TAG, triacylglycerol.

Supplementary Table 5.4 (A) Postprandial changes in a total 17/29 plasma DPA (22:5) TAG, DAG, and CE molecular species over the 5-hour period after krill oil or fish oil supplementation

DPA (22:5) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5				
TAG (56:6)	924.4	1262.4	1250.7	923.1	1262.6	1356.3	**0.001**	**0.002**	0.898	**0.001**	**0.000**	0.311	0.988	0.999	0.255	0.791	**0.000**	0.826	0.635
TAG (56:7)	425.0	609.7	582.9	442.6	598.9	634.8	**0.002**	**0.005**	0.595	**0.006**	**0.001**	0.478	0.727	0.831	0.308	0.883	**0.001**	0.845	0.675
TAG (54:5)	138.7	175.8	164.9	144.0	194.9	187.0	**0.039**	0.134	0.520	**0.007**	**0.019**	0.640	0.756	0.269	0.202	0.579	**0.013**	0.634	0.753
TAG (54:6)	133.5	191.4	156.2	128.0	205.4	182.4	**0.007**	0.253	0.083	**0.001**	**0.011**	0.245	0.778	0.474	0.189	0.427	**0.003**	0.712	0.512
TAG (58:7)	107.4	202.5	192.1	108.9	241.1	246.9	**0.001**	**0.003**	0.672	**0.000**	**0.000**	0.812	0.950	0.128	**0.036**	0.302	**0.000**	0.291	0.303
TAG (58:8)	63.4	106.6	113.5	70.8	113.2	135.5	**0.001**	**0.000**	0.506	**0.001**	**0.000**	**0.043**	0.479	0.522	**0.045**	0.809	**0.000**	0.510	0.502
TAG (52:5)	51.1	68.5	45.8	54.4	79.4	53.8	0.073	0.568	**0.023**	**0.014**	0.952	**0.012**	0.722	0.252	0.389	0.594	**0.042**	0.501	0.843
TAG (58:6)	47.0	62.6	60.2	45.8	68.3	69.1	**0.002**	**0.006**	0.575	**0.000**	**0.000**	0.851	0.766	0.194	**0.049**	0.250	**0.000**	0.534	0.245
TAG (56:8)	39.3	58.0	52.4	42.6	67.8	63.5	**0.004**	**0.034**	0.342	**0.000**	**0.002**	0.466	0.576	0.105	0.069	0.385	**0.001**	0.524	0.594
TAG (54:7)	20.8	33.0	22.2	20.1	36.7	30.4	**0.006**	0.735	**0.012**	**0.001**	**0.016**	0.126	0.851	0.353	**0.048**	0.377	**0.002**	0.529	0.286
DAG (18:1/22:5)	1074.8	1514.6	1652.3	1156.6	1675.6	1817.8	**0.004**	**0.000**	0.312	**0.001**	**0.000**	0.298	0.545	0.240	0.228	0.622	**0.001**	0.525	0.882
DAG (16:0/22:5)	451.3	584.8	599.7	476.3	709.7	663.8	**0.022**	**0.012**	0.784	**0.000**	**0.003**	0.402	0.646	**0.031**	0.245	0.347	**0.042**	0.532	0.428
DAG (18:2/22:5)	443.0	606.9	651.1	464.1	619.9	737.4	**0.003**	**0.000**	0.358	**0.004**	**0.000**	**0.022**	0.659	0.785	0.082	0.849	**0.001**	0.672	0.493
DAG (16:0/22:5)	362.2	467.2	423.7	396.5	459.2	464.3	**0.030**	0.142	0.420	0.161	0.058	0.582	0.297	0.864	0.136	0.741	0.134	0.635	0.618
DAG (16:1/22:5)	264.5	384.4	411.1	236.8	435.8	479.7	**0.005**	**0.001**	0.490	**0.000**	**0.000**	0.261	0.475	0.192	0.087	0.194	**0.001**	0.643	0.188
DAG (14:0/22:5)	131.1	353.6	292.9	152.3	693.7	630.4	0.055	0.153	0.583	**0.000**	**0.000**	0.567	0.847	**0.006**	**0.006**	0.082	**0.000**	**0.033**	0.083
CE (22:5)	1820.9	1779.9	2008.0	1910.5	1706.5	2021.9	0.575	**0.018**	**0.005**	**0.011**	0.138	**0.000**	0.228	0.320	0.848	**0.051**	**0.050**	0.944	0.299

Supplementary Table 5.4 (B) Postprandial changes in a total 12/29 plasma DPA (22:5) phospholipid molecular species over the 5-hour postprandial period after krill oil or fish oil supplementation

DPA (22:5) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5				
PE (P-16:0/22:5)	4136.4	4459.6	4439.5	4419.2	4307.2	4131.3	0.118	0.141	0.920	0.576	0.160	0.383	0.167	0.448	0.134	0.083	0.574	0.898	0.117
PC (18:0/22:5)	2703.2	2825.6	2836.2	2629.6	2666.1	2651.8	0.209	0.174	0.911	0.702	0.816	0.880	0.443	0.107	0.065	0.532	0.465	0.310	0.687
PE (P-18:0/22:5)	923.3	1029.2	1023.0	1000.5	970.4	979.0	0.060	0.075	0.907	0.575	0.688	0.872	0.160	0.279	0.415	**0.047**	0.628	0.940	0.164
PE (P-18:1/22:5) (a)	686.2	759.4	769.6	766.0	745.3	717.8	0.090	0.056	0.807	0.619	0.254	0.510	0.067	0.733	0.221	**0.034**	0.340	0.950	0.091
PE (18:0/22:5)	321.2	379.7	319.5	319.3	327.2	289.6	**0.043**	0.948	**0.037**	0.774	0.282	0.178	0.944	0.066	0.280	0.246	0.128	0.420	0.426
PI (18:0/22:5)	284.0	330.6	350.7	283.2	296.8	328.1	**0.038**	**0.005**	0.347	0.522	**0.045**	0.151	0.971	0.123	0.293	0.344	**0.000**	0.377	0.535
PC (P-18:0/22:5)	231.9	235.7	237.5	250.1	241.1	240.5	0.664	0.526	0.840	0.306	0.274	0.941	**0.048**	0.536	0.729	0.226	0.909	0.322	0.420
LPC (22:5) [sn1]/LPC (22:5) [sn2]	200.6	224.7	248.2	188.5	195.3	225.9	0.087	**0.001**	**0.044**	0.455	**0.004**	**0.019**	0.315	**0.052**	0.111	0.567	**0.005**	**0.049**	0.759
LPC (22:5) [sn1]	171.8	196.8	221.1	166.2	171.6	197.3	**0.047**	**0.000**	**0.020**	0.294	**0.003**	**0.029**	0.434	0.080	0.057	0.485	**0.003**	0.080	0.649
PE (O-18:0/22:5) (a)	139.3	142.9	139.7	127.6	120.1	118.6	0.453	1.000	0.453	0.265	0.265	1.000	**0.023**	**0.000**	**0.002**	0.195	0.734	0.253	0.412
PE (P-18:1/22:5) (b)	110.9	105.9	106.9	109.4	101.4	108.3	0.453	0.548	0.879	0.234	0.858	0.307	0.822	0.497	0.842	0.827	0.439	0.850	0.820
LPC (22:5) [sn2]	79.2	85.4	95.0	73.1	77.6	84.9	0.392	**0.003**	**0.020**	0.152	**0.006**	0.116	0.115	0.313	0.068	0.819	**0.010**	0.099	0.811

Values are expressed as mean of plasma DPA (22:5) molecular species concentration for 10 women. Two-way analysis of variance for repeated measurements was performed to analyse supplementation effect over time, supplement and interaction (time x supplement), difference between time point within supplementation (superscript with a) and each time point between the two supplementation (superscript with b). All *p* values (≤ 0.05) were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (hours from zero to five) of plasma lipid molecular species for 10 women was calculated using the trapezoid rule and compared between krill oil and fish oil supplementation using paired *t*-test. Abbreviation: CE, cholesterol ester; DAG, diacylglycerol; LPC, lyso-phosphatidylcholine; net iAUC, net incremental area under the curve from baseline (hours from zero to five); PC, phosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; T0, baseline; T3, 3 hours; T5, 5 hours; TAG, triacylglycerol.

Supplementary Table 5.5 (A) Postprandial changes in a total 10/61 plasma AA (20:4) TAG, DAG and sterol molecular species over the 5-hour period after krill oil or fish oil supplementation

AA (20:4) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time Time	Supple-ment	Inter-action
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5				
TAG	2764.9	3756.9	3700.1	3055.4	3840.4	3898.6	**0.000**	**0.000**	0.738	**0.000**	**0.000**	0.732	0.100	0.624	0.251	0.453	**0.000**	0.301	0.686
TAG	969.5	1064.7	1032.2	1153.7	1166.3	1097.6	**0.035**	0.149	0.446	0.766	0.195	0.116	**0.000**	**0.025**	0.134	0.064	0.348	0.152	0.147
DAG (18:1/20:4)	1990.2	2642.5	2500.7	1946.9	2797.4	2669.4	**0.002**	**0.012**	0.450	**0.000**	**0.001**	0.495	0.817	0.410	0.371	0.363	**0.001**	0.721	0.665
DAG (18:2/20:4)	875.2	1341.6	1251.2	942.5	1513.8	1454.1	**0.002**	**0.010**	0.494	**0.000**	**0.001**	0.651	0.610	0.201	0.135	0.499	**0.000**	0.436	0.744
DAG (18:0/20:4)	674.4	841.7	784.4	698.3	778.4	782.5	**0.003**	**0.036**	0.253	0.116	0.100	0.934	0.628	0.208	0.968	0.351	0.161	0.855	0.443
DAG (16:0/20:4)	454.4	481.3	456.3	458.7	565.6	495.9	0.543	0.965	0.572	**0.024**	0.402	0.125	0.922	0.067	0.373	0.255	0.414	0.609	0.441
CE (20:4)	297608.2	296042.8	315677.5	307907.3	289872.5	322438.9	0.888	0.117	0.090	0.118	0.202	**0.008**	0.360	0.581	0.545	0.284	0.109	0.805	0.547
Desmosterol (20:4)	1716.6	1600.7	1871.5	1864.4	1634.1	1847.8	0.117	**0.041**	**0.001**	**0.004**	0.816	**0.007**	**0.050**	0.641	0.740	0.147	0.083	0.774	0.240
OH-Cholesterol	370.2	368.2	377.9	382.8	373.2	375.4	0.895	0.609	0.521	0.528	0.626	0.884	0.407	0.738	0.869	0.680	0.820	0.764	0.775
OH-Cholesterol	180.7	181.4	176.2	195.9	164.3	183.1	0.954	0.737	0.694	**0.027**	0.340	0.169	0.260	0.208	0.606	0.170	0.838	0.915	0.221

Supplementary Table 5.5 (B.1) Postprandial changes in a total 51/61 plasma AA (20:4) phospholipid molecular species over the 5-hour period after krill oil or fish oil supplementation

AA (20:4) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-	Inter-
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5		Time	ment	action
PC (16:0/20:4)	52696.3	56630.8	58921.5	53279.4	52418.2	52863.6	**0.025**	**0.001**	0.170	0.598	0.798	0.784	0.720	**0.017**	**0.001**	**0.037**	**0.050**	0.169	**0.025**
PC (18:0/20:4)	24344.1	27024.9	26227.6	24886.1	25034.2	25046.2	**0.005**	**0.038**	0.356	0.862	0.851	0.989	0.528	**0.029**	0.177	0.097	0.149	0.493	0.123
PI (18:0/20:4)	19596.3	22515.6	24636.0	19437.7	21740.7	22669.2	**0.051**	**0.003**	0.215	0.276	0.070	0.433	0.752	0.529	0.276	0.506	**0.001**	0.496	0.591
PE (P-18:0/20:4)	13002.4	14669.5	14661.6	14022.3	13543.7	13100.9	0.059	0.060	0.993	0.570	0.280	0.599	0.234	0.191	0.076	0.060	0.548	0.695	0.088
PE (P-16:0/20:4)	11610.0	12867.2	12786.8	11369.1	11237.7	10456.2	0.137	0.162	0.922	0.873	0.273	0.346	0.769	0.059	**0.010**	0.158	0.472	0.350	0.205
PC (O-16:0/20:4)	9311.3	9558.6	9509.2	9405.1	9431.8	8816.6	0.387	0.487	0.861	0.925	**0.049**	**0.041**	0.740	0.655	**0.023**	0.357	0.356	0.560	0.149
PE (P-18:1/20:4) (a)	7862.7	8378.1	8753.8	7870.5	7575.6	7341.1	0.227	**0.044**	0.374	0.484	0.215	0.576	0.985	0.067	**0.003**	**0.036**	0.640	0.325	0.076
PE (18:0/20:4)	5369.7	6520.1	5815.4	5287.2	5609.8	5138.9	**0.009**	0.275	0.092	0.426	0.713	0.250	0.837	**0.034**	0.105	0.180	0.072	0.357	0.336
PC (P-16:0/20:4)	3660.1	3648.6	3670.1	3703.5	3577.6	3467.8	0.919	0.930	0.850	0.276	**0.050**	0.340	0.703	0.534	0.088	0.309	0.605	0.752	0.323
LPC (20:4) [sn1]	3264.6	3625.7	3922.2	3362.0	3288.3	3598.7	**0.047**	**0.001**	0.097	0.669	0.179	0.083	0.573	0.062	0.072	0.152	0.059	0.189	0.148
PE (16:0/20:4)	2464.6	2562.2	2353.9	2106.3	2224.2	2005.4	0.315	0.642	0.149	0.286	0.437	0.075	0.150	0.167	0.085	0.973	0.123	0.372	0.958
PC (38:4)	2191.4	2349.8	2391.4	2162.9	2203.1	2152.4	0.058	**0.020**	0.601	0.613	0.894	0.525	0.720	0.077	**0.007**	0.150	0.231	0.413	0.190
PC (O-18:0/20:4)	1945.2	2031.2	2056.0	2054.2	2034.0	1971.7	0.167	0.080	0.684	0.738	0.184	0.311	0.085	0.964	0.176	0.105	0.865	0.918	0.100
PI (16:0/20:4)	1600.4	1896.0	1945.4	1582.9	1739.7	1804.7	**0.008**	**0.003**	0.622	0.128	**0.037**	0.518	0.861	0.130	0.170	0.409	**0.000**	0.460	0.561
PE (O-16:0/20:4)	1383.3	1417.5	1397.7	1097.9	1043.3	995.6	0.537	0.794	0.720	0.328	0.076	0.392	**0.000**	**0.000**	**0.000**	0.212	0.328	**0.025**	0.308
LPC (20:4) [sn2]	1168.9	1272.3	1395.2	1141.5	1147.1	1231.5	**0.047**	**0.000**	**0.021**	0.909	0.080	0.099	0.578	**0.019**	**0.003**	0.210	**0.026**	**0.031**	0.152
PE (P-18:1/20:4) (b)	1075.0	1145.5	1059.0	1046.2	953.7	913.7	0.214	0.528	0.069	0.125	0.094	0.874	0.347	**0.001**	**0.051**	0.158	0.175	0.297	0.148
PC (17:0/20:4)	845.9	898.0	915.0	861.2	891.2	860.8	0.106	**0.036**	0.584	0.339	0.990	0.333	0.623	0.828	0.093	0.335	0.140	0.775	0.284
PC (P-18:0/20:4)	807.0	830.6	838.1	857.4	846.7	783.9	0.390	0.261	0.783	0.694	**0.014**	**0.031**	0.077	0.557	0.059	0.190	0.423	0.953	**0.038**
PS (38:4)	701.6	990.5	863.7	821.0	847.7	876.7	0.120	0.372	0.483	0.882	0.756	0.872	0.508	0.430	0.942	0.422	0.421	0.972	0.583
PC (15-MHDA/20:4)	677.5	735.1	781.2	701.9	735.1	718.7	0.091	**0.005**	0.170	0.316	0.608	0.617	0.458	1.000	0.068	0.359	**0.030**	0.848	0.173
PC (14:0/20:4)	612.7	703.8	681.9	569.9	598.0	580.5	**0.000**	**0.004**	0.306	0.193	0.615	0.411	**0.054**	**0.000**	**0.000**	0.067	**0.028**	0.272	0.083
PE (O-18:0/20:4)	607.6	598.8	598.0	431.7	397.8	386.0	0.732	0.707	0.973	0.196	0.087	0.645	**0.000**	**0.000**	**0.000**	0.464	0.253	0.124	0.594
LPE (20:4) [sn1]	599.5	646.8	717.8	539.2	658.0	683.9	0.163	**0.002**	**0.038**	**0.001**	**0.000**	0.410	0.085	0.625	0.382	0.240	**0.002**	0.568	0.281
PC (16:1/20:4)	582.5	673.0	699.2	541.4	592.5	593.9	**0.004**	**0.001**	0.358	0.083	0.075	0.959	0.156	**0.010**	**0.001**	0.298	**0.004**	0.058	0.283
PE (P-20:0/20:4)	488.2	604.7	571.3	561.3	537.7	557.0	**0.047**	0.146	0.550	0.671	0.937	0.728	0.198	0.236	0.796	0.128	0.395	0.933	0.215

Supplementary Table 5.5 (B.2) Postprandial changes in a total 51/61 plasma AA (20:4) phospholipid molecular species over the 5-hour period after krill oil or fish oil supplementation

AA (20:4) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time Time	Supple-ment	Inter-action
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5				
PC (15:0/20:4)	453.8	490.6	508.0	465.1	483.1	490.7	**0.045**	**0.005**	0.321	0.304	0.150	0.662	0.516	0.669	0.325	0.357	**0.029**	0.911	0.498
PE (P-17:0/20:4) (b)	430.3	466.9	471.4	488.6	479.6	457.2	0.253	0.203	0.888	0.774	0.325	0.480	0.077	0.689	0.653	0.138	0.793	0.752	0.274
PE (P-17:0/20:4) (a)	403.3	431.7	423.4	414.5	382.1	361.3	0.168	0.322	0.680	0.119	**0.015**	0.306	0.578	**0.022**	**0.006**	**0.019**	0.435	0.597	**0.038**
LPE (20:4) [sn2]	318.6	359.5	370.4	299.8	330.0	336.4	**0.007**	**0.001**	0.491	**0.038**	**0.010**	0.531	0.130	**0.027**	**0.024**	0.511	**0.007**	0.129	0.789
PE (P-20:1/20:4)	313.0	357.1	342.0	318.9	302.4	292.4	**0.005**	0.077	0.189	0.248	0.189	0.866	0.105	**0.014**	0.142	**0.015**	0.322	0.621	**0.016**
PC (P-17:0/20:4) (a)	286.3	286.3	297.4	304.0	299.7	285.7	0.998	0.352	0.353	0.722	0.135	0.245	0.147	0.265	0.328	0.479	0.922	0.811	0.185
PC (20:0/20:4)	125.2	133.7	132.3	128.5	130.7	130.7	0.133	0.203	0.802	0.691	0.685	0.994	0.549	0.582	0.769	0.403	0.486	0.941	0.691
PI (17:0/20:4)	120.0	142.2	149.6	134.0	146.4	148.0	**0.026**	**0.005**	0.432	0.190	0.144	0.869	0.146	0.652	0.861	0.438	**0.001**	0.602	0.493
PC (P-17:0/20:4) (b)	119.4	120.5	119.6	123.0	120.6	116.0	0.802	0.962	0.839	0.611	0.144	0.326	0.439	0.985	0.443	0.428	0.468	0.997	0.549
PE (17:0/20:4)	90.6	109.1	102.3	90.6	91.1	82.5	**0.033**	0.164	0.403	0.951	0.328	0.300	0.996	**0.037**	**0.024**	0.125	0.298	0.138	0.185
PC (O-35:4)	71.1	75.2	76.1	84.1	81.4	74.4	**0.038**	**0.013**	0.610	0.149	**0.000**	**0.001**	**0.000**	**0.003**	0.352	**0.012**	0.380	0.589	**0.000**
PE (P-19:0/20:4) (b)	67.9	74.2	71.4	82.6	74.5	74.0	0.347	0.600	0.671	0.232	0.210	0.949	**0.038**	0.970	0.691	0.164	0.816	0.232	0.274
PC (O-34:4)	67.7	107.9	161.9	110.7	104.6	97.8	**0.000**	**0.000**	**0.000**	0.463	0.129	0.410	**0.000**	0.690	**0.000**	**0.000**	**0.000**	0.630	**0.000**
PE (P-19:0/20:4) (a)	60.7	68.1	72.7	72.3	71.1	71.1	0.181	**0.036**	0.390	0.828	0.828	1.000	**0.042**	0.570	0.766	0.159	0.310	0.558	0.229
PE (P-15:0/20:4) (a)	57.9	59.3	57.9	55.9	64.4	54.8	0.819	0.990	0.829	0.189	0.869	0.143	0.754	0.426	0.624	0.555	0.464	1.000	0.611
LPI (20:4) [sn1]	52.4	60.8	69.6	57.7	53.5	59.6	0.082	**0.001**	0.070	0.375	0.669	0.196	0.267	0.125	**0.042**	0.117	**0.034**	0.262	0.066
PE (P-15:0/20:4) (b)	48.2	57.6	51.9	62.7	62.2	49.9	0.104	0.513	0.310	0.943	**0.032**	**0.037**	**0.017**	0.411	0.717	**0.049**	**0.027**	0.543	0.132
PE (20:0/20:4)	40.0	54.7	47.7	44.5	49.8	51.1	0.066	0.321	0.362	0.495	0.392	0.859	0.554	0.520	0.652	0.514	0.192	0.819	0.629
PE (15-MHDA/20:4)	38.0	43.4	40.0	34.8	39.6	35.4	0.190	0.619	0.402	0.242	0.888	0.300	0.439	0.357	0.263	0.865	0.317	0.544	0.968
PE (16:1/20:4)	28.4	37.4	35.5	27.4	33.9	28.6	**0.005**	**0.050**	0.306	**0.050**	0.679	0.109	0.917	0.356	0.132	0.380	**0.001**	0.551	0.498
PI (20:0/20:4)	28.2	34.3	36.5	29.1	32.9	30.8	**0.049**	**0.010**	0.460	0.205	0.558	0.483	0.753	0.637	0.067	0.423	**0.010**	0.499	0.290
PC (P-20:0/20:4)	21.4	24.6	24.6	25.0	24.7	22.8	0.084	0.081	0.987	0.865	0.229	0.297	0.057	0.978	0.302	0.143	0.581	0.553	0.114
PC (P-15:0/20:4) (b)	18.6	17.5	17.3	17.2	16.7	15.5	0.129	0.086	0.826	0.476	**0.028**	0.113	**0.051**	0.233	**0.015**	0.722	**0.027**	0.603	0.600
LPI (20:4) [sn2]	17.5	21.1	24.7	20.8	18.5	21.8	0.113	**0.004**	0.112	0.312	0.628	0.143	0.151	0.243	0.194	0.095	**0.026**	0.563	0.104
PC (P-15:0/20:4) (a)	13.1	13.0	12.9	11.2	10.6	9.5	0.191	0.303	0.770	0.294	**0.028**	0.206	**0.018**	**0.000**	**0.000**	0.105	0.552	0.308	0.067

Values are expressed as mean of plasma AA (20:4) molecular species concentration for 10 women. Two-way analysis of variance for repeated measurements was performed to analyse supplementation effect over time, supplement and interaction (time x supplement), difference between time point within supplementation (superscript with a) and each time point between the two supplementation (superscript with b). All p values (≤ 0.05) were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (hours from zero to five) of plasma lipid molecular species for 10 women was calculated using the trapezoid rule and compared between krill oil and fish oil supplementation using paired t-test. Abbreviation: CE, cholesterol ester; Desmosterol; DAG, diacylglycerol; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; LPI, lyso-phosphatidylinositol; net iAUC, net incremental area under the curve from baseline (hours from zero to five); OH-Cholesterol, hydroxycholesterol; PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; T0, baseline; T3, 3 hours; T5, 5 hours; TAG, triacylglycerol.

Supplementary Table 5.6 (A) Postprandial changes in a total of 28/101 plasma LA (18:2n-6) TAG, DAG and sterol molecular species over the 5-hour period after krill oil or fish oil supplementation

LA (18:2) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-	Inter-
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5		Time	ment	action
TAG (16:0/18:1/18:2)	84021.9	84270.7	81273.1	84238.6	81204.5	74777.1	0.919	0.266	0.227	0.222	**0.001**	**0.015**	0.929	0.217	**0.014**	0.102	0.139	0.566	0.170
TAG (16:0/18:2/18:2)	33027.9	32610.5	30233.5	36675.4	35409.4	29043.5	0.800	0.102	0.160	0.445	**0.000**	**0.001**	**0.037**	0.101	0.472	0.239	**0.017**	0.593	0.107
TAG (16:1/18:1/18:2)	24966.5	27142.0	24247.5	25981.5	27697.6	23194.1	0.194	0.661	0.089	0.301	0.101	**0.012**	0.537	0.734	0.522	0.676	0.072	0.948	0.642
TAG (18:1/18:2/18:2)	19345.5	23322.0	20576.1	24194.9	25652.8	21243.2	**0.036**	0.493	0.136	0.418	0.110	**0.022**	**0.013**	0.201	0.709	0.185	**0.030**	0.368	0.264
TAG (14:0/16:0/18:2)	14391.3	11294.6	8161.6	12962.8	10999.7	7014.1	**0.002**	**0.000**	**0.002**	**0.037**	**0.000**	**0.000**	0.118	0.739	0.204	0.480	**0.004**	0.368	0.639
TAG (16:0/16:0/18:2)	13398.9	11263.1	10366.7	14724.0	11924.6	10288.6	**0.015**	**0.001**	0.271	**0.002**	**0.000**	**0.053**	0.110	0.413	0.922	0.376	**0.000**	0.578	0.468
TAG (18:1/18:1/18:2)	9623.9	12345.7	11163.4	10944.7	12604.2	10626.6	**0.002**	**0.048**	0.121	**0.035**	0.667	**0.014**	0.086	0.726	0.470	0.240	**0.002**	0.728	0.221
TAG (14:0/16:1/18:2)	6775.3	5311.4	3436.1	6529.6	5261.3	2980.5	**0.007**	**0.000**	**0.001**	**0.017**	**0.000**	**0.000**	0.617	0.919	0.358	0.905	**0.004**	0.755	0.840
TAG (16:0/17:0/18:2)	5442.5	5089.9	4173.8	5100.6	4721.1	3599.0	0.371	**0.004**	**0.028**	0.336	**0.001**	**0.009**	0.385	0.350	0.152	0.887	**0.014**	0.397	0.896
TAG (14:0/18:2/18:2)	3818.1	3698.6	2462.8	4406.0	3970.8	2412.8	0.753	**0.002**	**0.004**	0.259	**0.000**	**0.001**	0.133	0.475	0.895	0.452	**0.004**	0.603	0.496
TAG (18:0/18:2/18:2)	3200.1	3447.6	2852.9	3867.4	3695.9	2963.4	0.436	0.279	0.072	0.588	**0.009**	**0.030**	**0.046**	0.435	0.726	0.343	**0.036**	0.351	0.436
TAG (18:2/18:2/18:2)	2685.4	2824.7	2579.1	3539.7	3361.6	2768.8	0.610	0.697	0.373	0.516	**0.010**	**0.041**	**0.005**	0.061	0.489	0.283	0.057	0.254	0.243
TAG (14:1/18:0/18:2)	929.6	1090.4	856.6	861.9	986.8	776.6	**0.052**	0.358	**0.007**	0.124	0.285	**0.014**	0.393	0.197	0.315	0.733	**0.030**	0.337	0.946
TAG (O-52:2)	155.4	171.4	169.4	154.5	169.3	151.8	0.067	0.104	0.813	0.090	0.740	**0.047**	0.921	0.799	**0.046**	0.605	0.246	0.427	0.298
DAG (18:1/20:4)	15512.0	15995.3	15132.0	16118.5	17670.0	14669.2	0.696	0.759	0.488	0.219	0.250	**0.024**	0.625	0.186	0.708	0.757	0.346	0.744	0.478
DAG (16:0/20:4)	3321.7	3444.5	3183.9	3582.9	3831.4	3099.7	0.658	0.620	0.352	0.375	0.094	**0.015**	0.351	0.173	0.761	0.978	0.372	0.761	0.465
DAG (18:0/20:4)	1680.2	1615.2	1408.2	1847.4	1947.9	1459.0	0.664	0.081	0.176	0.503	**0.016**	**0.004**	0.270	**0.036**	0.734	0.678	**0.044**	0.407	0.413
DAG (18:2/20:4)	1398.4	1493.4	1438.3	1617.9	1759.3	1395.8	0.474	0.762	0.676	0.290	0.104	**0.012**	0.108	0.055	0.747	0.740	0.388	0.551	0.221
DAG (14:0/20:4)	586.4	537.2	380.4	589.9	606.1	390.2	0.313	**0.000**	**0.004**	0.737	**0.001**	**0.000**	0.941	0.163	0.839	0.408	**0.046**	0.712	0.570
CE (18:2)	1169479.0	1089680.2	1111072.8	1148383.5	1073622.4	1141353.0	**0.009**	**0.046**	0.442	**0.013**	0.799	**0.023**	0.448	0.562	0.280	0.557	0.114	0.938	0.359
oxCE (18:2) [+O]	11684.9	10910.6	10973.9	12648.5	12028.1	11513.7	**0.018**	**0.028**	0.834	**0.052**	**0.001**	0.102	**0.005**	**0.002**	0.087	0.972	**0.010**	0.530	0.385
Desmosterol(18:2)	5851.4	5811.4	6115.9	6074.7	5689.3	6478.9	0.864	0.265	0.202	0.111	0.096	**0.003**	0.344	0.602	0.132	0.391	0.266	0.722	0.330
oxCE (18:2) [+2O]	1817.0	1885.9	1742.3	1911.9	1794.9	1802.4	0.272	0.235	**0.030**	0.070	0.088	0.903	0.136	0.152	0.336	0.127	0.356	0.797	0.099
OH-Cholesterol (18:2) (c)	776.9	710.0	717.9	777.5	756.6	747.0	0.081	0.120	0.831	0.570	0.410	0.794	0.987	0.214	0.431	0.485	0.426	0.764	0.668
OH-Cholesterol (18:2) (b)	571.8	455.5	438.4	572.9	402.6	461.4	0.216	**0.046**	0.399	0.110	0.139	0.896	0.900	0.604	0.645	0.918	0.556	0.914	0.780
OH-Cholesterol (18:2) (d)	320.8	317.7	336.2	317.9	321.9	321.3	0.814	0.253	0.172	0.758	0.795	0.961	0.822	0.749	0.266	0.929	0.723	0.728	0.585
OH-Cholesterol (18:2) (a)	98.0	88.6	90.7	104.4	100.3	104.8	0.426	0.540	0.852	0.728	0.968	0.699	0.588	0.324	0.239	0.731	0.445	0.105	0.891
OH-Cholesterol (18:2) (e)	78.4	60.3	64.0	65.9	62.7	65.1	**0.012**	**0.038**	0.577	0.626	0.903	0.715	0.069	0.713	0.861	0.116	0.326	0.663	0.222

Supplementary Table 5.6 (B) Postprandial changes in a total of 32/101 plasma LA (18:2n-6) sphingolipid and fatty acylcarnitine molecular species over the 5-hour period after krill oil or fish oil supplementation

LA (18:2) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-	Inter-
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5		Time	ment	action
SM (d18:2/16:0)	14121.8	15006.5	15511.8	15399.1	15284.7	14656.7	0.075	**0.008**	0.295	0.810	0.130	0.197	**0.014**	0.560	0.085	0.081	0.480	0.568	**0.017**
SM (d18:2/22:0)	12755.0	13463.0	13807.0	13535.3	13696.3	13397.1	0.092	**0.017**	0.399	0.691	0.733	0.462	0.066	0.565	0.317	0.231	0.158	0.597	0.135
SM (d18:2/24:0)	10794.9	11262.1	11601.6	11371.8	11260.7	11266.9	0.157	**0.020**	0.298	0.730	0.744	0.985	0.085	0.997	0.304	0.170	0.314	0.817	0.149
SM (d18:2/18:0)	8915.1	9474.2	9947.8	9652.7	9381.0	9110.2	0.144	**0.011**	0.212	0.468	0.156	0.469	0.059	0.802	**0.035**	0.100	0.559	0.917	**0.024**
SM (d18:2/23:0)	5140.4	5377.4	5527.5	5267.4	5337.9	5153.6	0.126	**0.017**	0.323	0.639	0.451	0.228	0.401	0.792	**0.021**	0.188	0.438	0.584	0.076
SM (d18:2/20:0)	4873.6	5019.1	5259.7	5159.4	5099.6	4985.1	0.425	**0.044**	0.193	0.741	0.341	0.528	0.126	0.657	0.140	0.238	0.762	0.822	0.107
Cer (18:2/24:0)	1183.5	1198.1	1354.7	1209.2	1203.8	1236.9	0.812	**0.003**	**0.005**	0.884	0.770	0.662	0.573	0.856	**0.021**	0.417	**0.042**	0.708	0.083
SM (d18:2/14:0)	991.9	1020.4	1045.6	1053.6	1022.6	994.1	0.375	0.103	0.429	0.334	0.073	0.374	0.064	0.944	0.116	0.130	0.994	0.947	0.061
SM (d18:2/18:1)	692.2	717.8	748.7	767.7	745.7	728.9	0.316	**0.035**	0.227	0.384	0.134	0.507	**0.007**	0.274	0.434	0.112	0.876	0.621	**0.045**
Hex1Cer (d18:2/24:0)	644.6	669.9	679.0	643.3	672.3	637.0	0.436	0.294	0.778	0.374	0.846	0.282	0.967	0.941	0.203	0.868	0.577	0.556	0.560
Sph (18:2)	492.8	556.3	540.8	510.7	500.4	531.2	**0.000**	**0.003**	0.262	0.069	0.450	**0.015**	**0.033**	**0.001**	0.704	**0.003**	0.523	0.520	**0.001**
Hex2Cer (d18:2/16:0)	474.6	482.7	499.3	491.7	478.6	472.6	0.696	0.239	0.422	0.528	0.359	0.770	0.411	0.845	0.204	0.411	0.961	0.830	0.334
Cer (d18:2/24:1)	435.8	453.5	496.3	451.6	435.9	474.9	0.276	**0.001**	**0.014**	0.330	0.158	**0.024**	0.326	0.279	0.191	0.168	**0.038**	0.815	0.211
SM (d18:2/17:0)	404.2	430.0	446.6	456.4	445.2	438.6	0.061	**0.002**	0.114	0.200	0.114	0.743	**0.002**	0.743	0.114	**0.029**	0.461	0.653	**0.005**
Cer (d18:2/22:0)	391.1	400.4	445.6	407.2	397.2	410.2	0.636	**0.011**	**0.031**	0.611	0.875	0.507	0.416	0.869	0.083	0.322	**0.051**	0.760	0.192
Cer (d18:2/23:0)	354.3	357.0	409.6	357.0	343.4	375.7	0.842	**0.001**	**0.001**	0.329	0.182	**0.028**	0.844	0.328	**0.022**	0.305	**0.008**	0.367	0.188
SM (37:2)	351.2	368.5	377.0	433.1	410.6	410.0	0.154	**0.040**	0.479	0.070	0.064	0.962	**0.000**	**0.002**	**0.011**	**0.036**	0.925	0.066	**0.019**
Hex2Cer (d18:2/24:0)	351.0	362.0	375.3	397.9	386.8	383.8	0.558	0.204	0.480	0.555	0.455	0.872	**0.021**	0.196	0.652	0.400	0.887	**0.035**	0.357
GM3 (d18:2/24:1)	339.8	358.9	380.8	408.1	361.8	353.3	0.431	0.101	0.369	0.067	**0.033**	0.725	**0.010**	0.905	0.262	**0.046**	0.564	0.425	**0.031**
Hex1Cer (d18:2/22:0)	311.6	308.4	321.9	318.8	319.4	304.5	0.828	0.479	0.358	0.969	0.330	0.311	0.619	0.453	0.239	0.854	0.973	0.982	0.335
S1P (d18:2)	272.6	276.7	263.2	286.1	267.2	281.0	0.740	0.437	0.273	0.131	0.672	0.264	0.274	0.439	0.153	0.316	0.573	0.428	0.248
SM (35:2) (b)	123.2	125.7	126.7	137.5	130.9	122.4	0.639	0.516	0.855	0.226	**0.010**	0.125	**0.014**	0.334	0.433	0.116	0.224	0.493	0.069
Hex1Cer (d18:2/18:0)	71.7	71.6	71.9	74.8	91.5	70.6	0.992	0.983	0.975	0.067	0.628	**0.025**	0.719	**0.032**	0.883	0.405	0.202	0.176	0.209
Cer (18:2/16:0)	49.6	51.6	56.7	54.8	51.2	58.1	0.317	**0.002**	**0.019**	0.089	0.104	**0.003**	**0.017**	0.842	0.481	**0.041**	**0.008**	0.304	0.154
Hex1Cer (d18:2/20:0)	44.6	52.4	55.8	47.6	47.8	55.6	0.090	**0.020**	0.454	0.977	0.085	0.090	0.497	0.298	0.966	0.363	0.131	0.842	0.471
Cer (d18:2/18:0)	22.6	20.6	23.7	22.4	20.9	22.5	0.075	0.404	**0.013**	0.248	0.560	0.091	0.482	0.984	0.340	0.769	0.122	0.736	0.785
Cer (d18:2/20:0)	21.4	21.1	23.1	21.5	22.0	21.7	0.856	0.180	0.132	0.723	0.918	0.800	0.906	0.514	0.257	0.988	0.544	0.831	0.428
Cer (d18:2/26:0)	17.4	16.9	16.6	15.8	16.3	17.7	0.603	0.432	0.786	0.582	0.058	0.162	0.114	0.575	0.260	0.277	0.624	0.704	0.160
Cer (d18:2/21:0)	10.0	10.7	10.9	7.7	8.8	9.5	0.403	0.237	0.718	0.145	**0.029**	0.410	**0.005**	**0.022**	0.058	0.521	0.111	0.088	0.724
Cer (d18:2/17:0)	5.3	5.9	5.8	6.4	5.5	6.0	0.152	0.263	0.738	**0.051**	0.301	0.321	**0.017**	0.353	0.688	0.301	0.938	0.373	0.061
Cer (d18:2/14:0)	5.0	4.6	4.7	4.2	4.1	4.0	0.299	0.377	0.871	0.766	0.627	0.850	**0.046**	0.187	0.101	0.643	0.503	0.183	0.864
Acylcarnitine (18:2)	236.7	220.2	247.0	245.2	221.2	236.7	0.111	0.314	**0.014**	**0.026**	0.402	0.133	0.403	0.921	0.313	0.509	**0.018**	0.980	0.420

Supplementary Table 5.6 (C.1) Postprandial changes in a total 41/101 plasma LA (18:2n-6) phospholipid molecular species over the 5-hour period after krill oil or fish oil supplementation

LA (18:2) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-	Inter-
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5		Time	ment	action
PC (16:0/18:2)	182938.3	189607.0	184193.6	182947.0	188398.6	177733.0	0.081	0.732	0.150	0.148	0.165	**0.008**	0.998	0.741	0.090	0.602	0.113	0.354	0.420
PC (18:0/18:2)	79480.3	82573.5	81202.3	80973.3	80448.8	78377.4	0.129	0.388	0.490	0.790	0.199	0.301	0.453	0.289	0.164	0.137	0.629	0.468	0.267
PC (18:1/18:2)	27277.4	28037.5	28603.7	28046.4	28440.2	27338.2	0.587	0.347	0.685	0.778	0.613	0.433	0.583	0.773	0.369	0.679	0.772	0.982	0.548
LPC(18:2) [sn1]	21687.3	24571.3	24600.9	21505.1	22604.0	24022.2	**0.031**	**0.029**	0.981	0.383	0.056	0.264	0.884	0.127	0.644	0.502	0.208	0.650	0.569
PC (18:2/18:2)	12209.7	12195.0	11462.6	12586.3	12175.5	11000.8	0.970	0.068	0.073	0.300	**0.001**	**0.007**	0.341	0.960	0.246	0.388	**0.014**	0.971	0.328
LPC (18:2) [sn2]	6501.2	7112.8	7248.0	6359.1	6703.5	6806.5	0.064	**0.027**	0.668	0.281	0.166	0.744	0.652	0.204	0.172	0.602	0.336	0.549	0.758
PI (36:2)	5735.0	6513.8	6995.8	6129.3	6722.8	7159.2	0.063	**0.008**	0.335	0.063	**0.006**	0.263	0.626	0.626	0.517	0.976	**0.000**	0.633	0.991
PE (18:0/18:2)	4539.4	5332.0	4246.0	4523.4	5110.9	4027.1	**0.003**	0.616	**0.001**	**0.007**	0.321	**0.001**	1.000	0.738	0.616	0.764	**0.017**	0.863	0.935
PE (P-16:0/18:2)	3636.0	3854.2	3906.1	4172.6	3954.4	3741.6	0.228	0.140	0.770	0.228	**0.024**	0.239	**0.007**	0.574	0.359	**0.021**	0.642	0.672	**0.034**
PC (P-16:0/18:2)	3615.8	3663.1	3718.9	3668.1	3560.2	3354.5	0.674	0.364	0.621	0.344	**0.011**	0.080	0.642	0.366	**0.004**	0.149	0.527	0.574	**0.048**
PE (P-18:0/18:2)	3579.4	3771.6	3737.9	3974.3	3832.0	3671.2	**0.041**	0.070	0.786	0.186	0.070	0.589	**0.004**	0.786	0.589	**0.019**	0.745	0.718	**0.024**
PC (17:0/18:2)	3457.5	3723.5	3554.4	3639.2	3774.5	3561.9	**0.009**	0.296	0.077	0.150	0.402	**0.030**	0.059	0.578	0.934	0.295	0.084	0.720	0.383
PC (16:1/18:2)	2186.0	2399.2	2389.1	2124.0	2297.2	2154.2	0.170	0.190	0.947	0.261	0.842	0.350	0.682	0.503	0.133	0.715	0.070	0.388	0.697
PE (P-18:1/18:2) (a)	2079.9	2248.0	2219.9	2244.3	2218.2	2105.8	0.193	0.274	0.824	0.836	0.279	0.377	0.202	0.813	0.370	0.142	0.456	0.967	0.291
PE (16:0/18:2)	2079.7	2274.7	1936.0	1962.2	2259.7	1743.4	**0.006**	0.091	**0.000**	**0.001**	**0.024**	**0.000**	0.383	0.826	0.135	0.576	**0.002**	0.797	0.458
PC(15-MHDA_18:2)	1876.8	2020.9	1966.9	1962.7	2101.4	1935.1	0.193	0.409	0.618	0.209	0.798	0.136	0.430	0.460	0.769	0.807	0.153	0.819	0.683
LPE (18:2) [sn1]	1552.5	1843.0	1747.0	1517.8	1834.7	1830.4	**0.006**	**0.053**	0.320	**0.003**	**0.004**	0.964	0.716	0.930	0.386	0.700	0.076	0.937	0.653
PC (38:2)	1398.6	1482.8	1475.8	1424.7	1459.0	1414.5	0.076	0.101	0.879	0.451	0.823	0.332	0.566	0.602	0.186	0.355	0.297	0.694	0.400
PE (18:1/18:2)	978.9	1268.5	1015.1	1069.6	1300.3	1016.1	**0.002**	0.656	**0.005**	**0.010**	0.512	**0.002**	0.271	0.695	0.989	0.519	**0.009**	0.770	0.726
PC (O-18:1/18:2)	838.3	871.2	885.1	897.3	869.2	838.7	0.185	0.066	0.568	0.255	**0.024**	0.217	**0.024**	0.936	0.068	**0.030**	0.912	0.916	**0.020**

Supplementary Table 5.6 (C.2) Postprandial changes in a total 41/101 plasma LA (18:2n-6) phospholipid molecular species over the 5-hour period after krill oil or fish oil supplementation

LA (18:2) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-	Inter-
	T = 0	T = 3	T = 5	T = 0	T = 3	T = 5	T0 : T3	T0 : T5	T3 : T5	T0 : T3	T0 : T5	T3 : T5	T = 0	T = 3	T = 5		Time	ment	action
PC (O-18:0/18:2)	688.5	692.5	695.6	700.5	701.4	675.2	0.855	0.747	0.889	0.966	0.259	0.242	0.588	0.687	0.359	0.658	0.771	0.996	0.519
PC (P-18:0/18:2)	633.3	637.7	649.4	673.7	631.2	617.6	0.619	0.369	0.682	**0.019**	**0.005**	0.506	**0.028**	0.504	0.093	**0.029**	0.443	0.994	**0.023**
LPE (18:2) [sn2]	602.7	735.0	718.5	588.3	710.8	711.9	**0.002**	**0.004**	0.648	**0.003**	**0.003**	0.977	0.688	0.505	0.854	0.922	**0.050**	0.824	0.941
PI (18:1/18:2)	448.6	482.4	510.0	518.2	514.9	526.4	0.362	0.106	0.454	0.928	0.822	0.753	0.070	0.381	0.656	0.474	**0.047**	0.564	0.576
PE (P-18:1/18:2) (b)	333.3	361.4	326.3	346.0	326.0	317.9	0.341	1.000	0.341	0.278	0.341	0.890	0.680	0.111	0.583	0.252	0.579	0.736	0.354
PE (P-20:0/18:2)	267.9	285.9	276.7	307.8	290.8	292.1	0.121	0.436	0.418	0.141	0.173	0.905	**0.002**	0.662	0.180	0.064	0.776	0.165	0.099
PE (O-16:0/18:2)	225.1	233.9	235.4	209.8	209.9	194.8	0.369	0.295	0.877	0.995	0.133	0.132	0.127	**0.022**	**0.001**	0.403	0.538	0.147	0.192
PC (P-35:2) (a)	204.7	205.1	201.8	207.7	204.6	184.5	0.957	0.658	0.620	0.650	**0.002**	**0.007**	0.663	0.943	**0.017**	0.337	0.135	0.858	0.093
PC (17:1/18:2)	194.9	212.9	219.6	202.5	207.2	194.2	0.098	**0.027**	0.517	0.650	0.426	0.219	0.470	0.591	**0.023**	0.206	0.348	0.677	0.100
PI (17:0/18:2)	176.6	198.2	196.1	196.4	189.1	191.7	**0.021**	**0.035**	0.806	0.406	0.589	0.768	**0.032**	0.303	0.616	0.056	0.221	0.803	0.061
PS (36:2)	133.5	170.0	158.5	159.8	154.3	156.3	0.231	0.407	0.700	0.853	0.907	0.946	0.383	0.600	0.942	0.377	0.718	0.889	0.596
PE (O-18:1/18:2)	91.1	93.1	95.4	85.9	84.3	81.7	0.650	0.321	0.583	0.708	0.338	0.554	0.227	**0.051**	**0.004**	0.328	0.999	0.067	0.383
PC (P-35:2) (b)	75.6	73.7	72.6	78.3	72.1	69.0	0.490	0.283	0.693	**0.032**	**0.003**	0.250	0.319	0.561	0.184	0.160	0.085	0.924	0.249
PE (17:0/18:2)	66.9	80.3	56.7	61.2	78.6	53.8	**0.016**	0.354	**0.002**	**0.009**	0.268	**0.001**	0.575	0.779	0.456	0.902	**0.004**	0.649	0.944
PG (36:2)	62.1	64.1	54.9	67.0	85.0	62.9	0.682	0.158	0.075	**0.002**	0.418	**0.000**	0.327	**0.000**	0.116	**0.043**	0.067	0.065	0.074
LPI (18:2) [sn1]	36.9	37.6	43.4	40.2	36.5	42.0	0.848	0.086	0.123	0.310	0.626	0.141	0.363	0.762	0.704	0.413	**0.037**	0.914	0.588
PE (15-MHDA_18:2)	34.1	45.3	31.7	37.4	36.4	31.9	**0.007**	0.525	**0.002**	0.791	0.147	0.229	0.375	**0.027**	0.966	0.079	0.067	0.692	0.077
PE (16:1/18:2)	27.1	29.2	24.0	27.2	25.3	22.2	0.838	0.478	0.611	0.542	0.115	0.314	0.314	0.164	0.065	0.701	0.327	0.430	0.806
LPS (18:2)	24.6	24.3	23.9	22.3	24.0	25.5	0.856	0.689	0.826	0.350	0.088	0.408	0.210	0.878	0.372	0.381	0.863	0.869	0.317
PG (34:2)	21.1	20.7	19.8	21.7	24.3	16.9	0.800	0.421	0.579	0.118	**0.007**	**0.000**	0.697	**0.034**	0.088	0.349	0.166	0.865	**0.032**
LPI (18:2) [sn2]	10.0	9.8	10.1	9.7	8.0	10.3	0.848	0.951	0.800	0.141	0.620	0.056	0.780	0.121	0.875	0.590	0.410	0.437	0.437

Values are expressed as mean of plasma LA (18:2) molecular species concentration for 10 women. Two-way analysis of variance for repeated measurements was performed to analyse supplementation effect over time, supplement and interaction (time x supplement), difference between time point within supplementation (superscript with a) and each time point between the two supplementation (superscript with b). All *p* values (≤ 0.05) were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (hours from zero to five) of plasma lipid molecular species for 10 women was calculated using the trapezoid rule and compared between krill oil and fish oil supplementation using paired *t*-test. Abbreviation: CE, cholesterol ester; Cer, ceramide; DAG, diacylglycerol; GM3, GM3 ganglioside; Hex1Cer, monohexosylceramide; Hex2Cer, dihexosylceramide; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; LPI, lyso-phosphatidylinositol; LPS, lyso-phosphatidylserine; net iAUC, net incremental area under the curve from baseline (hours from zero to five); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE,

phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; SM, sphingomyelin; Sph, Sphingosine; S1P: Sphingosine-1-Phosphate; TAG, triacylglycerol; T0, baseline; T3, 3 hours; T5, 5 hours.

Chapter 6: Comparison of a 30-day krill oil and fish oil supplementation on plasma omega-3 polyunsaturated fatty acids, triglycerides and inflammatory biomarkers in healthy women

6.1 ABSTRACT

Background: Fish oil and krill oil are two major supplemental forms of the long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) including eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). However, there has been conflicting findings over the bioavailability of LC n-3 PUFA from these two marine oils, with results from interventional and observational studies showing inconsistent outcomes, as described in C*hapter two* and detailed in section 2.4.

The literature contains a large number of references to the triglycerides (TAG) lowering effects as well as anti-inflammatory effects of LC n-3 from fish oil (as described in C*hapter two* and detailed in sections 2.3.2 and 2.3.3) in healthy and overweight/obese participants. However, little information is available on effects of krill oil.

The aim of this study was to compare the effects of krill oil versus fish oil on plasma PUFA, TAG and inflammatory biomarkers in healthy young women following a 30-day consumption of the omega-3 oils.

Methods: This was a randomised crossover study. Eleven healthy women aged 18-45 years consumed seven capsules of krill oil or five capsules of fish oil per day for 30 days each, separated by at least a 30-day washout period. These two intervention regimes gave 1,269 mg of total LC n-3 PUFA (EPA, DHA and docosapentaenoic acids (DPA, 22:5n-3)) from the krill oil and 1,441 mg from the fish oil. Fasting blood samples

were collected at days zero (baseline), 5, 10, 15 and 30. The plasma samples were analysed at all five-time points for fatty acids using gas chromatography, and at days zero, 15 and 30 for triglycerides using an auto-analyser. Additionally, plasma inflammatory cytokines, including interferon-gamma, tumor necrosis factor alpha and interleukins, were analysed using BioPlex-200 at day zero and 30.

Results: A thirty-day supplementation with krill oil and fish oil led to significant changes in plasma LC n-3 PUFA, TAG and multiple cytokines. The three main LC n-3 PUFA, including EPA, DHA and DPA, were significantly increased with both the krill oil and fish oil supplementation over the intervention period ($p < 0.001$). The increased plasma EPA with the 30-day krill oil supplementation had a significantly greater incremental area under the curve from the baseline (net iAUC $_{0\text{-}30\ d}$) than that of the fish oil supplementation ($p < 0.05$). Both the krill oil and fish oil supplementation significantly reduced plasma TAG after a period of 30 days ($p < 0.05$ and $p < 0.01$, respectively), although there was no significant difference between the two supplementation groups. Only the krill oil supplementation significantly decreased plasma interleukins (IL) including IL-1β, IL-10, IL-4 and IL-5 ($p \leq 0.05$) over the 30-day intervention. The levels of IL-10 and IL-5 at day 30 were significantly lower following the krill oil supplementation compared with the fish oil supplementation ($p \leq 0.05$).

6.2 INTRODUCTION

Lipids, as a constituent of various cell membranes, play critical and diverse biological roles in the human body, including distinctive cellular structuring, signalling molecules, and regulating hormones apart from energy storage, body insulation, and protection. Different dietary sources of the lipids have been demonstrated to investigate insights

of lipid dysregulation relevant to disease pathologies, particularly in metabolic disorders, cardiovascular events and inflammatory disorders, as well as various cancers (Chowdhury et al. 2014, Li et al. 2014, Mocellin et al. 2016). A substantial number of clinical studies have assessed the impacts of different dietary fats on human health (Linderborg et al. 2013, Meikle et al. 2015, Teng et al. 2016). Accumulated evidence have shown that low blood levels of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), particularly eicosapentaenoic acid (EPA,20:5n-3) and docosahexaenoic acid (DHA,22:6n-3), were found in individuals with type 2 diabetes, obesity, coronary heart disease (Chowdhury et al. 2014), degenerative cognitive impairment (Mocellin et al. 2016)and certain cancers (Mocellin et al. 2016). In contrast, enhanced LC n-3 PUFA derived from diets and/or supplementation have shown health benefits, including reduction in plasma triglyceride (TAG) and inflammation responses in various disease conditions, and improvement in cognitive functions related to degeneration and growth/development (Delgado-Lista et al. 2012, Leslie et al. 2015, Shearer et al. 2012, Zhang et al. 2016b). The effects of LC n-3 PUFA on various metabolic and immune responses in chronic metabolic syndrome (Leslie et al. 2015, Lopez-Huertas 2012), neurodegenerative conditions, (Dyall 2015) and related inflammation processes, have been well documented by epidemiological observations and clinical interventions (Eilat-Adar et al. 2013), as described in C*hapter two* and detailed in section 2.4. LC n-3 PUFA, particularly EPA and DHA, are found in cells in different lipid fractions (cholesterol ester, TAG, and phospholipids) where they are involved in lipid storage, lipid transport, and cell membrane function. Both of EPA and DHA, as precursors of potent lipid mediators, play a critical role in regulating lipid metabolism and in anti-inflammatory pathways (Calder 2015, Swanson et al. 2012). DHA has been recognised as a crucial fatty acid for human neurological development

and visual outcomes (Campoy et al. 2012). Clinical and experimental studies with EPA and/or DHA over the last decades have shown that they mediate significant reductions in the plasma TAG levels (Leslie et al. 2015), and can modulate inflammatory responses (Calder 2015, Haghiac et al. 2015, Li et al. 2014). These LC n-3 PUFA are mainly obtained from dietary sources due to an extremely inefficient conversion rate from alpha-linolenic acid, (ALA, 18:3n-3) to LC n-3 PUFA in humans. Marine dietary sources such as oily fish (mackerel, tuna, salmon) and krill are the major resources for these important EPA and DHA (Tou et al. 2007, U.S. Department of Agriculture 2014). It has been well reported that the consumption of seafood or supplementation with fish oil or algae oil can increase the level of EPA and/or DHA in the plasma and erythrocytes (Kagan et al. 2013, Maki et al. 2009, Purcell et al. 2014, Schuchardt et al. 2011b). Various national and international health authorisations have recommend LC n-3 PUFA with a combination of EPA and DHA for general population ranging from 250 mg to 2 g per day for general population (Kris-Etherton et al. 2009), while a recommended dose of LC n-3 PUFA is 1 g per day for individuals with elevated TAG level by the national heart foundation Australia (2009).

Krill oil extracted from crustaceans (*Euphausia superba*), a shrimp-like marine zooplankton living in the Antarctic, has been recognised as an important source of LC n-3 PUFA in the last decade (Tou et al. 2007). Krill oil is a rich source of LC n-3 PUFA, found in phospholipids (mainly phosphatidylcholine in both the sn-1 and sn-2 positions), TAG and in free fatty acids (Winther et al. 2011). The presence of LC n-3 PUFA in krill oil, mainly in the phospholipid form (predominantly phosphatidylcholine) is distinctive from fish oil where the LC n-3 PUFA are commonly found in the TAG form (Tou et al. 2007, Winther et al. 2011). Some human and animal studies have shown that krill oil containing LC n-3 PUFA found in phospholipids leads to a more efficient

incorporation of LC n-3 PUFA into the plasma and erythrocytes than that of fish oil (LC n-3 PUFA in TAG), as described in *Chapter two* and detailed in sections 2.4.

Investigations have been carried out to compare the bioavailability of LC n-3 PUFA from krill oil and fish oil in human and animal models over the last decades. Data from human clinical trials have shown that plasma EPA and DHA increased significantly following longer-term (ranging from four to seven weeks) consumption of supplementation with krill oil and fish oil, although there were no significant differences in the levels of these two fatty acids in the plasma or red blood cells between the two omega-3 oil supplements (Maki et al. 2009, Ulven et al. 2011, Yurko-Mauro et al. 2015). In a 4-week double-blind crossover randomised krill oil study by Ramprasath et al (2013), the plasma EPA in the krill oil supplementation increased significantly more than that in the fish oil supplementation. The increased levels of plasma EPA and DHA were also found in animal models after four to six weeks administration of krill oil and fish oil (Batetta et al. 2009, Ierna et al. 2010, Vigerust et al. 2013).

Three of the clinical studies, that showed a significant elevation of LC n-3 PUFA after krill oil supplementation, reported no significant changes in plasma TAG over the intervention periods , in which the daily dose of EPA and DHA ranged from 0.3 g to 0.9 g (Maki et al. 2009, Ramprasath et al. 2013, Ulven et al. 2011). However, Vigerust et al (2013) and Batetta et al (2009) suggested that krill oil seems to have a greater effect on lipid regulation in animal models compared with fish oil. They reported that krill oil significantly reduced the levels of TAG in the plasma (Vigerust et al. 2013), liver and heart (Batetta et al. 2009) compared with control, whereas fish oil significantly decreased only the level of TAG in liver (Batetta et al. 2009).

Two clinical studies have investigated the effect of krill oil supplementation on a commonly used plasma inflammatory biomarker, high-sensitivity C-reactive protein (hs-CRP) in individuals with and without dyslipidemia or obesity (Maki et al. 2009, Ulven et al. 2011). Both studies reported no difference in hs-CRP between krill oil, fish oil and control groups, although a significant increase in the plasma EPA and DHA was observed in krill oil and fish oil supplementation. These clinical findings were in line with animal studies revealing non-significant differences between the three groups (Batetta et al. 2009, Ierna et al. 2010, Vigerust et al. 2013). Interestingly, two of these animal studies also reported that the level of liver IL-17 and TNF-α in transgenic animal models (Vigerust et al. 2013), and serum IL-α and IL-13 in arthritic animal models (Ierna et al. 2010) were significantly elevated in the fish oil supplementation compared with control.

To date, there have been only a few human intervention studies comparing the effect of krill oil and fish oil on hypertriglyceridemia, inflammation, and bioavailability of plasma LC n-3 PUFA, and these studies (as reported above) have not shown consistent results. A systematic review of postprandial and long-term human studies with krill oil compared with fish oil strongly suggested standardised methodological approaches, such as providing equal amounts of LC n-3 PUFA and targeting the same blood compartments (Ghasemifard et al. 2014).

Many studies which have compared krill oil with fish oil have either included only male participants or both mixed gender in the same study and typically with a wide age range (Kohler et al. 2015, Ulven et al. 2011). A crossover randomised study design could help to eliminate possible genetic variances, particularly with only female participants. To the best of our knowledge, this is the first long-term study on krill oil

supplementation in young women. This study investigates the effect of a 30-day krill oil supplementation in comparison with fish oil on the plasma fatty acid composition and TAG as well as plasma inflammatory markers. It was hypothesized that krill oil (providing 1,1269 mg of total LC n-3 PUFA/day) would be more efficacious for incorporation of LC n-3 PUFA into the plasma, and for reducing plasma TAG inflammatory biomarkers when compared with fish oil (providing 1,441 mg of total LC n-3 PUFA/day).

6.3 MATERIAL AND METHODS

6.3.1 Study design

This is a randomised crossover study with krill oil and fish oil supplementation for 30 days. The two supplementation were separated by a minimum of 30-day washout period (Figure 6.1). During the study period, all participants were instructed to maintain their habitual diet and requested not to consume fish, seafood or omega-3 fortified foods more than once a week. For interventions, participants consumed daily 7 g of krill oil containing 1,269 mg of LC n-3 PUFA (EPA + DHA + DPA) or 5 g of fish oil containing 1,441 mg of LC n-3 PUFA for 30 days each. Participants were required to attend the clinic five times for data collection, at days 0 (baseline), 5, 10, 15 and 30 for each supplementation for blood samples collection. Prior to each clinic visit, participants were required to consume one of the most common low-fat dishes in their diet, to avoid drinking alcohol and strenuous physical activities, and to fast approximately 10 hours overnight. On each study day, standardised procedures were performed where participants arrived at the clinic between 7 am and 9 am, and a fasting blood sample (10 mL) was collected via a venepuncture by a qualified

practitioner. Following blood sample collection, the participants completed a 24-hour dietary recall and an electronic PUFA Food Frequency Questionnaire (FFQ) (Sullivan et al. 2008, Sullivan et al. 2006).

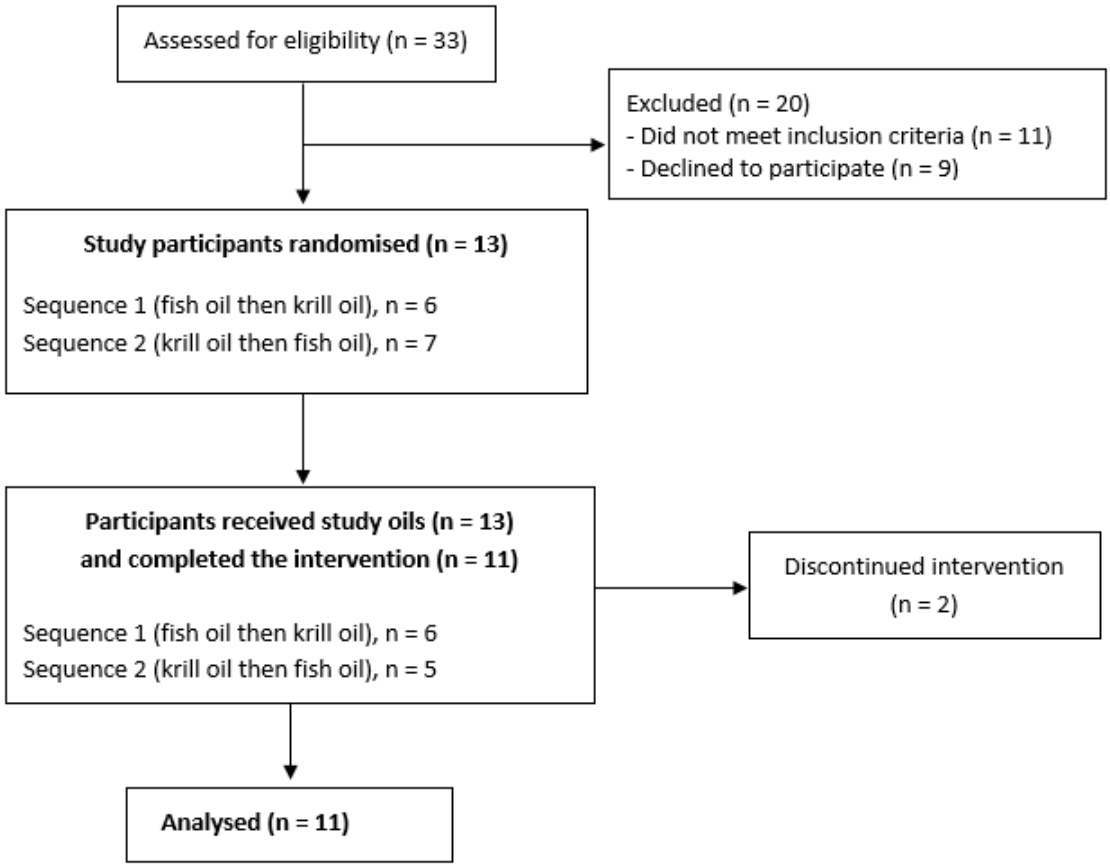


Figure 6.1 The 30-day crossover study flow diagram

n = the number of participants.

6.3.2 Study participants

A total of 11 healthy women aged between 18 and 50 years with BMI 20 - 35 (kg/m^2), who had not experienced menopause, were recruited through emails to all Victoria University staff and students; flyer advertisements via the Victoria University Nutritional Therapy Teaching Clinic, community centres, and local medical practices.

Participants were screened for their suitability for the 30-day study as they completed a medical questionnaire, anthropometric measurements, and the electronic PUFA FFQ prior to enrolling into the study. Participants were excluded if their daily LC n-3 PUFA was more than 500 mg based on results of the electronic PUFA FFQ (Sullivan et al. 2008, Sullivan et al. 2006, Swierk et al. 2011). Participants were also excluded if they were cigarette smokers; pregnant or lactating; or had heart, liver, kidney or inflammatory bowel disease, diabetes; or medications interfering with lipid metabolism or lowering blood lipids; allergy to fish or seafood; or intake of oily fish more than twice a week or supplements including omega-3 fatty acids in the past four weeks prior to the study.

6.3.3 Study supplements

The study supplements including krill oil (Swisse Wellness Pty Ltd., *Euphausia Superba* oil, Victoria, Australia) and fish oil (Swisse Wellness Pty Ltd., Natural fish oil, Victoria, Australia) were purchased from a local pharmacy. The fatty acid profiles of these oils were analysed prior to the commencement of intervention. The participants consumed study supplements, 7 x 1 g krill oil capsules or 5 x 1 g fish oil capsules, which equated to total EPA, DPA and DHA intakes of 1,269 mg for the krill oil and 1,441 mg for fish oil, as shown in Table 6.1. The single capsule fill weight was 1.054 g for krill oil and 1.063 g for fish oil, which was used to calculate the EPA, DPA and DHA contents.

Table 6.1 The study oils used in the 30-day intervention

(mg)	Krill oil		Fish oil	
	per capsule	**per day (7 capsules)**	per capsule	**per day (5 capsules)**
EPA (20:5 n-3)	108.4 (3.4)	**759**	157.3 (6.4)	**786**
DHA (22:6 n-3)	59.5 (2.1)	**417**	94.6 (6.7)	**473**
DPA (22:5 n-3)	13.4 (2.5)	**94**	36.3 (12.2)	**182**
Total LC n-3 PUFA	181.3 (2.7)	**1270**	288.2 (8.4)	**1441**

Values are expressed as mean (standard error mean) of five randomly chosen oil capsules mixed together and analysed for six times using gas chromatography. The total LC n-3 PUFA represents EPA, DHA and DPA. Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; mg, milligram.

6.3.4 Human ethics for the study

Ethics approval was obtained from the Victoria University Human Research Ethics Committee (HRE15-031). All experimental procedures were performed in accordance with the Declaration of Helsinki of the World Medical Association. Written informed consent was obtained from all participants prior to the study. This trial was registered with the Australian New Zealand Clinical Trial Registry (ACTRN 12615000472572).

6.3.5 Dietary assessment

Dietary fat intake was assessed at baseline, days 5, 10, 15 and 30, particularly for LC n-3 PUFA, using an electronic PUFA FFQ (PUFA Questionnaire, The University of Wollongong 2015) which contains data from NUTTAB 2010. A 24-hour dietary recall was also used to monitor individuals' food intake, particularly for the day before each study visit day. Data were analysed using *FoodWorks* version 8 (Xyris software, QLD, Australia) with NUTTAB 2010 and AUSNUT 2013 based on Australian Food Composition Database. The electronic PUFA FFQ provides automatic calculations of

estimated daily PUFA intake based on individuals' average food intake for the previous three months. The PUFA FFQ consists of 38 questions; frequency of consumption of common sources of omega-3 fatty acids such as meats, fresh fish and seafood, and vegetables as well as desserts as described in *Chapter three* and detailed in sections 3.3.2.4 (Sullivan et al. 2008, Sullivan et al. 2006, Swierk et al. 2011).

6.3.5 Plasma total fatty acid analysis

Modified transesterification method of Lepage and Roy (1986) was used in this study. Briefly, 200 µL of plasma samples or 25 µL of oil samples with an internal standard, tricosanoic acid, C23:0 (Nu-Chek Prep, Inc., Elysian, MN) in methanol: toluene (4:1 v/v) was reacted with 200 µL of acetyl chloride for 1 hr at 100 °C to form fatty acid methyl esters. Then 5 mL of 6% potassium carbonate (K_2CO_3) in distilled water was added and the blend was thoroughly vortexed prior to centrifugation at 3000 x *g* for 10 min to separate the layers, and the top toluene-rich layer was removed and evaporated to dryness under nitrogen.

Fatty acid methyl esters were isolated and identified using an Agilent Technologies 7890A gas chromatography (GC) System (Agilent Technologies; Santa Clara, CA, USA) equipped with a BPX70 capillary column (120 m × 0.25 mm internal diameter, 0.25 µm film thickness, SGE Analytical Science, Ringwood, VIC, Australia), a flame ionization detector (FID), an Agilent Technologies 7693 auto sampler, and a splitless injection system. The injection volume was 1 µL and the injector and detector temperatures were 300°C and 270°C, respectively. The temperature program was 60°C held for 2 min, then from 60°C to 150°C at 20°C min^{-1}, and held at 150°C for 2 min, then from 150°C to 205°C at 1.5°C min^{-1}, then from 205°C to 240°C at 5°C min^{-}

^{1}, and held at 240°C for 24 min. The carrier gas was helium at 1.5 mL min^{-1}, at a constant flow. Each of the fatty acids was identified relative to known external standards (a series of mixed and individual standards from Sigma-Aldrich, Inc., St. Louis, MO, USA and from Nu- Chek Prep Inc., Elysian, MN, USA), using the software GC ChemStation (Rev B.04.03; Agilent Technologies; Santa Clara, CA, USA). The resulting peaks were then corrected by the theoretical relative FID response factors (Ackman 2002) and quantified relative to the internal standard.

For purpose of the quality control, pooled plasma samples from five randomly chosen plasma samples obtained from a previous human study were applied every ten plasma samples, as well as daily calibration and control.

6.3.6 Plasma triglyceride

The concentration of TAG was measured on an auto-analyser (cobasIntegra® 400 Plus analyser, Roche Instrument Center, Rotkreuz, Switzerland) by enzymatic colorimetric methods using commercially available kits (TGIGL) as per the manufacturer's instructions (Thermo Fisher Scientific Inc., Victoria, Australia). Quality controls and calibration with reagents for TAG were undertaken prior to running plasma samples.

6.3.7 Plasma cytokines

The plasma inflammatory cytokines, including interferon-gamma (INF-γ), tumour necrosis factor alpha (TNF-α) and eight interleukins (IL), were analysed using the BioPlex-200 (Bio-Plex Precision Pro human cytokine assay, Bio-Rad Laboratories,

Munich, Germany) as per the manufacturer's instructions. In the Bio-Plex Precision Pro human cytokine assay (171A1001P, Bio-Rad Laboratories, Munich, Germany) microspheres (beads) were fluorescently dyed with a distinct colour for each cytokine. The antibody-conjugated beads were used to simultaneously detect different molecules for each cytokine in human plasma samples in a capture sandwich immunoassay. Finally, the assay solution was read by the Bio-Plex array reader and determined with the Bio-Plex Manager Software 4.1. For purpose of quality control for each assay, nine standards in different concentrations were used to produce a standard curve.

6.4 STATISTICAL ANALYSIS

The sample size was determined by statistical power analyses (two-tailed *t*-test at the 5% significance level for the power of 90%) to detect a difference in plasma total n-3 PUFA composition based on a previous 4-week study by Ramprasath et al (2013); the minimum required number of participants was eight in order to obtain a significant difference in outcome measures such as plasma EPA and total LC n-3 PUFA. Data, including plasma cytokines, TAG and fatty acids, were expressed as means ± standard error mean (SEM) and they were analysed using GraphPad Prism version 7.01. The normality of data distribution was checked using D'Agostino & Pearson normality test. Two-way analysis of variance (ANOVA) for repeated measurements was performed to assess supplementation effect over time (interaction time x supplementation), the difference between time point within supplementation, and the same time point between the two omega-3 oil supplementation groups. All *p* values were corrected for multiple comparisons using the Holm-Sidak method. The area under the curve from baseline to 30 days (net $iAUC_{0\text{-}30\ d}$) for plasma TAG and plasma

fatty acids was calculated using the trapezoid rule. The values of net iAUC $_{0\text{-}30\ d}$ were compared between the two omega-3 supplementation groups using a paired *t*-test. $P < 0.05$ was considered significant.

6.5 RESULTS

6.5.1 Participant's baseline characteristics and daily intake of LC n-3 PUFA

A total of eleven healthy women completed the 30-day crossover dietary intervention. The general baseline characteristics of study participants including anthropometric and blood pressure measurements, as well as the daily intake of total LC PUFA, are shown in Table 6.2. The daily intake of PUFA including LC n-3 PUFA based on food intake was estimated using PUFA FFQ over the 30-day intervention in both the krill oil and fish oil supplementation as shown in Table 6.3. The dietary intake of PUFA remained over the 30-day intervention when all participants were instructed to keep their own habitual diet. There were no significant changes from baseline to all time points within supplementation, as well as no significant difference at each time point between the two omega-3 supplementation groups.

Table 6.2 Baseline characteristics of study participants

Characteristics	Values
Age (year)	30.7 (7.1)
Height (m)	1.6 (0.06)
Weight (kg)	66.1 (14.9)
BMI (kg/m^2)	25.0 (5.2)
Waist circumference (cm)	82.6 (11.7)
Hip circumference (cm)	100.6 (10.2)
Sytolic blood pressure (mm Hg)	114.2 (7.2)
Diastolic blood pressure (mm Hg)	73.9 (5.9)
Total LC n-3 PUFA (mg/day)	239.4 (150.4)

Values are expressed as mean (standard deviation) for 11 healthy women. Abbreviations: cm, centimetres; BMI, body mass index (kg/m^2); kg, kilograms; kg/m^2, kilograms per meters squared; mg. milligrams; mm HG, millimetres of mercury. There were no significant differences between any of these parameters between the two baseline measurements.

6.5.2 Responses of plasma long-chain n-3 polyunsaturated fatty acids

Plasma PUFA were analysed at day zero (baseline), 5, 10, 15 and 30 over the intervention period, and the changes in plasma PUFA are shown in Table 6.4 and Figure 6.2. There were no significant differences in plasma PUFA between the two baseline measurements. Both the krill oil and fish oil supplementation groups significantly increased the level of plasma EPA, DHA and DPA over the 30-day intervention period, whereas there were no significant changes in arachidonic acid (AA, 20:4n-6), linoleic acid (LA, 18:2n-6) and ALA. The increased plasma EPA following the krill oil supplementation was significantly greater at 5, 10 and 30 days than that from the fish oil supplementation. These results are likely to have contributed to the significant increase in the total LC n-3 PUFA (including EPA, DHA and DPA) at 5, 10 and 30 days.

After the 30-day dietary intervention, there was a significant difference in plasma EPA concentration (net iAUC $_{0\text{-}30\ d}$) between the two supplementation groups in which the net iAUC $_{0\text{-}30\ d}$ in the krill oil supplementation was greater than that in the fish oil supplementation (p = 0.045), as shown in Figure 6.3. There were no significant differences in net iAUC $_{0\text{-}30\ d}$ between the two supplementation groups for DHA, DPA, total LC n-3 PUFA, AA, LA, or ALA.

6.5.3 Changes in TAG over the 30-day intervention period

Plasma TAG was analysed at day zero, 15 and 30 between the krill oil and fish oil supplementation groups over the 30-day intervention period, as shown in Table 6.5. There were no significant differences in plasma TAG concentration between the two baseline measurements. For both krill oil and fish oil, the level of plasma TAG decreased significantly over the intervention, with 0.3 mmol/L reduction by krill oil and 0.4 mmol/L reduction by fish oil. Neither the level of TAG at 30 days nor TAG net iAUC $_{0\text{-}30\ d}$, were significantly different between the krill oil and fish oil supplementation groups, as shown in Figure 6.4.

6.5.4 Different inflammatory responses in human plasma between krill oil and fish oil supplementation

Plasma cytokines were analysed at day zero (baseline) and day 30 only. The changes in the ten plasma inflammatory cytokines, including INF-γ, TNF-α and interleukins are shown in Table 6.6. There were no significant differences in plasma cytokines between the two baseline measurements. All ten plasma cytokines decreased following the krill oil and fish oil supplementation over the 30-day intervention period, except the

increase in the level of IL-6 in the fish oil supplementation although not all of these changes reached significant level. The krill oil supplementation significantly decreased the concentration of IL-1β, IL-4, IL-5 and IL-10 ($p \leq 0.05$) from baseline, as shown in Figure 6.5. However, there were no significant changes in inflammatory cytokines with the fish oil supplementation. The levels of IL-10 and IL-5 at day 30 were significantly lower following the krill oil supplementation compared with the fish oil supplementation ($p \leq 0.05$).

Table 6.3 Daily intake of PUFA assessed using PUFA questionnaire over a 30-day supplementation of krill oil and fish oil

(mg/day)	Estimated polyunsaturated fatty acids based on food intake									
	Krill oil					Fish oil				
	T = 0	T = 5	T = 10	T = 15	T = 30	T = 0	T = 5	T = 10	T = 15	T = 30
EPA (20:5)	89 (32)	69 (14)	65 (12)	63 (13)	71 (15)	62 (12)	57 (12)	56 (11)	60 (12)	62 (12)
DHA (22:6)	131 (46)	105 (20)	105 (19)	98 (19)	102 (21)	97 (19)	91 (21)	87 (20)	84 (15)	87 (17)
DPA (22:5)	71 (16)	64 (15)	58 (13)	55 (14)	58 (13)	67 (16)	54 (14)	54 (13)	57 (14)	55 (12)
AA (20:4)	150 (35)	146 (37)	139 (29)	125 (30)	130 (29)	162 (39)	127 (32)	152 (37)	132 (31)	124 (23)
LA (18:2)	14,747 (2,717)	12,929 (2,197)	12,344 (2,253)	12,945 (2,070)	11,700 (2,268)	18,464 (4,813)	13,904 (4,167)	16,240 (5,498)	15,911 (4,649)	14,544 (4,581)
ALA (18:3)	2,134 (795)	2,038 (812)	2,030 (738)	2,135 (758)	1,840 (757)	1,748 (385)	1,892 (769)	2,240 (976)	1,989 (737)	1,670 (760)
Total n-3 PUFA	291 (90)	239 (47)	228 (41)	215 (44)	232 (46)	226 (45)	202 (45)	196 (42)	201 (39)	205 (39)

Values are expressed as mean (standard error mean, SEM) of plasma fatty acids concentration (n = 11). Two-way ANOVA for repeated measurements was performed to assess supplementation effect over time (interaction time x supplementation), the difference between time point within supplementation and each time point between the two omega-3 supplementation groups (data not shown due to no significant changes over the intervention period). Abbreviation: T0, baseline; T15, 15 days; T30, 30 days; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; LA, linoleic acid; mg/day milligram per day. There were no significant differences between any of fatty acids between the two baseline measurements.

Table 6.4 A summary of plasma PUFA over the 30-day supplementation of krill oil and fish oil

Plasma fatty acids	Concentration (µg/mL)									
	Krill oil					Fish oil				
	T = 0	T = 5	T = 10	T = 15	T = 30	T = 0	T = 5	T = 10	T = 15	T = 30
EPA (20:5)	19.6 (3.2)	82.0 (9.8)	87.6 (8.1)	88.3 (10.3)	110.4 (13.4)	16.7 (2.5)	61.0 (6.5)	70.7 (6.5)	79.4 (9.3)	81.4 (8.2)
DHA (22:6)	42.7 (4.1)	60.6 (6.8)	66.0 (5.5)	64.9 (4.0)	74.2 (6.7)	38.6 (3.8)	57.6 (6.0)	59.9 (3.9)	67.6 (5.5)	71.6 (5.6)
DPA (22:5)	11.4 (1.0)	15.3 (1.6)	15.6 (1.5)	15.1 (1.4)	17.5 (1.9)	10.5 (1.2)	13.8 (1.2)	14.5 (1.1)	16.2 (1.5)	16.6 (1.2)
AA (20:4)	158.0 (12.6)	155.6 (12.9)	151.0 (10.2)	146.0 (9.0)	150.0 (9.3)	153.1 (14.0)	163.5 (13.8)	158.1 (10.3)	160.9 (11.0)	149.9 (10.6)
LA (18:2)	805.6 (42.7)	764.3 (29.2)	753.2 (44.0)	765.2 (47.3)	789.7 (51.7)	832.2 (64.7)	722.6 (55.1)	724.1 (49.5)	772.2 (57.9)	734.3 (37.2)
ALA (18:3)	19.6 (3.2)	29.5 (4.2)	34.4 (5.0)	29.9 (3.2)	25.7 (3.0)	27.9 (4.0)	26.2 (4.2)	26.9 (3.8)	22.5 (1.9)	24.2 (4.1)
Total LC n-3 PUFA	73.7 (7.9)	157.8 (17.3)	169.3 (12.8)	168.3 (14.5)	202.1 (21.0)	65.9 (7.0)	132.4 (13.0)	145.0 (10.9)	163.2 (15.5)	169.7 (14.7)

Plasma fatty acids	*P* value																
	Krill oil[a]				Fish oil[a]				Krill oil vs Fish oil[b]					Net iAUC	Time	Supple-ment	Inter-action
	T0 :T5	T0 : T10	T0 : T15	T0 : T30	T0 : T5	T0 : T10	T0 : T15	T0 : T30	T = 0	T = 5	T = 10	T = 15	T = 30				
EPA (20:5)	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.604	**0.002**	**0.012**	0.217	**0.000**	**0.045**	**0.000**	**0.000**	**0.018**
DHA (22:6)	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.739	0.818	0.440	0.818	0.818	0.589	**0.000**	0.224	0.559
DPA (22:5)	**0.001**	**0.000**	**0.002**	**0.000**	**0.007**	**0.001**	**0.000**	**0.000**	0.606	0.429	0.606	0.606	0.606	0.828	**0.000**	0.342	0.285
AA (20:4)	0.961	0.925	0.590	0.896	0.694	0.944	0.850	0.954	0.726	0.693	0.693	0.160	0.992	0.172	0.445	0.393	0.300
LA (18:2)	0.953	0.862	0.953	0.990	0.065	0.065	0.608	0.111	0.835	0.737	0.835	0.857	0.573	0.318	**0.006**	0.524	0.533
ALA (18:3)	0.987	0.739	0.987	0.872	0.990	0.990	0.809	0.957	0.907	0.748	0.217	0.217	0.907	0.295	0.198	0.142	0.569
Total LC n-3 PUFA	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.623	**0.027**	**0.028**	0.623	**0.004**	0.226	**0.000**	**0.000**	0.149

Values are expressed as mean (SEM) of plasma fatty acids concentration (n = 11). Two-way ANOVA for repeated measurements was performed to assess supplementation effect over time, supplement and interaction (time x supplement), the difference between time point within supplementation (superscript with a) and each time point between the two supplementation groups (superscript with b). All p values were corrected for multiple comparisons using the Holm-Sidak method. The net iAUC of plasma fatty acids concentration for 11 women was calculated using the trapezoid rule and compared between the two supplementation groups using a paired t-test. Abbreviation: T0, baseline; T15, 15 days; T30, 30 days; AA, arachidonic acid; ALA, alpha-linolenic acid; net iAUC, the incremental area under the curve from baseline to 30 days; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; LA, linoleic acid; µg/mL, microgram per millilitre. There were no significant differences in any of fatty acids between the two baseline measurements.

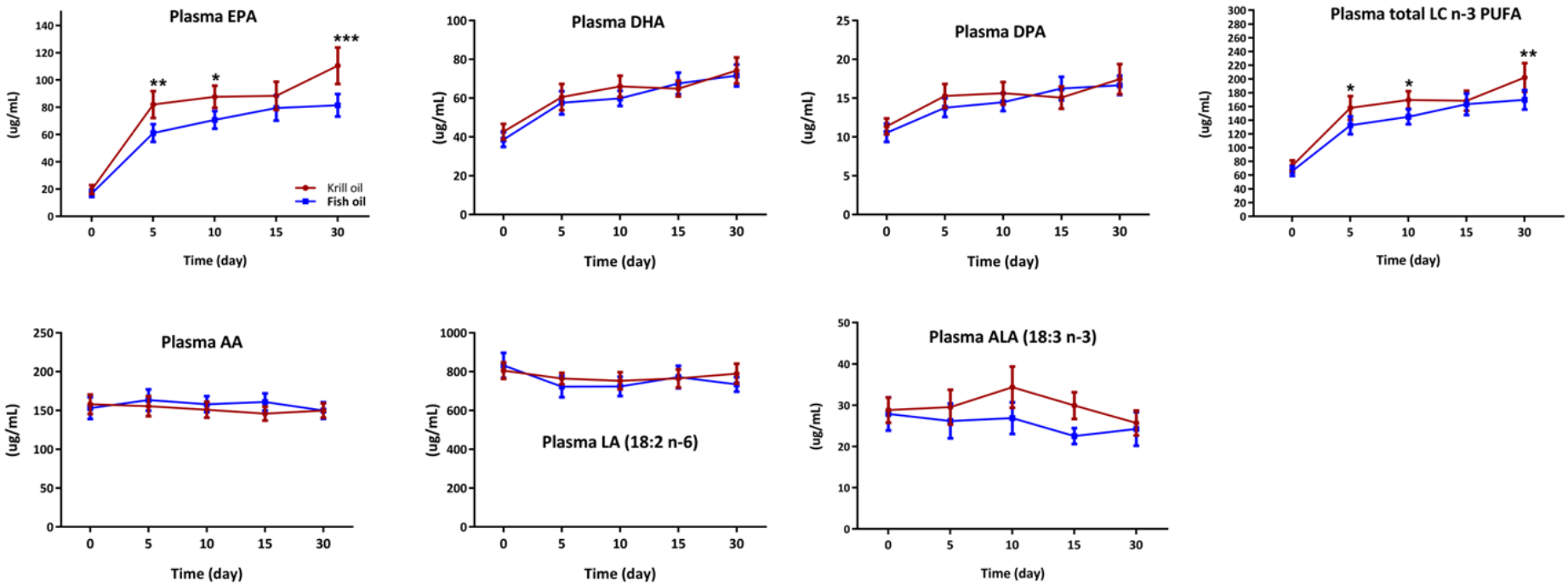


Figure 6.2 The concentration of plasma fatty acids over the 30-day supplementation of krill oil and fish oil

Values are expressed as mean ± SEM (µg/mL) (n = 11). Two-way ANOVA for repeated measurements was performed to assess significant differences at each time point between the krill oil and fish oil supplementation groups. All *p* values were corrected for multiple comparisons using the Holm-Sidak method. *P* values are represented as *** $p < 0.001$, ** $p < 0.01$ and *$p < 0.05$ (significant differences between the two supplementation groups). Abbreviation: AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; LA, linoleic acid; µg/mL, microgram per millilitre.

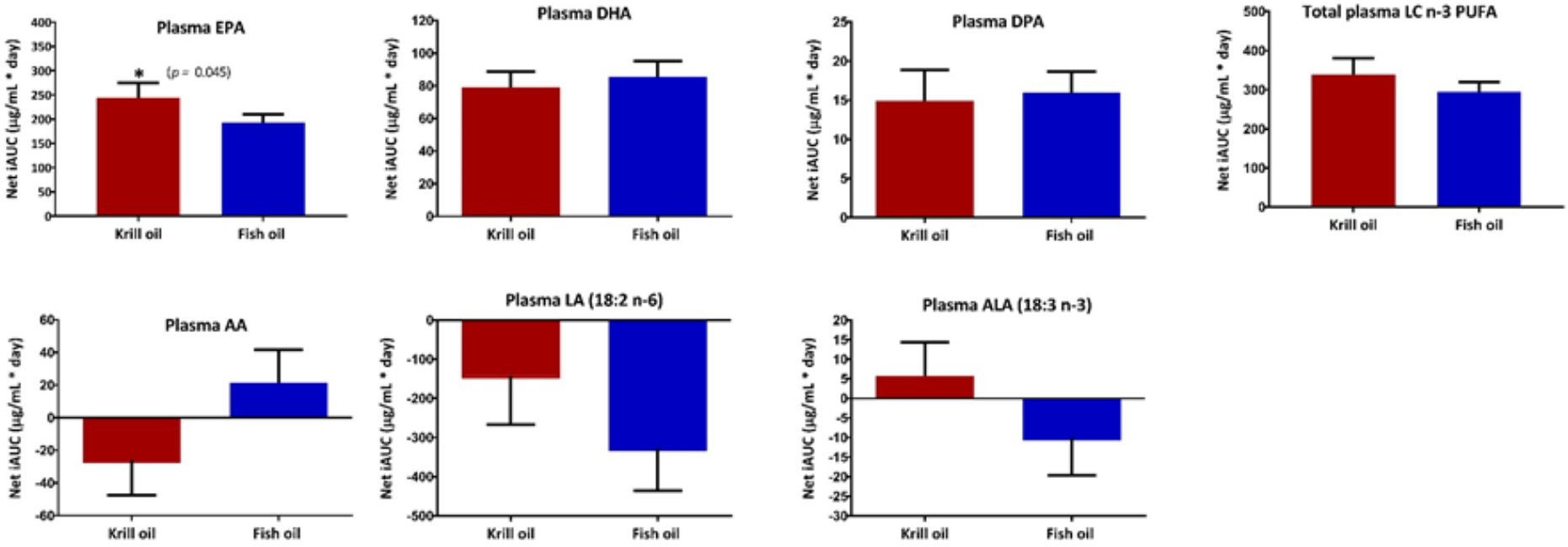


Figure 6.3 Net iAUC of plasma fatty acids over the 30-day supplementation of krill oil and fish oil

Values are expressed as mean ± SEM (µg/mL) (n = 11). The net iAUC (day zero to 30) of plasma fatty acids was calculated using the trapezoid rule and compared between the two supplementation groups using a paired *t*-test. *P* values are represented as *$p < 0.05$. Abbreviation: AA, arachidonic acid; ALA, alpha-linolenic acid; net iAUC, the incremental area under the curve from baseline to 30 days; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; LA, linoleic acid; µg/mL*day, microgram per millilitre times by day.

Table 6. 5 A summary of plasma triglyceride over the 30-day supplementation of krill oil and fish oil

Plasma TAG concentration (mmol/L)						*P* value											
Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil vs Fish oil[b]			Net iAUC	Time	supple-ment	Inter-action
T = 0	T = 15	T =30	T = 0	T = 15	T =30	T0 : T15	T0 : T30	T15 : T30	T0 : T15	T0 : T30	T15 : T30	T = 0	T = 15	T = 30				
1.0 (0.1)	0.9 (0.1)	0.7 (0.1)	1.2 (0.1)	0.9 (0.1)	0.8 (0.1)	0.198	**0.012**	0.198	**0.001**	**0.001**	0.741	0.070	0.934	0.371	0.221	**0.003**	0.189	0.272

Values are expressed as mean (SEM) of the concentration of plasma TAG (n = 11). Two-ANOVA for repeated measurements was performed to assess supplementation effect over time, supplement and interaction (time x supplement), the difference between time point within supplementation (superscript with *a*) and each time point between the two supplementation groups (superscript with *b*). All *p* values were corrected for multiple comparisons using the Holm-Sidak method. The net iAUC of plasma TAG for 11 women was calculated using the trapezoid rule and compared between the two supplementation groups using a paired *t*-test. Abbreviation: net iAUC, the incremental area under the curve from baseline to 30 days; mmol/L, millimole per litre; T0, baseline; T15, 15 days; T30, 30 days; TAG, triglyceride. There were no significant differences in plasma TAG between the two baseline measurements.

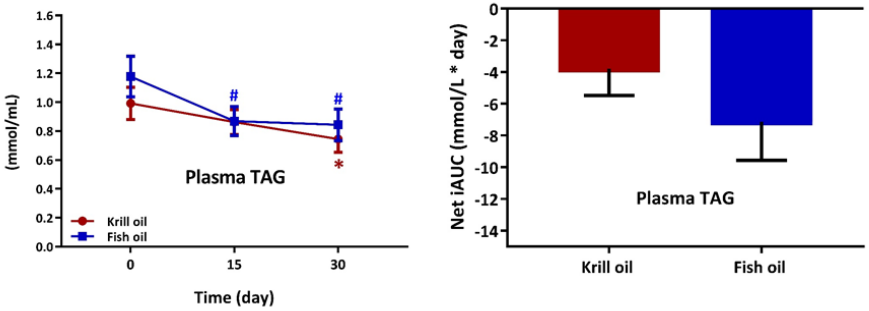


Figure 6.4 The plasma TAG and the area under the curve of TAG over the 30-day supplementation of krill oil and fish oil

Values are expressed as mean ± SEM (mmol/L) (n = 11). Two-way ANOVA for repeated measurements was performed to assess significant differences from the baseline for each supplementation and *p* values ($p < 0.05$) are represented as * for the krill oil supplementation and # for the fish oil supplementation. All *p* values were corrected for multiple comparisons using the Holm-Sidak method. The net iAUC of the concentration of plasma TAG was calculated using the trapezoid rule and compared between the two supplementation groups using a paired *t*-test. Abbreviation: T0, baseline; T15, 15 days; T30, 30 days; net iAUC, the incremental area under the curve from baseline to 30 days; TAG, triglyceride; mmol/mL, millimole per millilitre.

Table 6.6 A summary of blood parameters and inflammatory biomarkers over the 30-day supplementation of krill oil and fish oil

Blood cells and Inflammatory Biomarkers (n = 11)	% concentration within range (ng/L) and changes from baseline (Delta)						*P* value						
	Krill oil			Fish oil			Krill oil [a]	Fish oil[a]	Krill oil vs	Fish oil[b]	Time	Supple-	Inter-
	T = 0	T = 30	Delta	T = 0	T = 30	Delta	T0 : T30	T0 : T30	T = 0	T = 30		ment	action
Red blood cells (10^3µL)	4.0 (0.1)	4.2 (0.2)	0.20	4.2 (0.2)	4.1 (0.2)	-0.10	0.500	0.500	0.402	0.611	1.000	0.836	0.345
White blood cells (10^3µL)	5.3 (0.4)	4.8 (0.4)	-0.50	5.4 (0.4)	5.0 (0.2)	-0.40	**0.031**	0.058	0.691	0.457	**0.001**	0.641	0.802
Platelet (10^3µL)	227.4 (17.2)	212.1 (17.0)	-15.30	214 (15.1)	).5 (11.6)	-6.50	0.106	0.469	0.151	0.353	0.583	0.771	0.104
IL-6 (n = 10)	634.1 (113.4)	588.9 (194.3)	-45.20	696.1 (101.8)	0 (112.2)	162.90	0.834	0.704	0.773	0.405	**0.023**	0.243	0.205
IL-5 (n = 10)	617.5 (69.5)	303.7 (102.5)	-313.80	586.0 (83.5)	).5 (47.0)	-46.60	**0.012**	0.609	0.729	**0.050**	0.609	**0.006**	0.499
IL-2	435.5 (78.5)	283.9 (79.5)	-151.61	522.3 (55.8)	3.8 (40.3)	-28.51	0.387	0.809	0.468	0.186	0.066	0.464	0.084
IL-10 (n = 10)	406.5 (64.8)	213.6 (78.6)	-192.06	403.6 (87.1)	).4 (47.4)	-64.24	**0.005**	0.203	0.952	**0.049**	0.120	0.436	0.413
Interferon-gamma	256.8 (44.5)	168.1 (50.9)	-88.70	266.3 (48.0)	L.7 (86.0)	-34.80	0.144	0.452	0.835	0.337	0.182	0.365	0.428
IL-13	67.7 (11.2)	46.4 (14.1)	-21.30	72.0 (12.4)	63.2 (9.0)	-8.80	0.206	0.484	0.798	0.536	0.181	**0.020**	0.467
IL-1 beta	65.9 (11.5)	34.6 (42.3)	-31.27	68.5 (11.6)	60.5 (6.8)	-8.09	**0.053**	0.518	0.832	0.114	**0.024**	0.214	0.149
IL-4	49.2 (8.0)	23.91 (9.0)	-25.25	49.1 (8.1)	14.8 (5.1)	-4.28	**0.047**	0.661	0.993	0.101	**0.017**	0.197	0.060
IL-12 (P70) (n = 9)	42.9 (9.2)	33.2 (9.8)	-9.72	42.2 (8.7)	39.1 (3.7)	-3.06	0.523	0.741	0.938	0.775	0.393	0.750	0.613
Tumor necrosis factor-alpha	19.4 (3.3)	12.7 (4.0)	-6.65	20.7 (4.0)	18.1 (2.8)	-2.64	0.156	0.460	0.704	0.277	0.195	0.385	0.481

Values are expressed as mean ± SEM of blood parameters and plasma cytokines concentration (n = 11); otherwise, the number of participants was indicated e.g. (n = 10) due to data availability. Two-way ANOVA for repeated measurements was performed to assess supplement effect over time (interaction time x supplement), the difference between time point within supplementation (superscript with a) and each time point between the two supplementation groups (superscript with b). All *p* values were corrected for multiple comparisons using the Holm-Sidak method. There were no significant differences in any of these parameters between the two baseline measurements. Abbreviation: T0, baseline; T30, 30 days, FO, fish oil; IL, interleukin; IL-1β, interleukin-1 beta; KO, krill oil; ng/L, nanogram per litre.

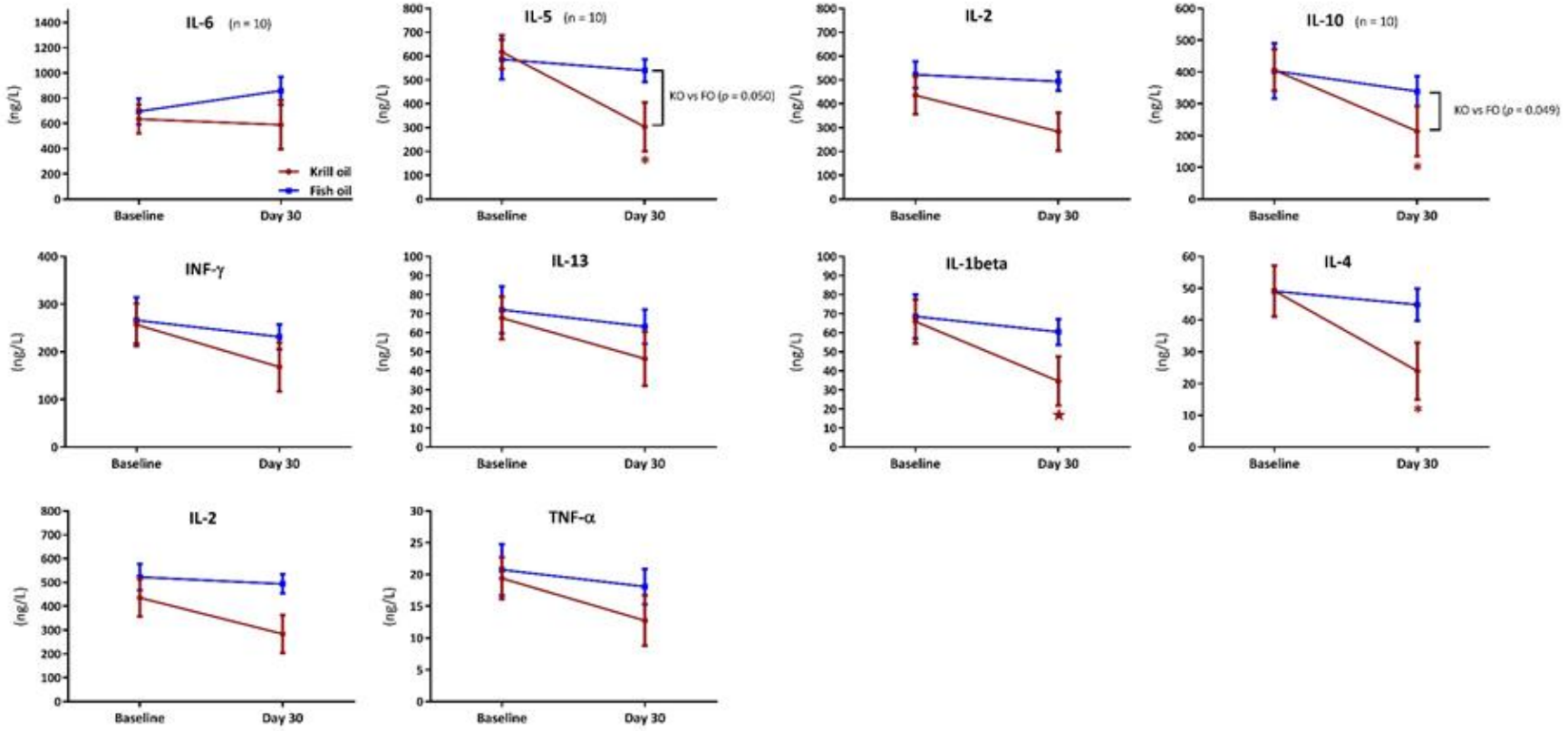


Figure 6.5 The concentration of inflammatory biomarkers over the 30-day supplementation of krill oil and fish oil

Values are expressed as mean ± SEM (ng/L) for 11 women; otherwise, the number of participants was indicated e.g. (n = 10) due to data availability. Two-way ANOVA for repeated measurements was performed to assess significant differences from the baseline for each supplementation ($p < 0.05$ with a closed star for krill oil and a closed square for fish oil) and significant differences at day zero and 30 between the krill oil and fish oil groups with * $p < 0.05$. All p values were corrected for multiple comparisons using the Holm-Sidak method. Abbreviation: IL, interleukin; IL-1β, interleukin-1 beta; ng/L, nanogram per litre; INF-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha.

6.6 DISCUSSION

The aim of this study was to investigate the efficacy of 30-day krill oil supplementation compared with fish oil in healthy young women, in terms of plasma fatty acid profiles, particularly for LC n-3 PUFA, plasma TAG levels and plasma inflammatory biomarkers. The results showed that there was a significantly greater level of EPA in the plasma after the krill oil supplementation compared with the fish oil supplementation. Also, krill oil significantly reduced the levels of inflammatory cytokines compared with fish oil.

The increase in plasma EPA concentration and EPA in net iAUC $_{0\text{-}30\ d}$ over the 30-day krill oil supplementation was significantly greater than that with fish oil supplementation. These results are consistent with our previous 5-hour postprandial study, reported in *Chapter four*, that krill oil, particularly with EPA, showed the evidence of a higher incorporation rate into the plasma compared with fish oil. This finding is also consistent with other human clinical trials with krill oil. Maki et al (2009) and Ulven et al (2011) reported similar findings in long-term clinical trials that there was a relatively greater rate of increase in the plasma EPA and DHA corresponding to the dose of LC n-3 PUFA consumed following krill oil compared with fish oil. These accumulated findings, particularly with the efficacious LC n-3 PUFA in plasma phospholipids, indicate a better absorption and/or transport into plasma lipids of EPA and DHA in krill oil compared with fish oil; the result may be due to the structural form of EPA and DHA found in phospholipids for krill oil, however the exact route of transport of fatty acids from phospholipid is still not yet well understood. It has been reported that the absorption and the incorporation of fatty acids in the phospholipid

form into various tissues were more efficient than the TAG form (Cook et al. 2016, Ramprasath et al. 2013, Wijendran et al. 2002). Ramprasath et al (2015) and Cook et al (2016) compared EPA and DHA in different forms between TAG and phospholipids, and high-phospholipid and low-phospholipid forms via randomised crossover clinical trials in healthy participants (4 weeks and 2 weeks, respectively). Their findings suggested that phospholipid and high-phospholipid sources (compared with TAG and low-phospholipid, respectively) for EPA and DHA could enhance the absorption and the incorporation of EPA and DHA into the plasma and red blood cells, and this was associated with better functional outcomes in circulating plasma TAG and HDL (Ramprasath et al. 2015).

Four long-term clinical studies have compared the effect of krill oil with fish oil supplementation on the plasma LC n-3 PUFA as shown in Table 6.7 (Maki et al. 2009, Ramprasath et al. 2013, Ulven et al. 2011, Yurko-Mauro et al. 2015). All studies reported that the plasma levels of EPA and DHA were significantly increased over the four to seven-week supplementation with both krill oil and fish oil. In three of these studies, there were no significant differences in LC n-3 PUFA between the krill oil and fish oil groups although the dose of LC n-3 PUFA in the krill oil supplementation was lower than that of the fish oil supplementation (Maki et al. 2009, Ulven et al. 2011, Yurko-Mauro et al. 2015), suggesting that a lower dose of krill oil was able to maintain the same plasma level of EPA or DHA as a higher dose of fish oil. In the third study, which was a randomised crossover trial by Ramprasath et al (2013), the increase of the plasma EPA with krill oil was significantly higher than that of fish oil (Table 6.7, Supplementary Table 6.1).

In the current study, a trend of decrease in the level of plasma AA as net iAUC $_{0\text{-}30\ d}$ may be reflected by the greater increase in plasma EPA level following the krill oil supplementation compared with fish oil. The two PUFAs, such as AA and EPA, are involved in the opposite enzymatic synthesis with cyclooxygenase and lipoxygenase which is associated with inflammatory pathways, as described in *Chapter two* and detailed in section 2.2.6). A significantly lower AA level in krill oil in animal models was associated with reductions in the levels of TAG and endocannabinoid in different tissues (Batetta et al. 2009, Tillander et al. 2014).

LC n-3 PUFA, as components of the cellular membranes, are involved in regulating lipid metabolism, membrane fluidity by regulating the activity of membrane proteins, and modulating lipid mediators related to inflammation and pathophysiologic events (Calder 2015). Due to the efficacious absorption of LC n-3 PUFA derived from krill oil, the enhanced LC n-3 PUFA in cell membrane phospholipids can lead to a shift in the lipid metabolism and the production of anti-inflammatory cytokines through an altered eicosanoid production, resulting in a suppressed inflammatory state as described in *Chapter two* and detailed in sections 2.3.2 and 2.3.3.

A number of systemic reviews on circulating TAG levels have shown that the serum TAG in individuals, ranging from healthy to elevated serum TAG, were significantly decreased following consumption of EPA and/or DHA, in doses ranging from 1 g to 5 g/d (Leslie et al. 2015, Lopez-Huertas 2012). Several animal model studies are consistent with human studies in which both krill oil and fish oil were associated with down-regulation of lipids including TAG and stimulating peroxisomal fatty acid β-oxidation (Burri et al. 2011, Vigerust et al. 2013). Some of these studies in animal models suggested that krill oil compared with fish oil was more effective on reduction

of plasma TAG (Batetta et al. 2009, Vigerust et al. 2013) and reducing hepatic lipogenesis (Ferramosca et al. 2012). Many of randomised clinical trials have suggested that a greater result of TAG-lowering can result from at least three months consumption of LC n-3 PUFA doses > 1 g (Lopez-Huertas 2012). Due to the consistent clinical and observational findings in significant TAG reduction with LC n-3 PUFA, doses of 2 to 4 g/d of dietary EPA and DHA intake have been recommended, to individuals with severe hypertriglyceridemia, by various national and international health authorities including American Heart Association (Davidson et al. 2012, McKenney and Sica 2007, Miller et al. 2011). In the present study, the plasma TAG levels significantly declined after both the 30-day krill oil and fish oil supplementation which were in line with a 4-week study by Cicero et al (2015), where the EPA and DHA dose in krill oil was only approximately 14% of that in the fish oil. While, other clinical trials (ranging from four to seven weeks duration) did not find a significant change in plasma TAG in which the daily EPA and DHA from the krill oil and fish oil supplementation were ranging from 0.3 g to 0.9 g (Maki et al. 2009, Ramprasath et al. 2013, Ulven et al. 2011). The mechanisms of TAG-lowering which have been proposed include enhanced TAG clearance, lipoprotein lipase-mediated conversion of very-low-density lipoprotein to low-density lipoprotein through peroxisome proliferator-activated receptors (PPARs) and increased fatty acid beta-oxidation (Jacobson 2008, Vigerust et al. 2013).

Following the significant elevation of plasma LC n-3 PUFA in the present study, the concentration of multiple inflammatory proteins was assessed. As shown in Table 6.6, IL-1β, lL-10, IL-4 and IL-5, as well as the total number of white blood cells were significantly reduced over the 30-day krill oil supplementation ($p \leq 0.05$), whereas fish oil did not change significantly any of these inflammatory cytokines. The plasma levels

of IL-10 and IL-5 with the krill oil supplementation were significantly decreased compared with the fish oil supplement. IL-10 is recognised to be an anti-inflammatory cytokine and the reason for the greater decreased following the krill oil supplementation compared with the fish oil supplementation has not been reported previously. This may be attributed to the significant increase in the plasma EPA by krill oil compared with fish oil, since the significantly increased EPA may impact on eicosanoids and other lipid mediator production modulating inflammation pathways. On the other hand, the moderately decreased plasma AA in the present study, as shown in Figure 6.3, may indicate the displacement by the increased plasma EPA from the krill oil supplementation , whereas the fish oil supplementation did not show a similar trend. This is in line with animal model studies where AA was significantly decreased by krill oil compared with fish oil (Batetta et al. 2009, Tillander et al. 2014).

Inflammation as an essential biological defence in the human body is underpinning of a range of acute and chronic human diseases including metabolic, systemic inflammatory responses and rheumatoid arthritis. Inflammation is characterised by the induced activation of leukocytes releasing chemical mediators such as lipid-derived mediators (prostaglandins, leukotrienes and other lipid mediators) and inflammatory cytokines (proteins) into the inflammatory sites (Calder 2012, Calder 2015). A number of observational and clinical studies have shown the consumption of EPA and DHA ameliorate inflammation states by protecting cells from damages and enhancing the clearance of debris from inflammatory sites which is linked to the complications of cardio-metabolic conditions, as well as cancers (as described in *Chapter two* and detailed in section 2.2.6). Therefore, the consumption of dietary EPA and DHA can contribute to a reduced disease burden resulting from excess inflammation.

There is consistent evidence of LC n-3 PUFA derived from marine sources for a significant reduction in fasting blood level of CRP, IL-6 and TNF-α in individuals with the chronic non-autoimmune disease and healthy individuals, particularly with a longer duration (≥ 12 weeks) reported by Li et al (2014) using a meta-analysis. Additionally, the most recent meta-analysis by Lin et al (2016) indicated that the level of CRP in individuals with type 2 diabetes was significantly decreased following six to 12 weeks of LC n-3 PUFA supplementation providing daily EPA and DHA ranging from 1 g to 6 g. However, several clinical studies, with marine LC n-3 PUFA supplementation providing daily EPA and/or DHA ranging from 0.12 g to 2.4 g for two to 26 weeks, have reported that none of the cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α, INF- γ) was significantly changed in healthy individuals (Rangel-Huerta et al. 2012). Additionally, animal model studies with krill oil also did not support the efficacy of krill oil for changes in the levels of cytokines compared with fish oil (Batetta et al. 2009, Ierna et al. 2010, Vigerust et al. 2013).

It is also possible that the presence of astaxanthin in krill oil, which is not present in fish oil, may be involved in anti-inflammatory responses (Grimstad et al. 2012, Tou et al. 2007). Astaxanthin, found in krill oil ranging from 0.2 mg to 2 mg/g (Tandy et al. 2009, Thomsen et al. 2013), has shown an anti-inflammatory and/or antioxidant effects in cardiovascular events (Pashkow et al. 2008), rheumatoid arthritis conditions (Deutsch 2007, Senftleber et al. 2017) and free radical-promoted neurodegenerative processes and cognition loss (Barros et al. 2014b) by modulating the levels of reactive oxygen species and possibly the formation of undesirable lipid oxidative products (Tandy et al. 2009, Thomsen et al. 2013).

Table 6.7 A summary of human clinical studies with krill oil compared with fish oil

	Study design	No of subjects	EPA+DHA from KO	EPA+DHA from FO	hs-CRP	Plasma TAG	Plasma EPA and DHA
Maki et al 2009	4 wks DB Parallel RCT	n = 76 obese subjects (n = 25 krill; n = 26 fish; n = 25 control)	306 mg/d	390 mg/d	No sig. changes in both groups	No sig. changes in groups	Sig. ↑ in both KO and FO where is no differences in groups
Ulven et al 2011	7 wks Parallel RCT	n = 113 subjects with moderately high TAG (n = 36 krill; n = 40 fish; n = 37 control)	543 mg/d	864 mg/d	No sig. changes in groups	No sig. changes in groups	Sig. ↑ in both KO and FO where is no differences in groups
Ramprasath et al 2013	4 wks semi DBCO RCT	n = 12 M and 12 F healthy subjects	585 mg/d	547 mg/d	NA	No sig. changes in groups	Sig. ↑ in both KO and FO where KO increased EPA and total n-3 PUFA greater than that for FO
Yurko-Mauro et al 2015	4 wks DB Parallel RT	n = 66 helathy subjects (n = 22 /krill, fish, control)	1296 mg/d	1380 mg/d	NA	NA	Sig. ↑ in both KO and FO where is no differences in groups

Abbreviation: CO, crossover; DB, double-blind; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; h, hours; KO, krill oil; mg/d, milligram per day; NA, not available; RCT, randomised control trial; RT, randomised trial; SBCO, single-blind; Sig., significant; TAG, triglyceride; wks, weeks; ↑, increase; ↓, decrease. (Maki et al. 2009, Ramprasath et al. 2013, Ulven et al. 2011, Yurko-Mauro et al. 2015).

6.7 CONCLUSION

In conclusion, the daily 1,269 mg of LC n-3 PUFA in the krill oil supplementation showed greater efficacy for the incorporation of plasma EPA, although the daily LC n-3 PUFA in the krill oil supplementation was 88% of that in the fish oil supplementation. The increased plasma EPA after the 30-day krill oil supplementation compared with the fish oil supplementation ($p = 0.045$) was associated with a significant reduction in plasma TAG, and a reduction in inflammatory biomarkers including IL-1 β, IL-4, IL-5 and IL-10 from the baseline, as well as a significant decrease in IL-5 and IL-10 compared with the fish oil supplementation ($p = 0.05$). Further large studies with more participants over a longer-term are required to verify the impacts of the krill oil supplementation on inflammation response in comparison with the fish oil supplementation.

6.8 STUDY SPECIFIC ACKNOWLEDGEMENTS

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Supplementary Table 6.1 Changes in the level EPA in clinical human trials with krill oil supplementation

	(mg)				Eicosapentaenoic acid (20:5n-3)									
Clinical trial; Study desigr	Krill oil EPA + DHA per day	Fish oil EPA + DHA per day	Krill oil EPA per day	Fish oil EPA per day	Krill oil Pre-	Krill oil Post-	Fish oil Pre-	Fish oil Post-	Krill oil Delta	Fish oil Delta	Krill oil delta per dose	Fish oil delta per dose	Krill oil Change mean %	Fish oil Change mean %
Cross-over 30 days[1]	1175	1441	**759**	**786**	20	110	17	81	90.8	64.7	0.120	0.082	463.3	387.4
DB Parallel RCT 4 weeks[2]	306	390	**216**	**212**	199	377	161	293	178.3	132.0	0.825	0.623	89.7	82.0
Parallel RCT 7 week[3]	543	864	**348**	**450**	30	75	31	76	44.5	45.1	0.128	0.100	146.4	144.6
Semi DBCO RCT 4 week[4]	586	546	**371**	**332**	0.8	2.0	0.8	1.5	1.15	0.72	0.003	0.002	140.2	87.8
DB Parallel RT 4 week[5]	1296	1380	**810**	**840**	10	57	10	50	47.4	40.5	0.058	0.048	492.4	420.5

Abbreviation: DB, double-blind; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; mg, milligram; RCT, randomised control trial; RT, randomised trial; SBCO, single-blind. The number of clinical trials present 1 (current study), 2 (An additional data for the level of EPA at baseline was obtained from the authors which was not described in their publication) (Maki et al. 2009), 3 (Ulven et al. 2011), 4 (Ramprasath et al. 2013) and 5 (Yurko-Mauro et al. 2015).

Chapter 7: The comparative effects of a 30-day krill oil and fish oil supplementation on plasma phospholipidomic profiles in healthy women: a randomised, crossover study

7.1 ABSTRACT

Background: Krill oil and fish oil are commonly used to formulate long-chain n-3 polyunsaturated fatty acid (LC n-3 PUFA) supplements. There is controversy over whether krill oil is more bioavailable than fish oil. This study aimed to compare plasma lipidomic profiles of phospholipid species between a 30-day krill oil and fish oil supplementation.

Methods: In a randomised crossover study, 11 healthy women (aged 22 – 45 years) consumed LC n-3 PUFA supplement with krill oil or fish oil for a period of 30 days, with at least a 30-day washout period between the two supplementation. The daily omega-3 oil supplements provided 1,269 mg/d of total LC n-3 PUFA from krill oil and 1,441mg/d from fish oil. Plasma phospholipidomics was conducted using a liquid chromatography electrospray ionisation-tandem mass spectrometry. Changes in molecular lipid species (plasma concentration (pmol/mL) following krill oil and fish oil supplementation were analysed using a two-way ANOVA for repeated measures. The differences between the two omega-3 supplementation groups were analysed using the paired *t*-test for the incremental area under the curve from baseline (net iAUC $_{0\text{-}30\ d}$, pmol/mL *day).

Results: Twenty four total lipid classes (≥ 500 pmol/mL) were identified in the plasma. The krill oil supplementation significantly increased 10/24 and decreased 3/24 lipid classes. Whereas the fish oil supplementation significantly increased only 1/24 and decreased 4/24 lipid class over a period of 30-days. For 5/24 total lipid classes, the net iAUC $_{0\text{-}30\ d}$ for the krill oil supplementation was significantly greater than the fish oil supplementation at each case.

Molecular species containing eicosapentaenoic acids (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3), 23 and 46 respectively, were significantly increased from the baseline after a 30-day krill oil and fish oil supplementation. Both krill oil and fish oil supplementation significantly increased molecular species containing EPA (21/23 and 18/23, respectively) and lipid species containing DHA (40/43 and 34/43, respectively). In a total of 53 arachidonic acid (AA, 20:4n-6)-containing molecular species, after the 30-day supplementation, the krill oil significantly increased 9/53 and decreased 10/53 AA-containing molecular species. Whereas, the fish oil significantly increased 1/53 and decreased 22/53 AA-containing molecular species. For the net $iAUC_{0\text{-}30\ d}$, there was a consistent trend of lipidomic responses to diacyl- and ether-phospholipid molecular species containing EPA and DHA. The net $iAUC_{0\text{-}30\ d}$ for diacyl-phospholipid molecular species containing EPA and DHA (1/23 and 3/46, respectively) following the fish oil supplementation was significantly greater than that for the krill oil supplementation ($p \leq 0.05$) although some other diacy-phospholipid species, including PC (38:5), PC (40:7) and PS following the krill oil supplementation was significantly greater than that from the fish oil supplementation. On the other hand, the net $iAUC_{0\text{-}30\ d}$ for ether-phospholipid molecular species containing EPA and DHA (4/22 and 8/43, respectively) following the krill oil supplementation was significantly greater than that from the fish oil supplementation at each case.

Conclusion: There was a significant increase in EPA and DHA containing molecular species following the krill oil and fish oil supplementation. The trends of lipidomic changes in diacyl- and ether-phospholipid species were similar for EPA and DHA molecular species although some LC n-3 and n-6 PUFA species responded differently to the supplementation. Therefore, these findings indicate that the 30-day supplementation with krill oil and fish oil do not have equivalent effects on all phospholipids molecular species.

7.2 INTRODUCTION

The findings on the bioavailability of LC n-3 PUFA between krill oil and fish oil are insufficient and conflicting although there was a number of studies to compare krill oil with fish oil in humans and animals over recent decades, as described in *Chapter two* and detailed in section 2.4. These inconsistent outcomes might be related to apparent limitations of study designs, including different doses, chemical forms of LC n-3 PUFA, and target tissues (Ghasemifard et al. 2014, Ulven and Holven 2015). Ghasemifard et al (2014) also pointed out the insufficient control of the matrix of the food and fat content of the test meal consumed by study participants leading to the conflicting outcomes. Moreover, dose rate related to body weight of the participants and absorption efficiency (faecal loss) might be considered due to variations between participants in such factors as ethnicity, gender, age and lipid metabolism associated with participants' lifestyle (Ghasemifard et al. 2014, Howe et al. 2014, Lohner et al. 2013). Moreover, the conventional analysis methods for fatty acids using lipid extraction and followed by consecutive chromatographic separation have been limited for only target lipid fractions, such as triglycerides (TAG) and phospholipids due to time-consuming and costly protocols involved when compared with a novel methodology, lipidomics. The application of lipidomics has been increased to study various lipid classes including glycerophospholipids, sphingolipids and ceramides which represent important signalling molecules for regulation of multiple cellular functions (Huston et al. 2016, Oliveira et al. 2016, Zhang et al. 2015). Due to the high sensitivity and specificity of lipidomics, the identification of individual plasma lipid molecules is highly valuable for understanding the insight into biochemical metabolic pathways, and this could lead to the discovery of various biomarkers in human health and disease (Murphy and Nicolaou 2013, Zhao et al. 2015).

Krill oil, extracted from crustaceans (*Euphausia superba*), is an important source of LC n-3 PUFA and contains a high amount of EPA and DHA (Tou et al. 2007). Attention to LC n-3 PUFA in krill oil has increased as these fatty acids are mainly bound in the phospholipid form (predominantly phosphatidylcholine), while in fish oil they are almost bound in the TAG form (Tou et al. 2007, Winther et al. 2011). In krill oil, the phospholipids are composed of a high proportion of ether-linked phospholipids as well as lyso-phospholipids (Winther et al. 2011). Due to a higher proportion of phospholipids bound LC n-3 PUFA in krill oil, an efficient incorporation of LC n-3 PUFA into the plasma, serum and/or erythrocytes have been reported in both human and animal studies (Schuchardt et al. 2011b, Skorve et al. 2015). There are several krill oil studies in humans compared with fish oil for the bioavailability of LC n-3 PUFA into the plasma and/or erythrocytes. These have reported that the levels of LC n-3 PUFA following a longer-term krill oil and fish oil supplementation (ranging from 4 to 7 weeks) were increased although there were no significant differences between the two omega-3 supplementation (Maki et al. 2009, Ulven et al. 2011, Yurko-Mauro et al. 2015). However, a 4-week randomised crossover study (Ramprasath et al. 2013) suggested that the plasma EPA in the krill oil supplementation increased significantly more than that in the fish oil supplementation.

Taken together, there is insufficient evidence, particularly with the study limitations to prove whether krill oil has different bioavailability of LC n-3 PUFA from fish oil over a longer-term period. There are only a few studies currently available to demonstrate the changes of plasma lipidomic responses following LC n-3 PUFA intake (Ottestad et al. 2012, Skorve et al. 2015). However, whether plasma lipidomic responses to krill oil consumption is different from those to fish oil consumption in humans has not yet been investigated. To the best of our knowledge, this is the first longer-term study to reveal lipidomic profiles with a krill oil supplementation compared with a fish oil supplementation. To minimise variability in study

participants, healthy young women were recruited for this study with a minimum 4-week washout period between the two omega-3 PUFA supplements. We investigated the effect of krill oil in comparison with fish oil on multiple lipid classes and lipid molecular species containing EPA, DHA and docosapentaenoic acids (DPA, 22:6n-3), particularly in phospholipid species. It was hypothesised that 1,269 mg/d krill oil supplementation, compared with 1,441 mg/d fish oil (the closest possible match to these fatty acids from the capsules), would result in different incorporation of LC n-3 PUFA molecular species into plasma lipids.

7.3 MATERIALS AND METHODS

7.3.1 Study design

The study was a randomised crossover design with krill oil and fish oil supplementation for 30 days in a randomised order with a minimum 30-day washout period between the two omega-3 oil supplementation as described in *Chapter 6* (Figure 6.1). In brief, all participants were instructed to maintain their habitual diet and requested not to consume fish/seafood or omega-3 fortified foods more than once a week during the study period. The participants consumed daily seven capsules (1g each) of krill oil (containing 1,269 mg of LC n-3 PUFA) or five capsules (1g each) of fish oil containing 1,441 mg of LC n-3 PUFA for 30 days each. The 30-day supplementation involved five visits, days zero (baseline), 5, 10, 15 and 30 to collect fasting blood samples. Prior to each study day, participants were required to consume one of the most common low-fat dishes in their diet, avoid drinking alcohol and strenuous physical activity, and fast approximately 10 hours overnight. On each study day, standardised procedures were performed where participants arrived at the clinic between 7 am and 9 am, and a fasting blood sample (10 mL) was collected via a venepuncture technique. Following

blood sample collection, the participants completed a 24-hour dietary recall and the online form of PUFA questionnaire (Sullivan et al. 2008, Sullivan et al. 2006, Swierk et al. 2011).

7.3.2 Human ethics for the study

This study was approved by the Victoria University Human Research Ethics Committee (HRE15-031) as described in *Chapter six*. All experimental procedures were performed in accordance with the Declaration of Helsinki of the World Medical Association (HRE15-031), and written informed consent was obtained from all participants prior to the study. This trial was registered with the Australian New Zealand Clinical Trial Registry (ACTRN 12615000472572).

7.3.3 Study participants

A total of 11 healthy women aged between 18 and 50 years within BMI 20 - 35 (kg/m^2), who had not experienced menopause, completed the 30-day supplementation with krill oil and fish oil, as described in *Chapter six*. A medical questionnaire, anthropometric measurements, and the online PUFA questionnaire were used to screen for the study criteria prior to enrolling into the study (Sullivan et al. 2008, Sullivan et al. 2006, Swierk et al. 2011). Participants were excluded if the daily LC n-3 PUFA consumed was more than 500 mg based on results of the online form of PUFA questionnaire.

7.3.4 Extraction of plasma lipids

Plasma lipids were isolated using a single-phase chloroform: methanol ($CHCl_3$:MeOH) extraction as previously described (Ghasemi Fard et al. 2014, Weir et al. 2013). Briefly,

randomised plasma samples (10 µL) were extracted in a single-phase extraction with 20 volumes of $CHCl_3$: MeOH (2:1) and 10 µL of an internal standard mix (in $CHCl_3$: MeOH (1:1)) containing between 50 and 1000 pmol each of 23 non-physiological or stable isotope-labelled lipid standards.

7.3.5 Lipid analysis

Lipid analysis was performed by high-performance liquid chromatography electrospray ionisation-tandem mass spectrometry (HPLC ESI-MS/MS) using an Agilent 1290 HPLC coupled to an Agilent 6490 triple quadrupole mass spectrometer. The details of settings of LC ESI-MS/MS were described in *Chapter three* and detailed in section 3.5.1.1, however the sensitivity was not sufficiently optimised to detect TAG species for this study.

7.3.6 Identification of plasma lipid species

A total of 522 lipid species were analysed using dynamic multiple reaction monitoring (dMRM) where data was collected for a retention time window specific to each lipid species (Supplementary Table 7.1). Results from the chromatographic data was analysed using Mass Hunter Quant where relative lipid abundances were calculated by relating each area under the chromatogram for each lipid species to the corresponding internal standard. Correction factors were applied to adjust for different response factors, where these were known, as indicated in Supplementary Table 7.1.

7.4 STATISTICAL ANALYSIS

The minimum number of participants was determined allowing an 90% power to detect a difference in plasma total EPA and DHA concentration based on a previous 4-week study (Ramprasath et al. 2013). Statistical analyses were performed to compare the significant effects of the 30-day supplementation on plasma lipid molecular species between the krill oil and fish oil supplementation groups. Values are expressed as mean of concentration ± standard error mean (SEM) for 11 participants. The normality of data distribution was checked using D'Agostino & Pearson normality test. Log-transformation of data was carried out where appropriate. Two-way analysis of variance (ANOVA) for repeated measurements was performed to analyse supplementation effect over time (interaction time x supplementation), differences between time point within supplementation and the same time point between the two omega-3 supplementation. All *p* values were corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR). The area under the curve from baseline (net iAUC $_{0\text{-}30\ d}$) of plasma lipid molecular species for 11 participants over the 30-day omega-3 supplementation was calculated using the trapezoid rule. The net iAUC $_{0\text{-}30\ d}$ was compared between the two supplementation groups using paired *t*-test. $P < 0.05$ was considered significant. The analyses were performed using GraphPad Prism version 7.01.

7.5 RESULTS

A total of 30 lipid classes and 522 molecular lipid species were identified and quantified in the plasma samples collected at days zero, 15 and 30 over the krill oil and fish oil supplementation. Of these lipid molecular species, glycerophospholipid species were

predominant among the identified five lipid categories, accounting for 65% of the total lipid molecular species, as shown in Table 7.1.

However, there was an arbitrary decision not to show the plasma total lipid classes under 500 pmol/mL. Even though, all LC PUFA-containing molecular species, including the concentration under 500 pmol/mL, are discussed since there was an attempt to differentiate the bioavailability of LC n-3 PUFA between the 30-day krill oil and fish oil supplementation. Therefore, herein a total of 24 total lipid classes, 23 EPA (20:5n-3) molecular species, 46 DHA (22:6n-3) molecular species, 19 DPA (22:5n-3) molecular species, 53 AA (20:4n-6) molecular specie and 63 LA (18:2n-6) molecular species are discussed (Supplementary Table 7.1 – 7.6).

It should be noted that the lipidomic technology is described as 'semi-quantitative' since the response factors remain for all species as mentioned in *Chapter three* and detailed in section 3.5.2.1 (personal communication, Peter Meikle, Baker Heart and Diabetes Institute, Melbourne, Australia). For the current 30-day lipidomic profiles, the instrument was able to detect mainly phospholipid species rather than TAG species.

7.5.1 Distribution of the total plasma lipid classes

As mentioned earlier, there were a total of 30 lipid classes detected. However, only 24 of them are described in details based on the arbitrary decision that consideration was given to those showing a concentration over 500 pmol/mL. All of these 24 lipid classes (> 500pmol/mL) showed significant changes over the 30-day supplementation period of krill oil or fish oil (Supplementary Table 7.1). The concentrations of total lipid classes were ranged from 1,555 to 1,338,614 pmol/mL. Cholesterol ether (CE) was the highest in concentration followed by

phosphatidylcholine (PC), cholesterol (COH), TAG, sphingomyelins (SM) and lyso-phosphatidylcholine (LPC). The 24 total lipid classes were classified as follows: eleven glycerophospholipids, seven sphingolipids, three sterols, two glycerolipids and one fatty acyl group.

There were significant changes in 13 of 24 total plasma lipid classes following 30-day the krill oil supplementation where 10 cases were significantly increased and three cases were significantly decreased. In contrast, only five of total 24 lipid classes in the fish oil supplementation were significantly changed over the 30-day supplementation in which only one case was significantly increased and four cases were significantly decreased.

In Figure 7.1, it is clear that there were different responses, particularly for ether-phospholipid species between the krill oil and fish oil supplementation. All ether-phospholipid species including alkylphosphatidylcholine (PC (O)), alkenylphosphatidylcholine (PC (P)), alkylphosphatidylethanolamine (PE (O)) and alkenylphosphatidylethanolamine (PE (P)) were significantly increased over the 30-day krill oil supplementation ($p < 0.001$). In contrast, the fish oil supplementation decreased PC (O), PE (O) and PE (P), and increased significantly PC (P) ($p > 0.05$). The changes in all ether-phospholipid classes were significantly different between the kill oil and fish oil supplementation at day 30 ($p < 0.005$).

For the glycerol species, two out of 24 total lipid classes were identified as TAG and DAG. Both krill oil and fish oil supplementation reduced the DAG class significantly ($p < 0.01$ and $p < 0.05$, respectively) over the 30-day supplementation; while no significant change was observed in the TAG class.

Figure 7.2 shows that five of 24 lipid classes in the net iAUC $_{0\text{-}30\ d}$ were significantly different following the two omega-3 oil supplementation. In all cases, including five cases of

phospholipid species and one case of ceramide species, the krill oil supplementation resulted in significantly greater net iAUC $_{0\text{-}30\ d}$ than the fish oil supplementation over the 30-day period ($p < 0.05$).

Table 7.1 Identification of lipid classes and molecular species in the plasma over the 30-day supplementation with krill oil or fish oil

Lipid classes	Abbreviation; Description	Number of identified molecular species (n = 522)
Glycerolipid		**64**
Diacylglycerol	DAG	20
Triaclyglycerol	TAG	44
Glycerophospholipids		**343**
Phosphatidylcholine	PC	65
Alkylphosphatidylcholine	PC (O)	20
Alkenylphosphatidylcholine	PC (P); plasmalogen	23
Lysophosphatidylcholine	LPC	50
Lysoalkylphosphatidylcholine	LPC (O); lysoplatelet activating factor	10
Lysoalkenylphosphatidylcholine	LPC (P); one vinyl ether linked acyl chain	4
Phosphatidylethanolamine	PE	35
Alkylphosphatidylethanolamine	PE (O); one ether linked acyl chain	14
Alkenylphosphatidylethanolamine	PE (P); plasmalogen one vinyl ether linked acyl chain	54
Lysophosphatidylethanolamine	LPE	14
Alkyllysophosphatidylethanolamine	LPE (P); plasmalogen one vinyl ether linked acyl chain	4
Phosphatidylinositol	PI	32
Lysophosphatidylinositol	LPI	8
Phosphatidylglycerol	PG	3
Phosphatidylserine	PS	7
Sphingolipids		**73**
Sphingomyelins	SM, SM (D)	30
Dihydroceramides	dhCer	4
Ceramides	Cer	6
Monohexosylceramide	Hex1Cer	6
Dihexosylceramide	Hex2Cer	6
Trihexosylcermide	Hex3Cer	6
GM1 ganglioside	GM1	6
GM3 ganglioside	GM3	3
Sulfoglycosphingolipids	Sulfatide	6
Sterols	**Sterols**	**29**
Cholesterol	COH	1
Cholesterol ester	CE	26
Oxidised cholesterol ester (fatty acid)	oxCE	2
Fatty acyls		**13**
Acylcarnitines		13

The lipids classes (n = 30) are presented for the all identified molecular species (n = 522) in the current study although only 24/30 of lipid classes (> 500 pmol/mL) are discussed in this Chapter.

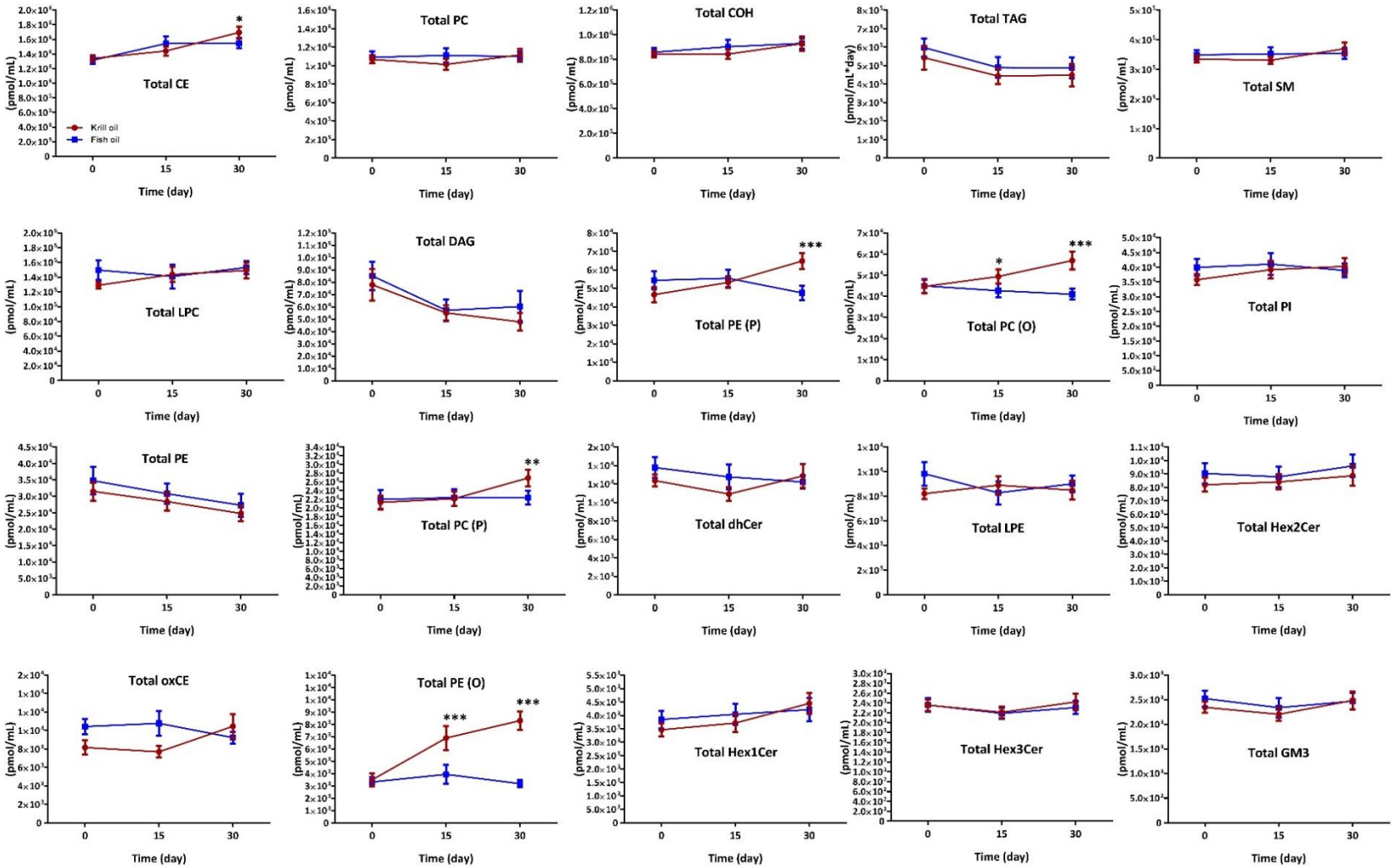

Figure 7.1 (A.1) Changes of 24 lipid classes over the 30-day krill oil and fish oil supplementation

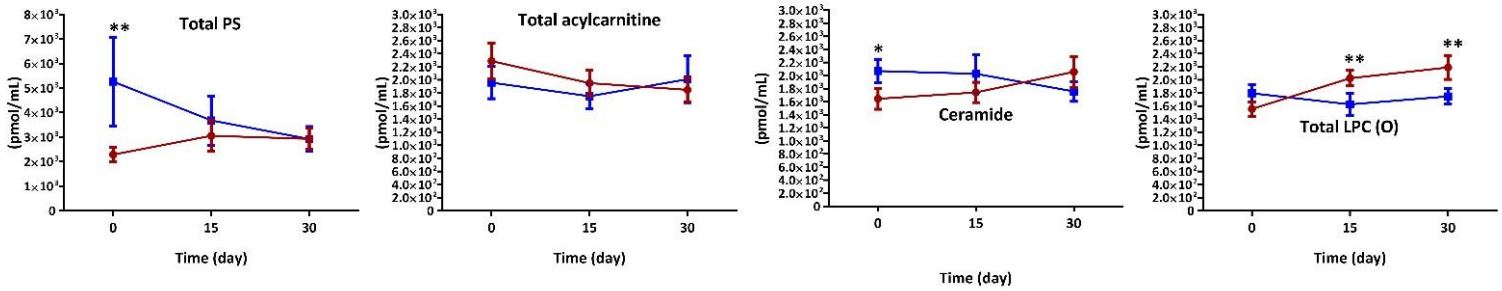

Figure 7.1 (A.2) Changes of 24 plasma lipid classes over the 30-day krill oil and fish oil supplementation

Values are expressed as mean of concentration (pmol/mL) ± SEM (n = 11). Two-way ANOVA for repeated measurements was performed to assess a significant difference at each time point between the krill oil and fish oil supplementation groups. All *p* values were corrected for multiple comparisons using the Benjamini-Hochberg FDR *P* values are represented as *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$. Abbreviation: CE, cholesterol ester; COH, cholesterol; DAG, diacylglycerol; dhCer, dihydroceramide; GM3, G_{M3} ganglioside; Hex1Cer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lyso-phosphatidylcholine; LPC (O), lyso-alkylphosphatidylcholine; LPE, lyso-phosphatidylethanolamine; oxCE, oxidised cholesterol ester(fatty acid); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; SM, sphingomyeline; TAG, triacylglycerol.

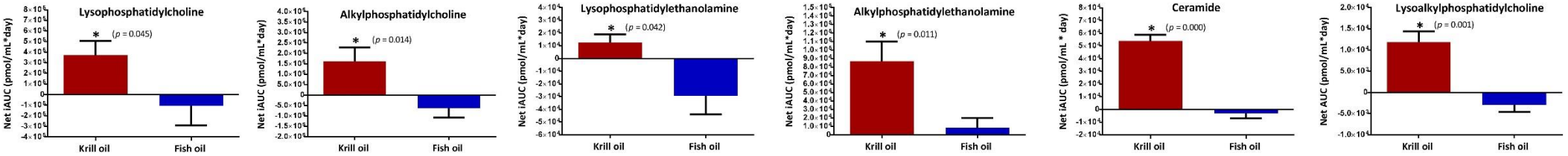

Figure 7.2 (A) Significant differences in the net iAUC of the total plasma lipid classes over the 30-day period between the krill oil and fish oil supplementation

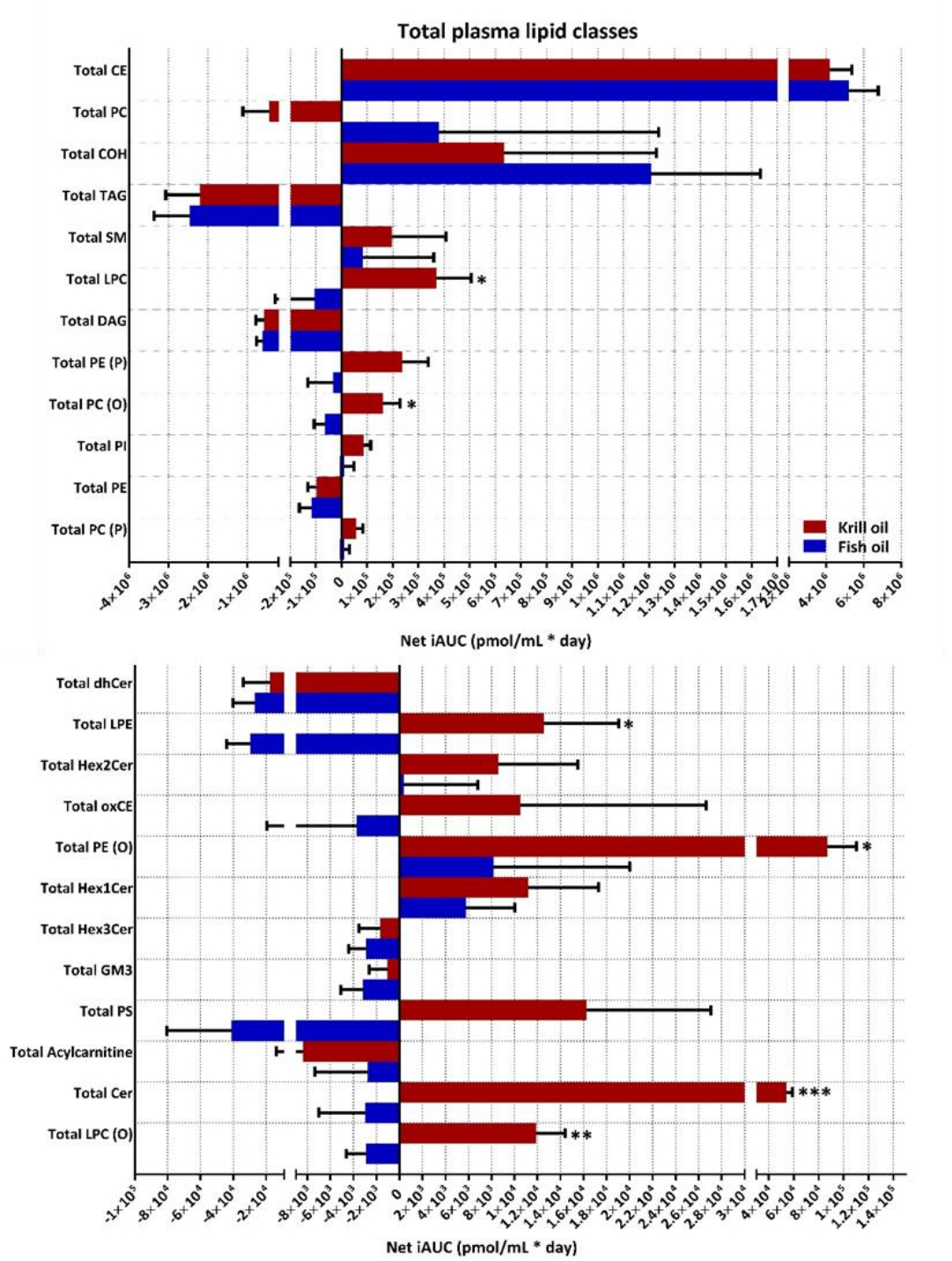

Figure 7.2 (B) The differences in the net iAUC of 24 plasma lipid classes between the 30-day krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) (n = 11). The net iAUC (days from zero to 30) of plasma total lipid classes was calculated using the trapezoid rule and compared between the krill oil and fish oil supplementation groups using paired t-test. *P* values are represented as *** $p < 0.001$, ** $p < 0.01$ and *$p < 0.05$. Abbreviation: net iAUC, incremental area under the curve (days from zero to 30); CE, cholesterol ester; COH, cholesterol; DAG, diacylglycerol; dhCer, dihydroceramide; GM3, G_{M3} ganglioside; Hex1Cer, monohexosylceramide; Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lyso-phosphatidylcholine; LPC (O), lyso-alkylphosphatidylcholine; LPE, lyso-phosphatidylethanolamine; oxCE, oxidised cholesterol ester(fatty acid); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; SM, sphingomyeline; TAG, triacylglycerol.

7.5.2 Distribution of plasma long-chain n-3 PUFA

There were three main LC n-3 PUFA including EPA, DPA and DHA which were identified in different number of lipid molecular species. Multiple molecular species showed significant changes over the 30-day krill oil and fish oil supplementation. The notation used in Figures 7.3 – 7.5 shows the lipid classes such as CE, PC and phosphatidylethanolamine (PE), followed by the molecular species.

As mentioned in *Chapter five*, plasma lipid molecular species are represented by the lipid class followed by one or more fatty acids, in which various degree of carbon chain lengths and double bonds are represented for the structures of esterified fatty acids. Additionally, the phospholipid species with an ether bond, in which either an alkyl bond (O) or an alkenyl bond (P) is linked to phospholipids, are referred to the ether-phospholipid species, as represented PC (P-XX/XX), PC (O-XX/XX), PE (P-XX/XX) or PE (O-XX/XX). Some molecular species are designated with (a) or (b) since the exact assignment of the fatty acids and fatty alkyl (alkenyl) moieties has not been determined yet, and the designation of (a) or (b) may be mixtures of two distinct molecular species.

The novel method, lipidomics using LC-MS/MS, is able to assign specific molecular species such as PC (16:0/20:5), which is considered as EPA molecular species. In other case, a molecular species can be also designated with only the carbon number and the number of double bonds e.g. PC (38:5). The molecular species, PC (38:5) can be assumed to contain both 18:0 and 20:5 as concentration increased over the 15 or 30-day period when there was the supplementation of krill oil and fish oil providing EPA (20:5). PC (38:5) is also considered

that it is composed of 18:1 and 20:4, however it would unlikely if the concentration of this species increased in both krill oil and fish oil supplementation.

7.5.3 Plasma eicosapentaenoic acid (20:5n-3)

A total of 23 EPA-containing molecular species were detected, as shown in Supplementary Table 7.2. The concentrations of plasma EPA (20:5n-3) molecular species ranged from 131 to 36,057 pmol/mL. The CE species was the highest in concentration with the remaining species detected being PC, PC (O), PC (P), PE, PE (O), PE (P) and PI species.

Over the 30-day supplementation, both krill oil and fish oil significantly increased 21/23 and 18/23 EPA-containing molecular species, respectively (Supplementary Table 7.2). For 15 of the 23 EPA-containing molecular species, there were significant differences in the concentration between the two omega-3 oil supplementation groups at 30 days in which the concentration with the krill oil supplementation has a higher concentration in each case when compared with the fish oil supplementation. This clearly indicates that the krill oil supplementation was more effective on incorporation of EPA-phospholipid molecular species than the fish oil supplementation.

The net iAUC $_{0\text{-}30\ d}$ for 5/23 EPA-containing phospholipid species were significantly changed following the two omega-3 oil supplementation ($p \leq 0.05$), as shown in Figure 7.3. The four EPA-containing ether-phospholipid molecular species following the krill oil supplementation showed a significantly greater net iAUC $_{0\text{-}30\ d}$ than the fish oil supplementation ($p \leq 0.05$). Whereas, in the other case, PC (38:5) (a), following the fish oil supplementation was significantly greater in net iAUC $_{0\text{-}30\ d}$ than the krill oil supplementation ($p = 0.053$). The trend of longer-term lipidomic responses was in line with the postprandial lipidomic responses

where the krill oil supplementation resulted in a more effective incorporation into ether-phospholipid molecular species when compared with the fish oil supplementation.

7.5.4 Plasma docosahexaenoic acid (22:6n-3)

A total of 46 DHA-containing molecular species including 43 phospholipid species were detected, as shown in Supplementary Table 7.3. The concentrations of plasma DHA (22:6n-3) molecular species ranged from 39 to 53,951 pmol/mL. PC (16:0/22:6) species was the highest in concentration with the remaining species detected being CE, PC, PC (O), PC (P), PE, PE (O), PE (P), PI, LPC, LPE and TAG species.

Both 30-day krill oil and fish oil supplementation increased significantly multiple molecular species (Supplementary Table 7.3). The krill oil supplementation increased significantly 40/46 DHA-containing molecular species, whereas the fish oil supplementation increased significantly 34/46 DHA-containing molecular species over the 30-day period. Particularly for phospholipid molecular species, the krill oil supplementation significantly increased 37/43 DHA-phospholipid molecular species. Whereas, the fish oil supplementation significantly increased 31/43 DHA-phospholipid molecular species and decreased 1/43 DHA-phospholipid molecular species over the postprandial period ($p \leq 0.05$).

It is clear that there were different lipidomic responses following the two omega-3 oil supplementation that the net iAUC $_{0\text{-}30\ d}$ for 14 of 46 DHA-containing molecular species, including 13/43 phospholipid species, were significantly different between the krill oil and fish oil supplementation groups (Figure 7.4). The net iAUC $_{0\text{-}30\ d}$ for 10/43 DHA-phospholipid molecular species, including eight ether-phospholipid species, following the krill oil supplementation was significantly greater than that of the fish oil supplementation ($p < 0.05$).

While, the fish oil supplementation showed a greater net iAUC $_{0\text{-}30\ d}$ for thee DHA-containing diacyl-phospholipid molecular species (Figure 7.4). The fish oil supplementation also resulted in a greater net iAUC $_{0\text{-}30\ d}$ for DHA-DAG molecular species than the krill oil supplementation.

7.5.5 Plasma docosapentaenoic acid (22:5n-3)

A total of 19 DPA-containing molecular species including 17 of phospholipid species were detected, as show in Supplementary Table 7.4. The concentrations of plasma DPA-containing molecular species ranged from 50 to 4,470 pmol/mL. PC (18:0/22:5 a) species was the highest in concentration with the remaining species detected being PE (P), CE, PC, and PI species.

The DPA-containing molecular species were significantly changed following the 30-day krill oil and fish oil supplementation, as shown in Supplementary Table 7.4. The 30-day krill oil supplementation significantly increased 8/19 and decreased 7/19 DHA-containing molecular species ($p \leq 0.05$). Whereas, the fish oil supplementation significantly increased 5/19 and decreased 8/19 DHA-containing molecular species. In particular, both omega-3 supplementation groups significantly increased 7/17 and 4/17 DPA-phospholipid molecular species, respectively. Both supplementation with krill oil and fish oil also significantly increased CE (22:5) (a) over the 30-day period ($p < 0.01$).

The supplementation with krill oil and fish oil did not result in a significant difference in the net iAUC $_{0\text{-}30\ d}$ of DPA-containing molecular species, except for a DPA-containing diacyl-phospholipid molecular species. As shown in Figure 7.5, the net iAUC $_{0\text{-}30\ d}$ of PE (18:/22:5) (b) following the fish oil supplementation was greater than that of the krill oil supplementation ($p < 0.05$).

7.5.6 Distribution of plasma n-6 PUFA

There were also omega-6 PUFA molecular species observed including arachidonic acid (AA, 20:4n-6) and linoleic acid (LA, 18:2n-6). There were a number of AA-containing and LA-containing lipid molecular species identified in different lipid classes.

7.5.7 Distribution of plasma arachidonic acid (20:4n-6)

A total of 53 AA-containing molecular species including 46 phospholipid species were detected, as show in Supplementary Table 7.5. The concentrations of plasma AA (20:4n-6) molecular species ranged from 17 to 133,379 pmol/mL. CE (20:4) species was the highest in concentration with the remaining species detected being PC, PC (O), PC (P), LPC, PE, PE (O), PE (P), LPE, PI, PS, DAG and TAG species.

As shown in Supplementary Table 7.5, there were differences in the lipidomic changes between the 30-day krill oil and fish oil supplementation groups. The krill oil supplementation significantly increased 19/53 and decreased 10/53 AA-containing molecular species ($p \leq 0.05$). Whereas, the fish oil supplementation significantly increased only 1/53 and decreased 22/53 AA-containing molecular species ($p \leq 0.05$). For the AA-phospholipid molecular species, the krill oil supplementation significantly increased 8/46 and decreased 10/46 molecular species. In contrast, the fish oil supplementation significantly decreased 22/46 AA-phospholipid molecular species over the 30-day period.

Ten out of 61 AA-containing molecular species were significantly different in the net $iAUC_{0\text{-}30\ d}$ between the two omega-3 oil supplementation groups. As shown in Figure 7.6, it is clear that there were different lipidomic responses between the krill oil and fish oil supplementation

groups over the 30-day period in which the net iAUC $_{0\text{-}30\ d}$ of 10/46 AA-phospholipid molecular species was significantly different between the two omega-3 oil supplementation groups. Seven of AA-phospholipid species, including six ether-phospholipid species, following the krill oil supplementation was significantly greater than that of the fish oil supplementation ($p < 0.05$). While, the 30-day fish oil supplementation resulted in a greater net iAUC $_{0\text{-}30\ d}$ for three out of four AA-containing diacyl-phospholipid molecular species than the krill oil supplementation.

7.5.8 Distribution of plasma linoleic acid (18:2n-6)

A total of 63 LA-containing molecular species including 33 phospholipid species were detected, as shown in Supplementary Table 7.6. The concentrations of plasma LA (18:2n-6) molecular species ranged from 8 to 576,675 pmol/mL. CE (18:2) species was the highest in concentration with the remaining species detected being PC, PC (O), PC (P), LPC, PE, PE (P), LPE, CE, ox CE, SM (d), DAG and TAG.

As shown in Supplementary Table 7.6, there were significant changes in LA-containing molecular species following the two omega-3 oil supplementation over the 30-day period. The krill oil supplementation resulted in significant changes in 19/63 LA-containing molecular species including an increase in 2/8 LA-sphingolipid species and 2/33 LA-phospholipid species. Whereas, the fish oil supplementation resulted in significant decrease in 33/63 LA-containing species including 21/33 LA-phospholipid species. Over the 30-day period, both krill oil and fish oil supplementation significantly decreased glycerol species such as LA-TAG molecular species, 4/13 and 8/13, respectively. Moreover, the concentrations of LA-DAG molecular species were significantly decreased by both krill oil and fish oil supplementation (3/5 and 4/5, respectively).

As shown in Figure 7.7, The net iAUC $_{0\text{-}30\ d}$ for two of 63 LA-containing molecular species in SM and ether-phospholipid species was significantly different between the krill oil and fish oil supplementation groups. The net iAUC $_{0\text{-}30\ d}$ of PE (O-16:0/18:2) following the krill oil supplementation was greater than the fish oil supplementation, whereas the net iAUC $_{0\text{-}30\ d}$ of SM (d 18:2/17:0) following the fish oil was greater than the krill oil supplementation over the 30-day period.

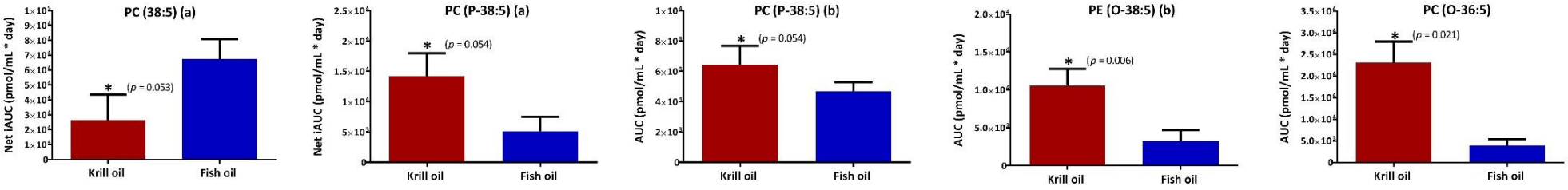

Figure 7.3 Significant differences in the net iAUCof EPA (20:5) molecular species over a 30-day period between the krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) (n = 11). The net iAUC from baseline (days from zero to 30) of plasma EPA molecular species was calculated using the trapezoid rule and compared between the two supplementation groups using paired *t*-test. ***** in graphs indicates significant differences ($p \leq 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: Net iAUC, incremental area under the curve (days from zero to 30); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE (O), alkylphosphatidylethanolamine; pmol/mL, picomole per millilitre.

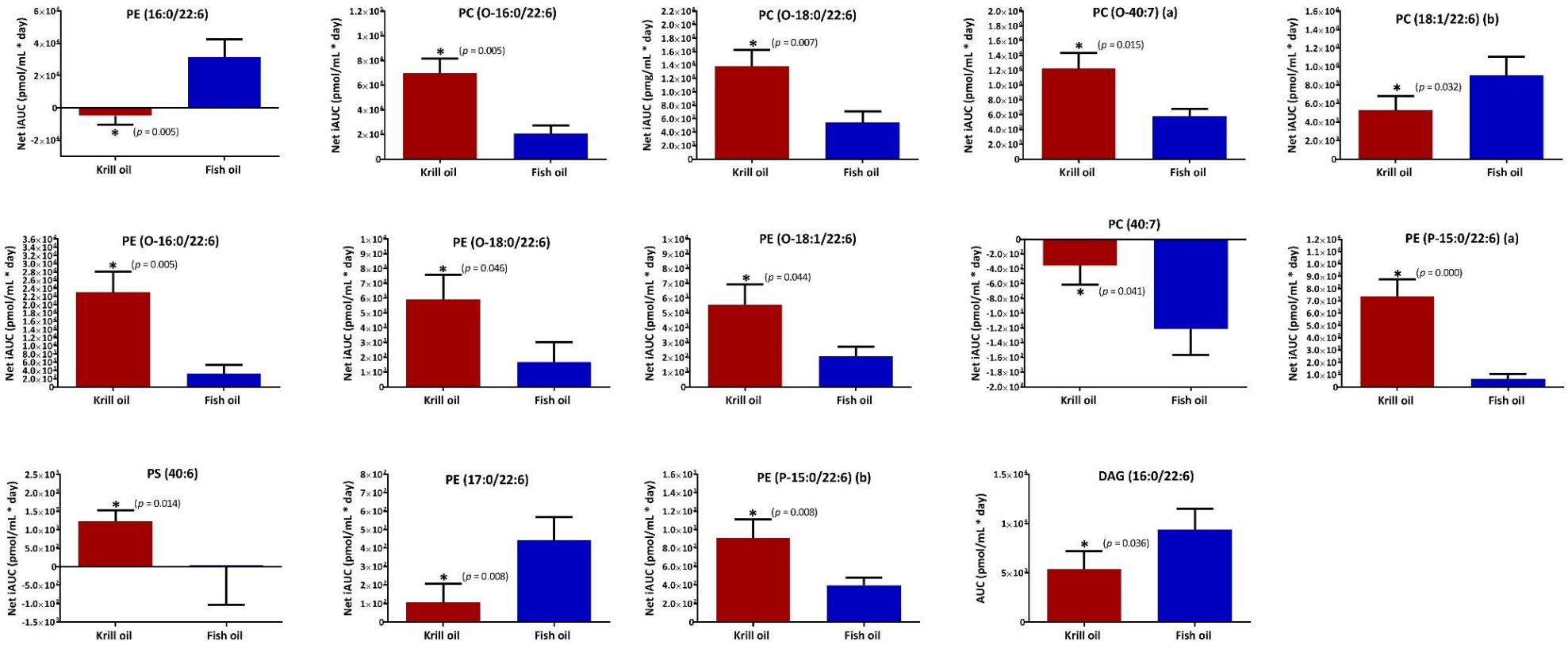

Figure 7.4 Significant differences in the net iAUC of DHA (22:6) molecular species over a 30-day period between the krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) (n = 11). The net iAUC from baseline (days from zero to 30) of plasma LA molecular species was calculated using the trapezoid rule and compared between the two supplementation groups using paired *t*-test. ***** in graphs indicates significant differences ($p < 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: DAG, diacylglycerol; Net iAUC, incremental area under the curve (days from zero to 30);

PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; pmol/mL, picomole per millilitre; PS, phosphatidylserine.

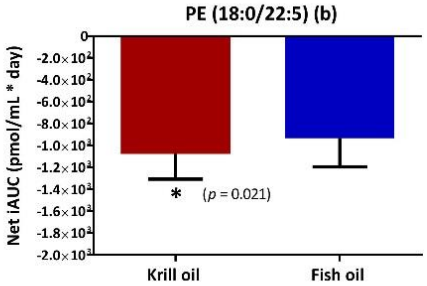

Figure 7.5 Significant differences in the net iAUC of DPA (22:5) molecular species over a 30-day period between the krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) (n = 11). The net iAUC from baseline (days from zero to 30) of plasma LA molecular species was calculated using the trapezoid rule and compared between the two supplementation groups using paired *t*-test. ***** in graphs indicates significant differences ($p < 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: Net iAUC, incremental area under the curve (days from zero to 30); PE, phosphatidylethanolamine; pmol/mL, picomole per millilitre.

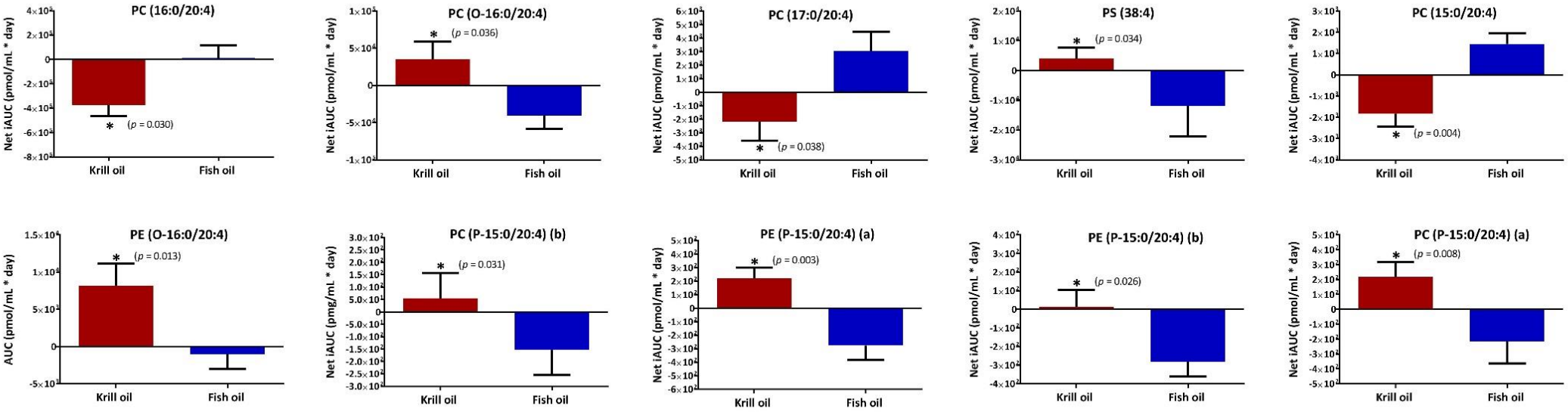

Figure 7.6 Significant differences in the net iAUC of AA (20:4) molecular species over a 30-day period between krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) (n = 11). The net iAUC from baseline (days from zero to 30) of plasma LA molecular species was calculated using the trapezoid rule and compared between the two supplementation groups using paired *t*-test. ***** in graphs indicates significant differences ($p < 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: Net iAUC, incremental area under the curve (days from zero to 30); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; pmol/mL, picomole per millilitre; PS, phosphatidylserine.

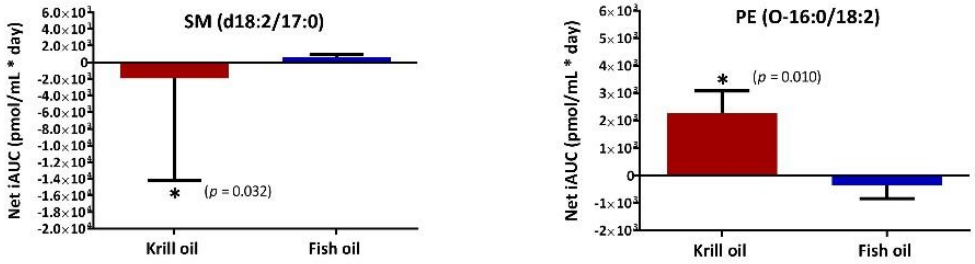

Figure 7.7 Significant differences in the net iAUC of linoleic acid (18:2) molecular species over a 30-day period between the krill oil and fish oil supplementation

Values are expressed as mean ± SEM (pmol/mL) (n = 11). The net iAUC from baseline (days from zero to 30) of plasma LA molecular species was calculated using the trapezoid rule and compared between the two supplementation groups using paired *t*-test. ***** in graphs indicates significant differences ($p < 0.05$) between the krill oil and fish oil supplementation groups. Abbreviation: Net iAUC, incremental area under the curve (days from zero to 30); PE (O), alkylphosphatidylethanolamine; pmol/mL, picomole per millilitre; SM, sphingomyeline; TAG, triacylglycerol.

7.6 DISCUSSION

This randomised crossover study investigated the lipidomic responses of a 30-day dietary supplementation with krill oil and fish oil in healthy women. The hypothesis was that there would be significant differences in the plasma lipidomic responses between the two omega-3 oil supplementation groups.

In the current study (Supplementary Table 7.1), the three most abundant fatty acid-containing lipid classes detected in the plasma were CE, PC and TAG, that was consistent with the postprandial lipidomic study as described in *Chapter five* and other studies (Quehenberger et al. 2010). The CE class was highly responsive to the longer-term supplementation with krill oil and fish oil in that there were significant increases in the concentration of all molecular species containing EPA, DHA and DPA which was in line with the postprandial lipidomic data. The concentration of the TAG class showed a non-significant decline following the 30-day supplementation with both krill oil and fish oil. Since the TAG concentrations were not optimised for HPLC analysis, these data are somewhat consistent with the significant reduction in plasma TAG, measured using an optimised auto-analyser method as described in *Chapter six* and detailed in section 6.4.3.

The lipidomic technology is recognised as a 'semi-quantitative' method since the response factors are not known for all species (personal communication, Peter Meikle, Baker Heart and Diabetes Institute, Melbourne, Australia). For the particular lipidomics method used for the current data, the complete range of TAG species containing LC PUFA were not identified, as the instrument was not specifically optimised for detecting them. Therefore, bearing in mind that the TAG species were not optimised for LC PUFA, the following discussion thus

considers only the differences in phospholipid molecular species between the krill oil and fish oil supplementation groups.

For the purpose of comparing changes in lipid molecular species between the krill oil and fish oil groups, a summary table (Table 7.2) is presented showing the differences in net iAUC $_{0\text{-}30\ d}$. There were six total lipid classes and 20 LC n-3 PUFA-containing molecular species which showed significant differences in net iAUC $_{0\text{-}30\ d}$ between the krill oil and fish oil groups ($p \leq 0.05$). Clearly, this indicates that there were significant differences in the lipidomic responses between the two omega-3 oil groups over the 30-day period.

As shown in Figure 7.2, the concentration of the PC class did not change, but the concentration of PE class significantly decreased following the 30-day krill oil and fish oil supplementation although there was no significant difference between the two omega-3 oil groups. However, many of the PC and PE molecular species containing EPA, DHA or DPA were significantly changed over the 30-day supplementation period (Table 7.2). For PC, which is the principal form of phospholipids circulating in the plasma, there were significant increases in a number of EPA, DHA or DPA molecular species in diacyl-phosphatidylcholine and ether-phosphatidylcholine species following the krill oil and fish oil supplementation.

As shown in Table 7.2, following the two supplementation for 30 days, distinctive patterns of incorporation of LC n-3 PUFA into the different plasma lipid molecular species with various structures were observed using the lipidomics technique. There was a highly significant incorporation of LC n-3 PUFA into ether-phospholipid species following the krill oil supplementation ($p \leq 0.05$). The net iAUC $_{0\text{-}30\ d}$ for 12 cases of the ether-phospholipid species containing EPA and DHA following the krill oil supplementation was significantly greater than the fish oil supplementation. In contrast, the net iAUC $_{0\text{-}30\ d}$ for five out of seven diacyl-

phospholipid molecular species containing LC n-3 PUFA following the fish oil supplementation was significantly greater than the krill oil supplementation. The mechanisms which account for these differences in the incorporation of LC n-3 PUFA into the plasma are not clear, with initial composition of the oils, digestive and absorptive processes and enzymatic remodelling preferences being some options for future investigations.

It was possible to differentiate between krill oil and fish oil in terms of incorporation of LC n-3 PUFA into the plasma due to the sensitivity and specificity of lipidomics. The findings that diacyl- and ether-phospholipid molecules showed different responses between the 30-day krill oil and fish oil supplementation are novel, and consistent in general with the postprandial lipidomic data, as described *Chapter five* and detailed in Table 5.2. However, there are no obvious explanations for these novel findings as it is the first time that the lipidomic responses between the krill oil and fish oil supplementation have been reported. Herein, several possible perspectives on the different lipidomic responses between the two omega-3 oil supplementation groups are outlined, as discussed in *Chapter five*. There is an obvious different changes in the lipid classes between the krill oil and fish oil groups in which the krill oil contains only about one quarter TAG (24% of total lipid classes), while the fish oil contains 98% TAG. Moreover, the krill oil contains a high proportion of phospholipids (61% of the total lipid classes), while the fish oil contains only 1% phospholipids (as described in *Chapter three* and detailed in Table 3.2). In this study, the total amount of phospholipids ingested in the krill oil arm was 774.1 mg/dose (calculated by 61% x 1,269 mg) compared with 14 mg/dose (calculated by 1% x 1,441 mg) for the fish oil. Therefore, the phospholipid digestion and absorption in the 30-day supplementation period would be different due to the distinctive pathways of digestion and absorption of phospholipids, particularly for diacyl and ether phospholipids, which are poorly understood (as described in *Chapter five* and described in Figure 5.8). In brief, it is suggested that dietary phospholipids are transported into the

bloodstream following digestion and absorption in chylomicron, in high-density lipoproteins or as lyso-phospholipids, which have a strong affinity to serum albumin. In contrast, dietary TAG can be taken up by the liver and/or incorporated into different lipids of exported liver lipoproteins (very low-density lipoproteins etc.).

The important issues to consider from the novel findings in this *Chapter* are that krill oil and fish oil have quite different lipid compositions – krill oil is phospholipid-rich, while fish oil is TAG-rich. Moreover, there are no obvious explanations why there were significant changes in the diacyl-phospholipid and ether-phospholipid species containing LC n-3 PUFA between the two omega-3 oil supplementation groups. A better understanding of dietary phospholipid digestion and absorption in health and disease could lead to the development of better diagnostic and prognostic biomarker which remains for the further studies.

Previous observational and experimental studies reported that reduced levels of ether-phospholipids, containing an alkyl (O) or an alkenyl (P) bond, was associated with various degenerative diseases, particularly impaired testis, lens and brain tissues (Brites et al. 2004, Gorgas et al. 2006). This was supported by other evidence from humans that ether-phospholipids such as PC (O), PC (P), PE (O) and PE (P) were negatively associated with type 2 diabetes (T2DM) and coronary artery disease as well as rheumatoid arthritis (Fang et al. 2016, 2014, 2013, Meikle et al. 2011). Moreover, the deficiency of ether-phospholipids, particularly plasmalogens, which contain a fatty alcohol with a vinyl-ether bond at the sn-1 position of glycerol backbone, and PUFA including AA (20:4n-6) and DHA (22:6n-3) at the sn-2 position, were found to be related with neural pathologies including Alzheimer's disease (Braverman and Moser 2012, Brites et al. 2009, Brites et al. 2004). Enhanced LC n-3 PUFA consumption has shown health benefits in human and animals (Block et al. 2010, Ottestad et al. 2012), such as the attenuation of atherosclerosis (Rasmiena et al 2015).

It has been reported that plasmalogens play a role in regulation of oxidative stress and anti-inflammatory activity (Gotoh et al. 2007, Oh et al. 2010, Wallner and Schmitz 2011). Previous evidence has shown that plasmalogen species, such as PC (P) and PE (P) are involved in a range of pathophysiological processes associated with the inflammation-related diseases by regulating membrane fluidity and permeability (Gorgas et al. 2006, Moraitou et al. 2014, Munn et al. 2003, Rasmiena et al. 2015). The presence of a vinyl-ether bond in plasmalogens species was also related to protecting cellular membranes from reactive oxygen species and reducing levels of oxidative stress and inflammation (Farooqui and Horrocks 2001, Moraitou et al. 2014, Rasmiena et al. 2015). It has been suggested that plasmalogens are susceptible to oxidation in that they oxidise themselves and spare the oxidation related to other membrane lipids (Gotoh et al. 2007, Oh et al. 2010, Wallner and Schmitz 2011).

In the current study, following the krill oil supplementation the alkylphosphatidycholine class, PC (O), was greatly increased at day 30 although it was decreased following the fish oil supplementation (Figure 7.1) and the changes in net iAUC $_{0\text{-}30\ d}$ of PC (O) class following the krill oil supplementation was significantly greater than the fish oil (Figure 7.2). PC (O), platelet-activating factor (PAF) containing an alkyl bond, has been found to be involved in a multiple inflammatory pathological states, particularly in relation to cardiovascular and central nervous system diseases (Liu et al. 2016, Mazereeuw et al. 2015). However, the Australian Diabetes, Obesity and Lifestyle Study reported that plasma PC (O) was negatively associated with prediabetes and T2DM (Meikle et al. 2013). It was suggested that there might be a complex relationship between the PAF and diseases, and different stage of disease progression should be considered.

In current study, the LPC class after the krill oil supplementation was significantly greater in net iAUC $_{0\text{-}30\ d}$ when compared with the fish oil supplementation, however individual LPC

molecular species did not show any significant differences in molecular species containing LC n-3 PUFA between the two omega-3 oil supplementation groups (Figure 7.2, Table 7.2). LPC (22:6) [sn1] was significantly increased in both krill oil and fish oil although LPC (20:4) [sn1] was not significantly changed over the 30-day supplementation period. A 4-week clinical study reported a similar finding in that the plasma LPC species containing EPA, DHA and DPA, following an omega-3 supplementation (3.4 g/d in 15 individuals), were significantly increased, and LPC species containing AA was significantly decreased (Block et al. 2010). The fact in the current study that levels of LPC species containing AA remained stable might be due to a lower dosage of LC n-3 PUFA/day, individual variability, and/or gender.

Elevated levels of LPC species have been observed in human and animal models with obesity and T2DM (Pietiläinen et al. 2007) and LPC species were positively correlated to oxidation of low-density lipoproteins (Matsumoto et al. 2007). However, Hung et al suggested that the presence of LPC species needs to be further discriminated by the degree of saturation and the length of carbon chains in relation to human pathophysiological conditions (Hung et al. 2012). Observational cohort studies have reported that the level of LPC species was reduced in individuals with obesity and/or T2DM compared with lean individuals (Barber et al. 2012, Lee et al. 2015).

Table 7.2 Summary of significant differences in the net iAUC between the 30-day krill oil and fish oil supplementation over the 30-day period (significant values less than $p \leq 0.05$ are shown)

Total lipid classes (n = 6)	*P* values	Comparison of the net iAUC $_{0\text{-}30\ d}$ for krill oil versus fish oil
Total LPC	0.045	krill oil > fish oil
Total PC (O)	0.014	krill oil > fish oil
Total LPE	0.042	krill oil > fish oil
Total PE (O)	0.011	krill oil > fish oil
Total Cer	0.000	krill oil > fish oil
Total LPC (O)	0.001	krill oil > fish oil
EPA (20:5n-3) molecular species (n = 5)	***P* values**	**Comparison of the net iAUC $_{0\text{-}30\ d}$ for krill oil versus fish oil**
PC (38:5) (a)	**0.053**	**krill oil < fish oil**
PC (P-38:5) (a)	0.054	krill oil > fish oil
PC (P-38:5) (b)	0.054	krill oil > fish oil
PE (P-38:5) (b)	0.006	krill oil > fish oil
PC (O-38:5) (b)	0.021	krill oil > fish oil
DHA (22:6n-3) molecular species (n = 14)	***P* values**	**Comparison of the net iAUC $_{0\text{-}30\ d}$ for krill oil versus fish oil**
PE (16:0/22:6)	**0.005**	**krill oil < fish oil**
PC (O-16:0/22:6)	0.001	krill oil > fish oil
PC (O-18:0/22:6)	0.007	krill oil > fish oil
PC (O-40:7) (a)	0.015	krill oil > fish oil
PC (18:1/22:6) (b)	**0.032**	**krill oil < fish oil**
PE (O-16:0/22:6)	0.005	krill oil > fish oil
PE (O-18:0/22:6)	0.046	krill oil > fish oil
PE (O-18:1/22:6)	0.044	krill oil > fish oil
PC (40:7)	0.041	krill oil > fish oil
PE (P-15:0/22:6) (a)	0.000	krill oil > fish oil
PS (40:6)	0.014	krill oil > fish oil
PE (17:0/22:6)	**0.008**	**krill oil < fish oil**
PE (P-15:0/22:6) (b)	0.008	krill oil > fish oil
DAG (16:0/22:6)	**0.036**	**krill oil < fish oil**
DPA (22:5n-3) molecular species (n = 1)	***P* values**	**Comparison of the net iAUC $_{0\text{-}30\ d}$ for krill oil versus fish oil**
PE (18:0/22:5) (b)	**0.021**	**krill oil < fish oil**

Abbreviation: net iAUC $_{0\text{-}30\ d}$, incremental area under the curve (days from zero to 30); Cer, ceramide; DAG, diacylglycerol; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; LPC (O), lyso-phosphatidylethanolamine PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PS, phosphatidylserine.

7.7 CONCLUSION

Overall, the current study demonstrated that the lower dose of LC n-3 PUFA from the krill oil supplementation (providing 1,269 mg/d) had a greater efficacy for the incorporation of the plasma lipid classes and a number of molecular species containing LC n-3 PUFA compared with the fish oil supplementation (which provided 1,441 mg/d of LC n-3 PUFA). The novel findings were that the 30-day krill oil supplementation was significantly effective on the incorporation of LC n-3 PUFA into the ether-phospholipid species compared with the fish oil supplementation ($p \leq 0.05$). Therefore, what is obvious from these novel findings is that LC n-3 PUFA from the krill oil are metabolised and processed in a different manner to LC n-3 PUFA from the fish oil. The significant changes in ether-phospholipid molecular species following the krill oil supplementation might be related to the presence of ether-phospholipids in the krill oil, although a clear understanding of the differences in lipidomic responses between the krill oil and fish oil supplementation groups is not yet clear.

Based on the current study, it was seen that lipidomic profile responses are complex and that much more research is needed to understand the causes of variations between individuals such as ethnicity, gender, age and lipid metabolism associated with individual's lifestyle.

7.8 STUDY SPECIFIC ACKNOWLEDGEMENTS

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Supplementary Table 7.1 Lipidomic changes in a total of 24 plasma lipid classes (> 500 pmol/mL) over the 30-day krill oil or fish oil supplementation

Total lipid class	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 15	T =30	T = 0	T = 15	T =30	T0:T15	T0:T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T =30				
Total CE	1338614.1	1440604.4	1694226.8	1313579.4	1541886.6	1549625.0	0.074	**0.000**	**0.000**	**0.000**	**0.000**	0.888	0.648	0.076	**0.015**	0.474	**0.000**	0.634	**0.016**
Total PC	1068351.8	1014170.1	1117596.5	1091017.7	1112252.4	1099238.0	0.303	0.348	0.057	0.683	0.874	0.802	0.663	0.070	0.724	0.459	0.399	0.384	0.287
Total COH	844849.2	844810.7	929460.8	859949.0	904892.9	931139.6	0.999	**0.033**	**0.033**	0.237	0.068	0.485	0.686	0.119	0.964	0.262	**0.019**	0.260	0.514
Total TAG	543938.5	444569.4	448743.9	598433.6	490505.1	487538.1	0.099	0.114	0.943	0.075	0.068	0.959	0.355	0.434	0.508	0.837	**0.018**	0.065	0.982
Total SM	335258.8	330835.6	370155.5	349326.9	352789.3	353438.2	0.757	**0.023**	**0.011**	0.809	0.774	0.964	0.331	0.136	0.250	0.669	0.118	0.566	0.149
Total LPC	129146.3	143826.6	149269.8	149823.3	141019.0	153307.2	0.265	0.132	0.675	0.499	0.788	0.348	0.122	0.829	0.756	**0.045**	0.274	0.393	0.426
Total DAG	77986.0	55142.9	47881.3	85126.5	57292.7	60287.2	**0.039**	**0.009**	0.490	**0.014**	**0.026**	0.775	0.497	0.837	0.244	0.900	**0.002**	0.309	0.784
Total PE (P)	46616.6	53234.5	64814.4	54343.8	55524.0	47550.3	0.135	**0.000**	**0.013**	0.784	0.125	0.075	0.084	0.596	**0.001**	0.081	0.163	0.386	**0.001**
Total PC (O)	44672.1	49360.7	56943.0	44955.6	42685.1	40969.9	0.089	**0.000**	**0.009**	0.397	0.145	0.521	0.915	**0.019**	**0.000**	**0.014**	0.076	**0.000**	**0.001**
Total PI	35765.6	39172.6	40317.6	39930.9	41059.6	38929.9	0.161	0.066	0.630	0.635	0.673	0.374	0.090	0.430	0.560	0.127	0.376	0.254	0.264
Total PE	31511.5	28419.4	24778.5	34788.7	30791.5	27271.3	0.184	**0.007**	0.121	0.090	**0.003**	0.133	0.160	0.303	0.280	0.694	**0.005**	0.316	0.953
Total PC (P)	21257.7	22134.1	26912.3	21948.0	22394.0	22369.6	0.494	**0.000**	**0.001**	0.727	0.741	0.985	0.589	0.838	**0.002**	0.223	**0.001**	0.228	**0.014**
Total dhCer	12386.3	10923.3	12881.2	13797.3	12781.6	12231.5	0.178	0.642	0.076	0.344	0.151	0.605	0.193	0.091	0.542	0.600	0.312	0.369	0.221
Total LPE	8221.3	8914.5	8509.5	9841.3	8290.5	9024.3	0.456	0.755	0.662	0.105	0.381	0.431	0.091	0.502	0.579	**0.042**	0.746	0.528	0.244
Total Hex2Cer	8187.4	8413.8	8871.2	9045.0	8789.7	9600.6	0.623	0.147	0.324	0.579	0.234	0.088	0.073	0.416	0.123	0.316	0.072	0.079	0.742
Total oxCE	8166.9	7698.5	10454.0	10427.2	10775.0	9233.2	0.660	**0.041**	**0.016**	0.744	0.268	0.157	0.201	0.086	0.379	0.476	0.726	0.147	**0.021**
Total PE (O)	3505.6	6894.8	8305.6	3325.6	3941.8	3181.6	**0.000**	**0.000**	0.146	0.602	0.809	0.447	0.983	**0.001**	**0.000**	**0.011**	**0.001**	**0.000**	**0.001**
Total Hex1Cer	3461.6	3713.1	4446.5	3846.1	4040.8	4213.5	0.312	**0.001**	**0.007**	0.432	0.146	0.486	0.129	0.192	0.349	0.456	**0.014**	0.329	0.165
Total Hex3Cer	2357.6	2210.9	2424.2	2360.1	2192.4	2308.6	0.096	0.439	**0.020**	0.060	0.546	0.182	0.978	0.827	0.184	0.507	**0.050**	0.596	0.579
Total GM3	2345.6	2204.5	2485.9	2523.8	2338.6	2470.4	0.260	0.263	**0.032**	0.144	0.667	0.292	0.827	0.827	0.946	0.173	**0.035**	0.248	0.511
Total PS	2292.3	3057.6	2929.6	5264.7	3681.9	2924.8	0.433	0.513	0.895	0.113	**0.024**	0.438	**0.006**	0.521	0.996	0.125	0.667	0.205	0.092
Total Acylcarnitine	2289.8	1952.3	1848.3	1959.4	1750.2	2010.2	0.217	0.111	0.699	0.439	0.850	0.338	0.227	0.455	0.548	0.374	0.428	0.515	0.411
Total Ceramide	1645.8	1744.3	2057.9	2070.4	2028.6	1757.8	0.510	**0.011**	**0.045**	0.778	**0.046**	0.080	**0.009**	0.067	0.054	**0.000**	0.931	0.582	**0.005**
Total LPC (O)	1555.1	2027.3	2191.7	1795.2	1625.1	1748.3	**0.003**	**0.000**	0.259	0.243	0.743	0.394	0.105	**0.010**	**0.005**	**0.001**	**0.002**	**0.051**	**0.004**

Values are expressed as mean of concentration of total lipid molecular classes and subclasses (n = 11). Two-way ANOVA for repeated measurements was performed to analyse supplementation effect over time, supplement and interaction (time x supplement), difference between time point within supplementation (superscript with a) and each time point between the two omega-3 supplementation groups (superscript with b). All *p* values were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (days from zero to 30) of plasma lipid molecular species was calculated using the trapezoid rule and compared between the two omega-3 supplementation groups using paired *t*-test. Abbreviation: net iAUC, incremental area under the curve (days from zero to 30); CE, cholesterol ester; COH, cholesterol; DAG, diacylglycerol; dhCer, dihydroceramide; GM3, GM3 ganglioside; Hex1Cer, monohexosylceramide;

Hex2Cer, dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lyso-phosphatidylcholine; LPC (O), lyso-alkylphosphatidylcholine; LPE, lyso-phosphatidylethanolamine; oxCE, oxidised cholesterol ester(fatty acid); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; SM, sphingomyeline; TAG, triacylglycerol; T0, baseline; T15, 15 days; T30, 30 days.

Supplementary Table 7.2 Lipidomic changes in a total of 23 plasma EPA (20:5) CE and phospholipid molecular species over the 30-day krill oil or fish oil supplementation

EPA (20:5) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 15	T = 30	T = 0	T = 15	T = 30	T0:T15	T0 : T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T = 30				
CE(20:5)	40691.8	155395.6	206129.0	31421.2	152180.7	158434.5	**0.000**	**0.000**	**0.007**	**0.000**	**0.000**	0.729	0.906	0.906	0.131	0.601	0.176	**0.000**	0.284
PC (38:5) (a)	16314.1	16624.3	19208.8	15719.5	18517.7	19095.6	0.700	**0.002**	**0.004**	**0.002**	**0.000**	0.474	0.462	**0.027**	0.888	**0.053**	**0.000**	0.536	0.086
PC (16:0/20:5)	14059.8	48818.8	60310.0	12143.3	48924.8	49923.0	**0.000**	**0.000**	**0.006**	**0.000**	**0.000**	0.794	0.617	0.978	**0.012**	0.772	**0.000**	**0.033**	0.139
PC (38:5) (b)	9893.2	24823.7	31027.8	9354.2	26468.4	26681.5	**0.000**	**0.000**	**0.004**	**0.000**	**0.000**	0.912	0.779	0.395	**0.032**	0.934	**0.000**	0.218	0.101
PC (O-38:5)	5144.7	4770.4	5401.2	4833.8	4592.2	4600.9	0.160	0.329	**0.023**	0.358	0.375	0.974	0.240	0.496	**0.005**	0.809	0.238	**0.036**	0.221
PC (P-38:5) (a)	2374.2	2836.9	3346.1	2423.5	2651.4	2653.9	**0.003**	**0.000**	**0.001**	0.114	0.110	0.987	0.724	0.193	**0.000**	0.054	**0.000**	**0.020**	**0.004**
PE (P-18:0/20:5)	1560.4	5487.3	7894.9	1583.0	5536.9	4806.0	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**	0.287	0.974	0.942	**0.000**	0.126	**0.000**	**0.001**	**0.004**
PE (P-16:0/20:5)	863.7	4170.8	4574.4	807.4	3001.1	2453.9	**0.000**	**0.000**	0.457	**0.000**	**0.000**	0.287	0.681	0.130	**0.003**	0.290	**0.000**	**0.001**	0.125
PE (O-38:5)	770.0	880.8	1137.9	684.7	665.2	539.3	0.324	**0.003**	**0.029**	0.860	0.199	0.264	0.445	0.063	**0.000**	0.144	0.412	**0.001**	**0.010**
PE (38:5) (b)	725.2	1457.0	1494.7	734.9	1474.3	1373.3	**0.000**	**0.000**	0.771	**0.000**	**0.000**	0.437	0.940	0.893	0.352	0.730	**0.000**	0.640	0.692
PC (O-40:5)	693.8	652.5	740.6	684.5	672.1	661.9	0.247	0.191	**0.019**	0.726	0.520	0.767	0.789	0.578	**0.034**	0.918	0.313	0.330	0.144
PC (P-16:0/20:5)	688.2	2220.9	3078.5	643.8	2075.8	2172.5	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**	0.692	0.855	0.553	**0.001**	0.087	**0.000**	**0.011**	**0.039**
PE (P-18:1/20:5) (a)	578.0	2242.0	2646.1	535.4	1759.8	1569.2	**0.000**	**0.000**	0.256	**0.000**	**0.000**	0.419	0.558	0.268	**0.005**	0.457	**0.000**	**0.013**	0.194
LPC (20:5) [sn1]	457.3	2035.9	2504.9	439.0	1823.3	2111.4	**0.000**	**0.000**	0.064	**0.000**	**0.000**	0.242	0.940	0.384	0.115	0.316	**0.000**	0.138	0.550
PI (38:5) (b)	391.8	563.7	565.2	417.5	564.8	536.8	**0.006**	**0.006**	0.981	**0.017**	**0.048**	0.624	0.655	0.986	0.620	0.559	**0.000**	0.979	0.797
PC (18:2/20:5)	320.4	1186.4	1543.9	346.5	1077.6	1123.5	**0.000**	**0.000**	**0.022**	**0.000**	**0.000**	0.754	0.858	0.459	**0.009**	0.149	**0.000**	0.103	0.105
PC (P-38:5) (b)	284.9	503.6	707.0	289.8	498.6	498.1	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.991	0.921	0.918	**0.000**	0.054	**0.000**	**0.049**	**0.007**
PE (16:0/20:5)	164.8	457.3	457.0	164.8	464.9	435.1	**0.000**	**0.000**	0.996	**0.000**	**0.000**	0.568	1.000	0.886	0.672	0.957	**0.000**	0.853	0.915
LPC (20:5) [sn2]	147.6	648.4	798.9	147.4	593.8	681.5	**0.000**	**0.000**	**0.042**	**0.000**	**0.000**	0.220	0.997	0.440	0.106	0.283	**0.000**	0.115	0.502
PE (O-38:5) (b)	140.5	571.1	686.1	131.4	300.6	228.9	**0.000**	**0.000**	0.184	**0.010**	**0.016**	0.831	0.944	**0.001**	**0.000**	**0.021**	**0.000**	**0.000**	**0.004**
PE (P-18:1/20:5) (b)	107.3	323.6	425.4	99.9	297.2	308.1	**0.000**	**0.000**	**0.010**	**0.000**	**0.000**	0.764	0.838	0.469	**0.004**	0.133	**0.000**	**0.032**	0.089
PE (O-36:5)	86.4	1084.7	1163.9	81.7	279.9	198.4	**0.000**	**0.000**	0.444	**0.001**	**0.002**	0.736	0.950	**0.000**	**0.000**	**0.006**	**0.000**	**0.000**	**0.000**
PS (40:5)	85.5	121.5	122.6	176.3	135.0	116.2	0.088	**0.047**	0.750	0.990	0.625	0.634	**0.029**	0.593	0.794	0.076	0.709	0.455	0.192

Values are expressed as the mean of plasma EPA (20:5) molecular species concentration (n = 11). Two-way analysis of variance for repeated measurements was performed to analyse supplementation effect over time (interaction time x supplementation), the difference between time point within supplementation (superscript with a) and each time point between the two omega-3 supplementation groups (superscript with b). All *p* values were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (days from zero to 30) of plasma lipid molecular species was calculated using the trapezoid rule and compared between the two omega-3 supplementation groups using paired

t-test. Abbreviation: net iAUC, the incremental area under the curve (days from zero to 30); LPC, lyso-phosphatidylcholine; LPC (O); PC, phosphatidylcholine; PC (O), alkylphosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkyl-phosphatidylethanolamine; PE (P), alkenyl-phosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; T0, baseline; T15, 15 days; T30, 30 days.

Supplementary Table 7.3 (A) Lipidomic changes in a total of 3/46 plasma DHA (22:6) CE, TAG and DAG molecular species over the 30-day krill oil or fish oil supplementation

DHA (22:6) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 15	T = 30	T = 0	T = 15	T = 30	T0:T15	T0 : T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T = 30				
CE (22:6)	25130	42451	60937	20933	46583	54470	**0.002**	**0.000**	**0.002**	**0.000**	**0.000**	0.139	0.328	0.335	0.775	0.358	**0.000**	0.601	0.194
TAG (18:1/18:1/22:6)	2165	3964	5226	1463	4911	5024	**0.001**	**0.000**	**0.013**	**0.000**	**0.000**	0.811	0.146	0.055	0.667	0.101	**0.000**	0.974	0.057
DAG (16:0/22:6)	275	491	559	255	621	773	**0.027**	**0.005**	0.467	**0.001**	**0.000**	0.109	0.825	0.168	**0.028**	**0.036**	0.633	**0.000**	0.348

Supplementary Table 7.3 (B.1) Lipidomic changes in a total of 43/46 plasma DHA (22:6) phospholipid molecular species over the 30-day krill oil and fish oil supplementation

DHA (22:6) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 15	T = 30	T = 0	T = 15	T = 30	T0:T15	T0 : T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T = 30				
PC (16:0/22:6)	56421	74143	84089	51481	83726	86864	**0.000**	**0.000**	**0.023**	**0.000**	**0.000**	0.446	0.235	**0.028**	0.500	0.060	**0.000**	0.425	0.060
PC (18:0/22:6)	11335	16397	18919	11228	17858	19596	**0.000**	**0.000**	**0.024**	**0.000**	**0.000**	0.107	0.918	0.171	0.518	0.330	**0.000**	0.134	0.569
PE (16:0/22:6)	6043	6148	5208	5100	6575	6346	0.852	0.148	0.106	**0.015**	**0.036**	0.684	0.105	0.451	**0.054**	**0.005**	0.199	0.653	**0.045**
PC (38:6) (a)	5011	8187	9645	5233	7741	7782	**0.000**	**0.000**	**0.019**	**0.000**	**0.000**	0.943	0.701	0.444	**0.004**	0.142	**0.000**	0.058	**0.051**
PE (P-16:0/22:6)	3271	5059	5852	3222	4296	3874	**0.000**	**0.000**	**0.037**	**0.007**	0.080	0.246	0.892	**0.043**	**0.000**	0.066	**0.000**	**0.000**	**0.004**
PC (18:1/22:6) (a)	3245	3698	3874	3184	3589	4083	**0.034**	**0.005**	0.388	0.056	**0.000**	**0.022**	0.763	0.590	0.306	0.811	**0.000**	0.926	0.489
PE (18:0/22:6)	2610	2975	2803	2351	3187	3111	0.140	0.426	0.476	**0.002**	**0.005**	0.753	0.289	0.384	0.210	0.059	**0.019**	0.570	0.221
PE (P-18:0/22:6)	2377	3033	3973	2439	3329	3000	**0.036**	**0.000**	**0.004**	**0.006**	0.068	0.272	0.833	0.321	**0.003**	0.522	**0.000**	0.228	**0.013**
PE (P-18:1/22:6) (a)	2270	3006	3470	2019	2811	2701	**0.002**	**0.000**	**0.037**	**0.001**	**0.004**	0.604	0.239	0.356	**0.001**	0.646	**0.000**	**0.029**	0.124
PC (O-16:0/22:6)	1832	4631	5523	1855	2852	2631	**0.000**	**0.000**	**0.023**	**0.012**	**0.045**	0.548	0.951	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**
PC (O-40:7) (b)	1087	1070	1249	1077	1101	1155	0.761	**0.010**	**0.005**	0.682	0.187	0.354	0.859	0.593	0.113	0.997	**0.001**	0.367	0.305
LPC (22:6) [sn1]	946	1505	1651	980	1560	1834	**0.002**	**0.000**	0.376	**0.002**	**0.000**	0.104	0.836	0.739	0.269	0.729	**0.000**	0.406	0.779
PC (O-18:0/22:6)	867	1385	1677	884	1122	1132	**0.000**	**0.000**	**0.003**	**0.011**	**0.008**	0.915	0.845	**0.006**	**0.000**	**0.007**	**0.000**	**0.000**	**0.001**
PC (P-16:0/22:6)	767	1049	1314	798	1064	1084	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.696	0.542	0.772	**0.000**	0.157	**0.000**	0.226	**0.003**
PC (O-40:7) (a)	759	1200	1509	734	982	1009	**0.000**	**0.000**	**0.001**	**0.004**	**0.002**	0.725	0.744	**0.009**	**0.000**	**0.015**	**0.000**	**0.000**	**0.001**
PC (18:1/22:6) (b)	758	948	1083	696	1070	1153	0.062	**0.003**	0.176	**0.001**	**0.000**	0.398	1.000	0.383	0.383	**0.032**	**0.000**	0.431	0.391
PI (18:0/22:6)	746	1175	1253	764	1241	1220	**0.000**	**0.000**	0.344	**0.000**	**0.000**	0.799	0.827	0.421	0.690	0.868	**0.000**	0.632	0.691
LPE (22:6) [sn1]	695	984	948	719	929	1075	**0.004**	**0.009**	0.685	**0.027**	**0.001**	0.112	0.790	0.536	0.164	0.835	**0.000**	0.537	0.358
PE (40:7)	591	630	558	529	639	603	0.594	0.663	0.337	0.151	0.330	0.625	0.413	0.909	0.553	0.189	0.190	0.948	0.593
PE (O-16:0/22:6)	522	1464	1711	485	662	559	**0.000**	**0.000**	0.238	0.205	0.401	0.656	0.938	**0.000**	**0.000**	**0.005**	**0.000**	**0.000**	**0.000**
PC (16:1/22:6)	511	627	760	454	681	727	**0.044**	**0.000**	**0.023**	**0.000**	**0.000**	0.407	0.304	0.326	0.551	0.207	**0.000**	0.722	0.329

Supplementary Table 7.3 (B.2) Lipidomic changes in a total of 43/46 plasma DHA (22:6) phospholipid molecular species over the 30-day krill oil or fish oil supplementation

DHA (22:6) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 15	T = 30	T = 0	T = 15	T = 30	T0:T15	T0 : T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T = 30				
PC (17:0/22:6)	449	669	766	410	693	752	**0.000**	**0.000**	0.058	**0.000**	**0.000**	0.239	0.428	0.623	0.771	0.341	**0.000**	0.752	0.654
PC (15-MHDA/22:6)	413	519	595	381	596	613	**0.047**	**0.002**	0.138	**0.000**	**0.000**	0.742	0.533	0.135	0.735	0.103	**0.000**	0.583	0.321
PE (P-18:1/22:6) (b)	389	453	578	393	474	476	0.091	**0.000**	**0.002**	**0.035**	**0.030**	0.942	0.924	0.565	**0.010**	0.644	**0.000**	0.377	**0.052**
LPE (22:6) [sn2]	381	524	523	381	504	536	**0.001**	**0.001**	0.978	**0.002**	**0.000**	0.372	0.984	0.572	0.717	0.787	**0.000**	0.939	0.799
PC (15:0/22:6)	360	469	550	312	534	571	**0.002**	**0.000**	**0.027**	**0.000**	**0.000**	0.874	0.750	0.238	0.196	0.592	**0.000**	0.846	0.216
PC (14:0/22:6)	355	558	696	337	628	619	**0.002**	**0.000**	**0.027**	**0.000**	**0.000**	0.874	0.750	0.238	0.196	0.592	**0.000**	0.846	0.216
LPC (22:6) [sn2]	333	518	561	331	530	629	**0.001**	**0.000**	0.361	**0.000**	**0.000**	**0.044**	0.967	0.797	0.156	0.541	**0.000**	0.472	0.537
PE (O-18:0/22:6)	288	514	626	304	404	332	**0.001**	**0.000**	0.064	0.096	0.639	0.219	0.769	0.068	**0.000**	**0.046**	**0.004**	**0.000**	**0.004**
PE (P-17:0/22:6)	275	371	476	266	344	320	**0.012**	**0.000**	**0.006**	**0.036**	0.133	0.504	0.793	0.452	**0.000**	0.112	**0.003**	**0.011**	**0.014**
PI (38:6)	274	406	408	285	446	429	**0.002**	**0.001**	0.953	**0.000**	**0.001**	0.633	0.770	0.280	0.578	0.543	**0.000**	0.141	0.843
PC (P-18:1/22:6)	241	301	389	238	301	325	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	0.132	0.871	0.962	**0.000**	0.327	**0.000**	0.152	**0.009**
PC (P-18:0/22:6)	230	302	399	237	318	328	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.576	0.658	0.323	**0.000**	0.178	**0.000**	0.439	**0.002**
PE (O-18:1/22:6)	226	443	535	190	287	270	**0.000**	**0.000**	0.065	**0.052**	0.102	0.729	0.457	**0.004**	**0.000**	**0.044**	**0.000**	**0.000**	**0.010**
PE (P-20:0/22:6)	198	273	311	206	293	277	**0.000**	**0.000**	**0.012**	**0.000**	**0.000**	0.274	0.567	0.158	**0.025**	0.775	**0.000**	0.869	**0.031**
PC (40:7)	192	168	192	228	177	170	0.068	0.960	0.062	**0.001**	**0.000**	0.585	**0.008**	0.465	0.089	**0.041**	**0.015**	0.540	**0.012**
PE (P-15-MHDA/22:6)	181	240	305	192	244	226	**0.017**	**0.000**	**0.010**	**0.033**	0.149	0.437	0.638	0.866	**0.002**	0.231	**0.000**	0.165	**0.020**
PE (P-20:1/22:6)	133	159	207	112	155	134	0.349	**0.014**	0.098	0.137	0.438	0.457	0.471	0.887	**0.016**	0.852	**0.004**	**0.032**	0.208
PE (P-15:0/22:6) (a)	96	400	474	93	131	106	**0.000**	**0.000**	0.057	0.304	0.733	0.486	0.922	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
PS (40:6)	80	130	145	153	165	127	**0.001**	**0.000**	0.444	0.204	0.661	0.395	**0.004**	0.433	0.406	**0.014**	0.130	0.382	**0.026**
PE (17:0/22:6)	62	69	62	51	71	69	0.239	0.951	0.217	**0.002**	**0.006**	0.657	0.072	0.679	0.228	**0.008**	0.099	0.895	0.095
PE (15-MHDA/22:6)	54	55	55	46	57	55	0.858	0.858	1.000	0.156	0.245	0.784	0.306	0.811	0.971	0.330	0.375	0.572	0.636
PE (P-15:0/22:6) (b)	45	77	103	33	55	44	**0.000**	**0.000**	**0.000**	**0.002**	0.084	0.084	**0.052**	**0.001**	**0.000**	**0.008**	**0.000**	**0.000**	**0.000**

Values are expressed as the mean of plasma DHA (22:6) molecular species concentration (n = 11). Two-way ANOVA for repeated measurements was performed to analyse supplementation effect over time (interaction time x supplementation), the difference between time point within supplementation (superscript with *a*) and each time point between the two omega-3 supplementation groups (superscript with *b*). All *p* values were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (days from zero to 30) of plasma lipid molecular species s was calculated using the trapezoid rule and compared between the two omega-3 supplementation groups using paired *t*-test. Abbreviation: net iAUC, incremental area under the curve (days from zero to 30); DAG, diacylglycerol; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; PC, phosphatidylcholine; PC (P), alkenyl-phosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkyl-phosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; T0, baseline; T15, 15 days; T30, 30 days.

Supplementary Table 7.4 Lipidomic changes in a total of 19 plasma DPA (22:5) CE and phospholipid molecular species over the 30-day krill or fish oil supplementation

DPA (22:5) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 15	T = 30	T = 0	T = 15	T = 30	T0:T15	T0 : T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T = 30				
CE(22:5) (a)	1173	1821	2592	1026	2038	2175	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.335	0.303	0.133	**0.007**	0.405	**0.000**	0.281	**0.015**
CE(22:5) (b)	674	568	717	649	733	628	0.215	0.612	0.088	0.319	0.802	0.217	0.760	0.060	0.293	0.372	0.935	0.780	0.103
PC(18:0/22:5) (a)	4432	5391	6616	4509	5964	6336	**0.040**	**0.000**	**0.011**	**0.003**	**0.001**	0.406	0.860	0.205	0.529	0.718	**0.000**	0.720	0.400
PE(P-16:0/22:5) (a)	1411	1537	1913	1718	1658	1462	0.347	**0.001**	**0.009**	0.649	0.064	0.150	**0.029**	0.365	**0.003**	0.076	0.485	0.933	**0.002**
PC(18:0/22:5 b)	615	413	458	636	545	496	**0.000**	**0.004**	0.269	0.106	**0.018**	0.385	0.742	**0.007**	0.328	0.130	**0.000**	0.093	0.166
PE(P-18:0/22:5) (a)	610	617	758	827	775	666	0.930	0.071	0.084	0.514	**0.052**	0.176	**0.011**	0.054	0.253	0.255	0.936	0.187	**0.025**
PI(18:0/22:5) (a)	528	761	898	551	868	795	**0.001**	**0.000**	**0.041**	**0.000**	**0.001**	0.255	0.724	0.103	0.115	0.778	**0.000**	0.802	0.081
PE(P-18:1/22:5) (a)	392	387	452	436	442	396	0.887	0.074	0.056	0.850	0.226	0.165	0.185	0.103	0.094	0.565	0.856	0.547	**0.044**
PE(18:0/22:5) (a)	368	379	348	468	433	375	0.777	0.580	0.406	0.347	**0.019**	0.127	**0.013**	0.153	0.465	0.292	0.405	0.292	0.381
PC(P-18:0/22:5)	305	373	441	298	353	365	**0.001**	**0.000**	**0.001**	**0.006**	**0.001**	0.497	0.724	0.254	**0.000**	0.216	**0.000**	**0.016**	**0.027**
LPC (22:5) [sn1[(a) /LPC (22:5) [sn2] (b)	222	291	322	231	308	355	0.077	**0.014**	0.413	0.054	**0.004**	0.223	0.797	0.666	0.401	0.766	**0.000**	0.422	0.910
PI(18:0/22:5) (b)	185	147	143	182	200	175	**0.052**	**0.031**	0.800	0.317	0.721	0.181	0.866	**0.009**	0.089	0.108	0.158	0.065	0.112
PE(P-16:0/22:5) (b)	176	121	127	196	153	113	**0.000**	**0.001**	0.763	**0.011**	**0.000**	**0.002**	0.255	**0.014**	0.235	0.847	**0.000**	0.368	**0.037**
PE(O-18:0/22:5) (a)	152	171	225	156	156	129	0.361	**0.002**	**0.019**	0.972	0.198	0.210	0.361	**0.002**	**0.019**	0.147	0.324	**0.012**	**0.007**
LPC (22:5) [sn2] (a)	94	124	138	95	136	153	**0.033**	**0.004**	0.342	**0.006**	**0.000**	0.242	0.930	0.376	0.269	0.406	**0.000**	0.348	0.742
PE(18:0/22:5) (b)	91	46	36	99	62	50	**0.000**	**0.000**	**0.053**	**0.000**	**0.000**	**0.029**	0.523	**0.001**	**0.002**	**0.021**	**0.000**	**0.032**	0.057
PE(P-18:0/22:5) (b)	81	52	60	92	71	52	**0.007**	**0.047**	0.390	**0.042**	**0.001**	0.072	0.278	0.067	0.410	0.941	**0.000**	0.347	0.157
PE(P-18:1/22:5) (b)	55	36	44	60	50	36	**0.002**	**0.050**	0.164	0.066	**0.000**	**0.024**	0.341	**0.019**	0.199	0.852	**0.002**	0.460	**0.040**
LPC (22:5) [sn1] (b)	46	30	31	53	39	39	**0.010**	**0.010**	0.987	**0.020**	**0.023**	0.961	0.231	0.130	0.122	0.798	**0.001**	0.054	0.957

Values are expressed as the mean of plasma DPA (22:5) molecular species concentration (n = 11). Two-way ANOVA for repeated measurements was performed to analyse supplementation effect over time (interaction time x supplementation), the difference between time point within supplementation (superscript with *a*) and each time point between the two omega-3 supplementation groups (superscript with *b*). All *p* values were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (days from zero to 30) of plasma lipid molecular species was calculated using the trapezoid rule and compared between the two omega-3 supplementation groups using paired *t*-test. Abbreviation: net iAUC, the incremental area under the curve (days from zero to 30); LPC, lyso-phosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; T0, baseline; T15, 15 days; T30, 30 days.

Supplementary Table 7.5 (A) Lipidomic changes in a total of 7/53 plasma AA (20:4) TAG, DAG and CE molecular species over the 30-day krill oil or fish oil supplementation

AA (20:4) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 15	T = 30	T = 0	T = 15	T = 30	T0:T15	T0 : T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T = 30				
CE (20:4)	139073	142517	170600	127684	163376	158475	0.659	**0.001**	**0.002**	**0.000**	**0.001**	0.531	0.154	**0.013**	0.131	0.061	**0.003**	0.916	**0.009**
TAG (18:1/18:1/20:4)	2668	2367	2541	2632	2753	2603	0.069	0.426	0.281	0.450	0.854	0.350	0.819	**0.023**	0.697	0.081	0.863	0.427	0.164
TAG (18:2/18:2/20:4)	747	663	745	909	833	787	0.241	0.978	0.252	0.283	0.093	0.514	**0.030**	**0.024**	0.556	0.729	0.310	0.103	0.361
DAG (18:1/20:4)	1986	1741	1736	2480	2070	2088	0.272	0.286	0.974	0.273	0.201	0.848	0.105	0.105	0.156	0.956	0.181	**0.051**	0.983
DAG (18:2/20:4)	1088	952	894	1483	1142	1142	0.190	0.084	0.649	0.330	0.121	0.540	0.103	**0.052**	0.071	0.845	0.165	0.104	0.968
DAG (18:0/20:4)	619	587	628	789	767	712	0.625	0.897	0.537	0.739	0.253	0.412	**0.018**	**0.012**	0.215	0.812	0.843	**0.044**	0.534
DAG (16:0/20:4)	419	355	325	560	383	453	0.368	0.196	0.682	**0.020**	0.142	0.326	0.057	0.691	0.083	0.486	0.058	0.152	0.472

Supplementary Table 7.5 (B.1) Lipidomic changes in a total of 46/53 plasma AA (20:4) phospholipid molecular species over the 30-day krill oil or fish oil supplementation

AA (20:4) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 15	T = 30	T = 0	T = 15	T = 30	T0:T15	T0 : T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T = 30				
PC (16:0/20:4)	106170	87078	94554	103133	105445	100060	**0.002**	**0.042**	0.176	0.669	0.571	0.325	0.576	**0.003**	0.314	**0.030**	**0.038**	0.087	**0.033**
PC (18:0/20:4)	39906	34917	39292	40345	41614	41077	**0.015**	0.746	**0.030**	0.505	0.699	0.777	0.817	**0.002**	0.351	0.084	0.325	0.070	0.067
PI (18:0/20:4)	17502	19328	19560	20071	20543	19440	0.088	0.057	0.822	0.648	0.542	0.291	**0.020**	0.247	0.907	0.143	0.340	0.175	0.200
PC (O-16:0/20:4)	12638	13599	15399	12955	11497	10519	0.315	**0.008**	0.067	0.133	**0.017**	0.307	0.738	**0.035**	**0.000**	**0.036**	0.777	**0.002**	**0.003**
PE (P-18:0/20:4)	8213	6458	8315	10638	8403	6841	**0.049**	0.904	**0.038**	**0.015**	**0.000**	0.077	**0.009**	**0.031**	0.094	0.164	**0.017**	0.227	**0.007**
PE (P-16:0/20:4)	6754	6167	6712	8463	7031	5533	0.335	0.944	0.370	**0.026**	**0.000**	**0.020**	**0.009**	0.161	0.061	0.127	0.089	0.416	**0.008**
PE (18:0/20:4)	5789	4831	3986	7583	5641	4631	0.132	**0.008**	0.181	**0.005**	**0.000**	0.113	**0.008**	0.199	0.303	0.285	**0.005**	0.173	0.373
PC (P-16:0/20:4)	4363	3839	4394	4543	4184	4099	**0.050**	0.900	**0.039**	0.168	0.092	0.737	0.481	0.184	0.253	0.886	**0.044**	0.721	0.198
PC (38:4) (b)	3933	2849	3197	4150	3027	2917	**0.001**	**0.018**	0.236	**0.001**	**0.000**	0.703	0.455	0.540	0.337	0.606	**0.000**	0.871	0.406
PE (P-18:1/20:4) (a)	3818	3052	3523	4383	3429	2936	**0.010**	0.283	0.094	**0.002**	**0.000**	0.080	**0.047**	0.174	**0.040**	0.255	**0.020**	0.712	**0.014**
LPC (20:4) [sn1]	2918	2760	2893	3083	3046	3265	0.604	0.937	0.660	0.903	0.551	0.474	0.586	0.350	0.229	0.846	0.570	0.107	0.888
PE (16:0/20:4)	2462	1690	1350	2730	1987	1575	**0.000**	**0.000**	0.057	**0.000**	**0.000**	**0.024**	0.126	0.093	0.195	0.983	**0.000**	0.221	0.956
PC (O-18:0/20:4)	2347	2023	2225	2371	2007	1870	**0.008**	0.284	0.082	**0.004**	**0.000**	0.229	0.825	0.883	**0.004**	0.363	**0.006**	0.260	**0.047**
PI (16:0/20:4)	1393	1217	1194	1496	1452	1298	0.065	**0.054**	0.925	0.500	0.127	0.375	0.426	**0.051**	0.220	0.395	0.174	0.067	0.669
PC (17:0/20:4)	1181	1073	1108	1093	1238	1210	0.164	0.340	0.645	0.067	0.134	0.714	0.252	**0.040**	0.189	**0.038**	0.908	0.148	0.067
LPC (20:4) [sn2]	1083	1078	1086	1185	1166	1234	0.965	0.974	0.939	0.861	0.639	0.521	0.336	0.404	0.168	0.956	0.784	0.107	0.913
PC (15/MHDA/20:4)	1030	934	1013	1019	1010	913	0.169	0.802	0.255	0.885	0.128	0.165	0.875	0.275	0.151	0.686	0.350	0.813	0.207
PC (P-18:0/20:4)	869	742	898	898	831	822	**0.034**	0.607	**0.011**	0.246	0.190	0.873	0.608	0.126	0.189	0.954	0.078	0.782	0.133
LPE (20:4) [sn1]	785	640	611	829	632	646	**0.030**	**0.011**	0.653	**0.005**	**0.008**	0.823	0.485	0.907	0.578	0.682	**0.000**	0.585	0.823
PC (16:1/20:4)	781	711	794	778	815	835	0.231	0.821	0.158	0.515	0.321	0.727	0.958	0.080	0.472	0.159	0.536	0.278	0.418
PS (38:4)	705	915	830	1560	1111	872	0.284	0.350	0.886	0.300	0.185	0.761	**0.013**	0.572	0.686	**0.034**	0.983	0.358	0.210
PC (P-20:0/20:4)	671	632	718	659	652	641	0.260	0.174	**0.018**	0.834	0.603	0.756	0.715	0.570	**0.032**	0.990	0.321	0.294	0.141
PE (P-15/MHDA/20:4)	600	414	571	726	565	450	**0.015**	0.683	**0.035**	**0.032**	**0.001**	0.113	0.087	**0.042**	0.097	0.491	**0.006**	0.430	**0.022**
PE (P-18:1/20:4) (b)	575	371	488	718	483	438	**0.001**	0.131	**0.046**	**0.000**	**0.000**	0.425	**0.017**	0.055	0.374	0.350	**0.006**	0.228	**0.048**
PC (14:0/20:4)	552	513	588	582	621	572	0.565	0.595	0.274	0.565	0.883	0.471	0.657	0.121	0.814	0.516	0.975	0.538	0.429
PC (15:0/20:4)	538	445	478	484	549	548	**0.040**	0.172	0.446	0.136	0.147	0.964	0.219	**0.022**	0.114	**0.004**	0.765	**0.041**	**0.038**
PE (O-16:0/20:4)	537	822	1059	513	511	384	**0.005**	**0.000**	0.151	0.623	0.085	0.205	0.850	**0.003**	**0.000**	**0.013**	0.131	**0.000**	**0.001**
LPE (20:4) [sn2]	504	409	408	546	428	402	**0.005**	**0.005**	0.967	**0.001**	**0.000**	0.403	0.179	0.558	0.832	0.370	**0.000**	0.649	0.535

Supplementary Table 7.5 (B.2) Lipidomic changes in a total of 46/53 plasma AA (20:4) phospholipid molecular species over the 30-day krill oil or fish oil supplementation

AA (20:4) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 15	T = 30	T = 0	T = 15	T = 30	T0:T15	T0 : T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T = 30				
PE (O-18:0/20:4)	408	392	509	405	347	285	0.721	**0.053**	0.105	0.110	**0.017**	0.368	0.648	0.132	**0.000**	0.075	0.685	**0.018**	**0.013**
PE (P-17:0/20:4)	393	294	405	436	347	287	**0.045**	0.797	**0.026**	0.070	**0.004**	0.208	0.367	0.263	**0.019**	0.447	0.062	0.834	**0.028**
PE (P-20:0/20:4)	388	370	393	609	479	421	0.419	0.941	0.462	0.204	**0.031**	0.328	**0.007**	**0.020**	0.443	0.464	0.550	0.083	0.272
PC (P-15/MHDA/20:4)	349	325	392	359	350	349	0.390	0.132	**0.024**	0.738	0.699	0.958	0.704	0.366	0.131	0.837	0.246	0.910	0.202
PC (20:0/20:4)#	173	171	173	214	196	190	0.872	0.978	0.894	0.170	0.068	0.621	**0.004**	0.066	0.205	0.376	0.460	**0.036**	0.410
PC (P-17:0/20:4)	153	141	176	151	155	156	0.278	**0.047**	**0.004**	0.745	0.653	0.901	0.841	0.229	0.077	0.773	0.135	0.728	0.115
PE (17:0/20:4)	125	94	84	136	110	91	**0.001**	**0.000**	0.215	**0.004**	**0.000**	**0.026**	0.193	0.062	0.406	0.835	**0.002**	0.254	0.728
LPI (20:4) [sn1]	103	101	111	98	116	112	0.784	0.312	0.203	**0.028**	0.069	0.651	0.514	0.060	0.830	0.119	0.194	0.586	0.186
PI (20:0/20:4)	44	48	46	55	53	49	0.478	0.659	0.786	0.754	0.330	0.505	0.079	0.428	0.690	0.413	0.734	0.155	0.582
PE (P-19:0/20:4) (a)	42	32	53	49	42	34	0.367	0.327	0.068	0.555	0.201	0.479	0.555	0.367	0.100	0.477	0.264	0.877	0.150
PE (P-19:0/20:4) (b)	39	34	47	50	42	32	0.454	0.299	0.082	0.255	**0.016**	0.159	0.139	0.271	**0.043**	0.140	0.338	0.798	**0.032**
PE (15/MHDA/20:4)	37	27	23	41	28	23	**0.004**	**0.000**	0.266	**0.000**	**0.000**	0.126	0.172	0.635	0.976	0.374	**0.001**	0.529	0.614
PC (P-15:0/20:4) (b)	33	32	42	35	28	29	0.577	**0.003**	**0.009**	**0.046**	0.099	0.694	0.477	0.063	**0.000**	**0.031**	0.405	0.057	**0.006**
PE (P-15:0/20:4) (a)	31	41	42	41	31	24	0.063	**0.047**	0.882	0.059	**0.002**	0.127	**0.053**	0.070	**0.002**	**0.003**	0.426	**0.024**	**0.002**
LPI (20:4) [sn2]	28	30	34	30	34	34	0.427	**0.038**	0.172	0.153	0.286	0.847	0.390	0.136	0.973	0.907	**0.027**	0.429	0.541
PE (P-15:0/20:4) (b)	24	23	28	31	20	16	0.848	0.423	0.323	**0.015**	**0.002**	0.365	0.117	0.423	**0.012**	**0.026**	0.097	0.188	**0.019**
PC (P-15:0/20:4) (a)	21	27	37	26	18	15	**0.017**	**0.000**	0.096	0.065	**0.025**	0.641	0.275	**0.003**	**0.000**	**0.008**	0.589	**0.003**	**0.000**
PE (16:1/20:4)	17	13	11	17	13	11	0.085	**0.022**	0.513	0.098	**0.024**	0.490	0.971	0.971	1.000	0.958	**0.045**	1.000	0.999

Values are expressed as the mean of plasma AA (20:4) molecular species concentration (n =11). Two-way ANOVA for repeated measurements was performed to analyse supplementation effect over time (interaction time x supplementation), the difference between time point within supplementation (superscript with *a*) and each time point between the two omega-3 supplementation groups (superscript with *b*). All *p* values were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (days from zero to 30) of plasma lipid molecular species was calculated using the trapezoid rule and compared between the two omega-3 supplementation groups using paired *t*-test. Abbreviation: net iAUC, the incremental area under the curve (days from zero to 30); DAG, diacylglycerol; LPI, lyso-phosphatidylinositol PC, phosphatidylcholine; PC (P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkylphosphatidylethanolamine; PE (P), alkenylphosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; T0, baseline; T15, 15 days; T30, 30 days.

Supplementary Table 7.6 (A) Lipidomic in a total of 30/63 plasma LA (18:2) sterol, TAG, DAG, sphingolipid and fatty acylcarnitine molecular species over the 30-day krill oil or fish oil supplementation

LA (18:2) Molecular species	Concentration (pmol/mL)						*P* value											
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-ment	Inter-action
	T = 0	T = 15	T = 30	T = 0	T = 15	T = 30	T0:T15	T0 : T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T = 30				
CE (18:2)	574035	560191	636882	579314	588130	580237	0.627	**0.037**	**0.013**	0.757	0.974	0.781	0.853	0.331	0.057	0.789	0.228	0.677	0.113
oxCE (18:2) [+O]	7256	6843	9345	9217	9710	8048	0.652	**0.031**	**0.012**	0.591	0.210	0.080	**0.042**	**0.005**	0.166	0.544	0.820	0.167	**0.010**
oxCE (18:2) [+2O]	911	856	1109	1210	1065	1186	0.785	0.337	0.222	0.479	0.903	0.557	0.152	0.309	0.707	0.392	0.191	0.318	0.737
TAG (16:0/18:1/18:2)	80082	69585	63912	83597	73328	70332	0.108	**0.017**	0.373	0.115	**0.046**	0.636	0.579	0.555	0.315	0.865	**0.003**	0.231	0.935
TAG (16:0/18:2/18:2)	34459	28363	25935	39221	29811	27382	0.072	**0.015**	0.457	**0.008**	**0.001**	0.457	0.153	0.656	0.657	0.457	**0.000**	0.334	0.704
TAG (16:1/18:1/18:2)	23856	18415	17832	24368	18624	18428	**0.033**	**0.019**	0.808	**0.025**	**0.021**	0.935	0.831	0.931	0.804	0.957	**0.001**	0.815	0.993
TAG (18:1/18:2/18:2)	20096	17276	17590	21863	16774	16116	0.146	0.094	0.807	0.182	**0.030**	0.353	0.905	0.806	0.655	0.914	**0.002**	0.976	0.870
TAG (16:0/16:0/18:2)	11255	9569	9045	14255	10604	10441	0.247	**0.038**	0.317	**0.010**	**0.010**	0.987	**0.025**	0.453	0.086	0.260	**0.003**	**0.042**	0.509
TAG (18:1/18:1/18:2)	9495	8321	8082	9696	8183	8040	0.361	0.275	0.852	0.243	0.203	0.911	0.875	0.914	0.974	0.800	**0.037**	0.996	0.981
TAG (14:0/16:0/18:2)	8917	6212	6468	11522	7740	7818	0.141	0.181	0.886	**0.045**	**0.049**	0.965	0.156	0.397	0.453	0.570	**0.053**	0.112	0.864
TAG (14:0/16:1/18:2)	3931	3005	2763	5462	4434	3426	0.394	0.285	0.822	0.345	0.070	0.354	0.165	0.194	0.540	0.711	0.118	0.195	0.821
TAG (16:0/17:0/18:2)	3698	2591	2984	3909	3053	3049	0.098	0.276	0.545	0.194	0.193	0.996	0.744	0.477	0.919	0.856	0.096	0.225	0.907
TAG (18:0/18:2/18:2)	3182	2917	3140	3546	3083	2744	0.512	0.915	0.582	0.257	0.057	0.404	0.370	0.681	0.332	0.352	0.184	0.860	0.392
TAG (14:0/18:2/18:2)	3031	2590	2642	3885	2945	2639	0.408	0.464	0.922	0.087	**0.027**	0.564	0.117	0.504	0.996	0.407	**0.034**	0.470	0.518
TAG (18:2/18:2/18:2)	2620	2450	2481	3089	2409	2253	0.318	0.334	0.974	0.239	**0.042**	0.349	0.478	0.600	0.651	0.586	**0.030**	0.768	0.674
TAG (14:1/18:0/18:2)	711	512	656	816	646	638	0.125	0.664	0.259	0.185	0.167	0.953	0.408	0.295	0.885	0.860	0.181	0.188	0.662
DAG (18:1/18:2)	14187	10342	8744	15401	10130	10414	0.056	**0.010**	0.409	**0.012**	**0.016**	0.882	0.529	0.912	0.389	0.338	**0.001**	0.510	0.768
DAG (18:2/18:2)	2667	1974	1775	3111	2042	2012	0.151	**0.009**	0.171	0.120	**0.023**	0.411	0.886	0.986	0.557	0.617	**0.001**	0.750	0.912
DAG (16:0/18:2)	2522	1783	1511	3038	1804	1938	**0.050**	**0.001**	0.119	**0.002**	**0.002**	0.952	0.150	0.976	0.101	0.458	**0.000**	0.192	0.446
DAG (18:0/18:2)	1251	880	883	1670	1002	1075	0.076	0.059	0.896	**0.036**	**0.033**	0.963	0.088	0.171	0.148	0.338	**0.016**	0.058	0.963
DAG (14:0/18:2)	243	190	175	327	196	244	0.361	0.246	0.797	**0.031**	0.158	0.403	0.153	0.922	0.239	0.425	0.061	0.405	0.592
SM (d 18:2/16:0)	13731	13051	14146	14084	14108	14073	0.211	0.440	**0.051**	0.964	0.984	0.948	0.510	0.058	0.891	0.457	0.359	0.296	0.329
SM (d 18:2/22:0)	13490	12820	13944	13787	13605	13941	0.297	0.477	0.088	0.774	0.808	0.597	0.640	0.224	0.997	0.659	0.319	0.454	0.673
SM (d 18:2/24:0)	10214	10128	11028	10825	10573	11318	0.832	0.054	**0.035**	0.533	0.230	0.076	0.140	0.277	0.475	0.619	0.078	0.156	0.851
SM (d 18:2/18:0)	9240	8986	9499	9455	8532	9413	0.676	0.673	0.404	0.141	0.945	0.159	0.726	0.460	0.888	0.421	0.337	0.822	0.738
SM (d 18:2/20:0)	5746	5431	6052	5821	5874	5979	0.258	0.273	**0.033**	0.847	0.566	0.701	0.786	0.118	0.791	0.473	0.156	0.436	0.400
SM (d 18:2/23:0)	5585	5457	6090	5655	5569	5792	0.571	**0.035**	**0.010**	0.706	0.545	0.330	0.758	0.618	0.196	0.592	0.127	0.820	0.376
SM (d 18:2/14:0)	802	765	880	811	828	801	0.401	0.086	**0.015**	0.705	0.809	0.537	0.839	0.163	0.080	0.852	0.211	0.933	0.087
SM (d 18:2/17:0)	446	481	549	424	450	446	0.163	**0.000**	**0.012**	0.288	0.375	0.855	0.381	0.225	**0.000**	**0.032**	**0.007**	**0.014**	0.057
Acylcarnitine (18:2)	143	141	130	137	132	128	0.836	0.262	0.356	0.641	0.456	0.777	0.604	0.438	0.896	0.978	0.494	0.573	0.896

Supplementary Table 7.6 (B) Lipidomic in a total of 33/63 plasma LA (18:2) phospholipid molecular species over the 30-day krill oil or fish oil supplementation

LA (18:2) Molecular species	Concentration (pmol/mL)						*P* value												
	Krill oil			Fish oil			Krill oil[a]			Fish oil[a]			Krill oil : Fish oil[b]			Net iAUC	Time	Supple-	Inter-
	T = 0	T = 15	T = 30	T = 0	T = 15	T = 30	T0:T15	T0 : T30	T15:T30	T0:T15	T0:T30	T15:T30	T = 0	T = 15	T = 30			ment	action
PC (16:0/18:2)	315619	281164	293470	328529	296136	286175	0.061	0.217	0.487	0.077	**0.024**	0.573	0.466	0.399	0.679	0.776	**0.005**	0.630	0.613
PC (18:0/18:2)	131565	119317	133997	141823	131548	124427	0.287	0.830	0.205	0.370	0.136	0.532	0.371	0.288	0.403	0.605	0.200	0.596	0.334
PC (18:1/18:2)	31900	29461	30010	34020	28047	27076	0.445	0.553	0.862	0.071	**0.038**	0.760	0.506	0.656	0.359	0.216	**0.038**	0.730	0.514
LPC (18:2) [sn1]	18427	20915	20168	20985	19712	20707	0.353	0.513	0.778	0.632	0.916	0.708	0.340	0.651	0.839	0.162	0.914	0.743	0.604
PC (18:2/18:2)	13197	11237	13575	15900	13384	11549	0.353	0.856	0.270	0.236	**0.047**	0.384	0.204	0.310	0.337	0.283	0.100	0.496	0.232
PC (17:0/18:2)	7816	6837	7501	7799	7179	7124	0.089	0.571	0.240	0.270	0.232	0.922	0.976	0.540	0.500	0.773	**0.041**	0.961	0.657
LPC (18:2) [sn2]	5867	6483	6222	6745	6208	6521	0.438	0.653	0.741	0.497	0.776	0.691	0.272	0.727	0.705	0.165	0.992	0.565	0.585
PC (15-MHDA/18:2)	5857	4838	5352	5763	4806	4549	**0.037**	0.280	0.272	**0.048**	**0.015**	0.579	0.838	0.945	0.093	0.720	**0.007**	0.394	0.428
PC (P-16:0/18:2)	4500	3886	4730	4731	3912	3794	0.099	0.524	**0.028**	**0.032**	**0.016**	0.742	0.522	0.943	**0.016**	0.307	**0.005**	0.331	0.068
PE (18:0/18:2)	3367	2441	2178	3997	2552	2214	**0.042**	**0.011**	0.543	**0.003**	**0.000**	0.435	0.154	0.796	0.934	0.306	**0.000**	0.422	0.570
PC (16:1/18:2)	2580	1931	2012	2397	1856	1922	**0.001**	**0.004**	0.648	**0.006**	**0.013**	0.708	0.305	0.672	0.613	0.666	**0.001**	0.415	0.893
PE (P-18:0/18:2)	2554	1601	2161	3037	1962	1659	**0.008**	0.240	0.100	**0.004**	**0.000**	0.362	0.152	0.279	0.138	0.355	**0.000**	0.594	0.089
PE (P-16:0/18:2)	2376	1846	2285	2756	1909	1558	0.148	0.799	0.227	**0.026**	**0.003**	0.332	0.295	0.858	**0.053**	0.288	**0.001**	0.652	0.099
PE (16:0/18:2)	2250	1535	1251	2557	1602	1275	**0.019**	**0.002**	0.322	**0.003**	**0.000**	0.256	0.285	0.814	0.934	0.536	**0.000**	0.564	0.745
LPE (18:2) [sn1]	1582	1419	1265	1898	1303	1350	0.458	0.156	0.481	**0.012**	**0.019**	0.829	0.157	0.594	0.697	0.114	**0.016**	0.653	0.380
PE (P-18:1/18:2) (a)	1364	939	1077	1480	952	871	**0.029**	0.126	0.455	**0.008**	**0.003**	0.660	0.529	0.943	0.269	0.535	**0.000**	0.820	0.453
PC (O-18:1/18:2)	1245	1234	1434	1305	987	930	0.937	0.197	0.172	**0.036**	**0.016**	0.694	0.678	0.096	**0.002**	0.069	0.070	**0.004**	**0.035**
PE (18:1/18:2)	937	796	672	1199	817	692	0.401	0.124	0.463	**0.031**	**0.006**	0.457	0.129	0.900	0.909	0.177	**0.001**	0.413	0.502
PC (P-18:0/18:2)	793	643	825	820	669	657	**0.021**	0.604	**0.007**	**0.021**	**0.013**	0.839	0.667	0.670	**0.011**	0.433	**0.006**	0.474	**0.050**
LPE (18:2) [sn2]	747	667	608	922	626	651	0.418	0.163	0.542	**0.006**	**0.010**	0.798	0.082	0.672	0.658	0.058	**0.017**	0.534	0.295
PI (18:1/18:2)	374	412	406	409	401	355	0.578	0.641	0.927	0.910	0.429	0.496	0.610	0.873	0.455	0.348	0.770	0.831	0.669
PC (17:1/18:2)	323	330	352	320	280	273	0.835	0.356	0.471	0.192	0.133	0.833	0.899	0.107	**0.016**	0.195	0.789	0.082	0.225
PE (P-18:1/18:2) (b)	256	149	193	291	162	153	**0.004**	0.067	0.187	**0.001**	**0.000**	0.791	0.297	0.691	0.232	0.448	**0.000**	0.909	0.269
PE (P-20:0/18:2)	164	125	139	194	148	139	**0.013**	0.093	0.345	**0.004**	**0.001**	0.528	**0.050**	0.129	0.980	0.413	**0.001**	0.122	0.318
PE (17:0/18:2)	111	80	65	124	79	74	**0.040**	**0.004**	0.277	**0.003**	**0.002**	0.769	0.339	0.886	0.508	0.513	**0.000**	0.501	0.717
PE (O-16:0/18:2)	85	178	200	90	85	54	**0.004**	**0.001**	0.457	0.396	0.059	0.271	0.887	**0.001**	**0.000**	**0.010**	0.176	**0.000**	**0.001**
LPI (18:2) [sn1]	60	57	61	55	66	61	0.655	0.868	0.541	0.142	0.417	0.492	0.492	0.214	0.969	0.297	0.842	0.853	0.379
PI (17:0/18:2) (b)	42	39	39	43	41	38	0.584	0.648	0.927	0.701	0.311	0.523	0.812	0.687	0.742	0.947	0.546	0.866	0.860
PE (15-MHDA/18:2)	32	24	18	36	21	18	**0.044**	**0.002**	0.140	**0.001**	**0.000**	0.430	0.353	0.403	0.904	0.157	**0.000**	0.991	0.453
PE (16:1/18:2)	19	12	9	22	11	9	**0.018**	**0.003**	0.423	**0.001**	**0.000**	0.591	0.359	0.834	0.952	0.516	**0.000**	0.696	0.701
PE (O-18:1/18:2)	15	13	21	17	13	11	0.516	0.096	**0.026**	0.148	0.061	0.636	0.535	0.835	**0.006**	0.154	**0.050**	**0.049**	**0.039**
LPI (18:2) [sn2]	14	14	14	13	16	15	0.763	1.000	0.763	0.196	0.523	0.499	0.548	0.314	0.970	0.383	0.625	0.817	0.515
PI (17:0/18:2) (a)	7	7	10	9	8	7	0.878	**0.049**	**0.035**	0.701	0.324	0.541	0.228	0.324	0.077	0.401	0.480	0.761	0.072

Values are expressed as the mean of plasma LA (18:2) molecular species concentration (n = 11). Two-way analysis of variance for repeated measurements was performed to analyse supplementation effect over time (interaction time x supplementation), the difference between time point within supplementation (superscript with *a*) and each time point between the two omega-3 supplementation groups (superscript with *b*). All *p* values were corrected for multiple comparisons using the Benjamini-Hochberg FDR. The net iAUC (days from zero to 30) of plasma lipid molecular species was calculated using the trapezoid rule and compared between the two omega-3 supplementation groups using paired *t*-test. Abbreviation: net iAUC, the incremental area under the curve (days from zero to 30); DAG, diacylglycerol; dhCer, dihydroceramide; LPI, lyso-phosphatidylinositol PC, phosphatidylcholine; PE, phosphatidylethanolamine; PE (O), alkyl-phosphatidylethanolamine; PE (P), alkenyl-phosphatidylethanolamine; PI, phosphatidylinositol; pmol/mL, picomole per millilitre; PS, phosphatidylserine; SM, sphingomyelin; T0, baseline; T15, 15 days; T30, 30 days.

Chapter 8: General discussion and concluding remarks

8.1 INTRODUCTION

Lipids, as abundant metabolic substrates in the human body, play an important role in fat metabolism and homeostatic regulation related to immune function and many other physiological functions related to human health. It has been well established that plasma lipids are critical biomarkers for the pathophysiology of a wide range of human diseases (Berge et al. 2014, Miller et al. 2011, Skulas-Ray et al. 2011). Circulating fatty acids in the blood stream are initially influenced by dietary sources, and they are further metabolised in the body and can be stored mainly in the adipose tissue. Therefore, dietary lipids are considered as the most important environmental factors in contributing to the development of lifestyle-related diseases including obesity, diabetes, cardiovascular diseases (CVD) and certain cancers. The increased prevalence of these diseases has been considered to be a clinical challenge and an urgent public health issue (described in C*hapter two*).

Over several decades, a number of observational and clinical studies have reported consistent evidence on the health benefits of dietary long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA), particularly eicosapentaenoic acids (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3) (Lian et al. 2017, Mocellin et al. 2016, Nelson et al. 2017, Senftleber et al. 2017, Siscovick et al. 2017, Zarate et al. 2017). Omega-3 PUFA are essential components of the cell membranes and they have to be obtained from the diet. The major dietary source of EPA and DHA are fish and other seafood (Food Standards Australia New Zealand 2015). The health benefits of LC n-3 PUFA include the reduction in plasma triglyceride (TAG) (Leslie et al. 2015)

and inflammatory responses (Li et al. 2014, Mocellin et al. 2016) associated with cardio-metabolic conditions (Nelson et al. 2017), rheumatoid arthritis (Senftleber et al. 2017) and cancers (Mocellin et al. 2016). Moreover, increased intakes of dietary EPA and/or DHA have shown an improvement in degenerative and behavioural neurological conditions (Canhada et al. 2017, Mocking et al. 2016). Some authors suggested that the efficacy of LC n-3 PUFA on cognitive conditions was related to a high dose of EPA and/or DHA consumption. Higher doses of EPA in participants with major depressive disorder was beneficial when taking in conjunction with anti-depressants, based on a meta-analysis of 13 randomised clinical trials (Mocking et al. 2016). A dose-response meta-analysis of 21 cohort studies also reported that a higher consumption of dietary DHA was associated with a lower risk of dementia and Alzheimer's disease (Zhang et al. 2016b).

Over the last decades, in addition to fish oil, krill oil has been used to formulate dietary LC n-3 PUFA supplements over-the-counter rather than plant sources which contain α-linolenic acids (18:3n-3) (Williams and Burdge 2006). Krill oil, derived from Antarctic krill (*Euphausia Superba*), contains both EPA and DHA found mainly in the phospholipids (predominantly phosphatidylcholine), whereas in fish oil these fatty acids are commonly found in the TAG (Tou et al. 2007, Winther et al. 2011).

Several studies investigated whether krill oil has a better bioavailability of LC n-3 PUFA compared with fish oil in humans and animals. However, the findings on the bioavailability of LC n-3 PUFA from these two omgega-3 oils remain insufficient, particularly with human trials. This might be related to the apparent limitations of the study designs including different doses, chemical forms of LC n-3 PUFA in the oils, and target tissues used in those studies (as described in *Chapter two*) (Ghasemifard

et al. 2014). The apparent limitations of study design also include lack of control study participants with their total deitary fat intake and dose of LC n-3 PUFA supplementation on a body weight basis, and the measurement of excretion (Ghasemifard et al. 2014).

This thesis investigated two randomised crossover interventions for a period of (1) 5 hours and (2) 30 days. The aims of intervention to compare the bioavailability of LC n-3 PUFA between krill oil and fish oil supplementation. The aims of these studies were to investigate the effect of krill oil supplementation on the incorporation of LC n-3 PUFA (EPA, DHA and docosapentaenoic acids (DPA) into the plasma and circulating plasma lipids (particularly TAG), as well as their impacts on the inflammatory biomarkers compared with the fish oil supplementation (as described in *Chapters four and six*). In addition, the plasma fatty acids from both studies were further analysed for the profiles of molecular lipid species using the novel lipidomic technology, and results are discussed for the perspective of the duration of intervention (as described in *Chapters five and seven*). Therefore, this chapter, based on the overall outcomes, particularly with lipidomic changes over two different study periods, provides an examination of links between the two studies, and also develops a collective efficacy perspective on dietary LC n-3 PUFA in the krill oil compared with the fish oil.

In particular, a postprandial and a 30-day studies herein, (n = 10 and n = 11, respectively), have considered to minimise the inter-individual variability by including a single gender participants at similar age and by keeping individual's routine lifestyle, including dietary intake and physical activity regime (as described in *Chapter three* and detailed in sections 3.2.1 and 3.2.2). Consequently, the findings from these

clinical interventions can be considered as a starting point for possible therapeutic implementation associated with the maintenance of optimal health.

8.2 GENERAL DISCUSSION

8.2.1 The effect of krill oil on the incorporation of LC n-3 PUFA into plasma

A comparison between krill oil and fish oil supplementation was made for the efficacy of the incorporation of dietary LC n-3 PUFA into the plasma over (1) a postprandial 5-hour and (2) 30-day intervention periods. As described in *Chapters four and six*, the significantly increased levels of circulating plasma LC n-3 PUFA following the krill oil and fish oil supplementation over both the postprandial and the longer-term interventions. These findings were consistent with previous studies through postprandial and longer-term dietary LC n-3 PUFA interventions (Kagan et al. 2013, Maki et al. 2009, Ramprasath et al. 2013, Ulven et al. 2011).

In the postprandial study, the similar incorporation of EPA into the plasma, as presented in the incremental area under the curve from the baseline (net iAUC $_{0\text{-}5\ h}$), suggested that there was an effective incorporation of EPA from krill oil compared with that from fish oil since there was a 31% lower dose of EPA in the krill oil than the dose in the fish oil. Moreover, in the 30-day intervention study, the incorporation of EPA into the plasma following the krill oil supplementation resulted in a significantly greater net iAUC $_{0\text{-}30\ d}$ than that of the fish oil supplementation ($p < 0.05$). Overall, these findings from both postprandial and longer-term studies suggest that the incorporation of EPA into the plasma following krill oil supplementation was more efficient than that from fish

oil. The reason for the efficient digestion and absorption of EPA from krill oil is probably due to the high content of phospholipids and free fatty acids in this oil. Given the well reported health benefits of EPA, these results implicate that the krill oil supplementation (with the same dose of EPA) may result in better health outcomes compared with the fish oil supplementation (Nelson et al. 2017).

8.2.2 The effect of krill oil on circulating plasma lipids, particularly TAG

Abnormal levels of circulating plasma lipids and lipoproteins have been recognised as critical risk factors for developing cardio-metabolic diseases. Accumulated evidence suggested that the acute response of TAG is associated with atherogenic risks in which significant changes in lipemic responses is attributed to a high-fat meal (≥ 40 g). However, the acute lipemic responses of individual fatty acids in different clinical studies remain controversial (Bonham et al. 2013, Teng et al. 2015a). The TAG-lowering effect has been one of the consistent effects of EPA and/or DHA consumption in doses ranging from 1 g to 5 g/d (Leslie et al. 2015, Lopez-Huertas 2012). Therefore, the current research investigated the responses of the plasma lipids, particularly TAG following the krill oil supplementation compared with the fish oil supplementation over the 5-hour postprandial and the 30-day periods (as discussed in *Chapters four and six*).

In the postprandial study, there were no significant differences in the levels of TAG, cholesterol and lipoproteins in either plasma or chylomicron fractions between the three oil supplementation (krill oil, fish oil and olive oil). A previous postprandial study also reported that there was no significant difference in the postprandial lipemic

changes between individuals with metabolic syndrome and those without metabolic syndrome following a high-fat meal (Bonham et al. 2013). The lack of significant postprandial responses in plasma lipid TAG and CM between the oil supplementation in the present study may be related to the lower content of dietary fats (20 g/meal) rather than the types of fatty acids in the oils, although it has been suggested that both amount and type of dietary fats are associated with the alteration of plasma lipids (TAG or cholesterols) (Teng et al. 2015a). It has been reported that the postprandial response of TAG in females was smaller than males, and individuals with diabetes had a greater alteration of plasma TAG than healthy individuals (Nappo et al. 2002, Sanders et al. 2011). Unlike the postprandial response of TAG, the 30-day krill oil and fish oil supplementation (containing 1,269 mg/d and 1,441 mg/d of LC n-3 PUFA, respectively) significantly reduced the plasma TAG although there was no evident differences between the two omega-3 oil supplementation groups. Other longer-term dietary intervention, ranging from 4 to 7 weeks, did not observe significant changes in the plasma TAG following either the krill oil or the fish oil supplementation providing EPA and DHA (ranging from 0.5 to 0.9 g/d) (Ramprasath et al. 2013, Ulven et al. 2011).

8.2.3 The effect of krill oil on circulating plasma pro-inflammatory biomarkers

Dietary LC n-3 PUFA have shown anti-inflammatory effects in terms of the development and progression of various inflammatory processes in rheumatoid arthritis, diabetes and CVD, as described in *Chapter two* and detailed in section 2.3.3. The defence mechanism in the human body increases various metabolic and immune responses such as elevated levels of eicosanoids, cytokines, and pro- and anti-

inflammatory molecules which are a part of inflammatory responses in various conditions. There has been consistent evidence that LC n-3 PUFA derived from marine sources can lead to a significant reduction in plasma pro-inflammatory biomarkers including C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in populations with the chronic cardio-metabolic conditions. A review of 68 studies (Li et al. 2014) reported that the efficacy of dietary LC n-3 PUFA on the levels of CRP, IL-6 and TNF-α level following longer-term supplementation (≥ 12 weeks) was greater in non-obese individuals than overweight or obese individuals.

In the current longer-term study, the level of plasma inflammatory biomarkers including interferon-gamma, TNF-α and interleukins following the 30-day supplementation with krill oil or fish oil were investigated (as discussed in *Chapter six*). Four of ten inflammatory cytokines including IL-1β, IL-10, IL-4 and IL-5 as well as the total number of white blood cells were significantly decreased from the baseline following the 30-day krill oil supplementation ($p \leq 0.05$). In contrast, none of the cytokines was significantly changed following the 30-day fish oil supplementation. The plasma levels of IL-5 and IL-10 at days 30 were significantly different between the two omega-3 oil supplementation groups ($p \leq 0.05$). For the greater decrease in plasma IL-10, recognised as an anti-inflammatory cytokine, following the krill oil supplementation compared with the fish oil supplementation, the mechanism of action has not been reported previously. Other longer-term studies (ranging from 4 to 7 weeks) did not also observe significant changes in inflammatory markers such as plasma hsCRP which may be related to the lower amount of LC n-3 PUFA intake (ranging from 309 to 645 mg/d) (Maki et al. 2009, Ulven et al. 2011).

8.2.4 The effect of krill oil on circulating plasma lipid molecular species containing LC n-3 PUFA

Lipids play a critical role as constituents of various membranes and precursors of lipid mediators. An enormous number of studies in biological samples have been investigated to determine the lipid status associated with pathophysiological relevant diseases, including CVD and diabetes (Diem et al. 2016). The development of lipid analysis has been in demand resulting in the recent innovation of mass spectrometry technique, namely lipidomics (as discussed in *Chapter three* and detailed in section 3.5). The application of lipidomics has been increased to study various lipid classes representing important signalling molecular species for regulation of multiple cellular functions (Oliveira et al. 2016, Zhang et al. 2015). Due to the high sensitivity and specificity of lipidomics, it is possible to understand the insight in biochemical metabolic pathways through identified lipid molecular species (Ekroos et al. 2010, Zhao et al. 2015). This can be further facilitated for the discovery of various human diseases at different stages.

In this thesis, lipidomic responses in the plasma were comparerd for significant differences in a number of molecular species containing LC n-3 PUFA between the two omega-3 oil supplementation groups over both (1) the postprandial (at zero, 3 and 5 hours) and (2) the 30-day (at zero, 15 and 30 days) periods. The novel findings of lipidomic responses from these two studies were consistent on that LC n-3 PUFA in ether-phospholipid molecular species, particularly alkenylphosphatidylethanolamine, were significantly increased following the krill oil supplementation when compared with the fish oil supplementation.

There were significant increases in the lipid molecular species containing EPA and DHA following krill oil and fish oil supplementation over both the postprandial and the longer-term periods. Other human and animal studies reported similar findings that the level of EPA- and DHA- containing lipid molecular species following dietary LC n-3 PUFA, derived from fish oil or fatty fish, were significantly increased over 4 - 8 weeks intervention periods (Block et al. 2010, Lankinen et al. 2009, Ottestad et al. 2012). Attributed to the sensitive settings of a high-performance liquid chromatography electrospray ionisation-tandem mass spectrometry, the lipidomics for the postprandial study were able to identify TAG species in more details compared with the longer-term data, in which mainly phospholipid species were detected. This limitation was due to the specific settings of the equipment available in the Metabolomics Laboratorylaboratory at Baker Heart and Diabetes Institute when the plasma samples were analysed.

In the 5-hour postprandial study, the krill oil supplementation (providing 907 mg/d of LC n-3 PUFA) demonstrated greater efficacy for the incorporation of plasma lipid classes, and molecular species containing LC n-3 PUFA, particularly EPA, compared with fish oil (providing 1,441 mg/d of LC n-3 PUFA). The krill oil supplementation had a significantly greater net $iAUC_{0\text{-}5\ h}$ for 16/33 EPA-phospholipid molecular species and 11/49 DHA-phospholipid molecular species than the fish oil supplementation (described in *Chapter five* and detailed in Table 5.2) ($p \leq 0.05$). On the other hand, the fish oil supplementation had a significantly greater net $iAUC_{0\text{-}5\ h}$ for 7/21 EPA-TAG and EPA-diacylglycerol (DAG) molecular species than the krill oil supplementation. This clearly indicates that the greater increases in EPA-phospholipid molecular species were associated with the biochemical form of EPA from krill oil, whereas the

EPA from fish oil was associated with increased EPA-TAG and EPA-DAG molecular species.

Likewise, in the 30-day intervention, both the krill oil (providing 1,269 mg/d of LC n-3 PUFA) and the fish oil supplementation (providing 1,441 mg/d of LC n-3 PUFA) showed a significant efficacy on the incorporation of some plasma lipid classes, and molecular species containing LC n-3 PUFA, particularly EPA. A total of 23 EPA-containing molecular species and a total of 46 DHA-containing molecular species were significantly increased, whereas there was a significant decrease in a total of 53 AA-containing molecular species after both 30-day krill oil and fish oil supplementation. The longer-term krill oil supplementation also resulted in a greater increase in net $iAUC_{0\text{-}30\ d}$ of ether-phospholipid species than the fish oil supplementation. The net $iAUC_{0\text{-}30\ d}$, of a total 20 lipid molecular species containing EPA, DHA and DPA, including 19/20 phospholipid species and 1/20 DAG species was significantly different between the two omega-3 oil supplementation groups (described in *Chapter seven* and detailed in Table 7.2). For 14 cases of 19 phospholipid molecular species including 12 ether-phospholipid species, the net $iAUC_{0\text{-}30\ d}$ following the krill oil supplementation was significantly greater than the fish oil supplementation. In contrast, five out of seven diacyl-phospholipid molecular species containing EPA, DHA and DPA following the fish supplementation oil was significantly greater in the net $iAUC_{0\text{-}30\ d}$ than the krill soil supplementation.

In summary of lipidomic data from the 5-hour postprandial and the 30-day supplementation studies, the findings might be associated with the form of LC n-3 PUFA. Krill oil contains a high content of phospholipids (predominantly phosphatidylcholine), while in fish oil, EPA and DHA are commonly found in TAG (Tou

et al. 2007, Winther et al. 2011). Our own data of study oil analysis (described in *Chapter three* and detailed Table 3.2) support that the krill oil contains a high proportion of phospholipids (61% of the total lipid classes) and TAG (24% of total lipid classes). Whereas, the fish oil contains 98% TAG, but only 1% phospholipids. In addition, there is evidence that krill oil contains a low level of ether-phospholipids, having an alkyl or an alkenyl bond. Several human lipidomic studies have evaluated the ether-phospholipid species status in relation to different health conditions. Four human lipidomic studies reported that there is an inverse association between ether-phospholipid species and various health status in individuals with obesity, diabetes, cardio-metabolic syndrome and/or rheumatoid arthritis (Fang et al 2016, Meikle et al 2014, Meikle et al 2013, Meikle et al 2011, Pietiläinen et al 2007). However, the interpretation of the findings is not necessarily clear due to different clinical endpoints (Meikle et al. 2014, Murphy and Nicolaou 2013, Wood et al. 2016, Wood et al. 2015). Therefore, further investigations with large number of participants are warranted to elucidate the differences in lipidomic responses between the krill oil and fish oil supplementation

8.3 GENERAL CONCLUDING REMARKS

One of the rich sources of EPA and DHA is the krill oil, extracted from the krill *(Euphausia Superba)* living in Southern oceans. In krill oil, EPA and DHA are found in phospholipids (predominantly phosphatidylcholine), free fatty acids, and in the triglycerides (TAG) whereas in fish oil they are mainly in TAG. Over the last decades, the efficacy of EPA and DHA in krill oil compared with fish oil has been explored although the outcomes from human and animal studies have been inconsistent. In this thesis, a 5-hour postprandial and a 30-day randomised crossover studies with healthy

women revealed remarkable differences between krill oil and fish oil supplementation. Importantly, the results, including the novel findings of lipidomic profiles, presented in this thesis make an important contribution to the literature and highlight the differences in the bioavailability of krill oil compared with fish oil from a combination of postprandial and longer-term observations in healthy young women.

In regards to the hypotheses in this thesis, the outcomes of incorporation of total plasma LC n-3 PUFA did support that both the postprandial and the longer-term supplementation with krill oil would be more effective on the incorporation of LC n-3 PUFA into plasma compared with the fish oil supplementation. The response of plasma TAG to the omega-3 oil supplementation did not show differences between krill oil and fish oil for either the postprandial or the long-term periods. The impact of supplementation on inflammatory biomarkers supported the hypothesis that the 30-day krill oil supplementation would show different effects on plasma cytokines compared with the fish oil supplementation. The lipidomic profiles in the longer-term study supported the hypothesis that there were significant differences between the krill oil and fish oil supplementation groups in terms of the incorporation of LC n-3 PUFA into different plasma molecular species.

In conclusion, the postprandial and the longer-term interventions presented in this thesis suggest that the krill oil supplementation is more potent than the fish oil supplementation in the incorporation of LC n-3 PUFA into the plasma, and reducing plasma pro-inflammatory cytokines although there was no significant difference of plasma TAG-lowering effect between the two oil supplementation. Moreover, lipidomic profiles in the postprandial and the longer-term intervention studies highlight that the incorporation of LC n-3 PUFA into different molecular species following the two

omega-3 oil supplementation groups. In particular, ether-phospholipids species containing LC n-3 PUFA, following the krill oil supplementation, was significantly different from the fish oil supplementation although the different lipidomic responses between the two omega-3 oil supplementation need to be further investigated in relation to the effect of n-3 PUFA in different lipid species. Taken together, the findings in this thesis might contribute to the current literature associated with potential preventative nutritional therapeutic implications for the general population related to the increased global prevalence of lifestyle-related diseases. Furthermore, the advantageous information of molecular lipid species of krill oil might provide important phenotype information to enhance understanding of lipid metabolic interactions, advancing cardio-metabolic and anti-inflammatory pathways.

Key messages from the research in this thesis are:

1. This thesis has shown clearly there are significant differences between krill oil and fish oil in terms of incorporation of LC n-3 PUFA into plasma lipids.

2. This thesis has demonstrated that such differences were only revealed by use of lipidomics, a highly sensitive technique.

3. This thesis has identified for the first time a connection between the krill oil and ether lipids. Ether lipids are becoming increasingly important in various disease states as low levels have been observed in a range of disorders. Researchers are looking for ways to increase ether lipid levels for disease prevention and treatment, and it is possible that krill oil might be one option.

4. Ether lipids and krill oil clearly a future research area.

Chapter 9: Strengths and limitations of the present research, and recommendations for future research

This thesis comprised two clinical interventions aimed at enhancing understanding of bioavailability of krill oil compared with fish oil in humans. Given the prominent role of dietary lipids, the present randomised cross-over studies in women have investigated the different responses between krill oil and fish oil supplementation over a postprandial and a longer-term periods. The findings in this thesis have made some important contributions to the literature, particularly in relation to women, and with the novel lipidomic data. Herein, based on the findings from the current clinical interventions, the following are summarised for the study strength and limitations which may be considered for future research in this area:

9.1 POSTPRANDIAL BIOAVAILABILITY OF KRILL OIL SUPPLEMENTATION

The **strength** of postprandial clinical intervention over the 5-hour period includes the following aspects:

(1) the first study showing that krill oil and fish oil are very different in terms of incorporation of LC n-3 PUFA into lipid classes in the body;

(2) novel data on molecular lipid species since lipidomics is a highly sensitive technique which allows the investigation of many different lipid classes and molecular species at the same time; and

(3) provision of insights into dynamic plasma responses by analysing plasma TAG and phospholipid or plasma total lipids rather than just plasma TAG. **Limitations** of the postprandial trial include:

(1) small number of participants, only 10, completed the trial. A larger number of participants would reduce the variability between individuals' responses;

(2) only 5-hour period was studied. A longer time frame should be a focus of future studies because the individual response in plasma fatty acid lipids (EPA and DHA) increase lasts for at least 24 hours after a single dose of supplementation (Kagan et al. 2013);

(3) only female participants were included. Future studies should include both male and female participants so a comparison can be made and that will help us to understand the gender difference in lipid dynamics in response to krill oil and fish oil supplementations;

(4) different amounts of LC n-3 PUFA from krill oil and fish oil were used. Future studies should use the matched dose of LC n-3 PUFA from two oils based on the body weight (Ghasemifard et al. 2014); and

(5) the total dietary fat intake needs to be controlled because dietary intake reflects the qualitative/quantitative status of plasma fatty acids (Lopes et al. 1991). Difficulties associated with this include: (i) the expense involved for a PhD project; (ii) keeping participants under study for 24 hours; and (iii) monitoring of fat intake in autonomous participants.

9.2 LONGER-TERM BIOAVAILABILITY OF KRILL OIL SUPPLEMENTATION

In addition to the postprandial clinical intervention, the **strength** of longer-term clinical intervention over 30-day period include the following aspects:

(1) the first time this study provided insights into dynamic plasma responses over the 30-day period, particularly with the findings in the incorporation of plasma LC n-3 PUFA, TAG and the sensitive and comprehensive lipid molecular species containing LC n-3 PUFA. It has been suggested that lipidomics should be added to the prognostic and diagnostic tools investigating the growing prevalence of lifestyle-related diseases including cardiovascular diseases and rheumatoid arthritis (Ekroos et al. 2010, Fang et al. 2016);

(2) the dynamic plasma responses by analysing both plasma TAG plus phospholipid and plasma total lipids were studied which is different from the current available literature, and

(3) the novelty of data attributed to advanced lipidomics. This highly sensitive technology generated a large number of data on lipid classes and lipid molecular species at the same time.

Limitations of longer-term intervention study are:

(1) only 11 participants were involved, therefore in the future studies a larger number of participants should be recruited in an attempt to reduce the effect of variations

between individuals. A recent lipidomic study with healthy participants reported a high degree of inter-individual variability in the lipemic responses (Nording et al. 2013);

(2) only female participants were studied, therefore in the future male participants should also be included;

(3) additional analysis could include the measurement of the n-3 index for a better understanding of longer-term lipid metabolism (Harris and von Schacky 2004, von Schacky 2014);

(4) future studies should consider recruiting participants with lower baseline levels of dietary LC n-3 PUFA intake (≤ 150 mg/d) in order to maximise the changes in plasma LC n-3 PUFA in the interventions and ideally with a limited range of age and/or a body mass index to minimise the variability between individuals. In the present longer-term study, the range of baseline LC n-3 PUFA was from 28 mg/d to 416 mg/d, and

(5) future studies could focus more on ether lipids in order to provide insight on diverse and complex biological lipids, particularly for plasmalogens which have been suggested to be associated with reduced levels of oxidative stress and inflammation, as well as improved cognitive and visual functions (Farooqui and Horrocks 2001, Rasmiena et al. 2015), and

(6) to take into account that krill oil contains astaxanthin, and therefore krill oil may be more oxidatively stable compared with fish oil (Barros et al. 2014a, Pashkow et al. 2008). Astaxanthin may influence the results since some studies on astaxanthin have suggested anti-inflammatory functions (Grimstad et al. 2012, Tou et al. 2007) and/or

antioxidant effects on cardiovascular events (Pashkow et al. 2008), as well as the potential to promote or maintain neural plasticity (Grimstad et al. 2012).

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