The effect of consuming farmed salmon compared to salmon oil capsules on long chain omega 3 fatty acid and selenium status in humans

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ABSTRACT

Salmon is a good source of long chain (LC) omega 3 fatty acids and selenium; these are well recognised for their health benefits. Recommendations for LC omega 3 fatty acid intakes presume equivalence between fish and fish oil. The aim of this research was to compare the effects of consuming salmon with salmon oil capsules on LC omega 3 fatty acid and selenium status.

Forty four healthy subjects were randomly assigned to consume either two servings of 120 g farmed New Zealand King (FNZK) salmon/week or 2, 4 or 6 capsules of salmon oil/day for 8 weeks. Fasting blood samples, anthropometric measures, food consumption habits information and blood pressure (BP) measurements were obtained at the study commencement and ending.

Each subject's intake of LC omega 3 fatty acids and selenium was determined by analysing the fatty acid and selenium content of duplicate portions of cooked salmon and capsules. The amount of salmon consumed was then calculated by subtracting unconsumed amounts of salmon and then calculating the intake of LC omega 3 fatty acids as grams of LC omega 3 fatty acids consumed per day. Percentage of compliance to capsule intake, based on counts of unconsumed capsules, was calculated to determine the amount of LC omega 3 fatty acids consumed per day from capsules. Change in red blood cells (RBC) LC omega 3 fatty acid levels from equivalent amounts of LC omega 3 fatty acids consumed from capsules and salmon were compared using linear regression analysis predictive models fitted to the capsule data. Omega 3 index was calculated.

LC omega 3 fatty acid intakes from salmon and 2, 4 and 6 capsules were 0.82, 0.24, 0.47 and 0.68 g/day, respectively. Equal amounts of LC omega 3 fatty acids consumed from salmon and capsules resulted in similar increases in RBC LC omega 3 fatty acids and omega 3 index (RBC eicosapentaenoic acid (EPA): 0.80 [0.58 – 1.02] vs. 1.00 [0.71 – 1.27] %; RBC docosahexaenoic acid (DHA): 0.93 [0.58 – 1.29] vs. 0.99 [0.68 – 1.31] %; omega 3 index: 1.92 [1.46 – 2.38] vs. 2.25 [1.65 – 2.83] %). The capsules did not contain selenium, but the salmon provided 6.84 μg selenium/day. Plasma selenium concentrations increased significantly in the salmon group compared to the capsule

group (0.16 ±0.13 vs. 0.02 ±0.13 μmol/l). Whole blood glutathione peroxidase (GPx) did not change with either treatment.

Salmon and capsules were equally effective in increasing RBC LC omega 3 fatty acid levels and omega 3 index. Consuming salmon also increased plasma selenium concentrations. Thus, omega 3 fatty acid status can be improved by either method according to consumer preference; however salmon has the added benefit of increasing plasma selenium status.

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TABLE OF CONTENTS

LISTS OF TABLES

LISTS OF FIGURES

LIST OF ABBREVIATIONS

Δ	end value – baseline value
AA	arachidonic acid
AI	adequate intake
ALA	α-linolenic acid
B	baseline value
BMI	body mass index
BP	blood pressure
CHD	coronary heart disease
CVD	cardiovascular disease
DART	diet and reinfarction trial
DART 2	diet and angina randomised trial
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
E	end value
EAR	estimated average requirement
EPA	eicosapentaenoic acid
FAME	fatty acids methyl esters
FFQ	food frequency questionnaire
FNZK salmon	farmed New Zealand King salmon
FSANZ	food standard Australia New Zealand
GC	gas chromatography
GISSI	gruppo Italiano per la sperimentazione della streptochinasi nell'Infarto miocardico
GPx	glutathione peroxidase
HDL-C	high density lipoprotein cholesterol
JELIS	Japan eicosapentaenoic acid lipid intervention study
LA	linoleic acid
LC	long chain
LDL-C	low density lipoprotein cholesterol
MI	myocardial infarction
MUFA	monounsaturated fatty acids
PUFA	polyunsaturated fatty acids
RBC	red blood cell
RCT	randomised controlled trial
RDI	recommended daily intake
SDT	suggested dietary target
SFA	saturated fatty acids
TC	total cholesterol
TC:HDL-C	total cholesterol to high density lipoprotein cholesterol ratio
TG	triacylglycerols
UL	upper level of intake
VLDL-C	very low density lipoprotein cholesterol
waist:hip	waist circumference to hip circumference ratio

CHAPTER 1

1. INTRODUCTION

Long chain (LC) omega 3 fatty acids have been shown to have many biological functions and affect several disease processes. They play a significant role in cellular membrane structure, are required for growth and are used in the development and function of the brain, neural tissue and eyes. Intake of LC omega 3 fatty acids have also been shown to play an important role in the prevention of cardiovascular disease (CVD), inflammatory disorders, behavioural disorders, osteoporosis and some cancers. A substantial amount of evidence exists to support the role that LC omega 3 fatty acids play in decreasing the risk of CVD. The potential mechanisms through which LC omega 3 fatty acids decrease the risk of CVD include reduction in triacylglycerol (TG) concentrations, blood pressure (BP), cardiac arrhythmia, platelet aggregation, inflammatory response, growth of atherosclerotic plaque and improved endothelial function (Kris-Etherton et al., 2002). The LC omega 3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from marine sources, have been shown to be largely responsible for these beneficial effects (Burr et al., 2005).

The omega 3 index has recently been introduced as a novel, physiologically relevant, modifiable and independent marker of risk for sudden cardiac death (Harris & von Schacky 2004). The omega 3 index can be calculated by the level of EPA and DHA in red blood cell (RBC) membranes expressed as a percentage of total fatty acids in RBC (Harris, 2008). An omega 3 index of greater than 8 % is associated with the highest cardio-protective effect (Harris & von Schacky, 2004).

Various recommendations for LC omega 3 fatty acid intake have been proposed by several organisations. On average an intake of 0.5 g LC omega 3 fatty acids per day is recommended for healthy individuals (Gebauer et al., 2006; Ministry of Health, 2006; Mozaffarian & Rimm, 2006). LC omega 3 fatty acids (specifically EPA and DHA) can be consumed from various different marine sources that obtain LC omega 3 fatty acids from single-cell phytoplankton and algae. Fish consume these single-cell phytoplankton and algae and LC omega 3 fatty acids are deposited into the fish tissue. Fish with high LC omega 3 fatty acid contents are often referred to as ‘fatty fish’ due to their significant fat content (≥ 12 % fat (Kolakowska et al., 2006)). The Heart Foundation of

New Zealand (Roberts, 1999) recommends consuming 1 to 2 servings of fatty fish per week. Fish oil supplements are also a significant source of LC omega 3 fatty acids. To meet the recommendation for omega 3 fatty acid intake from fish oil requires daily consumption. The recommended intakes for omega 3 fatty acids presume equivalence between fatty fish and fish oil supplements. However, there are few studies that have investigated the effects of different food matrices (fish and fish oil supplements) on LC omega 3 fatty acid status (Harris et al., 2007).

Visioli et al. (2003) demonstrated that omega 3 fatty acids from fish are more effectively incorporated into plasma lipids than when administered as capsules. The researchers speculated that most of the EPA and DHA is esterified in the sn-2 position of the TG in the fish. At this position EPA and DHA are, to a large extent, preserved from hydrolysis during digestion and intestinal absorption of exogenous fat. However in fish oil supplements EPA and DHA are found predominately in the sn-1 and sn-3 positions (Wijesundera & Abeywardena, 2004). Another study by Elvevoll et al. (2006) reported fish consumption caused a greater increase in serum omega 3 fatty acids when compared to consumption of cod liver oil. The authors proposed that consumption of fish created a dilute emulsion of omega 3 fatty acids causing a higher surface interaction between the food and intestinal wall. This may also create more favourable secretion of substances that aid the absorption of lipids (Elvevoll et al., 2006; Visioli et al., 2003). However two recent studies concluded that omega 3 fatty acids from fish or supplements were equally effective at increasing blood lipids with omega 3 fatty acids (Arterburn et al., 2008; Harris et al., 2007).

There were several design limitations in the above-mentioned similar studies, these included not matching the amount of omega 3 fatty acids present in capsules to those in the fish (Elvevoll et al., 2006) and not ensuring compliance of the subjects to the treatment (Elvevoll et al., 2006; Visioli et al., 2003). Between the different studies there were also variations in duration of supplementation and biomarkers used to measure the change that may have accounted for the difference in conclusions reached. These variations are important as changes in biomarkers can only be measured with different durations of supplementation. Neither did these studies investigate the recommended intake of fatty fish. In fact, Visioli et al. (2003) required subjects to consume 100g of

smoked salmon per day. This is an unrealistically high and costly intake that could not be maintained for extended periods of time.

Currently there is very limited data on the omega 3 fatty acid status of New Zealanders. Two studies that reported data on the omega 3 fatty acid status of the New Zealand population indicate that, like the rest of the Western World, their omega 3 fatty acid status is generally low (Hibbeln et al., 2006). Farmed New Zealand King (FNZK) salmon is a significant source of LC omega 3 fatty acids, but fish oil capsules could also be consumed to increase the LC omega 3 fatty acid status of the New Zealand population.

Whether consumers choose to consume fish or fish oil supplements depends on several factors other than the rate of omega 3 fatty acid incorporation into RBC membranes. These include the individual's tolerance of fish oil supplements and the cost of supplements versus fish.

Consuming salmon may be a more beneficial way of improving the omega 3 fatty acid status as it may replace other protein sources that contain high levels of saturated fatty acids (SFA). In addition, salmon contains not only omega 3 fatty acids, but many other beneficial nutrients such as selenium.

Research has shown that the selenium status of the New Zealand population is relatively low in comparison to other countries due to the low soil content (Thomson, 2004b). Selenium is used in several biological reactions through selenoproteins. Selenoproteins act as antioxidants and are involved in thyroid hormone metabolism, immune functionality and reproduction. Some research has shown selenium intake to be related to a decrease in the development of chronic diseases e.g. CVD (Froslie et al., 1985). Fatty fish (herring, salmon or mackerel) have previously been shown to increase selenium status (Thorngren & Akesson, 1987).

The research described herein investigated the effects that consumption of salmon and salmon oil supplements (available on the New Zealand market) had on healthy New Zealanders. The assumption that consuming LC omega 3 fatty acids from fatty fish and fish oil supplements are equivalent at increasing omega 3 fatty acid status was

investigated. This study also examined the effects of consuming the recommended 2 servings of fatty fish per week, including the impact this had on food consumption habits. The tolerance of the treatment regimens (salmon vs. salmon oil capsules) was also determined. As research indicates that the New Zealand population has low selenium status and salmon has been shown to be a good source of selenium, adding salmon to the diet was investigated as a way of improving selenium status within this population.

1.1 PURPOSE OF THE STUDY

1.1.1 AIM

To compare the effects of consuming LC omega 3 fatty acids from farmed salmon or salmon oil supplements. Furthermore, consideration was also given to other contributing factors related to consuming fatty fish, such as selenium status.

1.1.2 OBJECTIVES

1.1.2.1 PRIMARY OBJECTIVES

To compare the effects of consuming equivalent amounts of omega 3 fatty acids from weekly intakes of FNZK salmon with daily intakes of salmon oil capsules for a period of 8 weeks in healthy subjects aged between 21 – 45 years on:

- RBC levels of LC omega 3 fatty acids (EPA and DHA);
- the omega-3 index; and
- selenium status (plasma selenium and whole blood glutathione peroxidase (GPx)).

1.1.2.2 SECONDARY OBJECTIVES

- To determine the effects of consuming two servings (120 g/serving) of FNZK salmon per week with daily intake of salmon oil capsules (2, 4 or 6 capsules taken with a meal) for 8 weeks in healthy subjects aged between 21 – 45 years on:
  - blood lipid profiles (total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), ratio of total cholesterol to high density lipoprotein cholesterol (TC:HDL-C) and (TG); and
  - BP.

- To investigate the effect of consuming two servings (120 g/serving) of FNZK salmon per week on food consumption habits.

- To compare the tolerability of consuming two servings (120 g/serving) of FNZK salmon per week to a daily intake of salmon oil capsules.

1.1.3 HYPOTHESIS

H_1: It is hypothesised that the consumption of equivalent amounts of long chain omega 3 fatty acids from weekly intakes of FNZK salmon compared with daily intake of salmon oil capsules for 8 weeks in healthy New Zealand subjects aged between 21 – 45 years will result in significantly higher RBC levels of LC omega 3 fatty acids (EPA and DHA) and omega 3 index.

H_2: It is hypothesised that consumption of weekly intake of FNZK salmon per week will result in a significant increase in selenium status when compared with daily intake of salmon oil capsules for 8 weeks in healthy New Zealand subjects aged between 21 – 45 years.

1.1.4 STRUCTURE OF THE THESIS

This introductory section discussed the rationale for the investigation, including the aims and objectives of the study. In chapter 2 the literature relating to the description and classification of omega 3 fatty acids, including its effect on diseases and disorders and possible health benefits, is reviewed. This review also contains information on the effects diet, age, gender and lifestyle factors can have on omega 3 fatty acid status. The methods of consuming omega 3 fatty acids are also discussed including how the manufacturing process affects the source of omega 3 fatty acids and resulting physical and chemical changes between the methods of consumption. Literature has also been reviewed on selenium, and specifically its relevance to the New Zealand population including the contribution salmon may play in increasing selenium status.

A description of the methodology used during the study, including subject characteristics, treatments used, blood sampling method as well as the statistical

analysis is presented in chapter 3. The results have been reported in the form of tables and graphs in chapter 4. These results are discussed in detail in chapter 5 which includes a review of the factors known to influence omega 3 fatty acid and selenium status. Similar studies that have been conducted are also reviewed and their results are compared to the findings of the study reported here. Finally in chapter 6 conclusions are drawn and recommendations are made for future research and development.

CHAPTER 2

2. LITERATURE REVIEW

The purpose of the study reported herein was to compare the effects of consuming salmon or salmon oil capsules on LC omega 3 fatty acid and selenium status. The literature review focuses on methods of increasing omega 3 fatty acid status, including factors that influence omega 3 fatty acid bioavailability. Literature on the selenium status of the New Zealand population was specifically reviewed, along with information regarding the impact that fish consumption may have on the selenium status of this population.

2.1 OMEGA 3 FATTY ACIDS

2.1.1 OMEGA 3 FATTY ACIDS: CLASSIFICATION AND CHEMISTRY

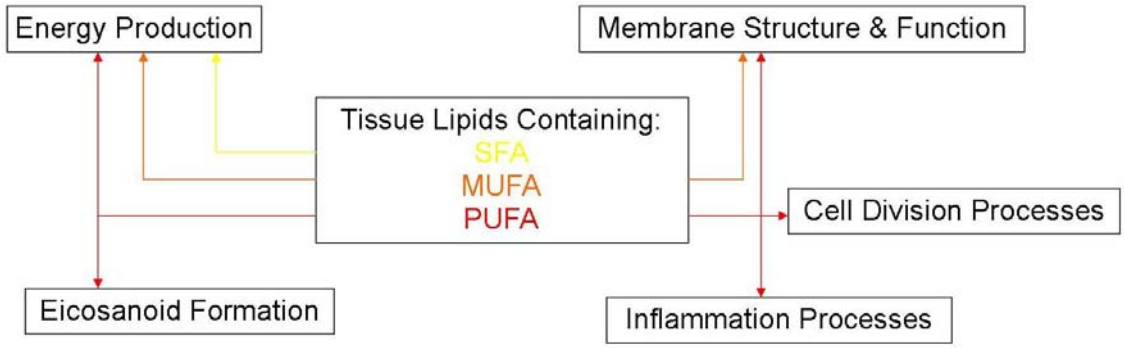
The role of lipids is not limited to providing the body with a rich energy source. Lipids also serve other purposes, including carrying fat soluble vitamins and providing essential fatty acids (Bockisch, 1993).

There are different categories of lipids that contain fatty acids:

1. TG – contain 3 fatty acids and glycerol. They are found in fats and food oils and are stored in adipose tissue of humans.
2. Glycolipids – contain fatty acids and sugars. Glycolipids are associated with cell surfaces.
3. Phospholipids – contain fatty acids, glycerol and phosphorus. These are structural components in cells and supply fatty acid precursors for the synthesis of eicosanoids.
4. Sphingolipids – contain fatty acids and LC amines. Sphingolipids are important components of brain tissue and the central nervous system.
5. Sterols – contain fatty acids as sterol esters. Cholesterol is the primary sterol in humans. It plays a structural role in cell membranes and is a precursor of steroid hormones, vitamin D and other fat emulsifying agents in bile (Wahlqvist, 2002).

TG are the most common (estimated to account for 95%) form of lipid found in food. This is also the form in which most fat is stored in the body (Thompson et al., 2008).

Fatty acids are involved in a number of diverse and important biological processes (see Figure 1). Unsaturated fatty acids play several important roles in the body, including maintaining membrane fluidity and structure. Short-chain fatty acids are considered to contain fewer than six carbons, medium-chain have between 6 and 12 carbons, and LC fatty acids have 14 or more carbons. Structural lipids in mammals consist of fatty acids containing between 10 – 24 carbons (Metherel, 2007; Thompson et al., 2008; Wahlqvist, 2002).



SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Figure 1. Biological roles of fatty acids (Wahlqvist, 2002)

In phospholipids fatty acids are found in the sn-1 and sn-2 positions, while the phosphate group is found in the sn-3 position on the glycerol backbone. Within TG the three fatty acids present on the glycerol backbone are esterified. Cholesteryl esters are synthesised from single fatty acids that have been esterified to form a hydroxyl group of cholesterol (Metherel, 2007).

On average individuals consume over 20 different types of fatty acids, which are classified as SFA, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Nair et al., 1997; Surette, 2008). Figure 2 illustrates how fats can be categorised according to the number of double bonds present. SFA have no carbon to carbon double bonds. While MUFA only contain one double and PUFA have more than one double bond (Thompson et al., 2008).

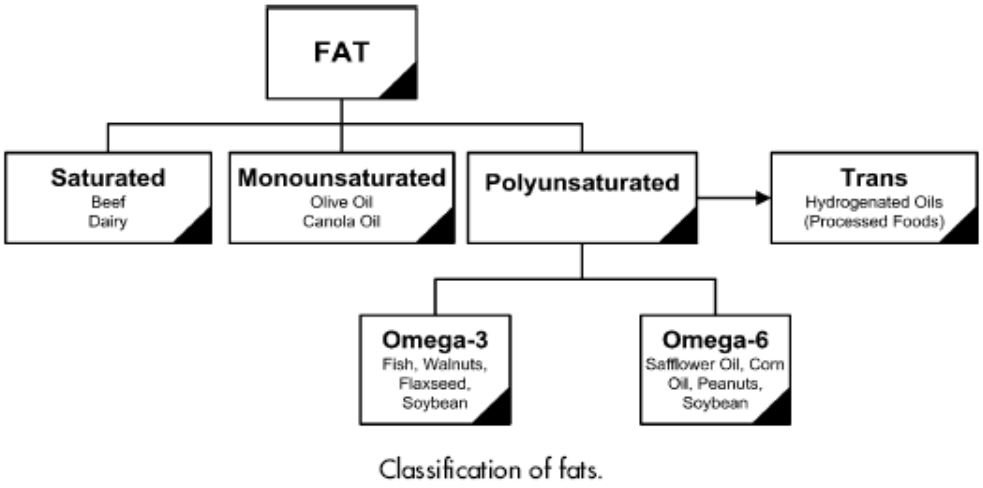
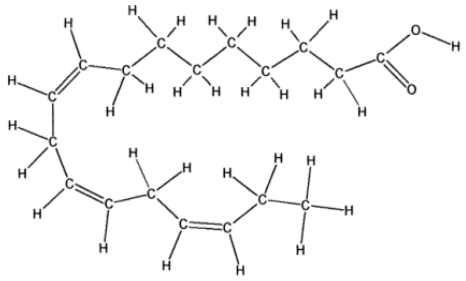
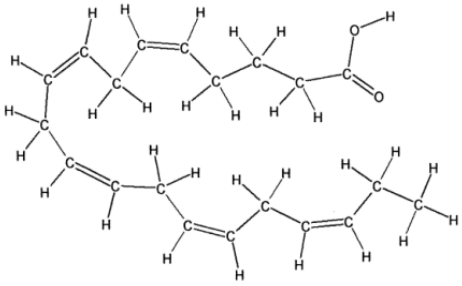


Figure 2. Classification of fat (DeFilippis & Sperling, 2006)

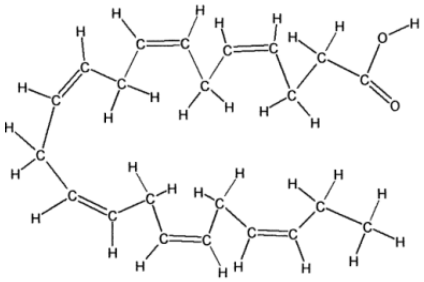
PUFA include omega 3 and omega 6 fatty acids, their names refer to the location of the first double bond from the methyl end of the fatty acid molecule (Dickinson, 2002; Kolanowski et al., 1999). Omega 3 fatty acids are very long hydrocarbon chains (consisting of 18 or more carbons) containing three or more double bonds. Figure 3 shows the three major types of omega 3 fatty acids: α-linolenic acid (ALA), EPA and DHA. ALA (18:3n-3) contains 18 carbons with three double bonds, EPA (20:5n-3) contains 20 carbons with five double bonds and DHA (22:6n-3) contains 22 carbons with six double bonds (Moyad, 2005). EPA and DHA make up approximately 70 to 85 % of omega 3 fatty acids in fish (Kolakowska et al., 2006).



LNA, Alpha-Linolenic Acid, 18:3ω3 (18:3n-3)(contains 18 carbon atoms and 3 double bonds/unsaturation sites)



EPA, Eicosapentaenoic Acid, 20:5ω3 (20:5n-3) (contains 20 carbon atoms and 5 double bonds/unsaturation sites)



DHA, Docosahexaenoic Acid, 22:6ω3 (22:6n-3) (contains 22 carbon atoms and 6 double bonds/unsaturation sites)

Figure 3. Chemical structure of omega 3 fatty acids (Holub & Holub, 2004)

MUFA and SFA are able to be synthesised by the body using dietary carbohydrates, proteins or other fats. However the body is not able to synthesise PUFA, specifically linoleic acid (LA) (C18:2n-6) and ALA, as it lacks the desaturase enzymes necessary to form the omega 3 and omega 6 fatty acid double bonds. This is due to the absence of desaturases, which are able to add a double bond at the C-15 position of a fatty acid carbon chain. EPA and DHA are considered to be conditionally essential fatty acids as they can be synthesised by the body if ALA is available. Despite this, LC omega 3 fatty acids are necessary for several bodily functions and although the body is able to produce them, conversion is limited (Gebauer et al., 2006; Holub & Holub, 2004).

Omega 3 fatty acids are involved in several cellular functions, such as being determinants of physiochemical properties of cell membranes, substrates for production of signalling molecules, modulation in the regulation of gene expression, inflammation

and the immune system (Kang, 2005; Leaf & Kang, 2001). The body can metabolise EPA to form eicosanoids that are components of short lived hormone-like lipids. Most eicosanoids formed from omega 3 fatty acids are anti-inflammatory and inhibit blood platelet aggregation (Hedelin et al., 2006; Kolanowski et al., 1999).

2.1.2 OMEGA 3 FATTY ACIDS: METABOLISM

Most lipids in food are in the TG form. As stated earlier TG are made up of a glycerol molecule attached to three fatty acids. The three fatty acids present on the TG molecule can include EPA and/or DHA. TG are digested in the small intestine by pancreatic lipase into monoglycerol and free fatty acids. The fatty acids can be incorporated into cell membranes (Calder & Burge, 2004; Frayn, 2003). Figure 4 illustrates how omega 3 fatty acids are incorporated into the phospholipid bilayer of cell membranes. Through this process omega 3 fatty acids can modify gene and protein expression, modulate membrane protein activity and act as a reservoir for bioactive molecules. Omega 3 fatty acids are not the only PUFA found in cell membranes, they compete with omega 6 fatty acids (particularly arachidonic acid (AA)) for incorporation into a cell membrane. Omega 3 fatty acids are able to displace AA within cell membranes and compete for the enzymes that catalyse the biosynthesis of eicosanoids (Surette, 2008).

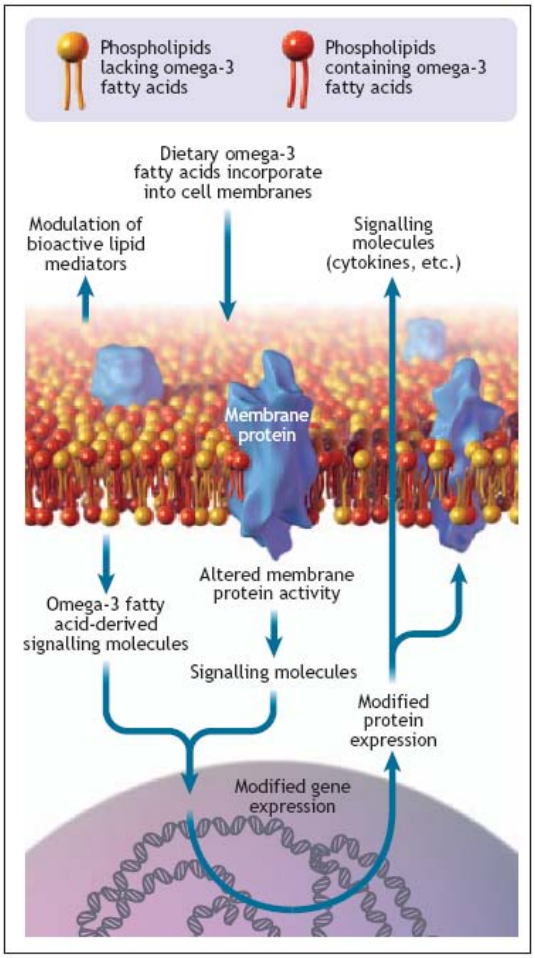
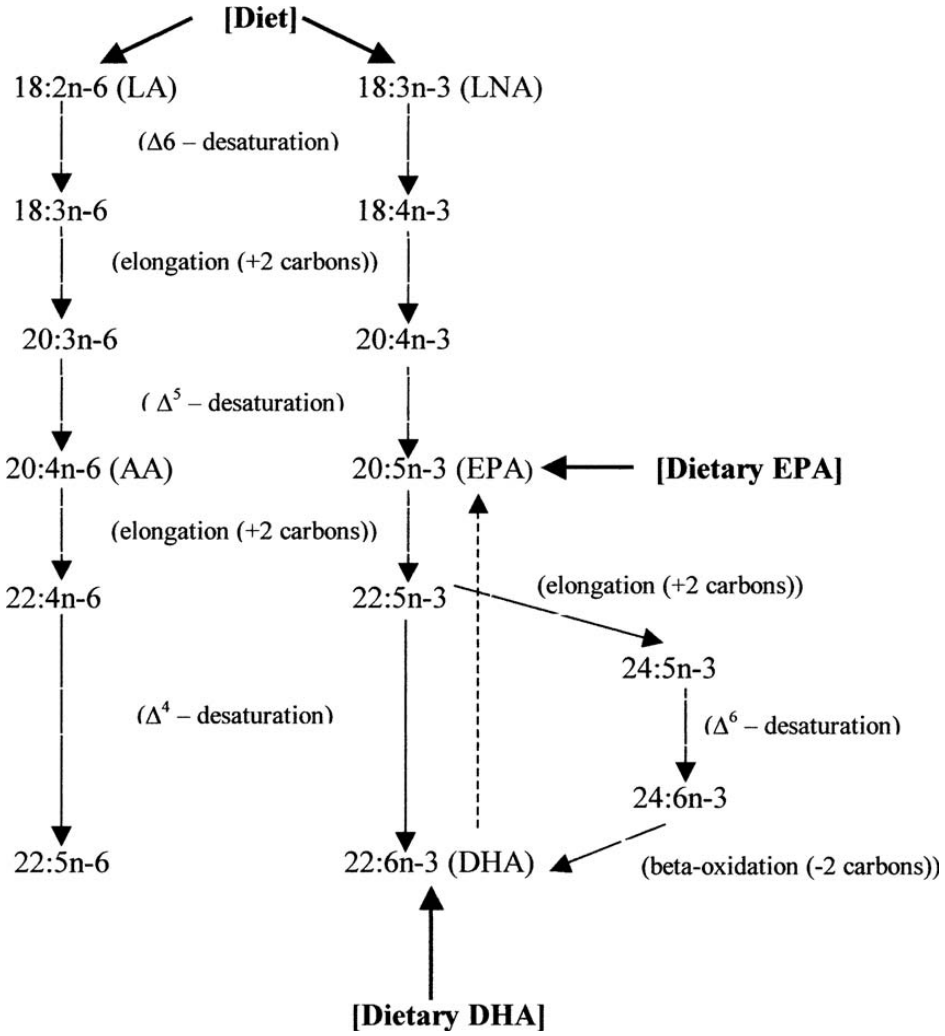


Figure 4. Omega 3 fatty acid membrane interaction (Surette, 2008)

Metabolism differs between individuals causing variations in digestion, absorption, tissue distribution and cellular metabolism of omega 3 fatty acids. Omega 3 fatty acid status is also influenced by in-vivo conversion of ALA into EPA and DHA, other dietary variables (such as energy intake), the health of the individual and intake of LA which requires the same enzymes for conversion to AA (Harris & von Schacky, 2004). Figure 5 illustrates that the body can obtain EPA and DHA directly from the diet or EPA and DHA can be metabolised through a series of enzymatic reactions. EPA can be metabolised from ALA through Δ6 and Δ5 desaturases and elongation of the carbon chain. From this docosapentaenoic acid (DPA) is formed, the addition of C_2 results in EPA. DHA can be synthesised from EPA by β-oxidation of the carbon chain by C_2 (Burdge & Wootton, 2002).

The process of conversion illustrated in Figure 5 has been shown to be inefficient and highly variable (Breslow, 2006). Based on isotope-labelled ALA feeding trials

approximately 0.2 to 21 % of ALA consumed is converted into EPA (Emken et al., 1994; Gerster, 1998). However less than 0.01 % of EPA is transformed to DHA (Nichols, 2007). This conversion process is also influenced by age (the conversion rate decreases with age) and gender (the conversion rate is higher in women) (Burdge & Calder, 2006).



LA, linoleic acid; LNA, alpha-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Figure 5. Desaturation, elongation and retroconversion of polyunsaturated fatty acids (Holub & Holub, 2004)

Cao et al. (2006) carried out a comparison between the direct supplementation of EPA and DHA or intake of ALA on the concentration of EPA and DHA in RBC and plasma phospholipids. Subjects were given either fish oil (1.30 g EPA and 0.86 g DHA/day) or flaxseed oil (3.51 g ALA and 0.90 g LA/day) to consume for 8 weeks. The authors reported that RBC EPA increased by 300 % and 33 % with supplementation of fish oil

and flaxseed oil, respectively. A 42 % increase in RBC DHA was reported in the fish oil group, while no significant increase was found in the flaxseed oil group. For every gram of EPA (from fish oil) consumed an increase of 1.4 % in RBC EPA was reported. An increase of 1.9 % in RBC DHA was reported for every gram of DHA (from fish oil) consumed. Lower individual variation was stated for EPA than DHA. The authors also reported that those with high RBC DHA levels at baseline incorporated DHA at a lower rate than those subjects with lower baseline levels. It was proposed that this was caused by the body regulating the incorporation of DHA into RBC. Every gram of ALA (from flaxseed oil) consumed resulted in an increase of 0.1 % in both RBC EPA and DHA. This study highlights the need for direct dietary intake of EPA and DHA.

As stated earlier, the conversion of ALA to EPA and DHA is influenced by gender, although no direct comparison between men and women has been carried out on plasma omega 3 fatty acids. In adult males the conversion of ALA to EPA was found to be approximately 8 %, for DHA this ranged from 0 – 4 % (Emken et al., 1994). The authors speculated that men may depend on pre-formed DHA. The conversion of ALA to DHA has been shown to be significantly greater in women. In women, plasma EPA and DHA conversion from ALA has been reported to be 21 % and 9.2 %, respectively (Burdge & Wootton, 2002). The explanation for the apparent synthesis of DHA in women may be because of an up-regulation of the desaturation and elongation pathway by estrogen. It is thought that estrogen may upregulate DHA biosynthesis and mobilise DHA into the blood (Jeong & Yoon, 2007). This proposition is supported by a study that reported synthesis of DHA to be three times greater in women who took oral contraceptive pills containing 17a-ethylnyloestradiol compared to those who did not (Burdge & Wootton, 2002).

Differences in ethnic background do not appear to influence the conversion of ALA to EPA and DHA. Chung et al. (2008) carried out research to determine the relationship between frequency and type of seafood consumed on plasma fatty acid concentrations in people from four different ethnicities (European origin, Chinese-American, African-American and Hispanic). They reported consumption of fish was positively correlated ($r = 0.24 – 0.46$) with EPA and DHA plasma phospholipids for all ethnic groups.

2.1.3 OMEGA 3 FATTY ACIDS: FOOD SOURCES

ALA is found in nuts, seeds and vegetable oils, it is especially high in flaxseeds. Trace amounts are also found in green leafy vegetables (Lee et al., 2008; Nair et al., 1997). Rich food sources of ALA are presented in Table 1.

Table 1. New Zealand food sources of alpha-linolenic acid (Food Standards Australia New Zealand (FSANZ), 2006)

Food Source	**ALA (g/100g)**
Flaxseed oil	53.3
Canola oil	9.98
Soyabean oil	9.05
Flaxseed	8.29
Walnuts	6.28
Butter	0.71
Olive oil	0.67
Sesame oil	0.48
Cheese	0.37
Grapeseed oil	0.29
Palm oil	0.19
Pumpkin seeds	0.18
Bread	0.12

ALA, alpha-linolenic acid.

Aquatic ecosystems are the principal source of LC omega 3 fatty acids. Different ecosystems produce different fatty acid compositions in the fish that reside there (Gladyshev et al., 2007). EPA and DHA are produced through microalgae species, such as algae and phytoplankton (Arts et al., 2001). In Table 2 the EPA and DHA levels of some New Zealand foods are displayed.

Table 2. New Zealand food sources of long chain omega 3 fatty acids (FSANZ, 2006)

Food Source	**EPA (mg/100g)**	**DHA**
Seafood		
Kahawai	480	1240
Tuna Albacore	250	950
Salmon	384	425
Trevally	160	290
Snapper	200	140
Eel	30	270
Tarakihi	30	200
Flounder Sand	110	110
Hoki	10	130
Meat		
Lamb	39	25
Beef	12	6
Veal	31	8
Pork	0	16
Chicken	0	7
Diary products		
Cream	47	0
Cheese	38	0
Other		
Tofu	35	0

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

The incorporation of omega 3 fatty acids into the body is influenced by the intake of omega 6 fatty acids. These are found in vegetable oils, such as corn, sunflower, soyabeans and safflower oils (Holub & Holub, 2004).

2.1.4 OMEGA 3 FATTY ACIDS: RECOMMENDATIONS FOR INTAKE

In New Zealand and Australia there is no recommended daily intake (RDI) set for omega 3 fatty acids. However an adequate intake (AI), an upper level of intake (UL) and a suggested dietary targets (SDT) have been set (see Table 3). SDT is the term used to describe "the daily average intake from food and beverages for certain nutrients that may help in prevention of chronic disease" (Ministry of Health, 2006, p. 3). This is equivalent to the 90th percentile of intake for the Australian and New Zealand populations.

Table 3. Nutrient reference values for long chain omega 3 fatty acids for the Australian and New Zealand population (Ministry of Health, 2006)

Age Group (years)		Recommendation (g/day)		
		AI	UL	SDT
Infants	0.0 – 0.5	-	Not possible to set	-
	0.6 – 1.0	-	Not possible to set	-
Children	1.0 – 3.0	0.04	3	-
	4.0 – 8.0	0.06	3	-
Boys	9.0 – 13	0.07	3	-
	14 – 18	0.13	3	0.61
Girls	9.0 – 13	0.07	3	-
	14 – 18	0.09	3	0.43
Men	> 19	0.16	3	0.61
Women	> 19	0.09	3	0.43
Pregnant	14 – 18	0.11	3	-
	> 19	0.12	3	-
Lactating	14 – 18	0.14	3	-
	> 19	0.15	3	-

AI, adequate intake; UL, upper limit intake; SDT, suggested dietary target.

A higher SDT, 1 – 2g per day, has been set for those suffering from coronary heart disease (CHD) (Ministry of Health, 2006). The New Zealand Heart Foundation (Roberts, 1999) recommends consuming 1 to 2 servings of fatty fish per week. Recommendations for omega 3 fatty acid intakes have been made by several organisations worldwide (see Table 4). Most of these sources recommend healthy individuals consume 0.5 g of omega 3 fatty acids per day.

Table 4. International recommendations for long chain omega 3 fatty acids (Gebauer et al., 2006)

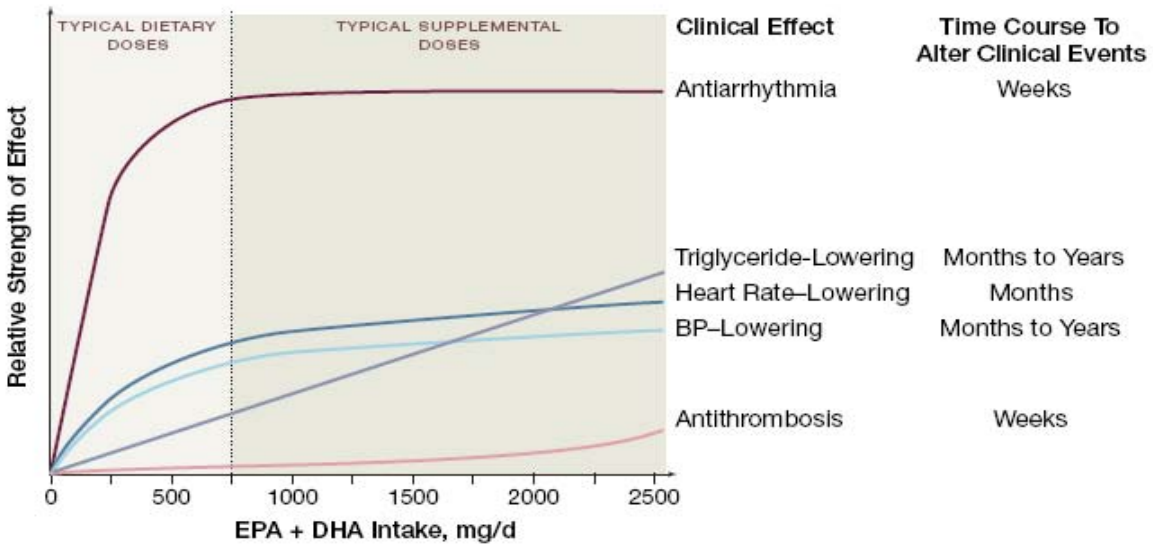
Source	Year Published	Recommendation		
		Total Omega 3 Fatty Acids (% of energy)	ALA (g)	EPA + DHA (g)
UK Committee on Medical Aspects of Food Policy	1994	0.2	-	0.10 – 0.20
US National Academics of Science, Institute of Medicine	2002	-	1.4	0.14
Eurodiet	2000	-	2	0.20
Health and Council of the Netherlands	2001	1	-	0.20
UK Dietary Guidelines		-	-	0.20
European Academy of Nutritional Sciences		-	-	0.20
New Zealand Heart Foundation	1999	-	-	0.20
Apports Nutritionnels Conseilles (France)	2001	0.8 – 1	1.8	0.45
UK Scientific Advisory Committee on Nutrition	2004	-	-	0.45
International Society for the Study of Fatty Acids and Lipids	2004	-	1.6	0.50
North Atlantic Treaty Organisation workshop	1989	-	3	0.80
World Health Organisation and Food and Agriculture Organization	2003	-	-	0.40 – 1.00
American Heart Association	2002	-	-	1 (CHD)
European Society of Cardiology	2003	-	-	1 (CHD)

ALA, alpha-linolenic acid; EPA , eicosapentaenoic acid; DHA, docosahexaenoic acid; CHD, coronary heart disease.

Another review of the recommended omega 3 fatty acid intake reported the level varies depending on the organisation and the health of the target population (Garg et al., 2006). A combined EPA and DHA intake of 0.18 g per day is recommended for healthy adults while 0.5 g per day is required to decrease heart disease and 1 g per day is required to decrease mental illness (Garg et al., 2006; Ruxton et al., 2004).

A review carried out by Mozaffarian & Rimm (2006) investigated the relative strength of effect EPA and DHA hav on risk factors relating to CVD. Figure 6 illustrates the relationship between EPA and DHA supplementation on several CVD risk factors. Anti-arrhythmia, lowering of heart rate and lowering of BP significantly increased in relative strength of effect with EPA and DHA intake from 0 – 0.5 g per day, a plateau was seen between 0.5 – 0.75 g per day. The authors stated that relative strength of effect is "estimated from effects of EPA and DHA on each risk factor and on the corresponding impact on cardiovascular risk" (Mozaffarian & Rimm, 2006, p. 1888).

The relative strength of effect on TG lowering and anti-thrombosis from EPA and DHA intake was linearly related. This suggests that at least 0.5 g per day EPA and DHA is required to assist in CVD prevention. The authors also stated that the duration of supplementation was important. To observe an effect of omega 3 fatty acids on the CVD risk factors, duration of supplementation from weeks to years is required, depending on the risk factor. This highlights the importance of ensuring that the duration of a study is long enough to ensure a noticeable clinical effect on the risk factor.



EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; BP, blood pressure.

Figure 6. Schema of potential dose responses and time courses for altering clinical events of physiologic effects of eicosapentaenoic acid and docosahexaenoic acid intake (Mozaffarian & Rimm, 2006)

When setting recommended intakes, potential negative effects from supplementation must be considered. Omega 3 fatty acids have been reported as having a dose-related effect on bleeding time although there are no documented cases of abnormal bleeding through the consumption of fish oil even at high dosages and in combination with anticoagulant medications (Garg et al., 2006; Sullivan et al., 2006).

2.1.5 OMEGA 3 FATTY ACIDS: BIOMARKERS

Omega 3 fatty acid status can be assessed by determining concentrations in adipose tissue, RBC, platelets, plasma, cholesterol esters, phospholipids and cheek cells (Sullivan et al., 2006). Table 5 shows the typical omega 3 fatty acid composition of adult human body tissues.

Table 5. Typical omega 3 fatty acid composition in adult human tissues (Burdge & Calder, 2006)

Lipid Fraction	Total Fatty Acids (%)		
	ALA	EPA	DHA
Plasma phosphatidylcholine	0.1	0.8	2.9
Plasma cholesteryl ester	0.4	0.8	0.5
Plasma TG	0.8	0.8	0.5
Platelet phosphatidylcholine	0.3	0.2	1.1
Platelet phosphatidylethanolamine	0.2	0.6	6.3
Mononuclear cell phospholipid	0.1	0.3	2.3
Neutrophil phospholipid	-	0.6	1.3
RBC phospholipid	-	0.8	3.5
Liver phosphatidylethanolamine	0.2	1.6	7.7
Brain grey matter phosphatidylethanolamine	0.1	-	24.3
Brain grey matter phosphatidylserine	-	-	36.6
Brain grey matter phosphatidylcholine	-	-	3.1
Brain white matter phosphatidylethanolamine	-	-	3.4
Retina phosphatidylcholine	-	-	22.2
Retina phosphatidylethanolamine	-	-	18.5
Retina phosphatidylserine	-	-	4.6
Testis total lipid extract	-	-	8.5
Sperm phospholipid	-	-	35.2
White adipose tissue	0.7	-	0.1

ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TG, triacylglycerol.

Serum or plasma fatty acids reflect omega 3 fatty acid intake over the last few days or meals. TG levels in serum have been shown to greatly fluctuate (coefficients of variance were reported to be 12 – 30 %) (Arab, 2003). Plasma phospholipids and cholesterol esters reflect dietary intake from the past few weeks, while RBC fatty acids can provide omega 3 fatty acid status based on the last 120 days (the approximate lifespan of a RBC) (Sullivan et al., 2006). RBC's stored at -80 °C have been shown to remain stable for more than 5 years (Arab, 2003). Adipose tissue (e.g. from the gluteal, abdominal or subscapular, or pectoral) reflects intake of the past 1 – 2 years (Andersen et al., 1996).

Consumption of fish is often measured through plasma phospholipid PUFA, however RBC membrane concentrations have been identified as a more accurate measure due to RBC being incapable of de novo fatty acid synthesis or modification of PUFA to form EPA and DHA. They are also not as influenced by the consumption of one isolated fish meal (Arab, 2003; Di Marino et al., 2000).

King et al. (2006) compared changes in fatty acids in RBC, plasma phospholipids and cholesterol esters after a low-fat diet (17 % energy intake from fat) or moderate-fat diet (34 % energy intake from fat) for 6 weeks. The changes in EPA and DHA concentrations in RBC, plasma phospholipids and cholesterol esters are shown in Table 6. The RBC change was less than in plasma phospholipids and cholesterol esters, due to the slow incorporation of EPA and DHA in the former.

Table 6. Fatty acid composition from low and medium fat diets (King et al., 2006)

Biomarker		**Low Fat Diet[a] (% total fatty acids)**	**Medium Fat Diet[b] (% total fatty acids)**
RBC	EPA	2.20	4.50
	DHA	19.4	19.1
Plasma phospholipids	EPA	5.70	11.9
	DHA	35.5	36.0
Cholesterol esters	EPA	12.7	15.8
	DHA	41.7	38.9

[a]17 % of energy intake from fat; [b]34 % of energy intake from fat.
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RBC, red blood cells.

In a study by Harris et al. (2004), RBC, cheek cell and plasma EPA and DHA concentrations were tested before and after 6 months of omega 3 fatty acid supplementation (0.3 g EPA and 0.2 g DHA/day). Cheek cells have been used as a non-invasive technique for testing omega 3 fatty acid levels. These cells changed by 0.14 % for EPA and 0.50 % for DHA after supplementation. This was not statistically different from the change reported for RBC or plasma. However the researcher found this method as more time consuming and complicated. A change of 1.28 % for EPA and 2.20 % for DHA was found in plasma. There was a higher coefficient of variance for EPA and DHA levels in the plasma (39 %) than the RBC (23 %), indicating higher inter-individual variability in plasma fatty acids. In the RBC an increase of 1.08 % for EPA and 0.50 % for DHA was established. The authors concluded that RBC EPA and DHA gave a better reflection of omega 3 fatty acid status.

The turnover of fatty acids in subcutaneous fat is slow. The use of this as a biomarker for omega 3 fatty acids is more appropriate as an average intake over longer periods of time. A study investigating the relationship between EPA and DHA concentrations in adipose tissue biopsies, reported the correlation between intake of EPA and adipose tissue EPA to be 0.40. A correlation of 0.66 for DHA intake and DHA adipose tissue was found. A strong linear relationship between adipose tissue DHA and fish intake was also observed (r = 0.55); adipose tissue DHA (%) = 0.15 + 0.0025 (g fish/day). The researchers reported that EPA is often the preferential fatty acid for measuring dietary intake effects on blood for short periods of time. However this study found that adipose DHA was a better indicator of long-term habitual intake of fish (Marckmann et al., 1995).

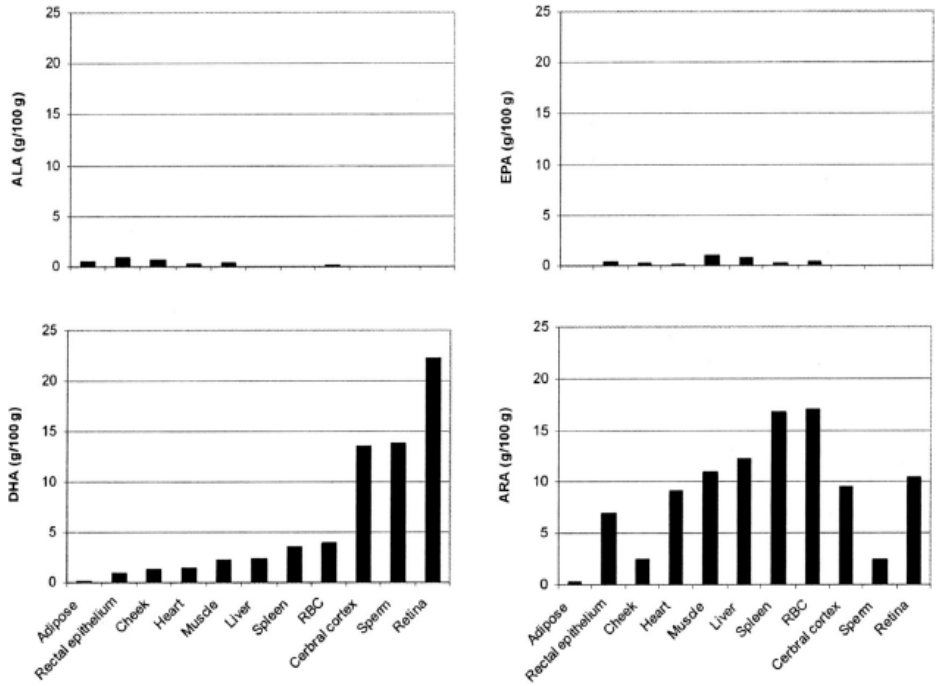
Sullivan et al. (2006) compared EPA and DHA intake based on a food frequency questionnaire (FFQ) with the EPA and DHA content of RBC and plasma. The correlation between EPA intake and RBC and plasma EPA were r = 0.40 and r = 0.54, respectively. For DHA intake, correlations with RBC and plasma DHA were r = 0.39 and r = 0.48, respectively.

Brown et al. (1991) illustrated the influence of LC omega 3 fatty acids on RBC EPA and DHA, by supplying subjects with one of three diets, a fish-free control diet, a fish diet of 200g lean fish per day (0.15 g EPA and 0.41 g DHA/day) or the fish diet with fish oil (0.99 g EPA and 0.99 g DHA/day) over 6 weeks. This was followed by a washout period (6 weeks) before the subjects where placed on a different treatment diet. The washout period was not long enough for RBC EPA or DHA to decrease to the baseline concentration. Findings indicated that the decline was much greater for EPA than DHA. The retention of EPA and DHA following 12 weeks of fish and fish-oil-free diets was 16 % and 44 %, respectively. The researchers of this study also reviewed ten other studies that gave subjects fish and/or fish oil (0.15 – 3.4 g EPA/day, 0.2 – 2.7 g DHA g/day) and enlisted these subjects to measure their RBC EPA and DHA over a period of time (2 – 20 weeks). They plotted the change in RBC EPA or DHA against the dietary intake and duration of treatment. They reported a linear relationship between the change in RBC LC omega 3 fatty acids and dietary intake for both EPA (change in RBC EPA = 1.22 x (dietary EPA (g/day)) – 0.230, r = 0.920) and DHA

(change in RBC DHA = 1.13 x (dietary DHA (g/day)) + 0.616, r = 0.572). A linear relationship was also found between change in RBC DHA and duration of supplementation (change in RBC DHA = 0.238 x (duration (weeks)) – 0.003, r = 0.776). The differences in response from EPA compared to DHA was described by the authors as being due to EPA's incorporation into the outer RBC membrane and DHA's distribution on the inner RBC monolayer. They speculated that EPA concentrations in RBC would be influenced by phospholipid exchange with plasma lipoproteins and is therefore better reflected by short term dietary amounts and biomarkers, such as plasma phospholipids. The incorporation of DHA into the inner monolayer of the RBC was hypothesised to occur during its development and therefore would be influenced by RBC turnover (~ 120 days).

Another study investigating the different rates of incorporation of EPA and DHA in various biomarkers after supplementation of fish oil was carried out by Katan et al. (1997). The authors examined the relationship between LC omega 3 fatty acid intakes (from either 0.81 g EPA and 0.16 g DHA, 1.62 g EPA and 0.33 g DHA or 2.43 g EPA and 0.49 g DHA/day) and LC omega 3 fatty acids incorporation into cholesteryl esters, RBC and adipose tissue over a year. The half life for reaching a steady state for cholesterol ester EPA was calculated to be 4.8 days, incorporation for the first month appeared to rapidly increase. For the rest of the year only slight increases were exhibited. The EPA levels during the washout period decreased as rapidly as it had increased, after 6 months levels were the same as at the baseline. Change in DHA with supplementation was not as great as that for EPA and was more erratic. A steady state in DHA cholesteryl ester levels was reached at 10.3 days, and like EPA, there was a rapid increase in DHA between the baseline and 1 month after supplementation. During the washout period there was a rapid decrease for the first month. A rapid increase in RBC EPA was shown in the first 2 months of supplementation. This continued to increase less rapidly until 6 months, when the concentration remained constant. Once supplementation had stopped there was a rapid decrease for the first month. It was reported that for every 1 g EPA per day consumed an increase of 2 % EPA RBC was measured. Intake of 1 g DHA per day resulted in a 1. 1 % increase in RBC DHA. EPA and DHA in adipose tissue from gluteal fat, did not plateau during the year of testing. Incorporation into the gluteal fat was greater for EPA than DHA.

Analysis of data from studies carried out in the United States, Canada, Australia, and Europe on concentrations of LC omega 3 fatty acids found in various body tissues is illustrated in Figure 7. AA was found in several tissues of the body. DHA was the most concentrated omega 3 fatty acid in body tissues, as it was present in all the membranes of all the organs that were tested. DHA is particularly high in the neural tissue, such as the brain and retina. Only small amounts of ALA and EPA were present in the bodily tissues examined, in fact, DHA concentration exceeds EPA 5 – 30 fold (Arterburn et al., 2006). The amount of DHA and EPA in adipose tissue is very small, suggesting that the storage of these fatty acids is limited and therefore they need to be replaced through regular dietary intake (von Schacky, 2006).



ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid.

Figure 7. Cross-study analysis of fatty acid levels (g/100g of total fatty acids) (Arterburn et al., 2006)

Fish and omega 3 fatty acid intakes have been shown to be more closely related to plasma or RBC DHA than EPA. EPA can be influenced by several non-dietary factors. It is preferentially mobilised from adipose tissue into the bloodstream at a much greater rate than DHA. EPA is also more likely to be distributed in the outer phospholipid layer of cell membranes. EPA has been shown to have higher incorporation and wash-out

rates than DHA showing that EPA may be metabolically more active and more available than DHA (Sun et al., 2008).

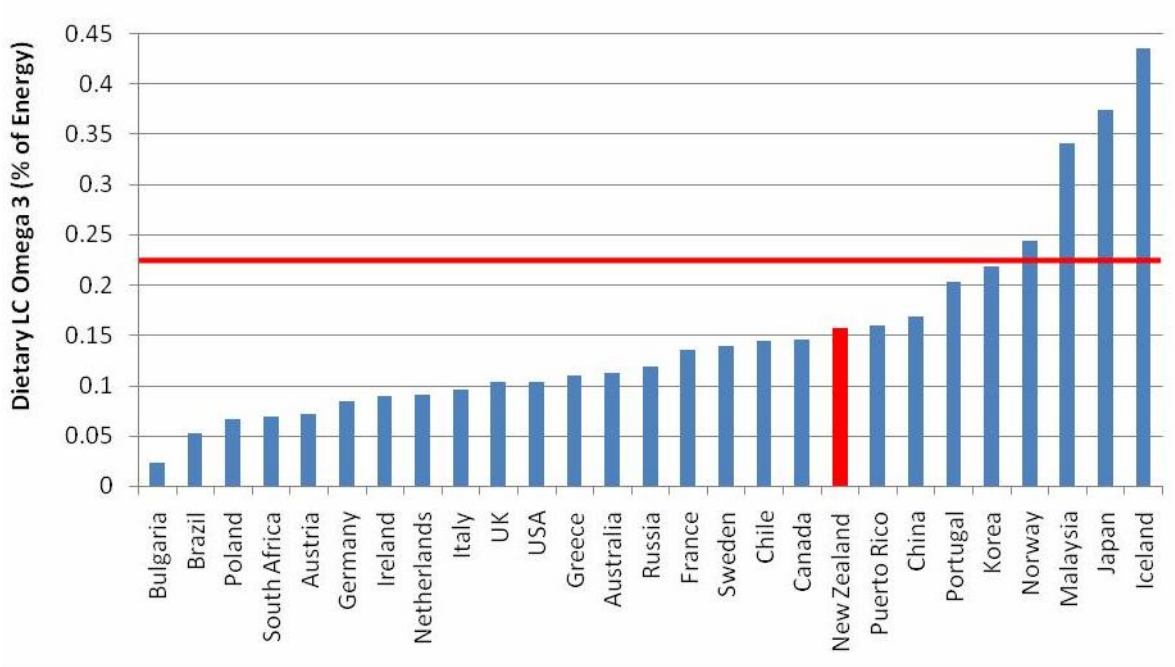
The literature reviewed in this section has shown that RBC omega 3 fatty acid concentration is the best biomarker for measuring omega 3 fatty acid status and intake (Harris et al., 2004; Sullivan et al., 2006). RBC contains a significant concentration of LC omega 3 fatty acids (Arterburn et al., 2006; Burdge & Calder, 2006), does not fluctuate significantly (Arab, 2003; Di Marino et al., 2000), has less inter-individual variability (Harris et al., 2004), reflects intake from the last 120 days (Sullivan et al., 2006) and remains stable at -80°C for years (Arab, 2003). A linear relationship has been reported between RBC EPA and DHA and intake of EPA and DHA (Brown et al., 1991; Katan et al., 1997; Sullivan et al., 2006).

2.1.6 OMEGA 3 FATTY ACIDS: STATUS IN NEW ZEALAND

Omega 3 fatty acid status can be determined from information on intakes of omega 3 fatty acids from food sources that are high in omega 3 fatty acids as well as from bodily tissue concentrations, as described in the previous section. The following sections will discuss the omega 3 fatty acid status of New Zealanders based on these two principles. There is limited information on the omega 3 fatty acid status of the New Zealand population. However it has been estimated that Australians and New Zealanders only consume approximately half of the recommended levels (Howe et al., 2006).

2.1.6.1 OMEGA 3 FATTY ACID STATUS IN NEW ZEALAND: DIETARY INTAKES

Hibbeln et al. (2006) reported the percentage of energy obtained from LC omega 3 fatty acids in several countries, including New Zealand, based on commodity data for the domestic supply of food available for human consumption, or disappearance data in 1995 (refer to Figure 8). The omega 3 fatty acid intake was based on poultry, pork, eggs, beef, goat and mutton, crustaceans, fish, molluscs, coconut oil, cottonseed oil, groundnut oil, maize germ oil, olive oil, palm oil, rape or mustard seed oil, rice bran oil, sesame oil, soyabean oil and sunflower oil as these foods were reported to account for 97 % of all dietary omega 3 fatty acids. The percentage of energy obtained from LC omega 3 fatty acids was based on an average intake of 2,000 kcal per day (8,400 kJ).



LC, long chain.

Figure 8. Percentage of energy consumed from long chain omega 3 fatty acids (Hibbeln et al., 2006)

The intake of LC omega 3 fatty acids by New Zealanders is reasonable compared to other Western countries, with 0.16 % of energy obtained from LC omega 3 fatty acids or 0.36 g LC omega 3 fatty acids per day. However consumption is less than the recommended amount of 0.5 g omega 3 fatty acids per day, which equates to 0.22 % of energy (based on an 8,400 kJ diet).

The New Zealand National Nutrition Survey (Russell et al., 1999) also investigated the frequency of fish and seafood consumption (see Table 7). Most battered, canned and fried fish does not contain a significant amount of omega 3 fatty acids, these groups were the most frequently consumed fish and seafood. Fish that was steamed, baked, grilled or raw was only consumed by 13 % of the population at least once a week. Lean and fatty fish were not differentiated between, therefore it is likely that even less than 13 % of the population consume fatty fish more than once a week. This indicated that most of the New Zealand population do not consume the recommended 2 servings of fatty fish per week (Roberts, 1999).

Table 7. Frequency of fish and seafood intake for the New Zealand population (Russell et al., 1999)

Fish/Seafood	**Percentage Consuming ≥ 1 serving/week**
Fish steamed/baked/grilled/raw	13
Fish battered	15
Fish canned	15
Fish fried	12
Shell fish	6
Other seafood	2
Eel	0

2.1.6.2 OMEGA 3 FATTY ACID STATUS IN NEW ZEALAND: TISSUE LEVELS

Only one study was found that examined tissue concentrations of omega 3 fatty acids in New Zealanders (Crowe et al., 2008) (details can be found in Table 8 and Table 9). The fatty acid composition of serum phospholipid, cholesteryl ester and TG was analysed from stored serum samples obtained during the 1997 National Nutrition Survey in New Zealand (Russell et al., 1999). Table 8 shows that the proportion of EPA in serum phospholipid, cholesteryl ester and TG increased significantly across the age categories for both men and women. The proportion of DHA in phospholipids for men and women, as well as cholesterol ester for men only, increased significantly across the age categories. The authors reported an increase in the ability to incorporate EPA into plasma phospholipids with age (Crowe et al., 2008).

Table 8. Long chain omega 3 polyunsaturated fatty acid composition of serum lipids for the New Zealand population as influenced by age (Crowe et al., 2008)

	Age (years), unadjusted means								
	15 – 24		**25 – 44**		**45 – 64**		**65 +**		***p***
	Mean	**Standard Error**	**Mean**	**Standard Error**	**Mean**	**Standard Error**	**Mean**	**Standard Error**	
EPA (mol %)									
Phospholipid									
Men	0.86	0.05	1.07	0.03	1.14	0.04	1.12	0.05	< 0.001
Women	0.78	0.06	0.93	0.02	1.08	0.03	1.14	0.05	< 0.001
Cholesterol ester									
Men	0.88	0.05	1.09	0.03	1.21	0.04	1.17	0.04	< 0.001
Women	0.77	0.05	0.94	0.02	1.12	0.03	1.22	0.06	< 0.001
TG									
Men	0.22	0.01	0.28	0.01	0.29	0.01	0.25	0.01	0.036
Women	0.20	0.02	0.26	0.01	0.28	0.01	0.29	0.02	< 0.001
DHA (mol %)									
Phospholipid									
Men	2.29	0.09	2.55	0.05	2.69	0.06	2.67	0.07	< 0.001
Women	2.58	0.12	2.67	0.04	2.81	0.07	2.79	0.08	0.035
Cholesterol ester									
Men	0.44	0.02	0.50	0.01	0.51	0.01	0.52	0.01	< 0.001
Women	0.50	0.03	0.51	0.01	0.54	0.01	0.54	0.02	0.097
TG									
Men	0.42	0.03	0.52	0.03	0.51	0.02	0.47	0.03	0.140
Women	0.49	0.07	0.53	0.02	0.52	0.02	0.52	0.03	0.795

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TG, triacylglycerols.

Table 9. Long chain omega 3 polyunsaturated fatty acid composition of serum lipids for the New Zealand population as influenced by gender (Crowe et al., 2008).

	Unadjusted Means			Adjusted Means[a]		
	Mean	**Standard Error**	***p***	**Mean**	**Standard Error**	***p***
EPA (mol %)						
Phospholipid						
Men	1.05	0.02		1.07	0.02	
Women	0.98	0.02	0.004	1.00	0.02	0.002
Cholesterol ester						
Men	1.09	0.02		1.12	0.02	
Women	1.00	0.02	< 0.001	1.02	0.02	<0.001
TG						
Men	0.27	0.01		0.27	0.01	
Women	0.26	0.01	0.224	0.26	0.01	0.205
DHA (mol %)						
Phospholipid						
Men	2.55	0.03		2.57	0.03	
Women	2.71	0.03	0.001	2.72	0.03	0.002
Cholesterol ester						
Men	0.50	0.01		0.50	0.01	
Women	0.52	0.01	0.007	0.52	0.01	0.036
TG						
Men	0.49	0.01		0.49	0.01	
Women	0.52	0.02	0.200	0.52	0.02	0.191

[a]Adjusted for age, body mass index, ethnicity, and smoking using multiple fractional polynomial regression.
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TG, triacylglycerols.

The authors also analysed the reported intake of fat from different food sources included in the 1997 National Nutrition Survey in New Zealand (Russell et al., 1999). They found that there were no differences ($p = 0.106$) in 'fish fat' intake between men (21 – 35 years, 1.8 ±0.2 g/day) or women (21 – 35 years, 1.3 ±0.2 g/day), however men (21 – 35 years, 17.9 ±0.7 g/day) consumed more 'meat fat' than women (21 – 35 years, 9.9 ±0.7 g/day) ($p < 0.001$). There was a significant difference between EPA and DHA concentrations in plasma phospholipids and cholesterol fatty acids between men and women. Men had higher EPA levels, while women had higher DHA levels (Crowe et al., 2008).

2.2 INCREASING OMEGA 3 FATTY ACID STATUS: FOOD VS. SUPPLEMENTS

2.2.1 INCREASING OMEGA 3 FATTY ACID STATUS: BIOAVAILABILITY FROM FISH VS. FISH OIL

There is some evidence to suggest that the bioavailability of omega 3 fatty acids from fish and fish oil supplements is different, although the recommendations for omega 3 fatty acids presume that bioavailability is the same. The term bioavailability describes how efficiently an ingested nutrient can be utilised by the organism in physiological processes (Fairweather-Tait, 1992; Ornsrud & Lorentzen, 2002). Biological availability of dietary fat is directly related to the chemical and physical properties of lipids, including chain length, degree of saturation and stereo-specific distribution of fatty acids on the TG structure (Bracco, 1994; Menoyo et al., 2007). It is important to note that absorption of omega 3 fatty acids can also be influenced by the composition of the background diet, droplet size of the oil emulsion, inherent resistance of longer chain fatty acids (especially EPA and DHA) to pancreatic lipase, delay in the enterocyte re-synthesis of TG and form of the lipids e.g. cholesterol ester, TG, phospholipids, and ethyl esters (Garaiova et al., 2007).

To date there have been only a few studies that have investigated whether LC omega 3 fatty acids from fish or fish oil supplements increase EPA and DHA status significantly. One of these is a study carried out by Visioli et al. (2003) who compared the effects of consuming smoked salmon (100 g/day (0.38 g EPA and 0.54 g DHA/day)) or ethyl ester capsules (1 capsule/day (0.15 g EPA and 0.11 g DHA/day) or 3 capsules per day (0.45 g EPA and 0.32 g DHA/day)). The authors measured the plasma omega 3 fatty acid levels in 16 healthy Italian men (10) and women (6) (26 – 38 years of age) over 6 weeks. Blood samples were drawn at -2, 0 and 6 weeks. Plasma EPA and DHA was linearly correlated with the dosages of LC omega 3 fatty acids from the treatment. Salmon consumption resulted in much higher LC omega 3 fatty acid levels, especially for DHA, compared to the equivalent amount of LC omega 3 fatty acids consumed from capsules. The change obtained was 2 to 9 fold higher for plasma EPA and DHA, respectively, if consumed as salmon compared to capsules. The authors suggested that this may have been a result of the capsules creating small lipidic boluses affecting the bioavailability of omega 3 fatty acids. In the salmon, omega 3 fatty acids are consumed with other nutrients present in the fish that dilute omega 3 fatty acids potentially increasing its bioavailability. EPA and DHA in the salmon are esterified to the sn-2

position of TG and phospholipids. At this position EPA and DHA are, to a large extent, preserved from hydrolysis during digestion and intestinal absorption of exogenous fat. However, during the processing of fish oil the fatty acids are randomly redistributed on the glycerol backbone of the TG. This results in an increase in the amount of EPA and DHA at the sn-1 and sn-3 positions of the glycerol molecule (Wijesundera & Abeywardena, 2004).

A study by Elvevoll et al. (2006) measured the change in serum EPA and DHA concentrations in healthy Norwegian subjects (71) after 8 weeks consuming 400 g of smoked salmon (0.47 g EPA and 0.71 g DHA/day), 400 g salmon fillet (0.45 g EPA and 0.72 g DHA/day), 400 g cod fillet (0.009 g EPA and 0.021 g DHA/day), or 105 g of cod liver oil (1.38 g EPA and 1.61 g DHA/day) per week. EPA and DHA in both the fish and cod liver oil were in the TG form. Intake of EPA and DHA from the fish supplied to the subjects was almost three times lower compared to the amount consumed by the cod liver oil group. However, the change in serum EPA and DHA in the fish group was almost as high as the change measured in the cod liver oil group. The authors concluded that fish was a more effective means of enhancing the serum concentration of LC omega 3 fatty acids compared to intakes of the same concentration via cod liver oil. Consuming a salmon fillet (57 g/day (0.45 g EPA and 0.72 g DHA)) was nearly twice as effective at increasing serum EPA compared with intake of cod liver oil (15 g/day (1.38 g EPA and 1.61 g DHA)). The authors suggested that the higher incorporation of EPA and DHA in serum from the consumption of fish may be due to omega 3 fatty acids in the fish being diluted by other nutrients present. This may cause a higher surface interaction between the food and intestinal wall, as well as a more favourable secretion of substances that aid in the absorption of lipids.

Harris et al. (2007) provided 23 American premenopausal women (21 – 49 years of age) with either fish (256.5 g canned albacore tuna and 85.5 g farmed Atlantic salmon per week (0.15 g EPA and 0.56 g DHA/day)) or TG capsules (0.10g EPA and 0.38g DHA/day) over 16 weeks. Fish recipes were provided for the subjects in the fish group. The omega 3 fatty acid concentrations of the treatments were measured using an internal standard and gas chromatography (GC). Subjects with a low intake of tuna or salmon (< 2 times/month) were chosen for the study. EPA and DHA concentration in RBC and plasma phospholipids were measured fortnightly. At the baseline the RBC

EPA concentrations were 0.80 ±0.12 and 0.99 ±0.17 % total fatty acids for the fish and capsule group, respectively. The RBC DHA concentrations were 3.22 ±0.58 and 3.34 ±0.79 % total fatty acids for the fish and capsule group, respectively. This study found that there was no difference in the change of RBC or plasma phospholipids EPA and DHA concentrations between consumption of fish or capsules. The consumption of 0.49 g of DHA from either fish or capsules per day produced a 40 – 50 % rise in RBC EPA and DHA and 60 – 80 % rise in phospholipid EPA and DHA. Harris et al. (2007) also stated that consumption of the allocated treatment was not directly monitored in the study. However, 97 ±8 % compliance to the fish oil capsule protocol was reported and all subjects in the fish group completed their treatment.

The form of the lipid containing omega 3 fatty acids may influence how effectively it is incorporated in body tissues. Nordoy et al. (1991) measured EPA and DHA concentrations of plasma phospholipids, cholesterol esters, TG and free fatty acids in five healthy subjects after consuming one of four treatment meals. The four treatment meals contained one of the following: 40 g of omega 3 fatty acids in triacylglyceride form, 28 g of omega 3 fatty acids in free fatty acid form with 12 g of olive oil, 28 g of omega 3 fatty acids as free fatty acids only or 40 g of olive oil. Blood samples were taken every 2 hours after the meal was consumed until 8 hours, then one final sample was taken after 24 hours. The authors concluded that EPA and DHA in either ester ethyl or TG form were equally well absorbed. A study by Harris et al. (1988) investigated the differences in omega 3 fatty acids in the TG or methyl ester form on the lipid profile in men with type IV hyperlipidemia. They reported that there was no significant difference between the change in EPA plasma phospholipid produced from either the TG (3.7 ±1.5 %) or methyl ester (3.8 ±1.1 %) forms. However the DHA plasma phospholipid concentrations were higher after consumption of the methyl esters (5.9 ±2.1 %) compared to the consumption of TG (4.2 ±1.9 %). The authors speculated that the difference in incorporation was due to methyl esters not needing to be hydrolysed before entering the RBC, consequently they can be absorbed more readily.

The use of capsules containing TG DHA from algal-oil (0.6 g DHA/day) was compared with intake of salmon (0.6 g DHA/day) to determine if the two sources caused differences in the incorporation of LC omega 3 fatty acids in plasma phospholipids and RBC (Arterburn et al., 2008). Salmon portions containing 0.6 g DHA were measured

based on analysis of the DHA content of salmon fillets using an internal standard and GC. The treatments were given to 29 healthy adults, 13 men and 16 women, for 2 weeks. Subjects with a low intake of DHA (< 0.2 g/day) were chosen and intakes were determined using a FFQ. At baseline, the fish group had RBC EPA and DHA concentrations of 0.53 ±0.05 and 5.74 ±0.21 % total fatty acids, respectively. The capsule group had RBC EPA and DHA concentrations of 0.61 ±0.04 and 5.82 ±0.17 % total fatty acids, respectively. The authors reported no difference in the change of LC omega 3 fatty acid plasma phospholipid and RBC concentrations with equivalent amounts of DHA from algal-oil capsules or salmon. The authors stated that subjects were required to consume more than 90 % of their allocated treatment to be considered compliant. However they did not report how compliance was determined.

Foods fortified with omega 3 fatty acids have been shown to significantly increase omega 3 fatty acid status. One study that examined the effects of consuming an omega 3 fatty acid enriched dip (100 g/day, 1.3 – 1.4g LC omega 3 fatty acids/day) on individuals with type II diabetes over a 6 week period reported an increase of 117 % in plasma EPA and 80 % for plasma DHA (Garg et al., 2007). Several studies have compared the consumption of fish oil supplements to omega 3 fatty acid enriched foods. Schrama et al. (2007) demonstrated differences in chylomicron fatty acid profiles after subjects consumed fish oil incorporated into different food matrices. The incorporation of both EPA and DHA into chylomricons was higher from the consumption of yoghurt containing fish oil than from fish oil capsules. This was probably due to yoghurt creating a lipid emulsion facilitating the absorption of lipids. The incorporation of EPA and DHA was slower for the fitness bar containing fish oil compared to yoghurt containing fish oil. The authors suggested that this was a result of the solid matrix creating a slower release of the lipids and therefore reducing the absorption rate. The incorporation of EPA and DHA from capsules appeared to be lower in comparison to the two other matrices. Another study (Higgins et al., 2000) looking at the incorporation of LC omega 3 fatty acids from the consumption of microencapsulated fish oil in a milkshake compared to fish oil capsules reported no significant difference between the LC omega 3 fatty acid response from the different treatments. This finding was also shown in a study by Wallace et al. (2000) who compared omega 3 fatty acid enriched bread, biscuits and soup to fish oil capsules.

Based on these studies there is no clear evidence as to whether the consumption of fatty fish or fish oil supplements is better at increasing omega 3 fatty acid status. All the previous research suffers from limitations in study design. In several of the studies the amount of omega 3 fatty acids present in capsules was not matched with the amount in the fish. The compliance of the subjects to the treatment was often not checked. Across the studies there were also variations in the duration of supplementation and the biomarkers used to measure LC omega 3 fatty acid concentrations. This may account for the differences in conclusions drawn between the studies.

2.2.2 INCREASING OMEGA 3 FATTY ACID STATUS: FOOD - SALMON

Salmon is characterised by its rayless fleshy, adipose fin which is located between its dorsal fin and its tail. There are three species of salmon in New Zealand, namely Chinook salmon (*Oncorhynchus tschawytscha)*, Sockeye salmon (*Oncorhynchus nerka)*, and Atlantic salmon (*Salmo salar)*. Chinook salmon is the species farmed within New Zealand and is produced by the New Zealand King Salmon Company Ltd, known as FNZK salmon (The New Zealand King Salmon Company Pty Limited, n.d.). In other countries it is mostly Atlantic salmon that is farmed with the exception of some Chinook salmon farmed in Canada and Coho salmon farmed in Chile. Table 10 displays a comparison of the omega 3 fatty acid content of different salmon species both farmed and wild. Wild Chinook salmon contains one of the highest concentrations of omega 3 fatty acids.

Table 10. Omega 3 fatty acid content of various species of wild and farmed salmon (Robson, 2006)

Salmon Species	**Omega 3 Fatty Acids (EPA + DHA/100g fish)**
Wild Chinook	2.0
Farmed Atlantic	2.0
Farmed Chinook	1.8[a]
Wild Atlantic	1.7
Farmed Coho	1.3
Wild Sockeye	1.3
Wild Coho	1.2
Wild Pink	1.0
Wild Chum	0.6

[a]value analysed from the present study.
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

The New Zealand King Salmon Company Ltd harvests approximately 6,000 metric tonnes (mt) of salmon per annum in the Marlborough Sounds of New Zealand for the brands King Salmon, Regal, Sea Smoke and Southern Ocean. Their salmon is free of diseases and genetic modification. They are fed on a balanced diet free of hormones, antibiotics, vaccines or chemicals. The salmon is slaughtered at 18 months old, when they reach approximately 3.4 – 4 kg (Steere, 2008).

The differences that exist in the fatty acid composition of fish flesh depend upon species, age, gender, size, reproduction period, fishing zone or breeding method, season and feed ingested. In fatty fish, such as salmon, there exists two major lipid depositions in the muscles, the anterior-posterior and the dorso-ventral areas. Therefore the location that the fillet is taken from may influence the fatty acid composition of the sample consumed (Sirot et al., 2008).

Lipids are considered to be indispensable nutrients in fish diets, making up an average of 28 % of the FNZK salmon diet. The average quantities of SFA, PUFA and omega 3 fatty acids in the feed are 6.2, 9.0 and 7.4 %, respectively (Couchman, 2004).

Salmon is considered to be a fatty fish as the fat content is greater than 7 %. Lean fish are categorised as having less than 1 % fat, while medium fat fish contain between 2 – 7 % fat (Kolakowska et al., 2006).

Salmon are usually farmed on the coastline where there is no effective barriers between the farm and the outside environment that can result in the many potentially detrimental effects on the environment (SeaWeb, n.d.). These effects include uneaten food and faecal matter from the farm contaminating the surrounding environment, a change of the biodiversity of the environment through attracting wild fish to the farm areas, and entanglement of wild fish in the farm nets. Farmed salmon can also directly influence wild fish by competing for resources, transferring pathogens and escapee farmed fish creating genetic variation within the wild fish population (Forrest et al., 2007; SeaWeb, n.d.).

Another issue that is important to consider with salmon consumption is sustainability. If everyone in the world was to consume 2 servings of fatty fish per week, supply would very rapidly run out (Garg et al., 2006).

2.2.2.1 FOOD- SALMON: NUTRIENT COMPOSITION

The nutrients in fish are influenced by the species, size, age, gender, sexual maturity, food source, water chemistry, environmental temperature, contaminants and methods of processing (Lall, 1995). Nutrients present in fish include unsaturated essential fatty acids, proteins (peptides and amino acids), vitamins, antioxidants, minerals and trace metals that have various beneficial effects on important physiological processes (Elvevoll et al., 2006). Table 11 shows the typical level of certain vitamins and minerals found in salmon.

Table 11. The typical vitamin and mineral composition of salmon and recommended daily intakes for Australia and New Zealand (Ministry of Health, 2006)

Nutrient		Salmon (per 100g)	RDI	
			Men	Women
Vitamin				
A[a]	µg	30	900	700
B1[a]	mg	0.14	1.2	1.1
B2[a]	mg	0.09	1.3	1.1
B3[a]	mg	6.8	16	14
B6[b]	mg	0.55	1.3	1.3
B12[a]	mg	0.5	2.4	2.4
C[a]	mg	2.6	45	45
D[b]	mg	10.7	-	-
E[b]	mg	3.2	-	-
Mineral	mg			
Calcium[a]	mg	8.8	1000	1000
Iodine[b]	mg	6.9	150	150
Iron[a]	mg	0.2	8	18
Magnesium[b]	mg	26	400	310
Potassium[b]	mg	306	-	-
Selenium[a]	mg	25.9	70	60
Sodium[b]	mg	30	-	-
Zinc[b]	mg	0.4	14	8

[a]Chinook salmon (The New Zealand King Salmon Company Ltd, 2008); [b]Atlantic salmon (FSANZ, 2006), data for Chinook salmon was not available.
RDI, recommended daily intake.

The protein content of fish is between 15 – 20 % of the total weight of the fish (Roberts, 1999). Amino acids in fish include taurine which has been used as a biomarker for fish intake and has been associated with a reduction in the risk of CVD. Arginine and glutamine, other amino acids present in fish, have been found to play a role in the prevention of CVD (He & Daviglus, 2005).

Although fish are rich in many nutrients some of these are sensitive and can be lost during processing, handling, storage, and cooking, through exposure to oxygen, light, heat and extreme pH (Lall, 1995).

Several researchers have speculated that fatty fish intake can have a beneficial effect on the body through replacing another food source in the diet (e.g. beef) that may be high in potentially detrimental nutrients, such as SFA (Din et al., 2004; Ismail, 2005; Kris-Etherton et al., 2002; Smith & Sahyoun, 2005).

2.2.2.2 FOOD- SALMON: CONTAMINANTS PRESENT

The significant beneficial effects from consuming fatty fish may be out-weighed by the potential harmful effects from contaminants present in the fish (Salonen et al., 1995).

Contaminant levels in salmon have been found to be associated with diet, geographic origin, maturation stage and harvest season. They have also been correlated to nutritional status, feed or food composition, fish size, age, marine residency and sexual maturation (Ikonomou et al., 2007).

Due to the pollution created by man there are a significant number of contaminants present in water ways and the ocean that are also found in some fish (e.g. shark and swordfish) that may be passed on to those who consume the fish. Fat-soluble environmental contaminants concentrate in fatty tissue of fish through bioaccumulation and biomagnification processes (Sidhu, 2003). Table 12 summaries common contaminants in Atlantic salmon; data on the contaminate levels in Chinook salmon could not be found. Salmon does not contain high levels of contaminants as it is not a predatory fish and does not have a long life span.

Table 12. Common contaminants in Atlantic salmon and their potential effect on humans (Wahlqvist, 2002; Ministry of Health, 2006)

Contaminant	Atlantic Salmon (mg/kg)	Maximum Acceptable Level (mg/kg)	Potential Effects
Lead	0.08[a]	0.5[a]	Learning and behavioural impediments in children and cardiovascular and kidney disease
Arsenic	1.49[a]	2.0[a]	Garlic smell to breath and tissue fluids, nausea, vomiting, diarrhoea, abdominal pain, delirium, coma, and seizures
Chromium	0.03[a]	0.1[a]	Irritation and ulcers in the stomach and small intestine and anaemia as well as infertility
Mercury	0.05[a]	0.5[a]	Bilateral visual fields, parasthesias of extremities and mouth, ataxia, un-coordination, tremor dysarthria and auditory impairments. Severe neurologic damage to infants whose mothers suffering mercury toxicity
Polychlorinated biphenyls	0.07 − 0.30[b]	0.2[a]	Chloracne lesions or rashes, elevated liver enzymes, possible increased risk of goiter and genetic alterations or mutations in foetuses
Dioxins	0.003 –0.009[c]	0.9[a]	Diabetes mellitus, endometriosis with infertility, abnormalities in thyroid function and impaired neurodevelopment of infants

[a]FSANZ, 2006; [b]Shaw et al., 2006 [c]Magnussen & Vang, 2006.

In studies on farmed salmon from Europe and North America it was found that relatively low numbers of salmon have elevated exposure to dioxins and dioxin-like compounds that could cause potential health risks. The authors reported that the risk of exposure to dioxins was offset by the significant health benefits of omega 3 fatty acids (Foran et al., 2005). This conclusion is supported by other researchers (Mozaffarian & Rimm, 2006).

2.2.2.3 FOOD- SALMON: FACTORS INFLUENCING OMEGA 3 FATTY ACID CONTENT

The lipid content of fish has been found to be variable based on numerous factors, including species, gender, age, season, location, cooking processes and freezing.

The lipid content of fish has been shown to be different depending on gender. In a study carried out on Baltic herring, females were found to have a higher PUFA and

omega 3 fatty acid content, as well as a lower SFA and MUFA content than males (Luzzana et al., 1996).

Variability in the fatty acid composition and lipid concentration in Baltic herring over a 12 month period has been demonstrated. This is thought to be related to an increase in energy and nutritional requirements for gonad development (Luzzana et al., 1996), temperature changes (Varljen et al., 2004) and diet (Bell et al., 2004). There was a significant reduction (13 %) in the concentration of fatty acid percentage of the fish from winter to spring 2004 (Kolakowska et al., 2006). Studies carried out in New Zealand have found that colder waters during autumn produce skipjack tuna with three times the amount of oil compared to those in warmer waters (Roberts, 1999).

Fish from the Southern Hemisphere have been shown to have higher concentrations of omega 3 fatty acids than those in the Northern Hemisphere (Mooney et al., 2002).

Heating of fish can result in progressive shrinkage and disintegration of the myofibril resulting in the expulsion of water soluble proteins and fats from the tissue (Konga et al., 2007). During cooking changes occur in the fatty acid composition of fish that are dependent upon the temperature, technique and medium used to apply heat (Kolakowska et al., 2006).

Larsen et al. (2008) compared the effects of poaching, steaming, microwaving, pan-frying, oven baking and deep frying on the LC omega 3 fatty acid content of FNZK salmon. Oven baked salmon was found to have the highest content of LC omega 3 fatty acids, while poached and microwaved salmon retained the least amount of omega 3 fatty acids. Interestingly the authors also stated that a sensory panel found the oven baked salmon had the most preferred taste.

The omega 3 fatty acid levels of fish that have been chilled for a few days are the same as those found in fresh fish. Some suggest that the fatty acid level may even increase due to enhanced extractability (and saponification) of the PUFAs in the fish tissue. Research carried out on cultured whole rainbow trout stored in ice at 2 °C found that the omega 3 fatty acid content of the fish increased on the third day after which the content decreased by 13.9 %. No lipid oxidation was observed at this temperature

(Kolodziejska et al., 2004). Another study carried out on minced herring meat kept at 4 °C for 4 days reported that no changes occurred in omega 3 fatty acids, however lipid oxidation increased by 1.5 (Kolakowska et al., 2006). Freezing of fish can cause changes in the lipid-protein binding and often releases lipids through the instability of PUFA (Kolakowska et al., 1995; Kolakowska & Szczygielski, 1994; Pokorny & Kolakowska, 2003)

2.2.2.4 FOOD- SALMON: COST

It was calculated that the intake of FNZK salmon needed to obtain the 0.5 g of omega 3 fatty acids per day as recommended is 150 g per week. The cost of salmon was determined to be between $20.90 – 32.95 per kg (Pakn'Save Albany and FoodTown Glenfield, March 2009), therefore it would cost $3.14 – 4.94 a week per person to achieve the recommended intake. A salmon meal would, however replace another protein source that would usually be consumed, such as beef steak. Beef steak costs between $14.99 – 20.99 per kg (Pakn'Save Albany and FoodTown Glenfield, March 2009), if 200g of steak was consumed in a meal it would cost $3.00 – 4.20 a week. Hence for an extra $0.14 – 1.94 a week per person salmon could be consumed instead of beef steak. This meal would contain all the omega 3 fatty acids required to meet the recommended level with the added benefit of containing less SFA and significant levels of selenium.

Salmon is an effective method of increasing omega 3 fatty acid status. Although it may contain very low-levels of contaminants, the risk is far outweighed by the beneficial effects from the omega 3 fatty acids and other nutrients such as protein, vitamins and minerals present in salmon. The storage, handling and cooking of salmon is important to the concentration of omega 3 fatty acids consumed in a meal. A barrier to consumers purchasing salmon is often the cost. However consuming salmon appears to be a more cost effective way of increasing omega 3 fatty acid status compared to consuming supplements (this is explored in more detail in section 2.2.4).

2.2.3 INCREASING OMEGA 3 FATTY ACID STATUS: SUPPLEMENT- FISH OIL

The term fish oil is generic and is defined as the class of lipids that originate from marine and freshwater bodies of the world. Fish oil is created from preparations of oil extracted from the body tissues of cold water fish or the livers of warmer water fish (Calder, 2001).

Oil extracted from fish is predominantly found in the TG form along with varying amounts of phospholipids, glycerol ethers and wax esters (Schmidtsdorff, 1995). In most fish oils EPA is the main fatty acid found, making up approximately 25 – 30 %, while DHA makes up only 8 – 20 % (Watkins & German, 1998). Tuna and salmon oil are higher in DHA than other fish oils.

2.2.3.1 SUPPLEMENT- FISH OIL: FAT OXIDATION

Fish oil is presented by some researchers as more beneficial than fish at increasing omega 3 fatty acid status as the extraction process can eliminate the contaminants found in fish (Willie & Gonus, 1988). However, the oil is prone to oxidation. This process creates contaminants from the by-products of fat oxidation.

Fish oil is very unstable and susceptible to oxidation giving it a rancid flavour causing it to be intolerable to some and resulting in health implications, including heart disease and cancer. Encapsulating purified fish oil in hard gelatin casings is able to protect them from oxygen and decrease the susceptibility to oxidation. Nevertheless, complete protection of encapsulated fish oil is difficult to achieve and therefore some oxidised compounds are often detected (Kolanowski et al., 2007).

Oxidation of PUFA occurs through the reaction of unsaturated fatty acids and oxygen. The oxidation of fat happens in three phases: initiation phase, a propagation phase and a termination phase (Kolanowski et al., 2007).

A hydrogen atom at the α-methylene group in double bonds of unsaturated fatty acid is removed, forming an alkyl radical. The oxidation that occurs is synthesised through a single oxygen molecule, the energy of the oxygen becomes metastably excited resulting in two unpaired electrons in the same orbital. This oxygen can be raised to an excited

state through light and the number, position and geometry of double bonds. It is also influenced by trace metals, antioxidants and fatty acid composition of the lipid. During the initiation phase oxygen attaches to unsaturated fatty acids producing peroxides and free radicals. For this to happen, oxygen and oxidative initiators must be present. This chain reaction causes further oxidation by propagation of a phase product which gives rise to anti-oxidation. Peroxides are particularly unstable due to the presence of active methylene groups that promote the formation of dihydroperoxides and decompose creating secondary oxidation products. In the final termination phase, relatively unreactive secondary oxidation products are formed, these include fatty acid esters, alcohol, lactones, hydrocarbons, aldehydes and ketones. Unsaturated aldehydes and ketones undergo further autoxidation (Kolanowski et al., 2007). The components created during fat oxidation are shown in Figure 9.

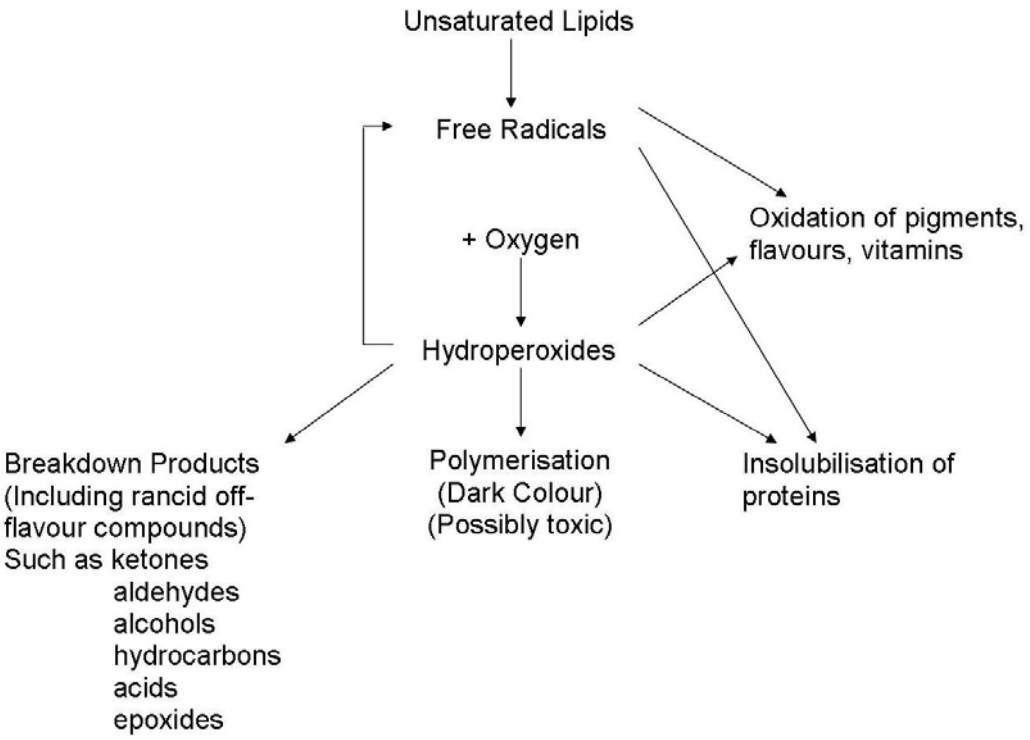


Figure 9. Oxidation of fat (Ohshima, 2005)

Fat oxidation has been shown to have a negative effect on the body as it results in the loss of omega 3 fatty acids and causes toxic oxidation products (Kolakowska et al., 2006). Fat oxidation has been associated with ageing, membrane damage, heart disease, and cancer (Kanner, 2006). Others have suggested it causes diarrhoea, liver enlargement and depression (Nwanguma et al., 1999; Pak, 2005).

Peroxide and anisidine values are often used to determine the overall oxidation value of a food or supplement (Allan, 2007). The peroxide value provides a measure of primary oxidation products (hydrogenperoxides) and the anisidine value provides a measure of carbonyl components (aldehydes and ketones) (O'Conor, 2007). The authors of a consumer study measuring the overall oxidation value of 29 fish oil supplements speculated that an acceptable level of 50 was too high and set a maximum acceptable oxidation value of 30. They categorised the fish oil supplements depending on their total oxidation values (< 20, 20 – 30, and > 30). Table 13 details total oxidation levels that were present in each of the categories of the 29 fish oil supplements analysed. They reported that four supplements had an oxidation value greater than 30 (Allan, 2007).

Table 13. Total oxidation value of fish oil supplements (Allan, 2007)

Brand	Total Oxidation[a]
Blackmores: Anti-inflammatory Evening Primrose + Fish Oil	< 20
Healtheries: Omega 3	< 20
Good Health: Health Guard Omega 3 fish oil	20 – 30
Thompson: Omega 3 Salmon Oil Capsules	20 – 30
Natures Own: Odourless Fish Oil Capsules	20 – 30
Clinicians: Omega 3	> 30

[a] Peroxide value + anisidine value

2.2.3.1.1 FAT OXIDATION: PEROXIDE VALUE

Peroxide value is one of the most common tests for determining oxidative rancidity. The primary products of fat oxidation are peroxides. These have low stability and decompose readily to secondary oxidative, volatile rancid flavours (Borneo et al., 2007). Peroxide value is determined by measuring iodine released from potassium iodide through titration with the iodide ion and is reported as milliequivalents of peroxide per kg of fat (Nawar, 1996).

Peroxide value increases significantly over time; therefore care must be taken in handling the test samples. High peroxide value is a definite indicator of the presence of

rancid fat. However moderate values may result from the depletion of peroxides after reaching high concentrations (Pak, 2005).

In New Zealand the peroxide value of fish oil is required to be less than 5 meq/kg fat (Ministry of Economic Development, 2009).

2.2.3.1.2 FAT OXIDATION: ANISIDINE VALUE

The breakdown of hydroperoxides during oxidation produce volatile aldehydes and a non-volatile portion of the fatty acid that remains as part of the glycerol molecule. The non-volatile product can be measured by reaction with anisidine (Nawar, 1996).

High anisidine values may be an indication that a fat has been oxidised even when aldehyde tests give low results as the volatile aldehydes may be removed during processing. Anisidine value is defined as 100 times the absorbance of a solution resulting from the reaction of 1 g of fat in 100 ml of solvent. There are no units for anisidine value (Nawar, 1996).

Anisidine value of less than 10 is required for fish oil within New Zealand (Ministry of Economic Development, 2009).

2.2.3.2 SUPPLEMENT- FISH OIL: TOLERANCE TO FISH OIL

Consumption of fish oil can potentially have unpleasant side effects. The most commonly reported adverse effects are nausea, gastrointestinal upset and 'fishy burps'. Side effects of omega 3 fatty acids are dose dependent. At 3 g or more per day gastrointestinal upset, clinical bleeding, fishy aftertaste, worsening hyperglycemia and increased LDL-C have been reported (Breslow, 2006).

Recommendations for reducing the incidence of burping and improving adherence to consumption of fish oil supplements include taking the fish oil at bedtime or with meals, keeping it in the fridge, or using enteric-coated products (Lee et al., 2008). Table 14 shows the number of subjects that suffered various side effects in a study providing 4 capsules (1.84 g EPA and DHA/day) or matching placebo (corn oil with 1 % fish oil) to

pregnant and postpartum women. There was no great difference in the complaints between the placebo and fish oil group (Freeman & Sinha, 2007).

Table 14. Pregnant versus postpartum subjects who experienced adverse events from omega 3 fatty acid supplements (Freeman & Sinha, 2007)

Subjects	**Placebo**	**Omega 3 Fatty Acids**
	(number of subjects)	
Pregnant (n=23)	3	3
Dizziness	0	0
Diarrhoea	0	0
Nausea	0	0
Burping	1	0
Difficulty breathing	1	0
Foul breath/bad taste	0	3
Heartburn/reflux	1	1
Tired	0	0
Postpartum (n=36)	4	3
Dizziness	1	0
Diarrhoea	1	0
Nausea	0	1
Burping	0	0
Difficulty breathing	0	0
Foul breath/bad taste	1	2
Heartburn/reflux	1	2
Tired	1	0

Harris et al. (2007) investigated the tolerability of fish and fish oil capsules after 16 weeks of supplementation. The authors asked participants if they had experienced any fishy aftertaste such as burping from the treatment and how frequent and unpleasant it was. Only one of the subjects in the fish group (out of 11) reported suffering this side effect, while almost all of those in the capsule group reported suffering fish aftertaste (10/12). Most suffered the side effect at least once a week and described it as mildly unpleasant.

2.2.3.3 SUPPLEMENT- FISH OIL: COST

Table 15 shows various fish oil supplements available on the market in New Zealand. Most are created from the oil of a variety of fish species and contain 0.18 g of EPA and 0.12 g of DHA per capsule. Based on this LC omega 3 fatty acid content, 2 capsules a day would be required to obtain 0.5 g of omega 3 fatty acids per day. This would cost between $2.52 – 5.88 per week depending on the brand of capsule consumed.

Table 15. Selected fish oil supplements on the market in New Zealand

Brand	EPA (g/g)	DHA (g/g)	Price ($/capsule)	Price ($/0.5 g omega 3 fatty acids/day)
Blackmores: Omega Daily Concentrated Omega 3	0.35	0.25	0.38	0.38
Healtheries: Omega 3	0.18	0.12	0.20	0.40
Red Seal: Omega 3	0.18	0.12	0.20	0.40
Good Health: Health Guard Omega 3 fish oil	0.18	0.12	0.25	0.50
Thompson: Omega 3 Salmon Oil Capsules	0.18	0.12	0.42	0.84
Natures Own: Odourless Fish Oil Capsules	0.18	0.12	0.18	0.36
Healtheries: Omega Advanced Pure Salmon Oil	0.06	0.10	0.29	1.16

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

The capsules used in the study reported herein were Healtheries omega advanced pure salmon oil. These cost $25.95 for a bottle of 90 capsules. To consume the recommended 0.5 g per day omega 3 fatty acids, 4 capsules per day are required. This would cost $8.12 per week.

Disparities in the omega 3 fatty acid content labelled on fish oil supplements and the content measured through independent testing have been reported in consumer studies. Consumer Magazine in New Zealand (Allan, 2007) reported that of the 29 different types of fish oil supplements tested, five were reported to contain less than the omega 3 fatty acids content stated on the label. These were Good Health omega 3 heart guard, Healtheries fish oil omega 3, Oils of Life omega 3 concentrate, Thompsons omega 3 fish oil and Thompsons omega 3 salmon oil. In Hong Kong the Consumer Council (Consumer Council, 2008) reported that out of 28 fish oil supplements tested, four contained less EPA and eight contained less DHA than the amounts stated on the label. Some products contained as little as 12 % of the EPA it was labelled as containing and 29 % of the DHA.

2.2.4 INCREASING OMEGA 3 FATTY ACID STATUS: SUMMARY

Both fatty fish (salmon) and fish oil supplements (capsules) have proven to be suitable for significantly increasing omega 3 fatty acid status. However, both of these have potential negative side effects. Salmon may contain contaminants, such as dioxins. Although fish oil does not have these contaminants it has potential to produce oxidative by-products which can have significant negative effects on the body. Salmon contains nutrients other than omega 3 fatty acids, such as selenium, which add to its beneficial health effects. To consume the recommended 0.5 g per day of omega 3 fatty acids, between two to four capsules must be consumed per day compared to one 150 g serving of FNZK salmon per week. It would cost between $3.14 – 4.20 a week to consume 150 g of FNZK salmon and between $2.52 – 8.07 per week to consume two to four capsules a day (based on pricing in New Zealand in March 2009). Therefore consuming salmon may be a more cost effective method of increasing the omega 3 fatty acid status than consuming supplements. It becomes even more cost effective if salmon replaces another protein source such as beef steak. The potential benefits and issues relating to the consumption of salmon versus capsules are summarised in Table 16.

Table 16. Potential benefits and issues from consumption of salmon versus capsules

Salmon		**Fish Oil Capsules**	
Benefits	**Potential Issues**	**Benefits**	**Potential Issues**
Provides other nutrients Replace other fat sources e.g. SFA Effectively increase omega 3 fatty acids	Cost Dislike taste Environmental impact	Convenient Effectively increase omega 3 fatty acids	Cost Fishy “burps” Oxidation Incorrect labelling

2.3 OMEGA 3 FATTY ACIDS & DISEASE PREVENTION

2.3.1 INTRODUCTION

LC omega 3 fatty acids have been shown to have many biological functions and affect several disease processes, including:

- pregnancy outcomes (postpartum depression and pregnancy duration);
- cognitive development and learning in infants and children;
- visual development;
- immune and inflammatory responses;
- rheumatoid arthritis, ulcerative colitis, Crohns disease, eczema, asthma and type 1 diabetes;
- metabolic syndrome, including type 2 diabetes and obesity;
- CVD and risk factors of CVD (e.g. thrombosis, blood lipid concentrations, vascular function, BP and cardiac arrhythmias);
- neurologic degeneration (dementia, including Alzheimer disease);
- mental health and mood disorders; and
- bone health (Akabas & Deckelbaum, 2006; Calder, 2008).

Omega 3 fatty acid intake has also been associated with a decrease in the incidence of many chronic diseases (Simopoulos, 1999). Chronic diseases are defined as diseases that occur over a long period of time with slow progression. These include heart disease, stroke, cancer, chronic respiratory diseases and diabetes. Chronic disease is the leading cause of death in the world (World Health Organisation, n.d.). Risk factors for chronic disease include consumption of less than 5 servings of fruit and vegetables per day, being over 45 years of age for males and over 55 years for females, having a BMI (body mass index) of greater than 25 kg/m^2, a BP exceeding 140/90 mmHg, a TC over 5.2 mmol/l, participating in less than 150 minutes of moderate-vigorous intensity physical activity a week and smoking (Kolbe-Alexander et al., 2008).

Over the last three decades numerous studies have been carried out on the effect of LC omega 3 fatty acids on CVD (Lee et al., 2008). A substantial amount of evidence exists to support the role that LC omega 3 fatty acids play in the prevention of many chronic diseases. The following sections review some of the research that has been carried out on the effect of LC omega 3 fatty acids on CVD.

2.3.2 OMEGA 3 FATTY ACIDS & DISEASE PREVENTION: CVD STUDIES

Evans and Burr were responsible for the discovery of essential fatty acids in 1929. The concept that some fatty acids might be related to risk of disease, such as ischaemic heart disease, was presented as early as 1937 by Hugh Sinclair. When he visited Eskimos in Greenland in 1944, he discovered their diet was linked to the low incidence of atherosclerosis. Further research by Bang and Dyerberg found that the Eskimos from the West Coast of Greenland had a low incidence of myocardial infarction (MI) and stroke. Their research led to the realisation that the Eskimos' high marine fat diet, which was largely derived from whale, fish and seal, protected them from thrombosis and atherosclerosis. Bang and Dyerberg reported the Eskimos had lower levels of plasma cholesterol and TG, increased bleeding times and plasma concentrations of omega 3 fatty acids as compared to the Danish population at that time (Goodnight, Harris, & Connor, 1981). Since then several studies have investigated fatty acids that are unique to marine sources, mainly DHA and EPA (Burr et al., 2005).

Figure 10 illustrates the relationship between LC omega 3 fatty acid concentrations in tissue and the incidence of mortality from CVD. High omega 3 fatty acid concentrations in tissue has a significant negative relationship with CVD mortality (Hibbeln et al., 2006).

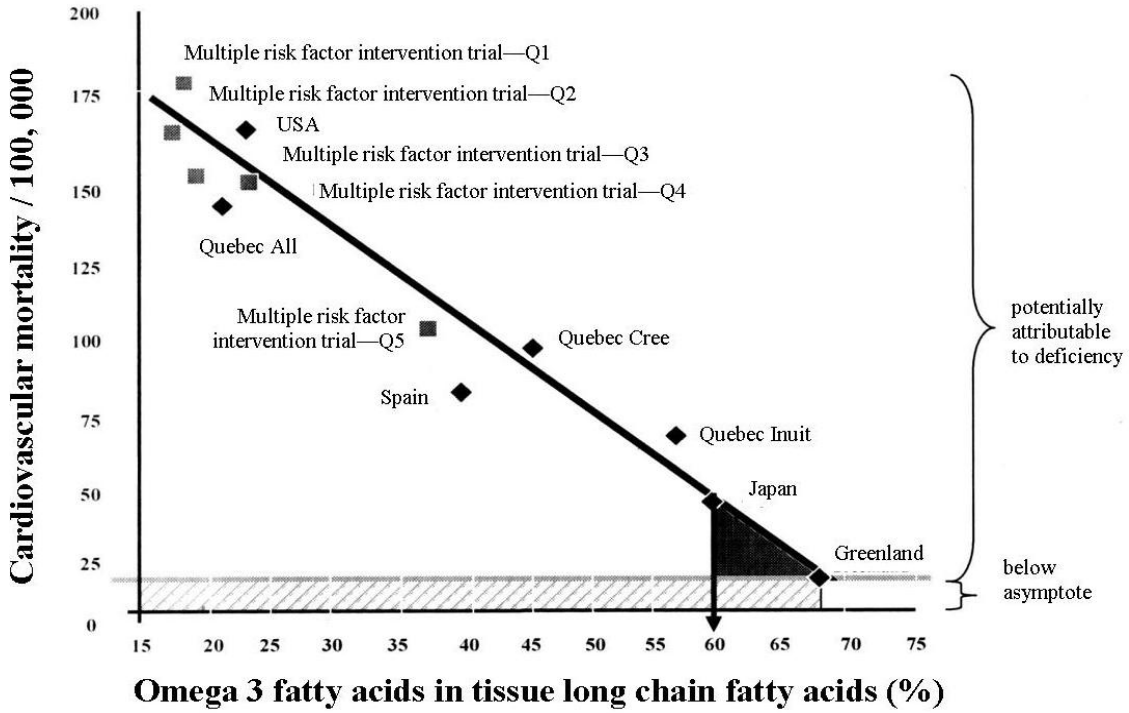


Figure 10. Tissue composition of long chain omega 3 fatty acids and cardiovascular mortality (Hibbeln et al., 2006)

Not all studies on omega 3 fatty acids and CVD risk factors have found a protective effect; however variations in results were based on differences in dose, sample size, treatment duration and subject selection (Rousseau et al., 2001).

2.3.2.1 OMEGA 3 FATTY ACIDS AND CVD STUDIES: META-ANALYSIS

A meta-analysis is a systematic technique for reviewing, analysing, and summarising quantitative research studies on specific topics or questions. It is done by analysing the results of two or more studies together to create greater accuracy and statistical power through a larger sample size. This is sometimes necessary as many studies have relatively small subject numbers that may not have enough power to show an effect (Eysenck, 1994).

This section will discuss meta-analysis and systematic reviews carried out on the relationship between CVD and omega 3 fatty acid status/intake, specifically LC omega 3 fatty acids. Table 17 summarises the results of a meta-analysis by (Mozaffarian & Rimm, 2006) of randomised controlled trials (RCT) and cohort studies that investigated the effect of omega 3 fatty acids on CVD. The authors demonstrated that there was strong evidence that omega 3 fatty acids decreased cardiovascular mortality.

Table 17. Summary of evidence for effects of consumption of fish or fish oil on cardiovascular outcomes (Mozaffarian & Rimm, 2006)

Outcome	Clinical Effect	Strength of Evidence
CHD mortality		
CHD death	35% decrease	Strong
Sudden death	50% decrease	Strong
Ischemic stroke	30% decrease	Moderate
Nonfatal CHD		
Nonfatal MI	Modest benefit	Equivocal
Progression of arthrosclerosis	Modest benefit	Equivocal
Post angioplasty restenosis	Modest benefit	Equivocal
Recurrent ventricular arrhythmia's	Modest benefit	Equivocal
Arterial fibrillation	30% + decrease	Limited
Congestive heart failure	30% + decrease	Limited

CHD, coronary heart disease; MI, myocardial infraction.

Table 18 highlights the overwhelming evidence of the protective effect of omega 3 fatty acids on reducing the risk of CVD. The meta-analysis/reviews examined RCT and observational studies that looked at omega 3 fatty acids obtained from fish, plant sources and fish oil supplements.

He et al. (2004) reported that even a small intake of fish had some benefit, for every 20 g of fish consumed per day the risk of CHD decreased by 7 %. Omega 3 fatty acids

were also reported to cause a decrease in the risk of stroke. However the authors of this study reported concern over the lack of differentiation between ischaemic and hemorrhagic stroke. Their results were thought to be based on risk of ischaemic stroke (RR = 0.65, CI 0.46 – 0.93) as this is the major type of stroke experienced in the Western World. They also pointed out that fish consumption may be a surrogate for some other underlying health lifestyle factors that protect against strokes and CHD, such as exercise, smoking and BMI.

Whelton et al. (2004) speculated that the reduced risk in sudden cardiac death with the consumption of omega 3 fatty acids was in their view due to increasing heart rate variability in survivors of MI, decreased resting heart rate and reduced damage to cardiac tissue. A greater inverse relationship was found between fish consumption and fatal CHD in those consuming 2 to 4 servings of fish per week compared to those consuming less than 2 servings per week. However a higher RR of fatal CHD in those consuming more than 4 servings of fish per week was reported. This finding was explained through potential increased blood mercury from the consumption of large amounts of fish which may increase the risk of CHD. They also reported that fish consumption may have slightly greater protective effect for women than for men.

Intakes of between 0.25 – 0.50 g per day of LC omega 3 fatty acids were reported by Mozaffarian & Rimm (2006) to be the threshold, at which point higher intakes do not substantially lower CHD morality. The researchers also stated that other protein foods, such as meat and dairy, could be replaced by consuming fish, which may contribute to the beneficial effects on the decrease in CVD.

Wang et al. (2006) concluded that evidence of the beneficial effects from fish oil was stronger in secondary than in primary prevention. The author speculated that this may have been due to the limited number of RCT carried out on primary prevention of CVD.

The systematic review carried out by Hooper et al. (2004) received much criticism. The authors stated that there was no clear evidence to suggest omega 3 fatty acids from fish or plant origin reduced mortality rates. However the inclusion of the Diet and Angina Randomised Trial (DART 2) study significantly influenced the results of this review.

By excluding the DART 2 study from this review findings show a reduction in risk of death (Burr et al., 2005). The DART 2 study has been reported by some to be an outlier study, its inclusion in Hooper et al. (2004) systematic review caused a positive test for heterogeneity in the data used in the review. The authors of the review have also been criticised for excluding biomarker studies, the exclusion of relevant cohort studies and inclusion of studies with questionable scientific integrity (von Schacky et al., 2006).

The systematic review carried out by Leon et al. (2008) was mostly driven by the large RCT Gruppo Italiano per la sperimentazione della streptochinasi nell'Infarto miocardico (GISSI) and Japan EPA Lipid Intervention Study (JELIS) studies, which together accounted for 92 % of the participants. Leon et al. (2008) reported that there may have been publication bias in their data as neutral or negative trials might not have been published.

In general the meta-analysis/reviews examined show that fish and fish oil ingestion resulted in a decrease in the risk of several cardiovascular events including MI, stroke and CHD. A significant amount of evidence suggests this can result in decreased risk of death from CHD, sudden death and overall mortality.

Table 18. Selected meta-analysis/reviews on the effect of omega 3 fatty acids on cardiovascular disease

Reference	Studies (n)	Subjects (n)	Treatment	Duration (years)	Conclusion
Bucher et al. (2002)	11 RCT	15 806	Omega 3 PUFA-enriched diets, control diets or placebo	≥ 0.5	Nonfatal MI in patients consuming omega 3 diets: RR 0.8 (CI 0.5-1.2). Fatal MI: RR 0.7 (CI 0.6-0.8). Sudden death: RR 0.7 (CI 0.6-0.9). Overall mortality: RR 0.8 (CI 0.7-0.9).
He et al. (2004)	9 cohort studies	200 575	Fish consumption	≥ 4.0	Compared with those who never consumed fish or ate fish less than once per month, individuals with higher fish intake had lower risk of total stroke. 2-4 servings/week: RR 0.82 (CI 0.72-0.94) for stroke.
Whelton et al. (2004)	14 cohort and 5 case-controlled studies	228 864	Fish consumption	>4.0	Fish consumption was associated with an approximately 20% reduction in the risk of fatal CHD: RR 0.83 (CI 0.76-0.90) and a 10% reduction in total CHD: RR 0.86 (CI 0.81-0.92).
Mozaffarian & Rimm (2006)	20 RCT and large prospective trials	-	Fish and fish oil consumption	-	At intakes up to 250mg/d, the relative risk of CHD death was 14.6% (CI 8-21%) lower per each 100mg/d of EPA and DHA for a total risk reduction of 36% (CI 20-50%).
Wang et al. (2006)	Primary prevention: 1 RCT, 25 prospective cohort studies and 7 case-controlled studies. Secondary prevention: 11 RCT and 1 prospective study.	19 403 and 12 015	Intake of omega 3 fatty acids (EPA, DHA and ALA supplements & diets) or fish	>1.0	Omega 3 fatty acids from fish or fish oil supplements, but not ALA, reduced the rates of all-cause mortality, cardiac and sudden death and possibly stroke.
Hooper et al. (2006)	48 RCT and 26 cohort studies	36 913	Dietary supplements, advice on eating oily fish and advice on diet and food supplements	≥ 0.5	Long chain and shorter chain omega 3 fats do not have a clear effect on total mortality: RR (0.86, CI 0.70-1.04), combined cardiovascular events: RR (0.95, CI 0.82-1.12).
Leon et al. (2008)	12 RCT	32 779	Fish oil	≥ 0.3	Reduction in deaths from cardiac causes but had no effect on arrhythmias or all cause mortality. Mortality: RR 0.92 (CI 0.82-1.03), sudden cardiac death: RR 0.81 (CI 0.52-1.25) and cardiovascular death: RR 0.80 (0.69-0.92).

RCT, randomised controlled trials; PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, alpha-linolenic acid; MI, myocardial infarction; CHD, coronary heart disease.

2.3.2.2 OMEGA 3 FATTY ACIDS AND CVD STUDIES: OBSERVATIONAL STUDIES

Observational studies look into the habitual behaviour of subjects. Selected observational studies investigating the relationship between omega 3 fatty acid intake and CVD are presented in Table 19.

The Chicago Western Electric study presented evidence that the consumption of fish decreased the risk of CHD mortality. An ecological study carried out by Zhang et al. (1999) reported a reduction in the risk of all-cause, ischemic heart disease and stroke mortality in 36 countries with increased consumption of fish. Mizushima et al. (1997) who investigated the influence of migration on dietary habits found a dose-response relationship between the frequency of fish consumption and the reduction in CVD risk factors. One study that is unique due to its focus on the effect of omega 3 fatty acids on women reported an inverse association between the amount of fish consumed and CHD especially CHD death (Hu et al., 2002). Several papers have been published from data collected during the US Physicians Study. A paper written after a 4 year follow up found no relationship between fish consumption and CVD (stroke or MI) (Morris et al., 1995). The reason behind this is uncertain, the study was carried out on a large population therefore there should have been sufficient statistical power and, in addition strict criteria were used to confirm all cardiovascular events. However they only looked at total fish consumption, not fatty fish, which may have had a significant effect on the results. In a paper published on data collected after a follow up of 11 years on the US Physicians Study an inverse relationship between sudden cardiac death and fish consumption was reported, however no relationship was found with MI (Albert et al., 1998). Albert et al. (1998) also reported a significant inverse relationship between blood omega 3 fatty acid levels and risk of sudden death.

Observational studies do not provide conclusive evidence that a causal relationship exists between the factors measured, as the studies are carried out in uncontrolled environments (Barton, 2000). However the studies reviewed indicated a significant decrease in the risk of several CVD's including CHD and stroke as well as death resulting from CHD.

Table 19. Selected prospective observational studies on the effect of fish consumption on cardiovascular disease

Reference	Subjects (n)	Gender	Age (years)	Follow-up (years)	Conclusion
Morris et al. (1995) (US Physicians Health Study)	20 551	Men	40 – 84	4	No relationship between cardiovascular endpoints, stroke or cardiovascular death and dietary intake of fish. Total MI for <1 meal/week: adjusted RR 1.0, 1 fish meal/week: adjusted RR 1.6 (Cl 1.1-2.3), 2-4 fish meals/week: adjusted RR 1.4 (Cl 1.0-2.0) and >5 fish meals/week: adjusted RR 1.2 (Cl 0.6-2.2)
Mizushima et al. (1997)	433 Japanese in Japan & 269 Japanese immigrants in Brazil	All	-	-	A striking negative gradient in the frequency of fish intake per week was found from group living in Japan (men/women, 3.8-4.7/3.6-4.8 servings/week) and those living in Brazil (0.5-1.9/0.5-1.6 servings/week). Possible association between fish intake and reduced cardiovascular risk, through the beneficial effects of omega 3 PUFA
Daviglus et al. (1997) (Chicago Western Electric Study)	2 107	Men	40 – 55	30	Inverse association between fish consumption and death from CHD RR: 0.62 (CI 0.40-0.94), especially non-sudden death from MI RR: 0.33 (CI 0.12- 0.91), total RR: 0.56 for total MI, sudden death RR: 0.68 (CI 0.37-1.25)
Albert et al. (1998) (US Physicians Health Study	20 551	Men	40 – 84	11	Fish consumption was inversely related to the risk of sudden cardiac death with a significant trend (p = 0.03) across 5 levels of dietary fish consumption (RR 0.34-0.68). Men who consumed any quantity of omega 3 fatty acids had a decreased RR (0.30-0.55) of sudden death (although this reduction was not significant in all quartiles). No association with MI was found
Zhang et al. (1999)	36 countries	All	45 – 74	30	Fish consumption was independently, significantly inversely associated with mortality from all causes (r = -0.47 to -0.62, P <0.01 to <0.001), ischemic heart disease (r = -0.38 to -0.51, P = 0.05 to <0.01) and stroke (r = -0.27 to -0.35, P <0.05) in both sexes
Hu et al. (2002) (Nurses Health Study)	84 688	Women	34 – 59	16	Higher consumption of fish and omega 3 fatty acids are associated with a lower risk of CHD: 1-3 servings fish/month: RR 0.79 (CI 0.64-0.97), 1/week: RR 0.71 (CI 0.58-0.87), 2-4/week: RR 0.69 (CI 0.55-0.88), and ≥5/week: RR 0.66 (CI 0.50-0.89). For CHD deaths: ≥5 servings of fish/week: RR 0.55 (CI 0.33-0.90)
Albert et al. (2002) (US Physicians Health Study follow up)	278	Men	40 – 84	17	Fish reduced the risk of sudden death from cardiac causes. Compared with men in the lowest quartile of blood omega 3 fatty acid levels men in the third quartile: RR 0.28 (CI 0.09-0.87) and the fourth quartile: RR 0.19 (CI 0.05-0.71)

PUFA, polyunsaturated fatty acid; MI, myocardial infarction; CHD, coronary heart disease; CVD, cardiovascular disease.

2.3.2.3 OMEGA 3 FATTY ACIDS AND CVD STUDIES: RANDOMISED CONTROLLED TRIAL

RCT are used to assess the efficiency of a substance or service through randomly assigning subjects to the treatment and a suitable control. Trials are used to determine if there is a cause-effect relation between the treatment and the outcome. The use of randomisation is important to ensure there are no systematic differences between intervention groups in factors that may affect the outcome (Sibbald & Roland, 1998).

The studies summarised in Table 20 demonstrate that increased intake of omega 3 fatty acids from fish or supplements reduces the risk of CHD events and mortality.

The only trial that did not report a reduction in risk of CHD events and mortality from omega 3 fatty acid consumption was the DART 2 trial (Burr, 2007). In fact the author reported a higher mortality rate in those who were advised to consume fish or fish oil. It was suggested that this may have occurred from an interaction between the fish oil intake and medication use. However the excess mortality in the fish group was restricted to those not taking medication, except those on Digoxin. It appeared in the follow-up that fish oil increased the risk of cardiac death in men with angina, this was attributed to fish oil in supplements rather than dietary fish. The authors proposed that this result was due to an increase in 'risk taking' behaviour as subjects may feel that the treatment protects them against heart disease. However investigations into weight, BMI, serum cholesterol, medication and modifications in behaviour did not find a significant difference. DART 2 can be considered an outlier study because, despite its reasonable study design, it was criticised for not being properly conducted or reported due to a lack of funding (von Schacky et al., 2006).

Table 20. Selected randomised controlled trials on the effect of omega 3 fatty acids on cardiovascular disease

Reference	Subjects (n)	Inclusion Criteria			Treatment	Duration (years)	Conclusion
		Gender	Age (years)	Health			
Burr et al. (1989) (Diet and Reinfarction Trial (DART))	2 033	Men	< 70	Nondiabetic recovering from an MI	Dietary advice to consume 200 – 400g fatty fish/week, 3 fish oil capsules/day or no advice	0.5 and 2.0	Men given fish advice had 29% lower two year all-cause mortality. 32 % decrease in fatal CHD, no change in non-fatal CHD.
Burr (2007) (Diet and Angina Randomised Trial (DART 2))	3 114	Men	< 70	Stable angina	Advice to eat fatty fish 2 times/week, take fish oil capsules or no advice	3.0 to 9.0	Advice to eat fatty fish or take fish oil did not affect all-cause mortality, but it was associated with a significant increase in sudden cardiac death (p = 0.018), and this effect was largely confined to the subgroup given fish oil capsules
Valagussa et al. (1999) (GISSI)	11 324	All		Survived acute MI	1 capsule/day (0.88g omega 3 fatty acids), omega 3 fatty acids and 300mg/day vitamin E, vitamin E only or control	3.5	20% decrease all-cause mortality 35% decrease fatal CHD, 45% decrease sudden death, no change in non-fatal CV events
Yokoyama et al. (2007) (Japan EPA Lipid Intervention Study (JELIS))	18 645	All	40 – 75	Hyperlipidemic, 3 664 with established CHD	1.8 g of EPA /day and statin or statin only	5.0	19% relative reduction in major coronary events. Sudden cardiac death and coronary death did not differ between groups. Patients with a history of coronary artery disease given EPA treatment, major coronary events reduced by 19%. Patients with no history of coronary artery disease, EPA treatment reduced major coronary events by 18%

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; MI, myocardial infarction; CVD, cardiovascular disease; CV, cardiovascular; CHD, coronary heart disease.

2.3.2.4 OMEGA 3 FATTY ACIDS AND CVD STUDIES: BIOMARKER STUDIES

The term biomarker (biological marker) was presented in 1989 as a medical topic used to describe "measureable and quantifiable biological parameters which serve as indices for health and physiology-related assessments, disease diagnosis, metabolic processes, substance abuse, pregnancy, cell line development, epidemiologic studies, etc" (Vasan, 2006, p. 2335). LC omega 3 fatty acids have been shown to decrease the risk of CVD through improvement in several CVD biomarkers, including cardiac arrhythmias, TG, BP, platelet aggregation, inflammation endothelial function and stabilisation of atherosclerotic plaque (von Schacky, 2006). Table 21 summaries the effects of EPA and DHA on several biomarkers related to CVD.

Table 21. Effects of eicosapentaenoic acid and docosahexaenoic acid on biomarkers related to cardiovascular disease (von Schacky, 2006)

Biomarker	**EPA**	**DHA**
TG	↓↓	↓↓
Cholesterol	↔	↔
LDL-C	↑	↑
HDL-C	↔	↑
Platelet aggregability	↓	↓
Mean platelet volume	↓	↔
BP	↔	↓
Heart rate	↓	↓↓
Endothelial function	↔	↑
Glucose metabolism	↔	↔

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TG, triacylglycerols; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; BP, blood pressure.

The following sections discuss the effect that omega 3 fatty acids have on lipid profile and BP as these biomarkers were investigated in the present study.

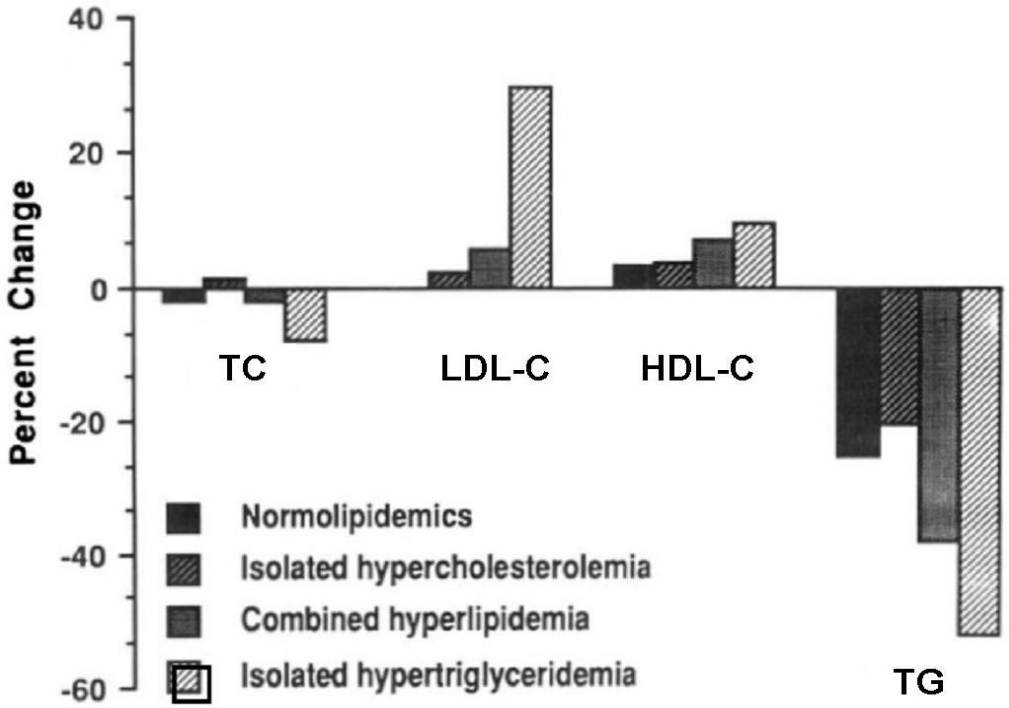
2.3.2.4.1 BIOMARKER STUDIES: LIPID PROFILE

High TG levels have been proven to be an independent risk factor for CHD (Hokanson, 2002). LC omega 3 fatty acids have consistently been shown to have a hypotriacylglycerolaemic effect, that is dose dependent and greater with higher baseline TG levels. However dosages of 3 – 5 g per day of omega 3 fatty acids may be required to be consumed before this effect is apparent (Kris-Etherton et al., 2002).

A meta-analysis by Harris (1997) investigated the results from 36 crossover and 29 parallel RCT on the benefits of consuming omega 3 fatty acids from marine sources. The author concluded that consumption of 3 – 4 g per day of LC omega 3 fatty acids for 2 weeks or longer resulted in a 25 % decrease in plasma TG in normolipidaemic subjects and 25 – 35 % in hyperlipidaemic subjects. A 5 % and 10 % increase in LDL-C was reported in normolipidaemic and hyperlipidaemia subjects, respectively. Although this would appear to have a negative effect on CVD, further research has shown omega 3 fatty acid increases larger LDL-C particles while decreasing small dense atherogenic very low density lipoprotein cholesterol (VLDL-C) resulting in a beneficial effect on the decreasing risk factors of CVD (Theobald et al., 2004). Harris (1997) reported that fish oil reduces the rate that hepatic VLDL-C is secreted; these VLDL-C molecules were also reported to be low in subjects consuming fish oil compared to individuals who did not.

These results have been supported via a meta-analysis by Balk et al. (2006) 10 years later. The authors reviewed 25 large RCT's that looked at the effect of omega 3 fatty acids from both plant and fish sources on the blood lipid profile of study participants. They reported that the effect from fish and fish oil on total, LDL-C and HDL-C was small. There was, however, a 15 % or greater reduction in TG. Fish oil caused a decrease of 0.70 mmol/l in TG and an increase of 0.04 mmol/l in HDL-C as well as an increase of 0.16 mmol/l LDL-C.

Figure 11 shows the effects of omega 3 fatty acids on subjects with different hyperlipidaemias that was created from a critical review by Harris (1989). A decrease in cholesterol was observed in hypertriacylglycerolaemic subjects, however this was not seen in hypercholesterolaemic and normolipidaemic subjects. The reason for the decrease in TC found in hypertriacylglycerolaemic subjects was reported by the author as being due to a fall in VLDL-C not LDL-C.



TC, total cholesterol; LDL-C, low-density lipoprotein; HDL-C, high-density lipoportein; TG, triaclygerides

Figure 11. Summary of the overall mean percentage changes in lipids and lipoproteins in fish oil trials according to lipid disorder (Harris, 1989)

Harris et al. (2007) reported that there was no significant difference in the blood lipid profile from the consumption of fish compared to fish oil. Both caused an increase from 2.74 to 2.97 mmol/l for LDL-C. There was no significant difference in HDL-C. However the plasma TG increased from 0.77 to 0.96 mmol/l in the capsule group, but decreased from 1.17 to 1.06 mmol/ in the fish group.

The studies on blood lipid profiles and omega 3 fatty acids have shown that omega 3 fatty acids improve the lipid profile through decreasing TG and VLDL-C, while increasing HDL-C. This can, in turn, result in a decrease in the risk of CVD.

2.3.2.4.2 BIOMARKERS: BLOOD PRESSURE

BP has been reported as being a risk factor in cardiovascular mortality. In fact research has shown that for every increase of 20/10 mm Hg the risk of death from ischaemic heart disease and stroke is doubled (Zatsick & Mayket, 2007).

A meta-analysis by Morris et al. (1993) that was done on 31 RCT reported consumption of 7.7 g omega 3 fatty acids per day lowered BP by 4/3 mm Hg in hypertensive subjects. Another meta-analysis found reductions of 5.5/3.5 mm Hg in BP when at least 3 g of fish oil was consumed per day (Appel et al., 1993).

Geleijnse et al. (2002) carried out a meta regression analysis on 90 RCT's investigating the effects of omega 3 fatty acids on BP. The authors found that consumption of approximately 4 g per day of omega 3 fatty acids was associated with a decrease of 1.7/1.5 mm Hg in BP. The reduction was greater in elderly and those with high baseline BP. The authors also reported that lowering systolic BP by as little as 2 mm Hg could cause a 4 % reduction in the risk of mortality due to CVD. Consuming 3.7 g per day of fish oil was reported to result in a reduction of 2.1/1.6 mmHg in BP. They also reported more significant effects on those over 45 years and those with a BP of 140/90 mmHg or greater.

Following their meta-analysis, Morris et al. (1993) reported a dose-response effect between BP and omega 3 fatty acids. Consumption of less than 3 g per day resulted in a decrease in BP of 13/0.7 mm Hg. At intakes between 3.3 – 7 g per day there was a decrease of 2.9/1.6 mm Hg and at 15 g per day the decrease was 8.1/5.8 mmHg. Both EPA and DHA were found to have an effect on BP. With a mean dose of 4.2 g per day of omega 3 fatty acids no effect on BP was found in healthy individuals.

2.4 OMEGA 3 INDEX

A new biomarker, the omega 3 index, has been introduced by von Schacky & Harris (2007) as a new risk factor for sudden cardiac death. It was proposed as being an independent risk factor that can quickly and easily increase through higher intakes of LC omega 3 fatty acids (Harris & von Schacky, 2004). The omega 3 index is defined as the level of EPA and DHA in RBC membranes expressed as a percentage of total fatty acids in RBC's (Harris, 2008). The formula for calculating the omega 3 index is: EPA + DHA fatty acids in RBC / total fatty acids in RBC x 100.

Harris & von Schacky (2004) compared the omega 3 index (calculated using RBC EPA and DHA) with plasma phospholipid and whole blood EPA and DHA as biomarkers of omega 3 fatty acids. They reported a correlation coefficient of greater than 0.9 between the omega 3 index and both whole blood and plasma phospholipid EPA and DHA. The information given in Table 22 suggests that the omega 3 index has the potential to be a significant biomarker in the future, due to its consistency, strong association with CVD, independence etc.

Table 22. Omega 3 index as a potential risk marker (Harris, 2007)

Consistency of epidemiological data for fish intake/omega 3 fatty acid biomarkers and CHD risk	
Between populations	Yes
Within populations	Yes
Prospective cohorts	Yes
Case-control studies	Yes
Raising biomarker levels with fish or omega 3 fatty acids reduce risk	Yes, No
Strong association between biomarker and disease	Yes
Biomarker independent of other known risk factors	Yes
Adds predictive value to currently available risk markers	Unknown
Biological plausibility for elevated biomarker levels	Yes
Biomarker is modifiable by diet	Yes
Omega 3 index is highly correlated with intake of fish and fish oil supplements	Yes
Advantages of using RBC	
Standardised methodology	Yes
Low biological variability	Yes
High analytical reproducibility	Yes
Pre-analytical stability	Yes
Measurable in fasting or fed samples	
RBC fatty acids composition stable for at least 4 years frozen at -80°C	
RBC fatty acids composition is less influenced by day-to-day variations and by dyslipidemias than are plasma	
The RBC is a readily available by-product of usual phlebotomy that, if shown to be useful, can be obtained without subjecting the patient to additional procedures	
The half-life of RBC EPA + DHA is 4 - 6 times longer than that of serum EPA + DHA	
RBC EPA + DHA is highly correlated with other omega 3 biomarkers such as whole serum, serum phospholipids and whole blood	

CHD, coronary heart disease; RBC, red blood cells; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Harris & von Schacky (2004) evaluated the relationship between omega 3 index and risk of CHD mortality in published observational trials and RCT's. They reported that an omega 3 index of 8 – 12 % or greater has been shown to have the highest cardio-protection, while concentrations of 4 % or less have the least cardiovascular protection. Individuals with an omega 3 index of 3.3 % have ten times the risk of dying due to a sudden cardiac episode than those with an omega 3 index of 7 % (von Schacky & Harris, 2007). Figure 12 illustrates the results of the dose-ranging studies. The 8 % or greater recommendation can be achieved through daily consumption of 2 g EPA and DHA per day for 8 – 20 weeks (Cao et al., 2006).

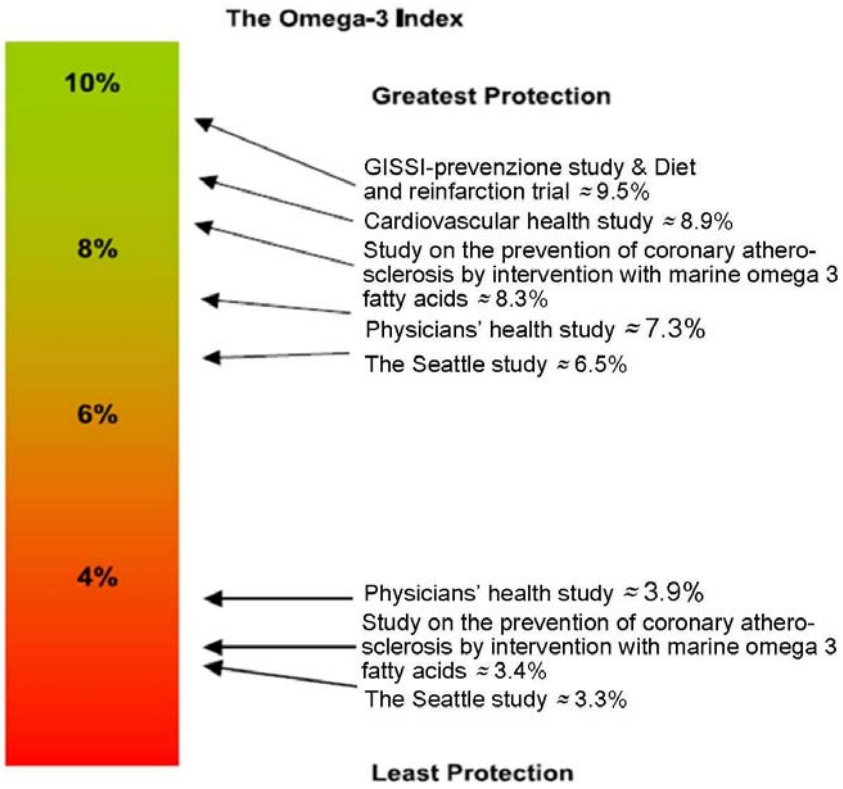


Figure 12. Summary of evidence for the proposed ranges of omega 3 index with greatest and least cardio-protection (Harris & von Schacky, 2004)

A study carried out on 23 healthy premenopausal women over 16 weeks reported that there was an increase in the subject's omega 3 index from treatment with both fish and fish oil capsules. An increase from 4.1 to 6.5 % in the fish group and 4.2 to 6.0 % in the capsule group was shown after 16 weeks. The authors also determined that the EPA and DHA levels in plasma phospholipids had a significantly higher coefficient of variance than the omega 3 index. The authors suggested that this shows that the omega 3 index is a more stable marker of EPA and DHA status (Arterburn et al., 2008; Harris et al., 2007).

The omega 3 index was criticised in one study by Hibbeln et al. (2006) for several reasons. It does not take into consideration the effects of omega 6 fatty acids on omega 3 fatty acid status, it is based largely on studies carried out on US subjects and does not reflect worldwide intakes of omega 3 or omega 6 fatty acids.

2.5 SELENIUM

Selenium is an essential trace element required in many biological reactions as a component of selenoproteins. It has been suggested that selenoproteins are involved in as many as 30 mammalian systems. These include in GPx enzyme systems which are involved in protection against oxidative damage through intracellular structures. The functional role of many selenoproteins is still unknown, however selenium is known to be essential in the functioning of at least two groups of enzymes (GPx and iodothyronine deiodinases). Selenoproteins can be used in the body as antioxidants; they are also used in thyroid hormone metabolism, immune function and reproduction. Selenium is able to repair cellular damage caused by free radicals (natural products created by oxygen metabolism) that have been shown to contribute to the development of chronic disease (Froslie et al., 1985).

In food, selenium is mostly bound to amino acids in the form of selenomethionine and selenocysteine (see Figure 13) where it replaces the sulphur atom (Wahlqvist, 2002).

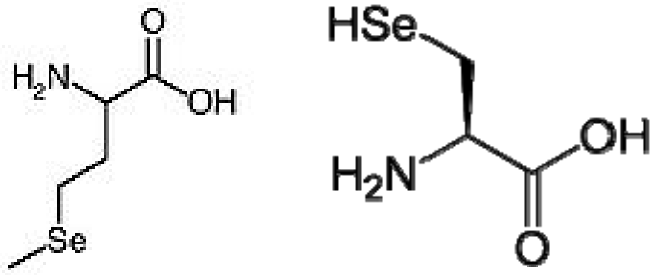


Figure 13. Structure of selenoproteins: selenomethionine and selenocysteine (European Virtual Institute for Speciation Analysis, 2008)

Selenocysteine is incorporated into proteins through genetic encoding (Thomson, 1998), whereas selenomethionine is randomly incorporated into methionine-containing proteins (Froslie et al., 1985).

Selenium deficiency in humans is rare, but when it does occur it may take one of two forms, that is, Keshan's disease and Kashin-Beck disease. Keshan's disease is an endemic cardiomyopathy that is seen during pre-adolescence and adolescence, while Kashin-Beck disease is an endemic osteoarthritis (Thomson, 2004a).

2.5.1 SELENIUM: FOOD SOURCES

Selenium is found in varying concentrations in soil, where it is taken up by plants. Therefore in areas with low soil selenium a higher prevalence of selenium deficiency is seen (World Cancer Research Fund / American Institute for Cancer Research, 2008).

Selenium is present in food as selenomethionine, which is found in plant and animal products, and selenocysteine, that is found only in animal products. Selenomethionine accounts for half of the available dietary selenium and has a bioavailability of greater than 90 %. Selenocysteine is also highly bioavailable (greater than 50 %) in inorganic forms, such as selenate and selenite (Thomson & Robinson, 1986).

Animal foods have higher concentrations of selenium than plant foods. Fish and organ meats are rich sources of selenium in New Zealand, followed by muscle meats. However the bioavailability of selenium is greatest in selenium-enriched yeast, cereals and grains. The poorest sources are dairy, fruit and vegetables due to selenium soil concentrations in New Zealand being low (Thomson & Robinson, 1986). Brazil nuts contain the highest amount of selenium, however the level varies significantly (2.35 – 10.2 µg selenium/g). A study by Thomson et al. (2008) investigating the effect of Brazil nuts on plasma selenium reported that 2 Brazil nuts per day increased plasma selenium by 62.4 %.

Food groups which contribute significantly to selenium intakes in New Zealand are highlighted in Table 23 and indicate that although fish/seafood intake is low it is still a significant contributor to selenium intake.

Table 23. Contribution of selected food groups to the total dietary selenium intake of New Zealand children (Thomson et al., 2007)

Food Groups	Selenium Intake (µg/day)	Total Intake (%)
Bread/grain	10.4	33.1
Meat	4.6	14.8
Bread	3.9	13.4
Poultry	4.4	11.2
Dairy	2.5	9.1
Fish/seafood	8.1	8.6
Fruit/vegetables	2.2	7.9

The processing of foods containing selenium has been shown to influence its bioavailability. Methods that degrade protein improve selenium's digestibility, this is thought to occur through the release of selenium bound to the protein (Shen et al., 1997; Shi & Spallholz, 1994). However heating of food may cause a loss in selenium (Higgs et al., 1972; Ornsrud & Lorentzen, 2002). Bioavailability is also influenced by other factors, including the biochemical form of selenium and the total amount of protein and fat present in the food. It has been found that diets high in protein produce better absorption of selenium (Greger & Marcus, 1981; Shen et al., 1997; Shi & Spallholz, 1994). The amount and type of fat also appears to affect the utilization of selenium. A study feeding chickens a diet contain selenium found that increasing the amount of SFA consumed from 4 to 20 % significantly enhanced the GPx activity, this was not found with consumption of unsaturated fats (Mutanen, 1984).

2.5.2 SELENIUM : INCREASING STATUS

A study by Duffield & Thomson (1999) reported that selenium intake can be increased by 33 % through the addition of fish, liver and kidney to the diet. However, in New Zealand these foods are not consumed frequently enough to have a significant effect on selenium status.

Fish is a rich source of selenium; studies have shown selenium concentration in fish to range from 200 to 600 µg per kg (Ornsrud & Lorentzen, 2002). However there are several studies on rats that have found the bioavailability of selenium in fish to be low compared to other selenium rich foods (Alexander et al., 1983; Douglass et al., 1981). In contrast, some studies have reported the bioavailability of selenium in fish to be high (Ornsrud & Lorentzen, 2002; Wen et al., 1997).

A study that investigated the effects of consuming 150 – 200 g of fatty fish per day (herring, salmon or mackerel (40 – 50 µg selenium/day)) on subjects' plasma selenium found that after 6 weeks of treatment there was an increase of 0.13 µmol/l. The authors reported large individual variation in selenium concentrations. The authors conclude that the selenium found in fatty fish is of relatively low bioavailability when compared to other forms of selenium (Thorngren & Akesson, 1987). Hagmar et al. (1998) reported a positive correlation between the number of fish meals consumed per month and plasma selenium. Subjects who consumed high amounts of fish had an 81% higher concentration compared to those with low intakes (Onning & Bergdahl, 1999). Fish intake increased plasma selenium, but had no effect on plasma GPx. The author claimed this was due to the chemical form of selenium in fish. The form of selenium varies in different species and different geographic areas (Thomson, 1998), but does not differ with the age of the fish (Froslie et al., 1985) or season (Vos & Hovens, 1986).

Selenium is an essential element for salmon; salmon that have low selenium levels have been shown to suffer from growth retardation, reduced packed cell volume, reduced selenium concentration in tissues and reduced GPx activity (Bell et al., 1985). Research has shown that wild salmon have higher selenium concentrations in the liver and fillets than farmed salmon (Maage et al., 1991; Poppe et al., 1985).

2.5.3 SELENIUM: BIOMARKERS

In general, blood selenium is a good indicator of dietary intake and selenium status, however other bodily tissues, such as hair and nails can also be used (Burk & Levander, 1999; Neve, 1991; Sheehan & Halls, 1999). Long term selenium status in humans can be measured by toenail and hair concentrations (Longnecker et al., 1996; Mannisto et al., 2000). However hair concentrations can be influenced by some shampoos which contain selenium. Urinary excretion of selenium has been associated with plasma selenium and dietary intakes in low selenium populations (Griffiths & Thomson, 1974). Short term selenium status (several days) can be measured by plasma or serum, whereas RBC selenium reflect longer term status (several weeks to months) as it is incorporated during the synthesis of RBC cells. Currently there are no ranges set for normal

selenium status as this varies greatly from country to country (Patching & Gardiner, 1999).

The concentration of selenium in tissue alone cannot always be depended on as it does not reflect functional activity, this instead depends on the form of selenium ingested (Neve, 1995; Thomson et al., 1993). If selenomethionine is ingested the tissue concentration will most likely be high, as selenomethionine is non-specifically incorporated into proteins in place of methioine (Behne et al., 1991). Organic selenium in the blood is non-specifically incorporated into haemoglobin in RBC and the plasma through albumin (Burk et al., 2001; Butler et al., 1991). True selenium status should reflect the amount of selenium that is available for the activity of the functional selenoproteins. Therefore selenoproteins in the body are a more appropriate measure of selenium status (Patching & Gardiner, 1999). Platelet GPx can detect increases in activity within 1 to 2 weeks (Alfthan et al., 1991; Levander et al., 1983; Neve et al., 1988; Thomson, 2004a; Thomson et al., 1985; Thomson et al., 1993). Measurement of GPx is a useful tool in areas of low selenium status and plasma or serum selenium is the favoured and most common measure to date (Patching & Gardiner, 1999).

2.5.4 SELENIUM: RECOMMENDATIONS FOR INTAKES

The RDI for selenium in Australia and New Zealand is 70 µg for men and 60 µg for women, which is based on amounts required to maintain adequate plasma GPx (Ministry of Health, 2006).

Recommendations for selenium intake and plasma concentrations based on selenium's function in the body are summarised in Table 24. Research carried out on New Zealanders found that most have adequate selenium concentrations to prevent Keshan's disease and meet the maximum requirement for GPx and selenoprotein P, however not enough for iodonthyroine 5' deiodinases or protection against some cancers (Thomson, 2004a).

Table 24. Estimates of requirements for dietary selenium and plasma selenium concentrations for adequate bodily function (Thomson, 2004a)

	Dietary Selenium Requirements (µg/day)	**Plasma Selenium (µmol/l)**
Minimum requirement for prevention of Keshan's disease	20	> 0.25
Physiological requirement (EAR) for maximal GPx and selenoprotein P	45 – 50	> 0.82
Maintain adequate plasma GPx (RDI)[a]	76 – 70	-
Requirement for iodothyroine 5'deiodinases	30	> 1.00 – 1.20
Protection against some cancers	120	> 1.50

[a]Ministry of Health, 2006.
EAR, estimated adequate requirement; GPx, glutathione peroxidase.
mg/dl = µmol x 76.3.

In a study carried out on New Zealanders, it was found that plasma selenium needs to be 1.14 µmol/L to achieve full expression of plasma GPx (Duffield et al., 1999). This is consistent with two previous studies that reported a value of 1.2 µmol/l for full expression (Thomson et al., 1993; Yang et al., 1988). Plasma GPx has been shown to plateau at a whole blood selenium concentration of 1.13 µmol/l (Alfthan et al., 1991). Neve (1995) demonstrated variations in GPx expression depending on the source of selenium. Supplementation of selenite or selenate resulted in saturation of platelet GPx activity at lower plasma selenium levels than consumption of organic forms of selenium.

The maximum safe intake of selenium is estimated to be 400 µg per day, however functional signs of toxicity are not seen till 750 – 850 µg per day (Thomson, 2004b). Toxicity causes garlicy odour in breath, fatigue, gastrointestinal disturbances, transverse lines on the nails, alopecia and peripheral neuropathy (Wahlqvist, 2002).

2.5.5 SELENIUM: DISEASE PREVENTION

Marginal selenium status can result in suboptimal amounts of one or more selenoproteins which may be associated with an increase in the risk of cancer, CVD, altered immune function, male infertility, inflammatory disorders, autoimmune thyroid disease and viral infections (Brown & Arthur, 2001; Thomson et al., 2008).

Some evidence suggests selenium has a beneficial role in CHD, however the research findings are conflicting. Two large cohort studies have highlighted low selenium concentrations as an independent risk factor for MI in populations with low selenium status (Salonen et al., 1995; Suadicani et al., 2002), however other studies did not find this result (Rayman, 2000). Inconsistency in results could potentially be due to lack of a standardised threshold for the protective effect of selenium (Thomson, 2004a).

A meta-analysis carried out on 14 cohort studies, 11 case-control studies and six RCTs relating selenium and CHD reported an inverse association between selenium concentrations and CHD incidence in observational studies. The authors estimated that a 25 % decrease in the risk of CHD could be seen with increasing selenium concentrations by 50 %. However this was not conclusive in the RCT review. An increase in selenium concentrations of 49 % has been shown with consuming 100 µg selenium per day. This could result in an 11 % reduction in the risk of CHD. The potential beneficial effect selenium has on decreased risk of CHD was proposed by the authors as being due to its use as an antioxidant through the selenoprotein, GPx. Selenium may also cause beneficial effects through neutralising peroxide intermediates which may cause a reduction in inflammatory leukotrienes and prostaglandins. Selenium supplementation of individuals previously deficient in selenium has been shown to increase their enzymatic antioxidant activity through decreasing lipid peroxidation (Flores-Mateo et al., 2006).

A review carried out by Rayman (2005) which investigated the protective effect selenium may have on cancer, reported that plasma selenium concentrations of 1.6 µmol/l (120 mg/l) were required to optimise selenium anti-cancer effects. The strongest evidence of the effect of selenium on cancer was reported for lung cancer, oesophageal cancer and gastric-cardia cancer, prostate cancer, and colorectal adenoma. The author suggested that there are several proposed mechanisms that selenium influences which may aid in the reduction in the risk of cancer. Selenium is present in the genetic code through the incorporation of selenocysteine. Selenocysteine controls cell redox status and protects tissues and membranes against oxidative stress. It is also used in methyl selenol a precursor to methyl seleninic acid. This has been shown to block the progression of the cell cycle, induce apoptosis of cancer cells and prevent the creation of new blood vessels which are required for the growth of tumours.

The largest systematic literature review on the effects of several factors including food on cancer also reviewed foods containing selenium. They concluded that selenium had a protective effect against prostate cancer. However there was only limited evidence to suggest that selenium is able to protect against stomach or colorectal cancers (World Cancer Research Fund / American Institute for Cancer Research, 2007).

A study by Akbaraly et al. (2005) found selenium status influenced the mortality rate for all forms of cancer. The study looked at the baseline plasma selenium levels of 1,389 subjects. In the 9 year follow-up 55 subjects had died from cancer. It was determined that the risk of mortality from cancer increased four fold in those in the lowest baseline (1.01 µmol/L) plasma selenium compared to those in the highest (1.10 µmol/L).

Selenium is thought to have a protective effect on mercury toxicity (Satoh et al., 1985) as the two are often negatively correlated (Caurant et al., 1994; Eisler, 1985; Kuehl & Haebler, 1995; Wagemann et al., 1996). The mechanisms of protection are unclear although it has been hypothesised that selenium and mercury form a biologically inactive compound, or that selenium plays an antioxidative role (Burger et al., 2001). Research carried out on fish living in severely polluted areas have found a possible link between mercury and selenium (Koeman et al., 1975). This has lead to the belief that selenium is able to "detoxify" the fish from the high concentration of mercury (Chvojka et al., 1990).

2.5.6 SELENIUM: STATUS IN NEW ZEALAND

The concentration of selenium in food is dictated by selenium levels present in the soil the plants are grown in (Thomson & Robinson, 1990). It has been found that the concentration of selenium in soil within a given geographical area roughly reflects the selenium status of the population within that region. Soil concentrations of selenium typically range from < 0.01 µg/g to > 1000 µg/g. This large range reflects why status is so variable across regions. The selenium status of the New Zealand population has been reported to be sub-optimal due to low concentrations of selenium in the soil (Thomson & Robinson, 1980; Whanger et al., 1988). In fact, the New Zealand population has one

of the lowest selenium intakes in the world. A study in Dunedin reported selenium intakes based on dietary records and a FFQ, to be between 29 – 30 µg per day (Duffield & Thomson, 1999). This intake is significantly less than the RDI for Australia and New Zealand.

Based on the 1997 National Nutrition Survey (Russell et al., 1999), men in New Zealand consumed an average of 56 µg of selenium a day and women 39 µg per day. The main sources of which were seafood, poultry and eggs (Thomson & Robinson, 1990, 1996). New Zealander's selenium intake has increased slightly over the years due to an increase in consumption of food imported from overseas and supplementation of selenium in farmed animal foods (Thomson, 2004b).

Plasma selenium varies in populations from different areas of New Zealand (see Table 25). A study investigating serum selenium concentrations in New Zealand children reported higher status in the North Island compared to the South Island (Thomson et al., 2007). The authors suggest that this was due to higher consumption of Australian bread and other bakery product by those in the North Island. Wheat from Australia has significantly more selenium than wheat from New Zealand.

Table 25. Plasma selenium concentration of adult residents of New Zealand (Thomson, 2004b)

New Zealand	Year	Plasma Selenium (µmol/l)
Otago	1994	0.9
Waikato & Taranaki	1994	1.08
Dunedin	1996	0.84
New Zealand	1997	1.03
South Island	1998 – 99	0.98
Dunedin		
Smokers		0.92
Non-smokers		1.12
Dunedin elderly	2000	0.9
Dunedin	2001 – 02	1.11

In New Zealand there have not been any clinical signs of selenium deficiency, apart from one patient that was on total parenteral nutrition (Thomson, 2004b). However the selenium status of the New Zealand population is marginal and remains lower than that reported in other Western countries (Thomson et al., 2008).

2.5.7 SELENIUM: SUMMARY

Selenium plays an important role in the human body through its antioxidant properties. It is of special relevance to the New Zealand population as food sources produced here are low in selenium. Fish is a significant source of selenium and has been shown to significantly increase selenium status in individuals who regularly consume fish. Adequate selenium intake is important as it is essential for normal function of the human body and has been reported as having an inverse relationship with several chronic diseases including heart disease and cancers such as prostate cancer.

CHAPTER 3

3. METHODS

3.1 FUNDING AND ETHICS APPROVAL

Funding for this project was obtained from the Institute of Food, Nutrition and Human Health, Massey University and the Foundation for Research Science and Technology. Additional support was provided by New Zealand King Salmon Company Ltd, who supplied the salmon fillets.

Ethical approval for the study was acquired from the Massey University Human Ethics Committee: Southern A (application 07/72). In accordance with this each of the subjects gave informed consent to participate in the study.

3.2 SUBJECTS

3.2.1 INCLUSION/EXCLUSION CRITERIA

Forty-four healthy volunteers, between 21 – 45 years of age were required for the study. The inclusion criteria were that subjects should:

- have a low habitual intake of fatty fish (< 2 servings per month);
- not smoke;
- not have taken fish oil or selenium supplements over the past 6 months;
- have no allergies to seafood;
- have no known condition or disease; and
- not have been taking any medication for chronic disease.

3.2.2 SUBJECT RECRUITMENT

Subjects were recruited via advertisements in the form of flyers and brochures that were distributed around the Auckland campus of Massey University (see Appendix A). Emails were sent to Auckland staff and students from Massey University and the study was also promoted during lecture times to university students. An advertisement was placed in the local North Shore Times newspaper (see Appendix B). Advertising material focused on the North Shore area, however subjects were recruited from various areas throughout greater Auckland.

Once subjects registered interest in the study they were sent further information in the form of an information sheet about the study (see Appendix C). This gave a brief description of omega 3 fatty acids and selenium, an outline of the study, an explanation of procedures involved, confidentiality measures and the rights of the subjects. If they volunteered to participate in the study they were given a screening questionnaire to complete and return (see Appendix D). This was designed to ensure that the applicants met the inclusion criteria of the study. Demographic information, including medical history, family medical history, and alcohol consumption habits were also collected in this screening questionnaire. If the applicants met the inclusion criteria and could be matched for age and gender with three other subjects (the following sections discuss this in detail) they were contacted via phone or email to set up an appointment time for their baseline measurements to be taken. Once an appointment was set up an email was sent to the subject that stated the time, date, and location of the appointment as well as instructions on how to prepare for the appointment (see Appendix E).

Applicants that did not fit the inclusion criteria were contacted via phone or email thanking them for their interest in the study and letting them know that they did not meet the inclusion criteria.

3.3 SETTING AND PROCEDURES

The study was carried out under free-living conditions. At the commencement and end of the study subjects were required to attend an hour and a half long appointment between 7:00 – 9:30 am at the Human Nutrition Research Unit, Massey University, Auckland. During the appointment fasting blood samples, anthropometric and BP measurements were obtained at the baseline and end. Subjects were then offered breakfast and asked to complete a FFQ on the computer after which they were advised the treatment group they had been assigned to. They were then given an explanation of the treatment intake requirements, storage and handling. Then instructed how to fill out the compliance booklet that was to be completed weekly over the course of the study. After 8 weeks of consuming the treatment each subject was again contacted via phone or email to set up another appointment. The same procedure was repeated to gather information for treatment outcomes. The final pages of the compliance booklets were also collected at this time for analysis.

3.3.1 STUDY DESIGN AND METHODS

A randomised comparative parallel study design was used, as shown in Figure 14 (discussed in more detail in the following sections). Subjects were matched for age and gender, based on the information provided in the screening questionnaire. Once four subjects of the same age and gender were recruited, each were randomly assigned to one of the four treatment groups, by blindly drawing names. The four different treatment regimens followed by subjects over 8 weeks are as follows:

- 2 servings of FNZK salmon (120 g/serving) per week (n = 11, ±0.36 g EPA/day and ±0.46 g DHA/day (Larsen et al., 2008)); or
- 2 salmon oil capsules per day (n = 11, ±0.12 g/day EPA and ±0.20 g/day DHA (Healtheries of New Zealand Ltd, 2008)); or
- 4 salmon oil capsules per day (n = 11, ±0.24 g/day EPA and ±0.40 g/day DHA (Healtheries of New Zealand Ltd, 2008)); or
- 6 salmon oil capsules per day (n = 11, ±0.36 g/day EPA and ±0.60 g/day DHA (Healtheries of New Zealand Ltd, 2008)).

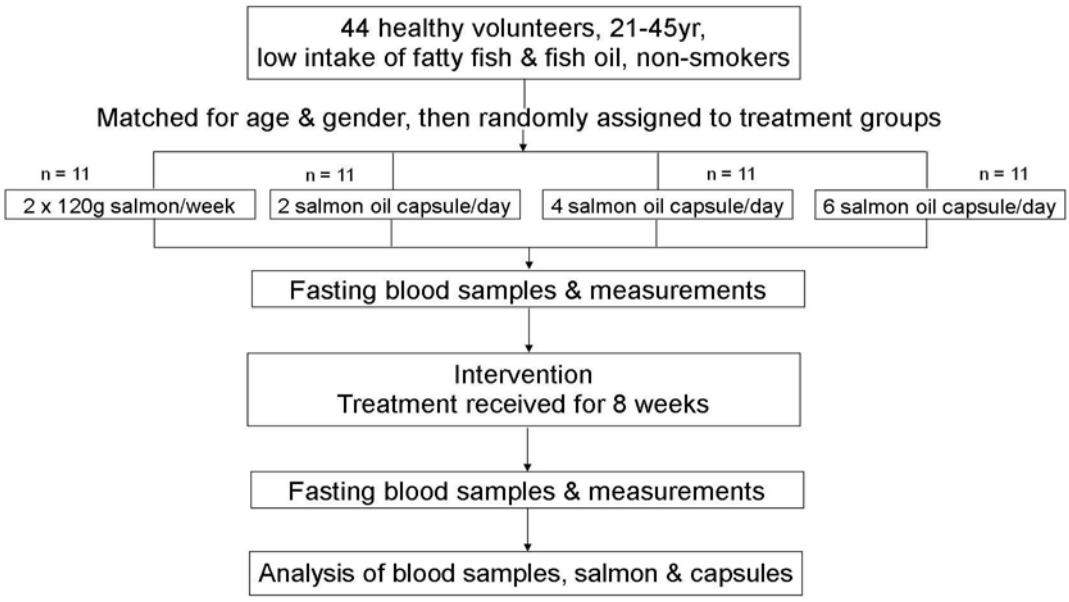


Figure 14. Study design

This study relied heavily on compliance (close as possible to 100 %) of the subject's to the treatment regimens. As compliance of subjects to treatment can decrease over time it was decided to use the shortest period of time possible to ensure useful results.

Although RBC LC omega 3 fatty acids only reach a plateau after 6 months, 8 weeks is sufficient to cause a significant increase in RBC LC omega 3 fatty acid levels (Katan et al., 1997). It is not possible to find salmon oil capsules that provide equivalent amounts of EPA and DHA contained in farmed salmon, as the amounts of EPA and DHA differ between batches of salmon and cooking methods used (Larsen et al., 2008). Therefore different dosages of capsules were provided in order to create a linear response model from which changes in RBC EPA and DHA with a dosage similar to the amount of EPA and DHA consumed from farmed salmon could be predicted.

3.3.2 INTERVENTION

The subjects were requested not to consume any fatty fish, omega 3 fatty acids or selenium supplements (other than those provided) and to maintain their normal daily routine (eating pattern, physical activity, and alcohol consumption) for the duration of the study. A compliance booklet (see Appendix F) was given to each of the subjects that contained:

- information on the study and contact information of the researchers;
- a calendar of important dates during the study;
- a weekly to-do list, storage and handling advice for the treatment; and
- a compliance diary that was to be completed on a weekly basis indicating each subject's compliance to the study protocol, including treatment intake, maintenance of normal daily routine, illness and medication use.

3.3.2.1 SALMON

The New Zealand King Salmon Company Limited provided the salmon fillets. The salmon fillets (120 g servings of fresh, boneless, vacuum packed salmon stored in polystyrene boxes containing ice packs) were delivered to Massey University, Auckland on a weekly basis via air freight from the New Zealand King Salmon factory in Nelson.

The subjects in the salmon group received a minimum of four pieces (two for consumption and two for duplicate preparation) of fresh chilled salmon on a weekly basis. Additional pieces were provided to subjects who wished to have a meal with their

partner. The fatty acid content of the cooked salmon consumed by the subjects was analysed as there can be differences in the fatty acid composition between batches and cooking methods used. This was done by providing the subjects with an additional portion of salmon for each meal. They were required to prepare both portions at the same time. For each salmon meal one portion was served and eaten, while the additional portion was stored in a coded bag (provided to them) and kept in the freezer. Subjects were also required to keep any leftover salmon that they were not able to consume. The fish were weighed and subtracted from the total amount of salmon consumed during the study. The duplicate portions were collected from the subjects on a weekly basis and stored at -80 °C until the end of the study when analysis was carried out on all the samples. At this time the 16 portions of cooked salmon obtained from each subject over the study period were pooled and homogenised. From this homogenised mix, three samples were taken for analysis of fatty acid and selenium content.

The compliance booklet provided to the subjects contained guidelines on how to incorporate salmon into their diet, recipes and recommended cooking methods. The cooking methods that the subjects were required to use were based on the results from a study by Larsen et al. (2008) who reported oven baking, pan frying and steaming cooking methods as producing the highest content of LC omega 3 fatty acids. Each week subjects were required to report any deviations from their normal routines including when they consumed the salmon (day and meal) (see Appendix F1).

3.3.2.2 CAPSULES

For this study it was important that the capsules and fish were from the same source. Therefore Healtheries “Omega 3 advanced pure salmon oil” was used as it contained only omega 3 fatty acids from salmon (1000 mg of salmon oil), whereas most other fish oil supplements contain oil from other fish or a mixture of fish. From correspondence with the Healtheries Regulatory Affairs Officer (Yates, personal communication, April 22, 2008) it was determined that the LC omega 3 fatty acids in this supplement were in the TG form, which is the same form as present in the salmon provided.

Labelling of the capsules stated the each one contained the equivalent of 60 mg of EPA and 100 mg of DHA, along with an encapsulating aid and d-α tocopherol. The capsules were well within their expiry date (01/2009) during the study period.

The salmon oil capsules were stored at 4 °C in opaque containers. The capsules were delivered to subjects fortnightly in 14 vials containing the exact number of capsules each subject was required to consume per day. At 0, 4 and 8 weeks into the intervention ten capsules were saved and stored at -80 °C for analysis of their fatty acid content and oxidation values at the end of the study.

The compliance booklet given to each subject in the capsule groups contained instructions requesting them to store the capsules in the fridge and out of the light. Capsules were requested to be consumed daily with a meal. Every week subjects were required to indicate any deviation from their normal routines or in the method they used to consume the capsules per day (with what meal and how many capsules they consumed in one sitting) (see Appendix F2).

Each compliance diary was analysed and the exact amount of omega 3 fatty acids consumed from the capsules was calculated in the following way: If a subject was in the 2 capsule group they were required to consume a total of 112 capsules over the study. If they missed 2 days then they would have only consumed 108 capsules. Therefore they only would have consumed 96 % (108 / 112 x 100) of the assigned treatment. The amount of omega 3 fatty acids required to be consumed by subjects in the 2 capsule group would then be multiplied by 96 % (0.96) to determine the omega 3 fatty acid intake for that subject. This was done for all the treatment groups.

3.3.3 TREATMENT TOLERANCE

At the end of the study each subject completed an online tolerance questionnaire to gather information on each tolerance factor of the treatment regimen. Initially each subject was asked if he/she experienced any side effects (mentioned without any probing to ensure responses were not led by suggestion). Next he/she was given a list of potential side effects that had been outlined in a study by Freeman & Sinha (2007), who measured the tolerability of omega 3 fatty acid supplements in perinatal women. For

each side effect listed a 9-point likert scale was available for subject's to rank how severe they perceived the side effect to be. Finally subjects were asked for suggestions and recommendations on what they felt was the best method for consuming the treatment they were assigned (see Appendix G).

3.3.4 FOOD CONSUMPTION HABITS

Information on each subject's dietary habits was gathered pre- and post- treatment using a semi-quantitative FFQ. This was done to identify factors that may have influenced their omega 3 fatty acid and selenium levels and monitor changes in their dietary habits that may have occurred during the course of the study. Subjects completed the FFQ online at baseline and end of the study. This FFQ was adapted from the validated FFQ of the New Zealand Adult Nutrition Survey (Russell et al., 1997). However the questionnaire was modified to include current brands and food products based on information gathered during visits to local food retailers (Pak'nSave Albany and Foodtown Glenfield, April 2008). Questions on fish consumption were added based on a validated semi-quantitative FFQ created in Australia (Mina et al., 2007). The Auckland fish markets were visited (April, 2008) and all fish species were noted, these were also added to the questionnaire. Questions that were directly related to foods high in omega 3 fatty acids and selenium, such as walnuts, Brazil nuts and omega 3 fatty acids fortified foods, were added. An additional question regarding fast food consumption was placed at the end of the questionnaire. Due to the incorporation of additional questions the order of the original FFQ was changed to create better continuity between questions. The final FFQ consisted of 61 questions and took subjects approximately half an hour to complete (see Appendix H).

3.3.4.1 CALCULATION OF NUMBER OF SERVINGS CONSUMED

The data collected in the FFQ was categorical data. In order to calculate the median number of servings consumed and to analyse differences between groups, the categorised data was analysed to determine the number of servings consumed for each individual subject. Servings were calculated on a per week basis as many of the foods were not consumed daily, while others were consumed several times a day. These calculations also allowed subjects' responses to be reviewed based on normal intakes

(e.g. 2 servings of protein/day) to determine whether there were any significant disparities.

An example of how the number of servings consumed was calculated (based on information provided in the FFQ) is shown in Table 26 and Table 27.

Table 26. Calculation of the number of servings per week of milk and bread consumed

Category (intake/day)	Calculation (number of servings consumed[a])	Consumption (servings/week)
None	0	0.00
< 1 serving	((0+1)/2) x 7	3.50
1 – 2 servings	((1+2)/2) x 7	10.5
3 – 4 servings	((3+4)/2) x 7	24.5
5 – 6 servings	((5+6)/2) x 7	38.5
≥ 7 servings	((7+8)/2) x7	52.5

[a]average serving consumed was calculated by adding the maximum number to the minimum number for each category, then dividing it by two to obtain the average per day and multiplying it by seven to obtain a serving per week.

Table 27. Calculation of the number of servings per week of foods high in omega 3 fatty acids and selenium consumed

Category (intake)	Calculation (number of servings consumed[a])	Consumption (servings/week)
Never	0	0.00
Less than once a month	¼	0.25
1 – 3 times per month	(1+3)/4	0.50
Once per week	1	1.00
2 – 4 times per week	(2+4)/2	3.00
5 – 6 times per week	(5+6)/2	5.50
Once per day	7	7.00
2 or more times per day	7 x 2	14.0

[a]if the answer referred to monthly intake the value was divided by four. If the answer referred to daily intake the value was multiplied by seven. Average serving consumed was calculated by adding the maximum number to the minimum number for the category, then dividing the answer by two to obtain the average intake per day and multiplying it by seven to obtain a serving per week.

More detailed information was gathered and analysed on food categories containing omega 3 fatty acids such as fish and other seafood, meat, poultry, soya, walnuts, and other nuts. Food sources of selenium that were analysed further included, fish and other seafood, meat, poultry, and Brazil nuts. Fish sources were categorised into lean, medium and fatty fish according to Kolakowska et al. (2006) (see Table 28). The fat

content of the fish consumed by subjects was categorised into lean, medium and fatty as determined by Quigley et al. (1995) and FSANZ (2006).

Table 28. Categorisation of fish based on fat content (Kolakowska et al., 2006; FSANZ, 2006; Quigley et al., 1995)

Fat Category	Total Fat Content (%)	Fish Sources
Lean fish	< 7	Barracuda, Bass, Baxter's Dogfish, Blue Mao Mao, Bluenose, Brill, Butterfish, Cardinal, Carp Grass, Carp Kio, Cod, Dogfish, Dory John, Dory Lookdown, Elephant fish, Flat fish, Flounder, Frostfish, Garfish, Grey Mullet, Groper, Gurnard, Hake, Hoki, Javelin, Kina, Kingfish, Leatherjacket, Ling, Mackerel Jack, Mackerel Peruvian Jack, Moki, Monkfish, Moonfish, Mullet, Oreo Dory, Parore, Perch, Pink Scorpion fish flesh, Porae, Rattail, Rays Bream, Ribaldo, Rig, Ruby, Sanma, Sea Perch, Shark, Skate, Slick head, Snapper, Sole, Southern Blue Whiting, Spiny dogfish, Sprat, Stargazer, Swordfish, Tarakihi, Tench, Toadfsih, Trevally, Trout, Trumpeter, Tuna Skipjack, Warehou Blue, Canned Tuna
Medium fat fish	7 – 11	Eel, Gemfish, Hapuku, Kahawai, Mackerel Blue, Marlin, Nuiean fish, Orange Roughy, Pilchard, Canned Anchovies, Canned Rollmops, Canned Salmon
Fatty fish	≥ 12	Carp Silver, Rudderfish, Salmon, Sardines, Toothfish, Tuna (Albacore, Butterfly, Southern Bluefin), Tuna Slender, Warehou (Silver/White), Canned Kipper, Canned Smoked Herring, Canned Herring, Canned Mackerel, Canned Sardines

3.3.5 ANTHROPOMETRY

Subject's height, weight and circumference measurements were taken in a private room. They were asked to remove their shoes and any items in their pockets before measurements were taken. Each subject was then required to stand in the middle of the scale and look straight ahead (Ministry of Health, 2008). The weight of the subject in kilograms (to the nearest 10 g) was taken using an electronic scale (August Sautr GmbH, Model D7470 Albstadt).

Height of subjects was measured with a stadiometer (Holtain Ltd). Each subject was required to stand on the surface with his/her feet together, heels, buttocks, shoulders and head pressed back against the back of the stadiometer (Ministry of Health, 2008). The plate was lowered onto the subject's head, and he/she was asked to take a deep breath and look straight ahead. The measurement was recorded in centimetres to the nearest millimetre (Ministry of Health, 2008).

Waist and hip circumferences were taken with a Lufkin measuring tape and recorded in centimetres to the nearest millimetre. The narrowest point of the waist between the lower rib cage and hip bone was used for the waist measurement. The hip circumference was taken over the largest area of the hips. These measurements were taken twice, with a third measurement taken only if the second measurement had a greater than one centimetre difference from the first. The third measurement was to check that the initial measurement was accurate.

3.3.6 BLOOD PRESSURE

BP was measured with a digital automatic BP monitor (Omron, Model HEM907) which records three consecutive measurements and reports the average of the three. The average measurement was recorded as systolic over diastolic BP in mm Hg.

3.3.7 BLOOD SAMPLE

A qualified phlebotomist collected fasting venous blood samples between 7:00 – 9:30 am, to avoid the effect of diurnal variation, at the beginning and end of the study using a sterile Vacutainer Flashback Precisionglyde needle and needle holder. The subjects were asked to fast overnight (at least 8 hours with no food or beverages, excluding water). A fasting blood sample of 10 ml EDTA blood was collected for the analysis of RBC fatty acids, plasma selenium concentrations, lipid profiles (TC, LDL-C, HDL-C and TG) and whole blood GPx activity.

Within 2 hours of taking the sample the following procedure was carried out: 200 µl of whole blood was transferred into a plastic microtube for GPx analysis. The original sample tube was then centrifuged for 10 minutes at 2500 xg at 4 °C. From this 500 µl of plasma was transferred into plastic microtubes for both the lipid profile and plasma selenium analysis. The remaining plasma was removed and the RBC were washed three times by adding an equal volume of ice cold isotonic saline (0.9 % sodium chloride) to the RBC. This was then centrifuged for 10 minutes and the supernatant removed.

This step was repeated two more times before 500 µl of packed RBC were transferred to a plastic micro-tube for fatty acid analysis. The samples were stored at -80 °C until the

analysis was done at the end of the study. The baseline and end samples for one subject were analysed at the same time. This was done to avoid day-to-day lab variation in the results.

3.4 BIOCHEMICAL ANALYSIS

3.4.1 RED BLOOD CELL FATTY ACIDS

The following solutions were prepared for the RBC fatty acids analysis.

- Internal standard stock: A bottle was rinsed with hexane and dried, before 0.0225 g of heptadechanoic acid and 0.33 g of butylated hydroxyl toluene were dissolved in 100 ml of methanol. The bottle was then covered with tinfoil and stored in the fridge at 4 °C.
- Internal standard: A glass pipette was used to measure 2.5 ml of internal standard stock into a 25 ml volumetric flask. To this 22.5 ml of methanol was added, before the solution was shaken well.

Two hundred µl of the internal standard was transferred into a silica glass tube with a screw cap and Teflon seal using a glass pipette. To this, 50 µl of RBC and 1 ml of methanolic hydrochloric acid was added using a plastic pipette. The screw cap was tightly closed before the solution was incubated for 4 hours in a 90 °C oven (Contherm Thermotec 2000 Model 2900). The sample was removed from the oven and allowed to cool to room temperature. A plastic pipette was then used to add 2 ml of hexane, before the tubes were vortexed (ZX3 Velp Scientifica Vortex) at 40 Hertz for 1 minute. The mixture was then centrifuged (Heraeus Labofuge 400R) at 2000 rpm for 3 minutes at 20 °C. The top layer was removed with a Pasteur pipette and placed in a clean silica glass tube. The silica glass tube was put in a nitrogen concentrator (Techne Sample Concentrator Model FSC400D and DB-3 Dri Block) at 4 psi. A Hamilton pipette was used to add 100 µl of hexane into the tubes. This liquid was then transferred with a Pasteur pipette into an insert of a vial. The lid of the vial was closed and the vial placed in the GC with the wash bottles filled with hexane. A 1 µl sample from the vial was injected into the GC-2010 Shimadzu GC and GC Mass Spectrometry QP2010 Plus with a flame ionization detector, an auto sampler (AOC-5000 Auto-injector) and a capillary column. Initially the GC oven was set to 70 °C. The detector temperature was set to 235

°C and the injector temperature was set to 250 °C, with a total running time of 36 minutes. The oven temperature programme was as follows:

Rate	Temperature (°C)	Hold Time (minutes)
	70	3
25	155	6
3	175	3
3	205	0
8	220	2

The quantifying of individual components was determined through both the fatty acid methyl esters (FAME) and mass spectrometry spectra. The individual fatty acids were identified by entering the concentration of internal standard (C17:0) as 22.50.

The coefficient of variance for RBC EPA and DHA were 2.67 and 3.58 %, respectively.

3.4.2 OMEGA 3 INDEX

The omega 3 index was calculated as follows:
((RBC EPA + DHA) / (RBC total fatty acids)) x 100 (Harris & von Schacky, 2004).

3.4.3 SELENIUM STATUS

Selenium biomarkers were analysed by the Human Nutrition Laboratory at Otago University, Dunedin.

A modified version of the method described by Jacobson & Lockitch (1988) was used to analyse the plasma selenium concentrations with the use of a graphite furnace atomic absorption spectrometer and a Zeeman background correction (Perkin-Elmer Model 3100; Norwalk, CT). External quality control materials (Utak Reference Plasma and Seronorm reference plasma) were analysed with each batch of analysis.

Whole blood GPx was determined using the method by Thomson et al. (1982) modified with the coupled enzyme procedure by Paglia & Valentine (1967) and a Cobas Fara autoanalyzer (Hoffman-La Roche, Basel, Switzerland).

3.4.4 PLASMA LIPID PROFILE

Plasma lipid profiles were measured by LabPLUS, Auckland City Hospital, Auckland, using the methods presented in Table 29.

Table 29. Testing methods for plasma lipid profile

Cholesterol	Method
TC	Enzymatic colourimetric method (Roeshclau and Allain). Roche Cholesterol reagent kit (Cat. No. 1491458)
HDL-C	Homogenous enzymatic colourmetric assay. Roche HDL-Cholesterol Plus reagent kit (Cat. No. 04713214)
LDL-C	Calculated: TC – HDL-C – (TG x 0.456)
TG	Enzymatic conversion. Roche Triacylglycerides reagent kit (Cat. No. 1727711)

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triacylglycerols.

3.4.5 SELENIUM CONTENT IN SALMON AND CAPSULES

The selenium content of the salmon and salmon oil capsules was analysed by the Massey University Nutrition Laboratory, Palmerston North.

Half a gram of sample (ground salmon or salmon oil) was weighed into a 68 ml polypropylene digestion vessel (Environmental Express, SC475). To this 5 ml of 12.5 % tetramethylammonium hydroxide pentahydrate solution was added. The vessel was then placed in a hot block (Environmental Express Hot Block SC154) at 90 °C for 1 hour. The digested material in the vessel was made up to 50 ml with double-distilled water before the vessel was shaken, and filtered through a cellulose acetate 0.45um syringe filter (Minisart Sartorius 1110647N). Samples were analysed on an Elan DRCII Inductively Coupled Plasma – Mass Spectrometer.

3.4.6 OXIDATION VALUE OF CAPSULES

At the end of the study period the capsules that had been stored in the -80 °C fridge at 0, 4 and 8 weeks into the intervention were removed and allowed to thaw in the dark before analysis was undertaken. Three capsules from each period of time were analysed individually to measure their oxidative value.

3.4.6.1 ANISIDINE VALUE

The following solution was prepared for anisidine analysis.

– p-anisidine: Reagent grade p-anisidine (0.25 g) was dissolved in 100 ml of glacial acetic acid. The solution was stored in a dark and dry place at room temperature.

In a 25 ml volumetric flask 1 g of salmon oil was weighed, this was then dissolved in iso-octane that had been filled to the 25 ml mark of the flask. The absorbance of the solution was measured at 350 nm in a glass curvette using a spectrophotometer (UV-1601 Shimadzu UV Visible Spectrophotometer) with iso-octane as a reference. Into one test tube 5 ml of the oil solution was pipetted and 5ml of iso-octane was pipetted into another. To each of the test tubes 1 ml of the p-anisidine solution was added and these were shaken. After exactly 10 minutes the absorbance of the fat solution was measured at the same wavelength using the iso-octane in the second test tube as a reference. The p-anisidine value was calculated using the following equation (AOCS Official Method):
(25 x (1.2 x As – Ab)) / (mass of the sample (g))
As = absorbance of the fat solution after reacting with p-anisidine solution
Ab = absorbance of the fat solution

The coefficient of variance was 5.07, 2.67 and 15.50 % for 0, 4 and 8 weeks, respectively.

3.4.6.2 PEROXIDE VALUE

The following solutions were prepared for the peroxide value analysis.

– 0.1 M sodium thiosulphate: To 1 L of water that had been boiled for at least 5 minutes and allowed to cool, 15.81 g sodium thiosulphate and 0.05 g sodium carbonate were added and dissolved. This was transferred into a clean stoppered bottle.

– 0.002 N sodium thiosulphate: In a 100 ml flask 0.1 mol/l of sodium thiosulphate solution was diluted to 0.002 N by transferring 2 ml of the above 0.1 M sodium thiosulphate.

– Saturated potassium iodide: To 5 ml of water 10 g potassium iodide was added and stirred thoroughly. Some undissolved crystals were present after stirring, indicating the solution was saturated.

– 1 % (W/V) starch indicator: To approximately 2 g of cold water 1 g of unmodified starch was added and mixed to form a slurry. To this 100 ml of distilled water was added and boiled for 2 minutes before it was cooled and stored in stopped bottle at 4°C.

– Acetic acid/dichloromethane (3/2 V/V): Three volumes of glacial acetic acid and 2 volumes of dichloromethane were mixed.

Sodium thiosulphate was standardised by drying the primary standard grade potassium idoate in a 110 °C oven for 1 hour then allowing it to cool in a desiccator. Into a 250 ml conical flask 0.10 – 0.15 g of potassium iodate was weighed and then dissolved in 75 ml of water to which 2 g of free potassium iodide was added. Then 2 ml of 6 M hydrochloric acid was added and this was titrated with sodium thiosulphate solution until the solution was pale yellow. To this 0.5 ml of the 1 % starch indicator solution was added and the titration was continued. Sodium thiosulphate was added drop-wise until the violet colour disappeared.

The volume of sodium thiosulphate used was recorded. The volume of sodium thiosulphate solution was calculated using:
$(28.07 \times W) / (S - B)$
W = weight of potassium iodate (g)
S = volume (ml) of the sodium thiosulphate required to the titrate the sample
B = volume (ml) of the sodium thiosulphate required for the blank

Into a 100 Erlenmeyer flask 2 g of salmon oil was weighed and 10 ml of the acetic acid/dichloromethane solvent was added to dissolve the oil. Two drops of potassium iodide solution was added, swirled and left in a dark cupboard for 2 minutes. Upon removing the solution from the cupboard sodium thiosulphate was titrated into the solution until the yellow colour disappeared. To this 1 ml of 1 % starch solution was

added. At this point the mixture was a dark blue-black colour. Sodium thiosulphate was again titrated until the blue-black colour of the iodised indicator turned colourless.

The peroxide value was calculated using the following equation:
((S – B) x N x 1000) / W = (meq active oxygen/kg sample)
S = volume of sodium thiosulphate required to titrate the sample
B = volume of sodium thiosulphate required for the blank
N = calculated normality of the standardized thiosulphate solution
W = weight of the sample (g)

The coefficient of variance was 2.37, 3.01 and 5.10 % for 0, 4 and 8 weeks, respectively.

3.4.7 Fatty Acid Analysis of Salmon and Capsules

3.4.7.1 Oil Extraction from Salmon

The duplicate portions of salmon were analysed for each individual subject using the following procedure. The 16 duplicate portions of salmon returned by each subject were removed from the -80 °C fridge and left to thaw. Once thawed, each portion was cut width-wise every 0.5 cm. The pieces were manually mixed and then ground in a meat grinder. The ground salmon was finally mixed thoroughly for 5 minutes with a large stainless steel spoon.

Ten ±0.2 g of ground salmon was weighed into a centrifuge tube and the weight of the sample was recorded to three decimal places. To the ground salmon, 16 ml of isopropanol and 20 ml of cyclohexane was added. This was homogenised (IKA Ultra Turrax Z506753) for 2 minutes at level 1. To this mixture 22 ml of distilled water was added and the mixture was blended for 1 minute at level 1 with the Ultra turrax homogeniser. The blended mixture was then transferred into a centrifuge tube before being centrifuged (Thermo Scientific IEC Centra CL3R Refrigerated Centrifuge) for 5 minutes at 2000 rpm. A pear shaped flask was weighed and this value was recorded. The top layer of organic phase from the centrifuged sample was transferred into the pear shaped flask with a Pasteur pipette. Once this was removed 20 ml of cyclohexane:isopropanol (87:13 v/v) mixture was added, and blended for another 2

minutes before it was centrifuged again. The organic phase was again removed and added to the pear shaped flask with the first extraction. This step was again repeated to ensure that all the organic phase was removed. Once the organic phase from the third extraction was removed the pear shaped flask was attached to the rota-evaporator (Buchi Rotavapor R-144 with Buchi Waterbath B-480) at 35 °C until all the solvent had evaporated. Further solvent was removed by flushing the flask with nitrogen and agitating it until no more bubbles were created. This procedure was repeated twice. The pear shaped flask was then weighed before the oil was transferred from the flask into 10 ml vials with screw cap lids. The vials were flushed with nitrogen before being stored at -80 °C for further analysis of fatty acids.

3.4.7.2 FATTY ACID ANALYSIS OF SALMON AND CAPSULES

Three samples of the oil extracted from the cooked salmon were analysed for each subject. The capsules that had been stored in the -80 °C fridge at 0, 4 and 8 weeks into the intervention were removed and allowed to thaw in the dark before analysis was done. Three capsules from each period of time were analysed individually.

The following solutions were prepared for the fatty acid analysis:

- Saponifying agent (0.5 M sodium methoxide solution): To 50 ml of methanol, 1 g of sodium hydroxide was added. This was sonicated until all the solid dissolved, the solution was then stored in an airtight container.

- Methylating agent: In a round bottomed flask 2 g of ammonium chloride was weighed to which 60 ml of methanol and 3 ml of concentrated sulphuric acid were added. This mixture was heated to 50 °C for 10 minutes and then refluxed for 25 minutes with water. The mixture was then stored in an airtight container.

- Internal standards: To 1 mg of internal standard (C13:0 and C23:0) 25 ml of hexane was added.
- Saturated sodium chloride solution: Enough sodium chloride was added to 50 ml of distilled water so that the crystals no longer dissolved in the water. This was then stored in an airtight container.

Into a 15 ml Kimax tube, 50 mg of salmon oil was weighed to which 300 µl of internal standard and 0.5 ml saponifying agent was added. This mixture was heated to 65 °C for 5 minutes, then 3 ml of methylating agent was added before the mixture was heated again for a further 3 minutes. The mixture was then cooled to room temperature in cold water before adding 10 ml of hexane, this mixture was vortexed (ZX3 Velp Scientifica Vortex) for 2 minutes. The hexane layer was removed and transferred into a GC vial and sealed for GC analysis.

The methylated lipid samples containing the FAME (Supelco 37 Comp) was analysed using a GC-2010 Shimadzu GC and a GC Mass Spectrometer QP2010 Plus with a flame ionization detector, an auto sampler (AOC- 5000 Auto-injector) and a capillary column. Initially the GC oven was set to 70 °C. The detector temperature was set to 235 °C and the injector temperature was set to 250 °C, with a total running time of 36 minutes. The oven temperature programme was as follows:

Rate	**Temperature (°C)**	**Hold Time (minutes)**
	70	3
25	155	6
3	175	3
3	205	0
8	220	2

The quantifying of individual components was determined through both the FAME and mass spectrometry spectra. The individual fatty acids were identified by entering the concentration of internal standards (C13:0 and C23:0) concentration (1200).

The coefficient of variance for the content of EPA and DHA in the oil extracted from the salmon was 2.52 and 1.74 %, respectively. The coefficient of variance for the content of EPA and DHA in the salmon oil compared to the capsule was 2.62 and 1.94 %, respectively.

3.5 DATA HANDLING AND ANALYSIS

It was calculated that a sample size of ten per group would provide 80 % power to detect a significant difference ($\alpha = 0.05$, two-tail) between groups of one unit in omega 3 index and in total RBC omega 3 fatty acid levels. Each group consisted of 11 subjects to allow for a 10 % dropout rate in each group.

Analysis employed standard statistical software, Statistical Package for the Social Sciences (SPSS) v.16 (SPSS Inc., Chicago, IL, USA). Normally distributed variables are presented as mean ±standard deviation and non-normally distributed variables as median (25^{th}, 75^{th} percentile). A *p* value of < 0.05 was considered to be statistically significant. The change in each variable from baseline to end was calculated by end value minus baseline value.

Variables were tested to see if they were normally distributed using Kolmogorov-Smirnov and Shapiro-Wilk tests together with examining Normal Q-Q, box and steam and leaf plots. Differences within groups between baseline and end values were analysed using a paired *t*-test for parametric variables and the Wilcoxon ranked-sum test for non-parametric variables. The one-way analysis of variance (ANOVA) with post-hoc tests (Tukey's Honest significance difference test) were used to determine differences between groups for parametric variables. Differences between groups for non-parametric variables were analysed using the Kruskal-Wallis test with post-hoc analysis and Bonferroni adjustments. The differences in plasma selenium between the salmon and the capsule (three groups combined) groups were analysed while controlling for baseline selenium using the analysis of covariance (ANCOVA) test.

Associations between omega 3 fatty acid rich food sources and RBC EPA and DHA concentrations were determined by calculating Spearmans correlations coefficients.

Linear regression analysis predictive models were fitted to the capsule data. The residual values produced from the linear regression analysis were tested for independence using the Durbin-Watson test, normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests together with examining Normal Q-Q, box and steam and leaf plots. Homoscedacity and multiple colinearity were evaluated by plotting standardised residuals against the predicted values. Linear regression analysis was carried out with a

dummy variable to calculate the predicted change and 95 % confidence interval in RBC omega 3 fatty acid levels with intakes of LC omega 3 fatty acids from capsules in amounts equivalent to that consumed from salmon.

CHAPTER 4

4. RESULTS

4.1 SUBJECT CHARACTERISTICS

A total of 44 subjects were recruited for this study. Baseline characteristics of the subjects are summarised in Table 30. Two subjects withdrew from the study. One due to illness (6 capsule group) and the other was for personal reasons (4 capsule group); 42 subjects completed the study.

More females (23) than males (19) participated in the study. The mean age of the subjects was 30 ±5.6 years. The mean baseline anthropometric measurements (BMI and waist circumference to hip circumference ratio (waist:hip) and BP were within the ideal healthy ranges. Three of the five blood lipid profile average concentrations were also within the ideal ranges (TC, HDL-C, and TG). The mean baseline TC and LDL-C concentrations of the subjects were higher than the ideal range.

There was no significant difference between the groups at baseline for gender, age, BMI, waist:hip, TC, HDL-C, LDL-C, TC:HDL-C and BP. The 4 capsule group had significantly higher baseline TG concentrations than the 6 capsule group (p = 0.025). RBC EPA and DHA did not significantly differ between groups at the baseline of the study.

One of the inclusion criteria for the study was a low habitual intake of fatty fish (< 2 servings/month). Based on the FFQ half a serving of fatty fish per week was the average amount consumed by the subject group. There was no difference in the amount of fish consumed at the baseline of the study between groups.

Table 30. Baseline characteristics of subjects

	Ideal Range	Total Group N = 42	Salmon n = 11	2 Capsules n = 11	4 Capsules n = 10	6 Capsules n = 10	*p**
Gender (*male/female)*		19/23	5/6	5/6	5/5	4/6	0.820
Age (*yr)*		30.0 ±5.53	29.5 ±5.17	30.5 ±6.50	30.3 ±5.74	29.6 ±6.09	0.982
BMI (*kg/m^2)*	≤ 25[c]	24.3 ±3.32	24.0 ±2.54	24.1 ±2.07	25.3 ±5.00	23.0 ±2.21	0.463
waist:hip	< 0.90/0.80[d]	0.78 ±0.08	0.78 ±0.07	0.76 ±0.07	0.82 ±0.11	0.77 ±0.08	0.659
TC *(mmol/l)*	< 4[c]	4.70 ±0.91	4.73 ±1.10	4.77 ±0.26	4.97 ±1.12	4.46 ±0.61	0.680
HDL-C *(mmol/l)*	> 1[c]	1.39 ±0.36	1.28 ±0.35	1.42 ±0.28	1.26 ±0.45	1.58 ±0.31	0.424
LDL-C *(mmol/l)*	< 2.5[c]	2.84 ±0.76	2.91 ±0.85	2.98 ±0.67	3.01 ±0.89	2.51 ±0.57	0.169
TC:HDL-C	< 4.5[c]	3.63 ±1.28	3.92 ±1.21	3.48 ±0.83	4.41 ±1.92	2.91 ±0.54	0.058
TG *(mmol/l)*	< 1.7[c]	1.09 ±0.62	1.18 ±0.66	0.91 ±0.26	1.60 ±0.93[a]	0.81 ±0.26[b]	0.022
Systolic BP *(mm Hg)*	< 130[c]	117 ±11.5	112 ±10.84	117 ±9.41	128 ±5.07	115 ±9.70	0.170
Diastolic BP (*mm Hg)*	< 80[c]	71.6 ±7.11	73.6 ±8.03	74.8 ±5.67	80.0 ±8.14	75.9 ±8.28	0.104
RBC EPA *(%)*		0.82 ±0.24	0.78 ±0.20	0.90 ±0.24	0.84 ±0.23	0.78 ±0.31	0.644
RBC DHA *(%)*		5.20 ±0.62	5.06 ±0.70	5.21 ±0.46	5.13 ±0.46	5.43 ±0.79	0.581
Omega 3 Index *(%)*	> 8%[e]	6.02 ±0.73	5.84 ±0.72	6.10 ±0.63	5.94 ±0.54	6.20 ±1.01	0.682
Fish intake[#] *(servings/week)*							
Lean		2.25 (1.25, 3.75)	2.00 (0.75, 3.00)	3.25 (1.50, 3.75)	3.13 (1.50, 4.00)	1.88 (1.25, 2.50)	0.427
Medium		0.00 (0.00, 0.25)	0.00 (0.00, 0.00)	0.25 (0.00, 0.25)	0.13 (0.00, 1.00)	0.00 (0.00, 0.25)	0.264
Fatty		0.50 (0.25, 1.00)	0.50 (0.25, 0.75)	0.75 (0.50, 1.00)	0.63 (0.25, 1.00)	0.50 (0.25, 1.00)	0.247

Results expressed as mean ±SD; [#]Results expressed as median (25th, 75th percentile); * Difference between groups (Chi-square test, ANOVA, Kruskal Wallis).
[a, b]: different symbols indicate significant differences ($p < 0.05$); [c]New Zealand Guidelines Group, 2003; [d]male/female Russell et al., 1999; [e]Harris & von Schacky, 2004.
BMI, body mass index; waist:hip, waist circumference to hip circumference ratio; TC, TC; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triacylglyercols; BP, blood pressure.

4.1.1 ANTHROPOMETRIC MEASURES

Table 31 summarises the anthropometric measures of the subjects that participated in the study. There was no significant difference from baseline to end or between groups at the end of the study.

Table 31. Anthropometric measures

		Salmon n = 11	**2 Capsules n = 11**	**4 Capsules n = 10**	**6 Capsules n = 10**	***p****
BMI (kg/m^2)	E	24.6 ±3.18	24.3 ±2.15	25.3 ±4.96	23.2 ±0.20	0.524
	Δ	-0.15 ±0.50	-0.25 ±1.16	-0.04 ±0.43	0.02 ±0.59	0.533
	*p***	0.167	1.000	0.343	0.279	
Waist:hip	E	0.80 ±0.00	0.78 ±0.06	0.81 ±0.09	0.77 ±0.07	0.670
	Δ	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.02	0.734
	*p***	0.810	0.324	0.104	0.775	

Results expressed as mean ±SD; *Difference between groups (ANOVA); ** Difference between baseline and end (Paired t-test)
BMI, body mass index; waist:hip, waist circumference to hip circumference ratio.
E, end value; Δ, end value – baseline value.

4.2 TREATMENTS

4.2.1 FATTY ACID AND SELENIUM COMPOSITION OF TREATMENTS

Analysis carried out on the treatments is shown in Table 32. The LC omega 3 fatty acid content was higher in the cooked salmon than in the raw salmon. Selenium was only found in the salmon and was at higher concentrations in the cooked portions compared to raw salmon.

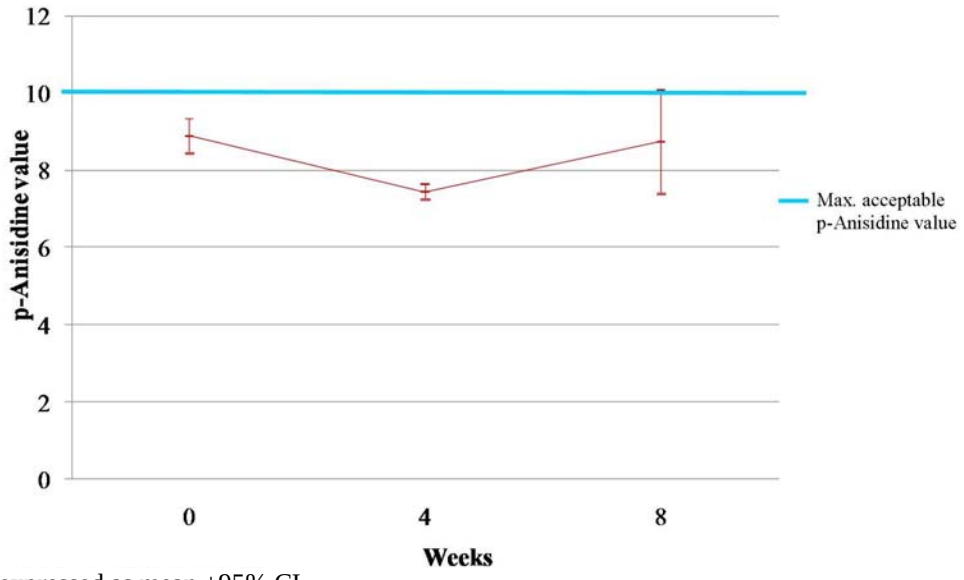
Table 32. Fatty acid composition and selenium content of salmon and capsules

Fatty Acid	Cooked Salmon [a, b]		Raw Salmon [b, c]		Capsules	
	(g/100g extracted oil)		*(g/100g extracted oil)*		*(g/100g oil)*	
C10:0	0.018	±0.002	0.019	±0.007	0.243	±0.016
C11:0	0.002	±0.002	0.003	±0.000	0.000	±0.000
C12:0	0.148	±0.010	0.135	±0.014	0.097	±0.005
C14:0	3.586	±0.075	1.751	±0.120	3.943	±0.198
C14:1	0.150	±0.010	2.923	±0.186	0.109	±0.004
C15:0	0.311	±0.019	0.139	±0.013	0.398	±0.015
C16:0	12.077	±0.411	9.825	±0.738	8.923	±0.506
C16:1	4.818	±0.156	3.900	±0.275	4.067	±0.208
C17:0	0.504	±0.025	0.458	±0.044	0.484	±0.026
C17:1	0.195	±0.084	0.202	±0.028	0.220	±0.015
C18:0	4.860	±0.147	4.157	±0.354	2.784	±0.168
C18:1-t9	3.797	±2.741	2.545	±0.051	5.336	±2.454
C18:1-t11	0.237	±0.643	0.094	±0.009	9.739	±4.493
C18:2-t9,12	6.224	±0.259	4.981	±0.392	0.164	±0.007
C18:2-c9,12	0.180	±0.010	0.168	±0.016	3.987	±0.218
C20:0	0.147	±0.008	0.132	±0.014	0.207	±0.006
C18:3	0.853	±0.046	0.728	±0.071	0.099	±0.006
C20:1	1.460	±0.073	1.215	±0.123	0.161	±0.002
C20:2	0.238	±0.013	0.202	±0.020	0.513	±0.012
C20:3n3	0.212	±0.014	0.068	±0.008	0.148	±0.007
C22:0	0.073	±0.004	0.583	±0.053	0.071	±0.001
C20:4	0.635	±0.027	0.146	±0.014	0.681	±0.013
C22:1	0.165	±0.015	1.751	±0.120	0.834	±0.018
C20:5n3	3.837	±0.126	3.193	±0.208	4.461	±0.128
C22:2	0.932	±0.029	0.776	±0.050	0.725	±0.527
C24:0	0.029	±0.007	0.030	±0.002	0.018	±0.014
C24:1	0.213	±0.015	0.193	±0.017	0.629	±0.016
C22:5n3	1.242	±0.052	1.062	±0.084	1.438	±0.041
C22:6n3	4.225	±0.150	3.416	±0.201	5.975	±0.127
Selenium *(mg/kg)*	0.203	±0.016	0.180		<0.02	

Results expressed as mean ±SD; [a] Fatty acid content of cooked salmon was determined from portions cooked by the subjects during the study; [b] 26 g extracted oil per 100 g of cooked salmon; [c] 22 g extracted oil per 100 g of raw salmon.
C20:5n3, eicosapentaenoic acid; C22:6n3, docosahexasenoic acid.

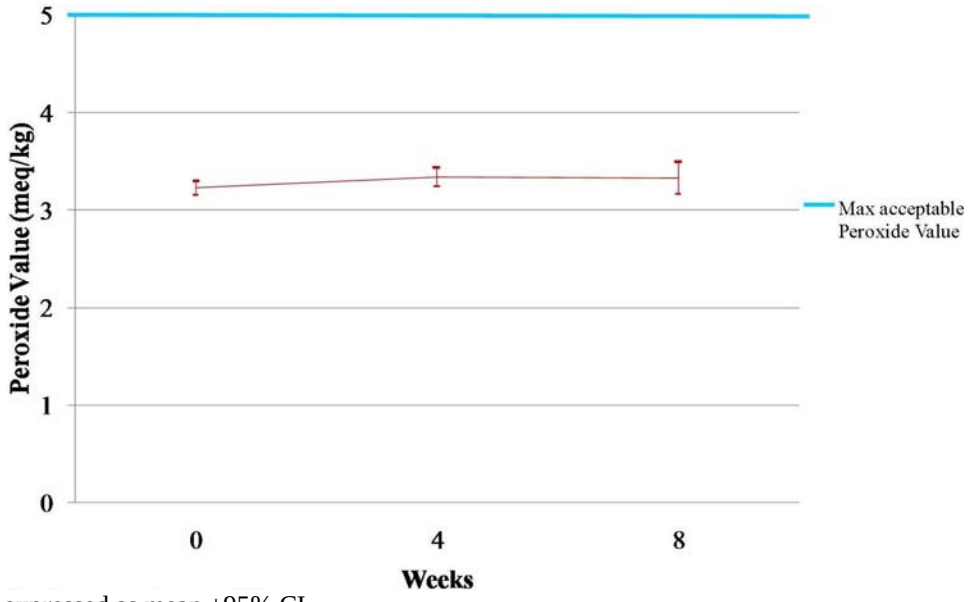
4.2.2 OXIDATION VALUES OF CAPSULES

Figures 15 and 16 show that the p-anisidine and peroxide values were significantly lower than the maximum oxidation levels permitted for all fish oil products within New Zealand (Ministry of Economic Development, 2009). There did not appear to be any trend in the oxidation values over time.



Results expressed as mean ±95% CI.

Figure 15. p-Anisidine value of the capsule oil during the study



Results expressed as mean ±95% CI.

Figure 16. Peroxide value of the capsule oil during the study

4.2.3 OMEGA 3 FATTY ACID AND SELENIUM INTAKES FROM THE TREATMENTS

Table 33 summarises the amount of omega 3 fatty acids and selenium consumed by subjects in the different treatment groups. Subjects in the salmon group had a higher omega 3 fatty acid intake from the treatment than subjects in the group consuming the highest number of capsules (6 capsules). The amount of EPA and DHA displayed on the label of the capsules was greater than the amount contained in the capsules based on analysis. The ratio of EPA to DHA was higher in the salmon group. Selenium was present in salmon only; no selenium was detected in capsules.

Table 33. Omega 3 fatty acid and selenium intakes from the treatments based on chemical analysis

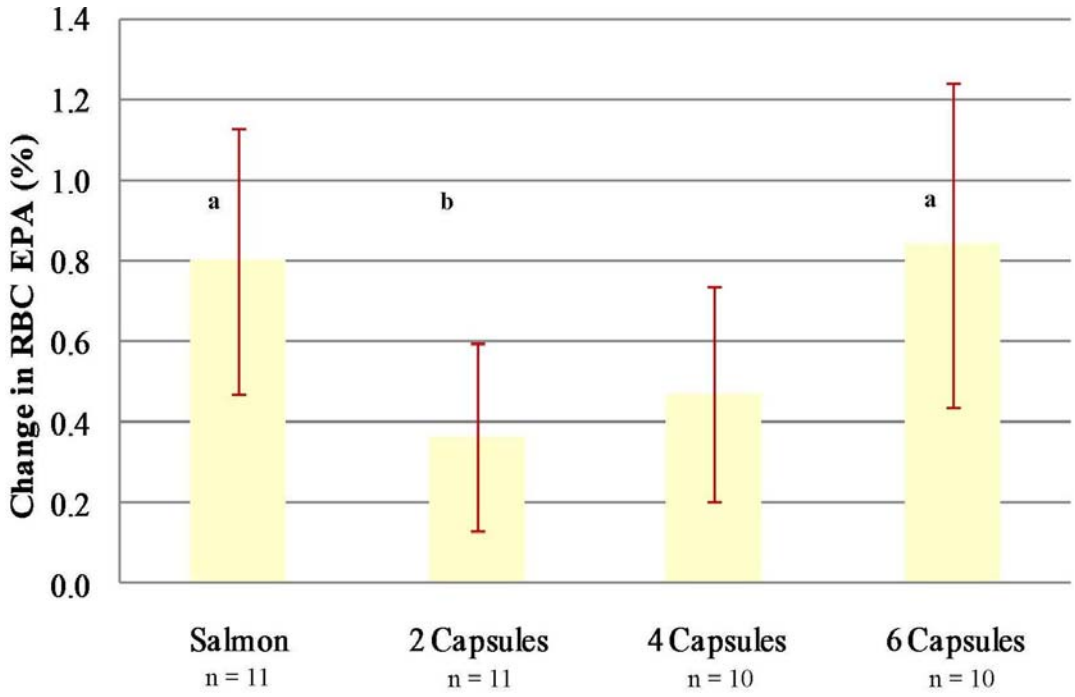
		Salmon	**2 Capsules**	**4 Capsules**	**6 Capsules**
		n = 11	**n = 11**	**n = 10**	**n = 10**
EPA	*(g/day)*	0.337 ±0.224	0.090 ±0.001	0.181 ±0.001	0.264 ±0.008
DHA	*(g/day)*	0.370 ±0.024	0.120 ±0.002	0.239 ±0.002	0.349 ±0.011
DPA	*(g/day)*	0.109 ±0.008	0.029 ±0.000	0.058 ±0.000	0.076 ±0.002
Total omega 3 fatty acids	*(g/day)*	0.816 ±0.055	0.239 ±0.004	0.479 ±0.004	0.689 ±0.022
EPA:DHA		0.911 ±0.006	0.757 ±0.000	0.757 ±0.000	0.756 ±0.000
Selenium	*(µg/day)*	6.840 ±0.272	<0.02	<0.02	<0.02

Results expressed as mean ±SD; Average intake based on compliance to treatment.
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid.

4.3 RED BLOOD CELL OMEGA 3 FATTY ACIDS

4.3.1 RED BLOOD CELL EPA

The subjects' average baseline RBC EPA for each treatment group is shown in Table 30. Figure 17 displays the change from baseline to end in RBC EPA following consumption of the different treatments regimens. Significant increases were shown from baseline to end in RBC EPA for all four groups ($p < 0.05$), the greatest increase was found in the salmon and 6 capsule groups. There were no significant differences in the EPA RBC levels between groups at the baseline of the study ($p = 0.664$). A significant difference was found in the increase in RBC EPA ($p = 0.002$) between groups. The increase in RBC EPA in the 2 capsule group was significantly less compared to both the 6 capsule ($p = 0.005$) and salmon ($p = 0.010$) groups.

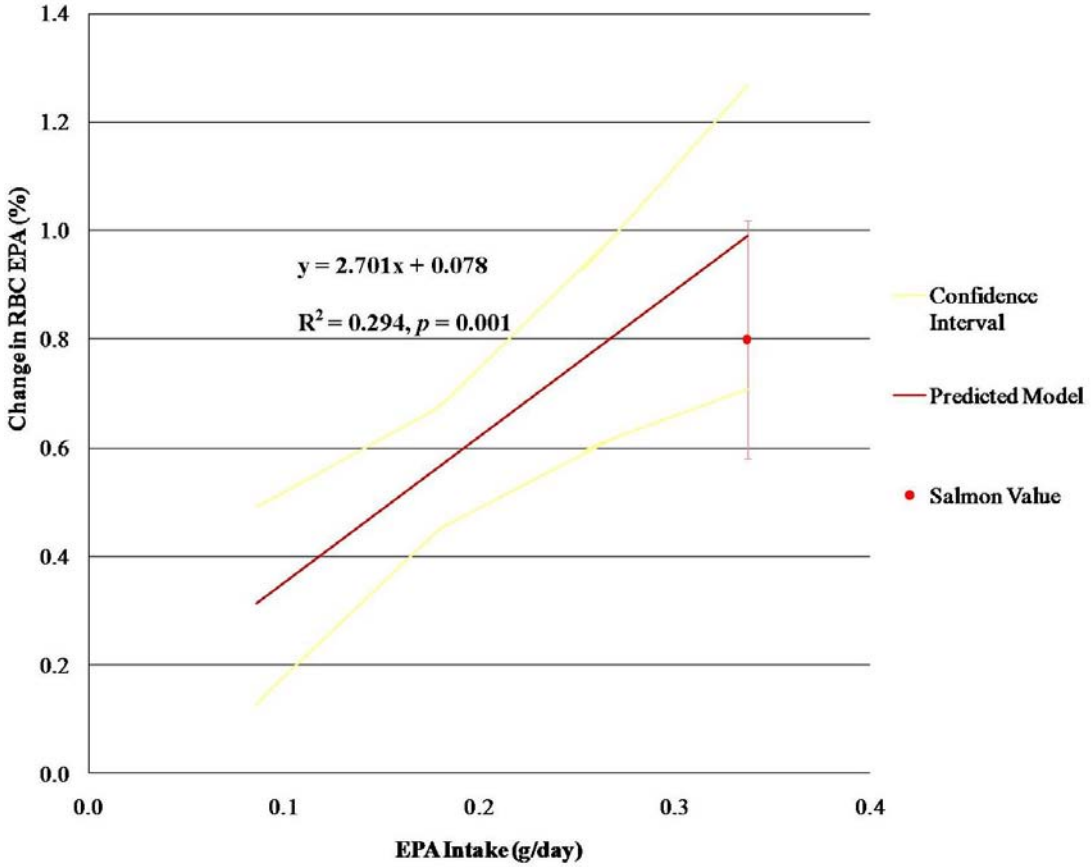


Results expressed as mean ±95 % CI.
a,b: different symbols indicate significant differences (p <0.05).
RBC, red blood cell; EPA, eicosapentaenoic acid.

Figure 17. Change in red blood cell eicosapentaenoic acid levels in different treatment groups

The predictive model of change in RBC EPA concentrations with increased intakes of EPA is illustrated in Figure 18. Thirty percent (29.4 %) of the variance in the RBC EPA was explained by EPA intake from the treatment. Increases in RBC EPA concentrations were similar with intakes from salmon oil capsules and salmon as the salmon value fell within the 95 % confidence interval of the predicted capsule values. From the predictive

model it was calculated that an intake of 0.34 g per day of EPA from capsules would result in an increase of RBC EPA of 1.00 [0.71 – 1.27] %. This increase is similar to that from salmon (0.80 [0.58 – 1.02] %).

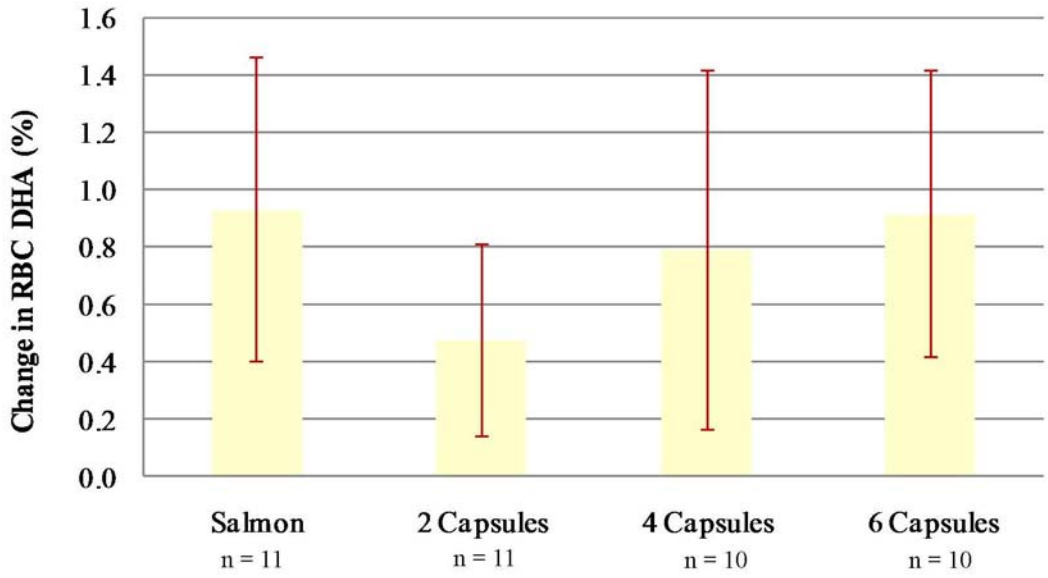


Results expressed as mean ±95 % CI.
RBC, red blood cell; EPA, eicosapentaenoic acid.

Figure 18. Predictive model of change in red blood cell eicosapentaenoic acid levels from capsules compared to eicosapentaenoic acid from salmon

4.3.2 RED BLOOD CELL DHA

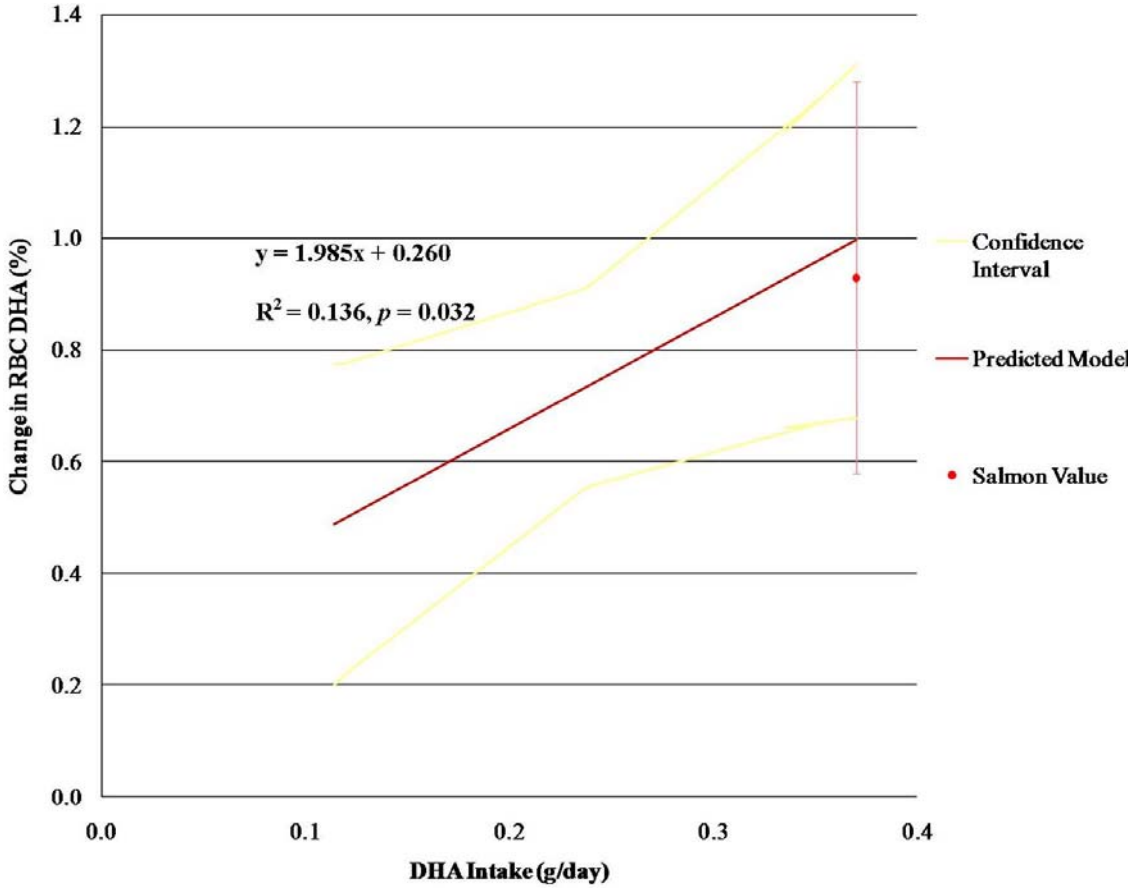
The subjects' average baseline RBC DHA for each treatment group is shown in Table 30. The RBC DHA levels were not significantly different between groups at the baseline (p = 0.581), end (p = 0.123) or the change from baseline to end (p = 0.142). There was however a significant increase from baseline to end in all four groups (p < 0.001), see Figure 19.



Results expressed as mean ±95 % CI.
a,b: different symbols indicate significant differences (p <0.05).
RBC, red blood cell; DHA, docoahexaenoic acid.

Figure 19. Change in red blood cell docoahexaenoic acid levels in different treatment groups

The predictive model of change in RBC DHA concentrations with increased intake of DHA is illustrated in Figure 20. Fourteen percent (13.6 %) of the variance in the RBC DHA was explained by DHA intake from the treatment. Increases in RBC DHA concentrations were similar with intakes of salmon oil capsules and salmon as the salmon value fell within the 95 % confidence interval of the predicted capsule values. There was a notable 95 % confidence interval in the salmon group indicating substantial individual variations in change in RBC DHA within this groups. From the predictive model it was calculated that an intake of 0.37 g per day of DHA from capsules would result in an increase of RBC DHA of 0.99 [0.68 – 1.31] %. This increase is similar to that from salmon (0.93 [0.58 – 1.29] %).

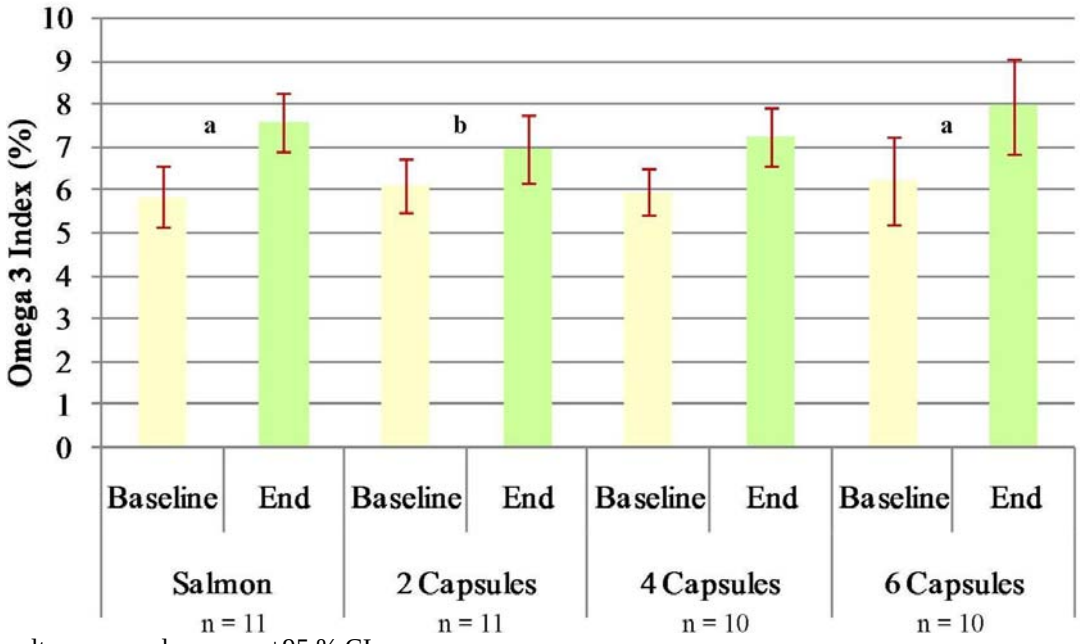


Results expressed as mean ±95 % CI.
RBC, red blood cell; DHA, docoahexaenoic acid.

Figure 20. Predictive model of change in red blood cell docoahexaenoic acid levels from capsules compared to docoahexaenoic acid from salmon

4.3.3 OMEGA 3 INDEX

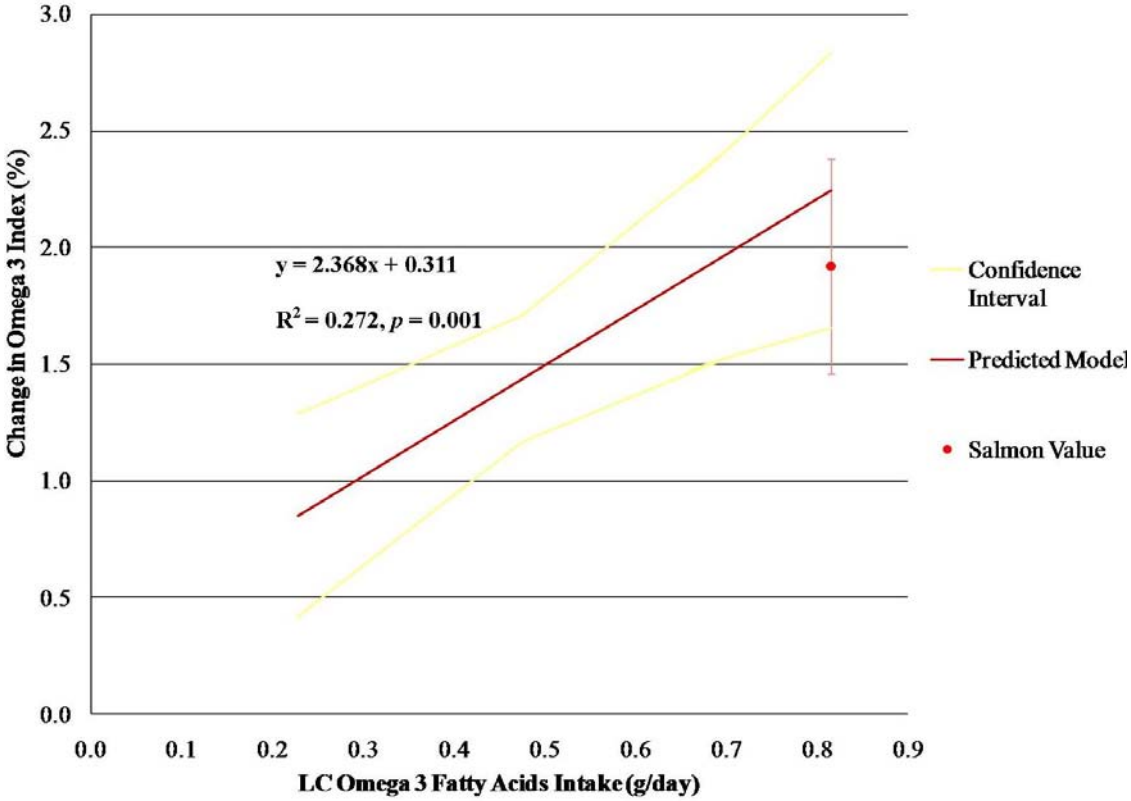
The subjects' average baseline omega 3 index for each treatment group is shown in Table 30. No significant difference was seen in the omega 3 index between groups at baseline (p = 0.682). Omega 3 index increased significantly from baseline to end in all the groups (p < 0.001). There was also a significant difference between groups for change in the omega 3 index (p = 0.011), this lay between the 2 capsule group and both the 6 capsule (p = 0.015) and salmon groups (p = 0.016), see Figure 21. At the end of the study 30.95 % of subjects reached the recommended 8 % or greater omega 3 index.



Results expressed as mean ±95 % CI.
a,b: different symbols indicate significant differences (p <0.05).

Figure 21. Omega 3 index at the baseline and end of the study

The predictive model of change in the omega 3 index with increased intake of LC omega 3 fatty acids is illustrated in Figure 22. Twenty seven percent (27.2 %) of the variance in the omega 3 index was explained by LC omega 3 fatty acid intake from the treatment. As the salmon value is within the 95 % confidence interval of the predictive model no significant difference existed between consuming LC omega 3 fatty acids from salmon or salmon oil capsules with regards to the omega 3 index. From the predictive model it was calculated that an intake of 0.82 g per day of LC omega 3 fatty acids from capsules would result in an increase of omega 3 index of 2.25 [1.65 – 2.83] %. This increase is similar to that from salmon 1.92 [1.46 – 2.38] %.



Results expressed as mean ±95 % CI.

Figure 22. Predictive model of omega 3 index from capsules compared to omega 3 fatty acids from salmon

4.4 PLASMA SELENIUM AND WHOLE BLOOD GLUTATHIONE PEROXIDASE

The plasma selenium and whole blood GPx concentrations of subjects are presented in Table 34. No selenium was detected in the capsules therefore the salmon group was compared to all the capsule groups combined. Participants in this study were found to have adequate selenium status at the commencement of the study as the mean baseline plasma selenium concentration of subjects was greater than the value recommended for protection against cancer (> 1.5 μmol/l) and for full expression of GPx (> 0.82 μmol/l) (Thomson, 2004). There was no significant difference in plasma selenium at baseline between the groups, however the salmon group had statistically significantly higher plasma selenium concentrations at the end compared to the capsule group levels. After controlling for each subject's baseline plasma selenium the increase in plasma selenium for those in the salmon group was statistically significantly greater compared to those in the capsule group.

No significant difference was found in the whole blood GPx concentrations at baseline or end of the study. There was also no significant difference between baseline and end within or between groups.

Table 34. Selenium status of subjects

		Salmon n = 11	**Capsules n = 26**	***p****
Plasma Selenium *(μmol/l)*	B	1.63 ±0.30	1.57 ±0.26	0.461
	E	1.77 ±0.27	1.59 ±0.18	0.024
	Δ[a]	0.16 ±0.13	0.02 ±0.13	0.007[a]
	*p***	0.016	0.144	
Whole blood GPx[b] *(units/g Hb)*	B	46.9 (39.8, 53.1)	44.6 (37.5, 55.3)	0.573
	E	44.8 (38.8, 53.1)	43.9 (37.9, 57.4)	0.944
	Δ	0.26 (-2.44, 4.24)	-0.58 (-1.57, 2.77)	0.573
	*p***	0.799	0.754	

Results expressed as mean ±SD; [a] ANCOVA adjusted for baseline plasma selenium, values expressed as adjusted mean ±SD; [b]Results expressed as median (25,75[th] percentile); * Difference between groups (ANOVA or Krusal-Wallis); ** Difference between baseline and end (Paired t-test or Wilcoxon).
GPx, glutathione peroxidase; B, baseline value; E, end value; Δ, end value – baseline value.
mg/dl = μmol x 76.3.

4.5 LIPID PROFILE

The subjects' average baseline lipid profile levels for each treatment group are summarised in Table 30. HDL-C concentrations were significantly higher in the 6 capsule group at the end of the study compared to the salmon and 4 capsule groups. However, the change from baseline to end did not differ between groups. The 6 capsule group was also the only group that showed a significant increase in HDL-C over the study. The ratio of TC to HDL-C at the end of the study was significantly higher in the 6 capsule group compared to the 4 capsule group. No statistically significant difference was found in TC, LDL-C and TG concentrations at baseline or end of the study; neither was there any change in these concentrations between groups. There was also no significant difference between baseline and end within groups in these variables There was no statistically significant change in the plasma lipid profile in the 2 capsule group (see Table 35).

Table 35. Plasma lipid profile

		Salmon n = 11	**2 Capsules n = 11**	**4 Capsules n = 10**	**6 Capsules n = 10**	***p****
TC	E	4.70 ±0.80	4.90 ±0.90	5.00 ±1.10	4.80 ±1.00	0.877
(mmol/l)	Δ	0.00 ±0.80	0.10 ±0.40	0.10 ±0.30	0.40 ±0.70	0.493
	*p***	0.907	0.358	0.515	0.151	
HDL-C	E	1.30 ±0.30 [b]	1.40 ±0.20	1.30 ±0.50 [b]	1.70 ±0.30 [a]	0.022
(mmol/l)	Δ	0.00 ±0.20	0.00 ±0.20	0.00 ±0.20	0.12 ±0.20	0.215
	*p***	0.895	0.625	0.494	0.038	
LDL-C	E	2.90 ±0.80	3.10 ±0.70	3.00 ±1.00	2.70 ±0.80	0.674
(mmol/l)	Δ	0.00 ±0.70	0.10 ±0.40	0.00 ±0.30	0.10 ±0.60	0.936
	*p***	0.788	0.428	0.763	0.421	
TC:HDL-C	E	3.80 ±1.30	3.60 ±0.70	4.20 ±1.40[a]	2.90 ±0.60[b]	0.045
(mmol/l)	Δ	-0.10 ±0.40	0.10 ±0.50	-0.20 ±0.80	0.00 ±0.30	0.609
	*p***	0.422	0.482	0.438	0.651	
TG	E	1.00 ±0.50	0.90 ±0.30	1.50 ±0.90	0.90 ±0.40	0.580
(mmol/l)	Δ	0.00 ±1.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.484
	*p***	0.399	0.921	0.538	0.191	

Results expressed as mean ±SD; * Difference between groups (ANOVA and post-hoc analysis); ** Difference between baseline and end (Paired t-test).
a, b: different symbols indicate significant differences (p <0.05).
TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC:HDL-C, total cholesterol:high density lipoprotein cholesterol; TG, triacylglycerols; E, end value; Δ, end value – baseline value.

4.6 BLOOD PRESSURE

The subjects' mean baseline BP for each treatment group are summarised in Table 30. No significant differences were found between the baseline, end or change in BP between the treatment groups. BP did not change significantly ($p > 0.05$) from baseline to end within groups (see Table 36).

Table 36. Blood pressure

		Salmon n = 11	**2 Capsules n = 11**	**4 Capsules n = 10**	**6 Capsules n = 10**	***p****
Systolic BP *(mm Hg)*	E	112.19 ±10.84	117 ±9.11	124 ±12.4	115 ±11.2	0.106
	Δ	-4.36 ±6.82	-0.55 ±7.29	-2.67 ±14.0	-0.60 ±10.1	0.764
	*p***	0.060	0.809	0.582	0.855	
Diastolic BP *(mm Hg)*	E	73.55 ±8.03	75.7 ±8.64	78.7 ±7.33	76.3 ±10.6	0.607
	Δ	1.91 ±6.25	0.91 ±6.70	-1.44 ±8.25	0.40 ±8.73	0.794
	*p***	0.335	0.662	0.614	0.888	

Results expressed as mean ±SD; * Difference between groups (ANOVA); ** Difference between baseline and end (Paired *t*-test).
BP, blood pressure; E, end value; Δ, end value – baseline value.

4.7 TOLERANCE OF TREATMENT

Nine subjects reported without being prompted having side effects from the treatment, compared to 21 who reported side effects once asked specific questions on potential side effects, see Figure 23.

One subject consuming salmon complained of burping from treatment, no other complaints were reported by any other subjects in the salmon group. Burping was the most common side effect reported overall. There did not appear to be any correlation between amount of capsules consumed and percentage of subjects reporting side effects. Subjects in the 4 capsule group reported the most side effects.

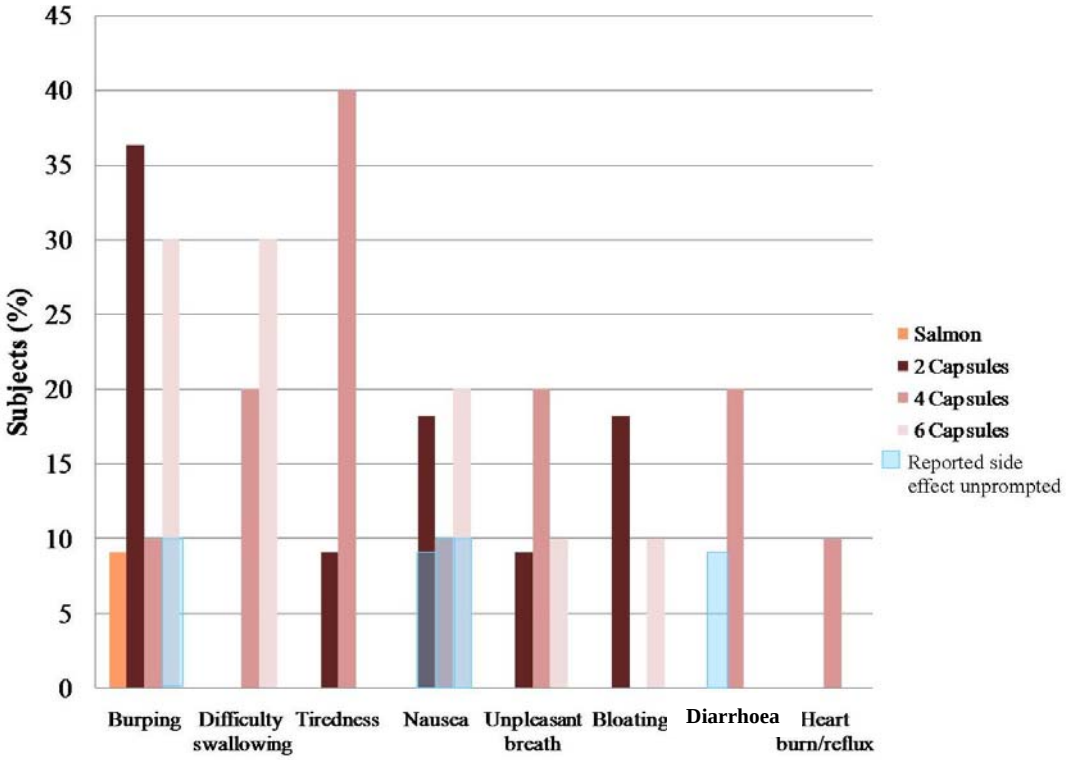


Figure 23. Reporting of side effects from treatment

Subjects who experienced side effects were asked to rank how significant they felt each effect to be using a 9-point likert scale ranging from insignificant to very severe. Participants reported that the majority of side effects experienced were insignificant. However one subject in the 4 capsule group reported severe diarrhoea that had a significant impact upon the tolerability of the treatment.

An open-ended question on the best method for consuming the treatment was presented to the subjects in the tolerance questionnaire. Answering this question was not mandatory, so many of the subjects did not provide an answer however 21 did respond. Table 37 lists the responses in categories and shows that there was no clear indication of a best method for consumption of the capsules. Much of the advice given was conflicting as others suggested consuming the capsules together, while some suggested separately. Interestingly 50 % of those in the 6 capsule group suggested that consuming all the capsules together was the best method.

Table 37. Recommendations made by subjects for consuming capsules

Consume:	**Total (%) N = 21**	**2 Capsules (%) n = 8**	**4 Capsules (%) n = 4**	**6 Capsules (%) n = 9**
At breakfast	9.5	0.0	33.3	11.1
At dinner	9.5	25.0	0.0	0.0
Before meals	4.8	0.0	0.0	11.1
Together	42.9	37.5	33.3	55.6
Distributed throughout the day	19.0	12.5	33.3	22.2
With a cold drink	14.3	25.0	33.3	0.0

4.8 FOOD CONSUMPTION HABITS

The average intake of the main food categories for each treatment group at baseline and end along with the change from baseline to end is displayed in Table 38. Median milk intake reported at baseline was significantly lower in the salmon group compared to the 4 capsule group ($p = 0.016$). No other differences between groups or changes during the study in any of the food groups were seen. Meat, poultry and fish intake did not change with the inclusion of salmon in the salmon group.

Table 38. Intakes of main food categories expressed as servings per week

		Salmon n = 11	2 Capsules n = 11	4 Capsules n = 10	6 Capsules n = 10	*p**
Milk	B	3.5 (3.5, 10.5)[b]	10.5 (3.5, 10.5)	10.5 (10.5, 10.5)[a]	10.5 (3.5, 10.5)	0.047
	E	10.5 (3.5, 10.5)	10.5 (3.5, 10.5)	10.5 (10.5, 10.5)	10.5 (10.5, 10.5)	0.454
(servings/week)	Δ	0.0 (0.0, 7.0)	0.0 (0.0, 7.0)	0.0 (-7.0, 0.0)	0.0 (0.0, 7.0)	0.587
	*p***	1.000	0.461	0.357	0.396	
Bread	B	10.5 (3.5, 24.5)	10.5 (3.5, 24.5)	10.5 (10.5, 24.5)	17.5 (3.5, 24.5)	0.952
	E	10.5 (3.5, 10.5)	10.5 (3.5, 10.5)	10.5 (10.5, 24.5)	10.5 (3.5, 10.5)	0.245
(servings/week)	Δ	0.0 (-14.0, 0.0)	0.0 (-7.0, 0.0)	0.0 (0.0, 7.0)	-2.5 (-14.0, 0.0)	0.257
	*p***	0.131	0.176	0.450	0.268	
Butter, margarine/ spread	B	35.0 (14.0, 77.0)	14.0 (0.0, 77.0)	77.0 (35.0, 119.0)	24.5 (14.0, 56.0)	0.082
	E	35.0 (14.0, 56.0)	14.0 (14.0, 35.0)	56.0 (14.0, 56.0)	24.5 (14.0, 35.0)	0.564
(servings/week)	Δ	0.0 (-35.0, 21.0)	0.0 (0.0, 21.0)	-21.0 (-63.0, 0.0)	0.0 (0.0,0.0)	0.204
	*p***	0.670	0.892	0.067	0.854	
Breakfast cereal	B	5.0 (2.0, 8.0)	5.0 (2.0, 8.0)	5.0 (5.0, 8.0)	3.5 (2.0, 5.0)	0.452
	E	4.0 (4.0, 10.0)	5.0 (2.0, 8.0)	5.0 (5.0, 8.0)	3.5 (2.0, 5.0)	0.312
(servings/week)	Δ	0.0 (0.0, 0.0)	0.0 (0.0, 2.0)	0.0 (-3.0, 0.0)	0.0 (0.0, 3.0)	0.619
	*p***	0.317	0.450	0.655	0.684	
Starches	B	2.0 (2.0, 8.0)	2.0 (2.0, 2.0)	5.0 (5.0, 8.0)	5.0 (2.0, 8.0)	0.055
	E	5.0 (2.0, 8.0)	5.0 (2.0, 5.0)	5.0 (5.0, 8.0)	3.5 (2.0, 5.0)	0.477
(servings/week)	Δ	0.0 (-3.0, 6.0)	0.0 (0.0, 3.0)	0.0 (0.0, 3.0)	0.0 (-3.0, 0.0)	0.520
	*p***	0.457	0.096	0.414	0.48	
Meat	B	2.0 (2.0, 5.0)	5.0 (2.0, 8.0)	6.5 (5.0, 8.0)	3.5 (2.0, 5.0)	0.067
	E	2.0 (2.0, 5.0)	5.0 (2.0, 5.0)	5.0 (2.0, 8.0)	3.5 (2.0, 8.0)	0.589
(servings/week)	Δ	0.0 (-2, 3)	0.0 (0, 0)	0.0 (-3, 0)	0.0 (-1.5, 1.5)	0.799
	*p***	0.739	1.000	0.194	0.832	
Poultry	B	2.0 (2.0, 5.0)	2.0 (2.0, 5.0)	3.5 (2.0, 5.0)	3.5 (2.0, 5.0)	0.777
	E	2.0 (2.0, 5.0)	2.0 (2.0, 2.0)	2.0 (2.0, 5.0)	2.0 (2.0, 5.0)	0.658
(servings/week)	Δ	0.0 (0.0, 0.0)	0.0 (-3.0, 0.0)	0.0 (-3.0, 0.0)	0.0 (0.0, 0.0)	0.707
	*p***	0.655	0.187	0.655	0.414	
Fish & seafood	B	2.0 (2.0, 2.0)	2.0 (0.5, 2.0)	2.0 (0.5, 2.0)	2.0 (0.5, 2.0)	0.379
	E	2.0 (2.0, 2.0)	2.0 (0.5, 2.0)	2.0 (2.0, 5.0)	2.0 (2.0, 2.0)	0.052
(servings/week)	Δ	0.0 (0, 0)	0.0 (0, 0)	0.0 (0, 1.5)	0.0 (0, 1.5)	0.189
	*p***	0.414	0.157	0.059	0.336	

		Salmon n = 11	2 Capsules n = 11	4 Capsules n = 10	6 Capsules n = 10	*p**
Fat[c]	B	5.5 (2.0, 5.5)	2.0 (0.5, 5.5)	5.5 (5.5, 9.0)	2.0 (2.0, 5.5)	0.084
	E	5.5 (2.0, 5.5)	5.5 (2.0, 5.5)	5.5 (2.0, 9.0)	5.5 (2.0, 5.5)	0.429
(servings/week)	Δ	0.0 (-3.5, 3.5)	0.0 (0.0, 1.5)	0.0 (0.0, 0.0)	1.8 (0.0, 3.5)	0.762
	*p***	0.928	0.279	1.000	0.257	
Food dressing[d]	B	2.0 (0.5, 2.0)	0.5 (0.0, 5.5)	2.0 (2.0, 5.5)	1.3 (0.0, 2.0)	0.218
	E	0.5 (0.5, 2.0)	0.5 (0.5, 2.0)	2.0 (2.0, 2.0)	0.5 (0.0, 2.0)	0.495
(servings/week)	Δ	0.0 (-1.5, 0.0)	0.0 (0.0, 0.5)	0.0 (-3.5, 0.0)	0.0 (-1.5, 1.50	0.53
	*p***	0.161	1.000	0.141	0.516	
Eggs	B	3.0 (2.0, 4.0)	2.0 (0.5, 3.0)	1.5 (0.5, 2.0)	1.5 (1.0, 3.0)	0.428
	E	2.0 (1.0, 4.0)	2.0 (0.5, 4.0)	2.0 (1.0, 3.0)	2.0 (1.0, 2.0)	0.828
(servings/week)	Δ	0.0 (-1.0, 0.0)	0.0 (0.0, 1.0)	0.0 (0.0, 1.0)	0.0 (-1.0, 1.0)	0.449
	*p***	0.496	0.078	0.334	0.571	
Vegetables	B	21.0 (14.0, 21.0)	21.0 (14.0, 21.0)	21.0 (14.0, 21.0)	21.0 (14.0, 21.0)	0.993
	E	21.0 (14.0, 21.0)	21.0 (7.0, 21.0)	24.5 (21.0, 28.0)	21.0 (14.0, 28.0)	0.099
(servings/week)	Δ	0.0 (0.0, 7.0)	0.0 (-7.0, 7.0)	7.0 (0.0, 7.0)	0.0 (-7, 7)	0.194
	*p***	0.655	0.271	0.058	0.739	
Legumes	B	3.5 (0.0, 3.5)	3.5 (0.0, 7.0)	3.5 (0.0, 3.5)	2.1 (0.0, 3.5)	0.752
	E	3.5 (0.0, 3.5)	3.5 (0.0, 3.5)	3.5 (3.5, 3.5)	3.5 (3.5, 3.5)	0.872
(servings/week)	Δ	0.0 (0, 0)	0.0 (0.0, 0.0)	0.0 (0.0, 3.5)	0.0 (0.0, 3.5)	0.541
	*p***	0.564	0.655	0.157	0.257	
Fresh fruit	B	7.0 (7.0, 14.0)	7.0 (7.0, 21.0)	14.0 (14.0, 21.0)	10.5 (7.0, 14.0)	0.524
	E	14.0 (7.0, 14.0)	14.0 (7.0, 21.0)	15.0 (7.0, 21.0)	14.0 (14.0, 21.0)	0.511
(servings/week)	Δ	0.0 (0.0, 7.0)	0.0 (-7.0, 7.0)	0.0 (0.0, 7.0)	0.0 (0.0, 7.0)	0.589
	*p***	0.317	1.000	0.655	0.066	
Preserved fruit[e]	B	14.0 (3.5, 14.0)	14.0 (14, 35.0)	14.0 (14, 35.0)	14.0 (3.5, 35.0)	0.183
	E	14.0 (14.0, 14.0)	14.0 (3.5, 14.0)	14.0 (3.5, 14.0)	14.0 (14.0, 35.0)	0.507
(servings/week)	Δ	0.0 (0.0, 10.5)	0.0 (0.0, 0.0)	-5.6 (-10.5, 0.0)	0.0 (0.0, 10.5)	0.198
	*p***	0.257	0.854	0.084	0.680	
Beverages	B	10.5 (3.5, 10.5)	10.5 (3.5 10.5)	3.5 (3.5, 10.5)	7.0 (3.5, 10.5)	0.873
	E	10.5 (3.5, 10.5)	3.5 (3.5, 10.5)	3.5 (3.5, 10.5)	3.5 (3.5, 10.5)	0.741
(servings/week)	Δ	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.716
	*p***	0.705	0.157	0.581	0.180	
Takeaways	B	2.0 (1.0, 2.0)	1.0 (1.0, 2.0)	2.0 (1.0, 2.0)	1.5 (1.0, 2.0)	0.258
	E	2.0 (1.0, 2.0)	1.0 (1.0, 2.0)	2.0 (1.0, 2.0)	1.0 (1.0, 2.0)	0.257
(servings/week)	Δ	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (-1.0, 0.0)	0.961
	*p***	1.000	0.414	0.564	0.317	

Results expressed as median (25, 75th percentile); *Difference between groups (Kruskal-Wallis); ** Difference between baseline and end (Wilcoxon).

[a, b]: different symbols indicate significant differences (p <0.05); [c]cooking fat or oil; [d]condiments; [e]dried, canned and stewed.

B, baseline value; E, end value; Δ, end value – baseline value.

4.8.1 CONSUMPTION OF FOODS CONTAINING OMEGA 3 FATTY ACIDS

Several foods that may influence the omega 3 fatty acid status of individuals were analysed. Table 39 displays the average intake of these foods per treatment group. There was no significant difference in intake of lean and medium fat fish from baseline to end or between groups during the study. As expected, the intake of fatty fish increased significantly in the salmon group. This increase was significantly greater compared to the capsule groups. Fresh and processed fish as well as frozen fish decreased in the salmon group during the study, although this decrease was not significant. Fatty fish intake increased significantly in the 2 capsule group. There was also a significant decrease in PUFA rich spreads and oils consumed between baseline and end in the 2 capsule group. At the end of the study only one subject reported consuming milk fortified with omega 3 fatty acids. No other omega 3 fatty acid fortified foods were consumed by the subjects. None of the other omega 3 fatty acid rich foods were found to have significantly differed between baseline and end or between groups, during the study period.

Table 39. Intakes of foods containing omega 3 fatty acids

		Salmon **n = 11**	**2 Capsules** **n = 11**	**4 Capsules** **n = 10**	**6 Capsules** **n = 10**	***p****
Fresh & processed lean fish	B	3.3 (1.0, 1.8)	4.0 (2.0, 4.8)	4.0 (2.0, 5.3)	3.0 (2.0, 4.5)	0.387
	E	2.5 (1.5, 4.5)	4.3 (1.8, 5.3)	4.5 (2.3, 7.3)	3.3 (1.8, 4.8)	0.447
(servings/week)	Δ	-0.3 (-0.5, 2.3)	0.8 (-0.8, 1.2)	0.6 (-0.8, 2.0)	0.9 (-1.0, 1.3)	0.974
	*p***	0.812	0.674	0.745	0.639	
Fresh & processed medium fish	B	0.0 (0.0, 0.0)	0.3 (0.0, 0.3)	0.1 (0.0, 1.0)	0.0 (0.0, 0.3)	0.201
	E	0.0 (0.0, 0.3)	0.3 (0.0, 0.3)	0.4 (0.0, 1.0)	0.1 (0.0, 0.5)	0.447
(servings/week)	Δ	0.1 (0.0, 0.2)	0.0 (-0.3, 0.1)	0.3 (0.0, 0.8)	0.0 (0.0, 0.1)	0.864
	*p***	0.655	0.739	0.713	0.167	
Fresh & processed fatty fish	B	0.5 (0.3, 0.8)	0.8 (0.5, 1.0)	0.6 (0.3, 1.0)	0.5 (0.3, 1.0)	0.335
	E	3.0 (1.0, 3.5)	1.0 (0.5, 1.3)	0.9 (0.5, 1.3)	1.1 (0.5, 1.5)	0.121
(servings/week)	Δ	2.5 (0.5, 2.8)[a]	0.3 (0.0, 0.5)[b]	0.1 (0.0, 0.5)[b]	0.1 (0.0, 0.8)[b]	0.025
	*p***	0.007	0.047	0.610	0.397	
Frozen fish	B	1.3 (0.3, 1.8)	0.8 (0.5, 1.0)	0.9 (0.5, 1.3)	1.3 (0.8, 2.0)	0.700
(servings/week)	E	0.8 (0.5, 2.0)	1.3 (0.8, 1.5)	1.4 (0.5, 2.0)	1.3 (0.5, 1.8)	0.845
	Δ	-0.5 (-0.6, 0.3)	0.3 (0.0, 1.0)	0.5 (0.1, 1.0)	-0.3 (-0.5, 0.4)	0.703
	*p***	0.263	0.212	0.043	0.511	
Crustaceans & molluscs	B	1.5 (0.8, 3.3)	1.5 (1.0, 2.0)	1.9 (0.8, 2.8)	1.5 (0.0, 3.0)	0.908
	E	1.5 (1.0, 2.5)	1.3 (0.3, 2.0)	2.1 (1.0, 2.8)	1.4 (0.5, 2.5)	0.724
(servings/week)	Δ	0.0 (-0.1, 0.3)	0.0 (-0.8, 0.6)	1.3 (0.0, 0.6)	0.0 (-0.4, 0.3)	0.075
	*p***	0.831	0.765	0.121	0.609	
Meat	B	5.0 (3.3, 7.0)	5.0 (2.3, 8.0)	9.0 (5.0, 10.8)	12.3 (4.5, 14.5)	0.060

		Salmon n = 11	2 Capsules n = 11	4 Capsules n = 10	6 Capsules n = 10	*p**
(servings/week)	E	4.0 (3.3, 6.0)	5.8 (4.0, 10.5)	5.5 (4.0, 8.0)	8.8 (5.8, 9.8)	0.068
	Δ	-0.3 (-3.3, 2.9)	-0.5 (-2.9, 3.8)	-0.8 (-3.3, 1.4)	-2.1 (-5.2, 1.2)	0.169
	*p***	0.859	0.306	0.126	0.083	
Poultry	B	1.8 (1.3, 3.8)	1.8 (1.3, 3.3)	3.0 (1.8, 6.0)	1.9 (1.3, 3.8)	0.661
(servings/week)	E	1.5 (1.0, 2.0)	1.8 (1.3, 4.5)	2.3 (1.5, 3.3)	3.0 (1.3, 4.0)	0.393
	Δ	-0.8 (-1.3, 0.3)	0.3 (-0.4, 1.3)	-1.1 (-3.5, 1)	0.0 (-1.0, 2.6)	0.414
	*p***	0.304	0.539	0.359	0.528	
Soy (beans, tofu & soy beverages)	B	0.0 (0.0, 0.3)	0.3 (0.0, 0.5)	0.1 (0.0, 1.0)	0.4 (0.3, 0.5)	0.329
	E	0.0 (0.0, 0.5)	0.0 (0.0, 0.3)	0.1 (0, 0.5)	0.1 (0.0, 0.5)	0.887
(servings/week)	Δ	0.0 (0.0, 0.0)	0.0 (-0.1, 0.0)	0.0 (-0.2, 0.0)	-0.1 (-0.3, 0.0)	0.492
	*p***	0.564	0.257	0.577	0.344	
Walnuts	B	0.3 (0, 0.5)	0.0 (0, 0.3)	0.1 (0, 0.3)	0.3 (0.3, 0.3)	0.307
(servings/week)	E	0.3 (0, 0.5)	0.3 (0, 0.5)	0.1 (0, 0.3)	0.3 (0.3, 0.5)	0.541
	Δ	0.0 (-0.1, 0.1)	0.0 (0.0, 0.3)	0.0 (0.0, 0.0)	0.0 (-0.2, 0.3)	0.649
	*p***	0.914	0.180	0.414	0.380	
Other nuts	B	0.5 (0.5, 7.0)	1.0 (0.3, 5.5)	0.5 (0.3, 0.5)	0.5 (0.5, 3.0)	0.622
(servings/week)	E	0.5 (0.5, 3.0)	0.5 (0.3, 1.0)	0.4 (0.3, 0.5)	0.5 (0.5, 3.0)	0.475
	Δ	0.0 (-0.1, 0.0)	-0.5 (-1.4, 0.0)	0.0 (-0.3, 0.0)	0.0 (0.0, 0.0)	0.384
	*p***	0.498	0.127	0.317	0.854	
SFA rich spread & oil	B	7.0 (0.0, 28.0)	7.0 (0.0, 28.0)	14.0 (7.0, 21.0)	14.0 (7.0, 14.0)	0.951
	E	7.0 (0.0, 14.0)	21.0 (0.0, 35.0)	7.0 (7.0, 21.0)	7.0 (0.0, 14.0)	0.409
(servings/week)	Δ	0.0 (-7.0, -7.0)	7.0 (0.7, 14.0)	-7.0 (-7.0, 0.0)	-7.0 (-7.0, -0.0)	0.662
	*p***	0.202	0.491	1.000	0.414	
MUFA rich spread & oil	B	21.0 (14.0, 28.0)	21.0 (14.0, 28.0)	17.5 (7.0, 21.0)	17.5 (14.0, 28.0)	0.639
	E	21.0 (0.0, 35.0)	21.0 (14.0, 28.0)	17.5 (7.0, 28.0)	14.0 (7.0, 14.0)	0.237
(servings/week)	Δ	0.0 (-7.0, 7.0)	0.0 (-7.0, 7.0)	0.0 (0.0, 7.0)	-7.0 (-14.0, 0.0)	0.764
	*p***	0.918	0.731	0.167	0.066	
PUFA rich spread & oil	B	7.0 (0.0, 21.0)	14.0 (0.0, 21.0)	7.0 (0.0, 14.0)	7.0 (0.0, 7.0)	0.388
	E	7.0 (0.0, 21.0)	7.0 (0.0, 7.0)	7.0 (7.0, 14.0)	0.0 (0.0, 14.0)	0.522
(servings/week)	Δ	0.0 (-7.0, 0.0)	-7.0 (-14.0, 0.0)	0.0 (0.0, 7.0)	0.0 (-7.0, 0.0)	0.104
	*p***	0.862	0.028	0.336	1.000	

Results expressed as median (25,75th percentile); * Difference between groups (Kruskal Wallis); ** Difference between baseline and end (Wilcoxon); $^{a, b}$: different symbol indicate significant differences (p <0.05).
B, baseline value; E, end value; Δ, end value – baseline value; SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Fatty fish intake at the end of the study was correlated with RBC EPA (r = 0.478, p = 0.001) and omega 3 index (r = 0.327, p = 0.034) at the end of the study.

4.8.2 SELENIUM CONSUMPTION

The intake of fish, meat and eggs which are good sources of selenium have been discussed in the previous section. Brazil nuts are a very good source of selenium. During the study period there was no significant difference in the consumption of Brazil nuts by subjects. There was no significant difference found between groups or at the baseline versus study end (see Table 40).

Table 40. Intake of Brazil nuts

		Salmon n=11		Capsules n=11		*p**
Brazil	B	0.3	(0, 0.5)	0.0	(0, 0.38)	0.224
(servings/week)	E	0.3	(0, 0.5)	0.0	(0, 0.25)	0.150
	Δ	0.0		0.0		
	*p***	0.854		0.280		

Results expressed as median (25,75th percentile);
*Difference between groups (Kruskal Wallis); **Difference between baseline and end (Wilcoxon).
B, baseline value; E, end value; Δ, end value – baseline.

CHAPTER 5

5 DISCUSSION

The main purpose of this study was to investigate whether salmon or salmon oil capsules were a more effective method of increasing RBC LC omega 3 fatty acids, the omega 3 index and selenium status in healthy adults. This was determined by comparing the consumption of 2 weekly servings of FNZK salmon with a daily intake of salmon oil capsules. The findings from this study establish whether the recommendations for omega 3 fatty acid intake should continue to assume equivalence between fish and fish oil consumption. The study also determines if the consumption of fatty fish is an appropriate recommendation for increasing selenium status. This section will discuss ways in which the treatment influenced LC omega 3 fatty acids, omega 3 index, selenium status, anthropometric measures, blood lipid profile and BP in a healthy adult population. Discussion also includes how the consumption of the recommended intake of 2 servings of fatty fish per week affected subjects' food consumption habits. The tolerance of the treatment is also discussed. Finally the overall conclusions of the study are reported along with recommendations for future research.

5.1 SUBJECT CHARACTERISTICS

This research explored the effects of the treatment on a healthy adult population. A healthy population group was defined as those without any known health condition or disease. Healthy subjects were selected through recruiting men and women between 21 – 45 years of age, who did not smoke and who had no known health condition or disease. The reasons for recruiting this population was firstly due to the increased incidence of chronic disease after 45 years of age and secondly because of the established correlation between chronic disease and smoking (Kolbe-Alexander et al., 2008). The average baseline measurements indicated that the subjects who participated in this study were healthy. They were non-smoking, most had a BMI of less than or equal to 25 kg/m2 and a waist:hip ratio equal to or less than 0.8. Both of these measurements are used to assess obesity, waist:hip also gives an indication of fat distribution. The mean lipid profile concentrations of the subjects were within the recommended ideal range. TC and LDL-C concentrations were above the optimal recommended concentrations. Despite this the average HDL-C was high and TC:HDL-

C was low indicating a low risk lipid profile. The average BP measurements of the subjects were at levels that are generally considered to be healthy. This was expected as the inclusion criteria for the study required subjects to be healthy. Based on the measurements taken subjects used in the present study were found to be healthier than the average New Zealand population group for their age and gender (Russell et al., 1999). To ensure that a significant increase in RBC LC omega 3 fatty acids could be obtained, individuals with high habitual intake (> 2 servings/month) of fatty fish and/or those who consumed omega 3 fatty acid supplements in the last 6 months were excluded from the study. Two similar studies also selected individuals with a low omega 3 fatty acid intake. Harris et al. (2007) excluded individuals who consumed greater than 2 servings of tuna or salmon per month or consumed fish or flaxseed oil. Arterburn et al. (2008) selected subjects who consumed less than 0.2 g of DHA per day based on information provided in a FFQ. The levels of EPA and DHA in RBC at baseline in all three studies are displayed in Table 41. The baseline RBC EPA and DHA levels in the present study were similar to those reported by Arterburn et al. (2008) and Harris et al. (2007).

Table 41. Baseline red blood cell long chain omega 3 fatty acids from studies comparing the effects of fish and fish oil capsules

Study	**Country**	**EPA** (% fatty acids)	**DHA** (% fatty acids)
Present study	New Zealand	0.78 ±0.20 (fish) 0.84 ±0.26 (capsule)	5.06 ±0.67 (fish) 5.25 ±0.56 (capsule)
Arterburn et al., 2008	US	0.53 ±0.06 (fish) 0.61 ±0.04 (capsule)	5.74 ±0.21 (fish) 5.82 ±0.17 (capsule)
Harris et al. (2007)	US	0.80 ±0.12 (fish) 0.99 ±0.17 (capsule)	3.22 ±0.58 (fish) 3.34 ±0.79 (capsule)

Expressed as mean ±SD
EPA , eicosapentaenoic acid; DHA, docosahexaenoic acid.

Despite selecting individuals with low habitual intake of fatty fish the baseline omega 3 index was higher than expected (5 – 7 %) for this study. A value of 4 % was associated with the least cardioprotection and a value of 8 % with the greatest carioprotection (Harris & von Schacky, 2004). The baseline FFQ was examined to determine reasons for this. It was found that only one subject in this study used omega 3 fatty acid fortified

products (omega 3 fatty acid fortified milk). However intake of meat and poultry may have contributed to the LC omega 3 fatty acid status (Howe et al., 2006).

5.1.1 Anthropometric Measures

As healthy subjects were recruited and they maintained their normal routine throughout the study period (determined using the compliance diaries), no significant variations were expected from the baseline to end anthropometric measures. The BMI and waist:hip did not change from baseline to end, indicating subjects probably continued their usual routines during the study.

5.2 Treatments

5.2.1 Fatty Acids Composition of Treatments

The treatments given to subjects were based on recommendations by the Heart Foundation of New Zealand for fatty fish intake (2 120 g servings/week) (Roberts, 1999). This recommendation is a realistic expectation for fish intake. Other similar studies have not taken this into consideration. In fact Visioli et al. (2003) required subjects to consume 100g of smoked salmon per day, which is unrealistic for an ordinary diet and very costly to consume for extended periods of time.

The present study examined the effects of consuming LC omega 3 fatty acids in whole foods (salmon) compared to supplements (salmon oil) on the incorporation of RBC LC omega 3 fatty acids into the body. Therefore it was important that other treatment variables did not affect omega 3 fatty acids absorption. Consequently treatments containing omega 3 fatty acids from the same source were chosen; in both the fish and capsules omega 3 fatty acids were from salmon and were present in the TG form.

Analysis was carried out to determine the EPA and DHA content of the cooked salmon portions (after each portion was cooked by the subject), raw salmon portions and capsules. Both the salmon and capsules contained more DHA than EPA. The salmon oil from the capsules contained greater amounts of EPA and DHA than the oil extracted from both the cooked and raw salmon.

A greater concentration of LC omega 3 fatty acids was present in the cooked salmon compared to the raw salmon. Heating can result in progressive shrinkage and disintegration of the myofibril resulting in the expulsion of water soluble proteins and fats from the tissue (Konga et al., 2007). Therefore it is important that studies with similar designs carried out analysis of cooked portions and not raw. Larsen et al. (2008) reported that the omega 3 fatty acid content of fish can vary significantly depending on cooking methods. The omega 3 fatty acid content of the cooked salmon was high, this is probably due to the cooking methods required to be used by the subjects. The cooking methods (oven baking, pan frying or steaming) used in this study were outlined in a study by Larsen et al. (2008), who reported these three methods maintained the highest omega 3 fatty acid levels in the salmon.

Based on the method of analysis employed in this study, the fish oil capsules used were found to contain lower amounts of EPA and DHA than stated on the label, the capsules contained 65.2 % of the EPA and 37.5 % of the DHA. Analysis of oil in the capsules involved using an internal standard to compare the amount of fatty acids present in the treatment. This is a more accurate method of analysing fatty acids, as the internal standard is prepared in the same way as the sample, removing any variations that may have been created from external factors, such as the machinery used or the operator's handling methods. Harris et al. (2007) and Arterburn et al. (2008) also used an internal standard to analyse the fatty acid content of the fish and capsule treatments used in their studies.

It is speculated that Healtheries may have used a different method for analysing the fatty acid content of the capsule which has given rise to disparities in the two results. These findings emphasise the need for the development of international standardised methods for the analysis of fatty acids.

Other research has found that some of the fish oil supplements on the market do not contain the quantity of omega 3 fatty acids stated on the label. A study in New Zealand reported 17 % (5/29) of the supplements were mislabelled (Allan, 2007). A Hong Kong study found 29 % (8/28) of the supplements misreported the omega 3 fatty acids content of fish oil supplements (Consumer Council, 2008).

The species of salmon used in this study was Chinook. This species contains significantly higher amounts of omega 3 fatty acids when compared to the Atlantic salmon used in similar studies (Arterburn et al., 2008; Elvevoll et al., 2006; Harris et al., 2007); see Table 42 for comparisons. Authors in these other studies did not consider the cooking method employed by their subjects (Arterburn et al., 2008; Elvevoll et al., 2006; Harris et al., 2007; Visioli et al., 2003). However Harris et al. (2007) reported supplying subjects with recipes.

In this study the capsules, like most fish oil capsules used in the similar studies, were in the TG form. The study by Visioli et al. (2003) was the only one that compared ethyl ester capsules on LC omega 3 fatty acid status. This may account for the difference in conclusions found in the study by Visioli et al. (2003).

Table 42. Studies comparing the effects of consuming fish with omega 3 fatty acid supplements on long chain omega 3 fatty acid status

Study	Subjects (n)	Gender	Age (years)	Duration (weeks)	Biomarker/s Measured	Fish Consumed	EPA (g/day)	DHA (g/day)	EPA+DHA (g/100g)	Capsules Consumed	EPA (g/day)	DHA (g/day)	EPA+DHA (g/capsule)
Present study	42, healthy	19 males 23 females	21 – 45	8	RBC	240 g Chinook salmon/week	0.337	0.370	2.062	Healtheries omega advanced pure salmon oil 2, 4 or 6 capsules/day	0.090 0.181 0.294	0.120 0.239 0.349	0.105
Visioli et al. (2003)	8, healthy	10 males 6 females	26 – 38	6	Plasma	100g smoked salmon/day (700g/week)	0.383	0.544	0.927	Ethyl ester (fish oil) capsules 1 or 3 capsules/day	0.150 0.450	0.110 0.320	0.260
Elvevoll et al. (2006)	71, healthy	NR	NR	8	Serum	400 g smoked salmon/week or 400 g Atlantic salmon fillet/week or 400 g cod fillet/week	0.470 0.450 0.009	0.710 0.720 0.021	2.062 2.048 0.052	Cod liver oil 15ml/day	1.380[†]	1.610[†]	2.990[a]
Harris et al. (2007)	23, premenopausal	23 females	21 – 49	16	RBC Plasma phospholipids	3 x 171g of canned albacore tuna & 171 g Atlantic salmon/ fortnight (257 g tuna/ week & 85 g salmon/ week)	0.095	0.390	0.993	CardioTabs omega 3 (fish oil) capsules 1-2 capsules /day	0.104	0.378	0.397
Arterburn et al. (2008)	32, healthy	13 males 16 females	20 – 65	2	RBC Plasma phospholipids	27 – 60 g Atlantic salmon/day (378 – 840/week)	NR	0.600	NR	DHASCO-T algal-oil capsules 3 capsules/day	NR	0.600	NR

[a] per 15 ml.

EPA , eicosapentaenoic acid; DHA, docosahexaenoic acid; RBC, red blood cells; NR, not reported.

5.2.2 OXIDATION VALUE OF CAPSULES

LC omega 3 fatty acids are vulnerable to oxidation due to the presence of double bonds (Kolanowski et al., 2007). Oxidation has been associated with increased ageing, membrane damage, heart disease and cancer (Kanner, 2006). Oxidation also causes a decrease in omega 3 fatty acids (Kolakowska et al., 2006). Capsules used in the present study were analysed to ensure that they were within the safe level of oxidation and did not cause any negative effects on the subjects. The capsules were found to be within the safe oxidation parameters set for New Zealand (peroxide value < 5 meq/kg fat and anisidine < 10) Ministry of Economic Development, 2009). A consumer study that analysed fish oil supplements in New Zealand did not analyse the capsules used in this study (Healtheries omega advanced pure salmon oil capsules), however they did analyse another type of Healtheries capsule that contained fish oil. They reported these capsules had an oxidation value of less than 20 (Allan, 2007). The authors defined the oxidation value as the peroxide value plus the anisidine value and stated that an oxidation value of less than 30 was acceptable. The capsules from the present study were stored in the fridge and out of the light which may explain the low values.

5.2.3 SELENIUM INTAKE FROM TREATMENTS

Following analysis, no selenium was detected in the capsules. However selenium was found to be higher in the cooked salmon than the raw salmon. This could be due to the degradation of protein during cooking, as cooking has been reported to increase selenium levels (Shen, Hook-van Nieuwenhuizen, & Luten, 1997; Shi & Spallholz, 1994). The salmon portions consumed in the present study provided 6.84 μg selenium per day. This intake would account for approximately 10 % of the New Zealand RDI for selenium (Ministry of Health, 2006). It was reported by the 1997 National Nutrition Survey (Russell et al., 1999) that men in New Zealand consumed an average of 56 μg per day and women 39 μg per day. The addition of two servings of salmon per week would increase these amounts to meet 90 % and 77 % of the selenium RDI (70 μg for men and 60 μg for women) for men and women, respectively.

5.3 RED BLOOD CELL OMEGA 3 FATTY ACIDS

5.3.1 RED BLOOD CELL EPA AND DHA

As expected a significant increase in RBC EPA and DHA was seen in all treatment groups. Consumption of 0.34 g of EPA per day from either salmon or salmon oil capsules resulted in similar increases in RBC EPA (capsules 1.0 [0.71 – 1.27] % and salmon 0.8 [0.58 – 1.02] %). RBC DHA showed a similar increase (capsules 0.99 [0.68 – 1.31] % and salmon 0.93 [0.58 – 1.29] %) with the consumption of 0.37 g of DHA per day from salmon or salmon oil capsules. This was not seen with two similar studies by Visioli et al. (2003) and Elvevoll et al. (2006). Table 42 gives a comparison of study design between the four similar studies and the present study.

Visioli et al. (2003) reported that smoked salmon increased plasma EPA and DHA by 2 and 9 fold, respectively, compared with consumption of fish oil capsules. Elvevoll et al. (2006) compared a variety of fish types with cod liver oil and concluded that serum EPA and DHA were almost three times higher with the consumption of fish. However, Harris et al. (2007) and Arterburn et al. (2008) did not find any significant difference between the incorporation of omega 3 fatty acids from the consumption of fish compared to fish oil supplements. Harris et al. (2007) compared the change in RBC and plasma phospholipid EPA and DHA from supplementation of canned tuna and Atlantic salmon to fish oil capsules. Arterburn et al. (2008) compared the effects of consuming salmon and algal-oil capsules containing DHA on RBC and plasma phospholipid EPA and DHA.

Elvevoll et al. (2006) did not report matching intakes of EPA and DHA from the fish and capsules. Harris et al. (2007) compared equivalent levels of total omega 3 fatty acids from fish and fish oil capsules, but EPA and DHA levels were not equivalent. Arterburn et al. (2006) provided portions of salmon and capsules that contained exactly 0.6 g of DHA to be consumed daily. The authors reported that they analysed the DHA content of cooked Atlantic salmon fillets and based on their findings weighed out salmon portions with exactly 0.6g of DHA which were then immediately frozen and provided to subjects. Visioli et al. (2003) and this present study were the only ones that used a dose-response model to compare the effects of fish and fish oil capsules on LC omega 3 fatty acid status. However Visioli et al. (2003) used data from a previous study to complete their response model.

Similar studies used different LC omega 3 fatty acid biomarkers. RBC have been shown to contain significant concentrations of EPA and DHA compared to other bodily tissues, they reflect the last 120 days of intake and vary to a lesser degree compared to other omega 3 fatty acid biomarkers (Arterburn et al., 2008; Harris et al., 2004; Sullivan et al., 2006). RBC LC omega 3 fatty acid levels are therefore considered as a good biomarker for LC omega 3 fatty acid intake.

Visioli et al. (2003) and Elvevoll et al. (2006) looked at plasma and serum EPA and DHA, not RBC levels. Plasma and serum fatty acids reflect the short term omega 3 fatty acid status, RBC omega 3 fatty acids have been reported to be a more accurate measure of omega 3 fatty acid status and reflect longer term status (Arab, 2003; Di Marino et al., 2000). Harris et al. (2007) and Arterburn et al. (2008) who measured both plasma phospholipids and RBC EPA and DHA, did not report a significant difference between the increase in omega 3 fatty acids from fish compared to fish oil capsules. The lipids in both the fish and capsules were in the TG form. Harris et al. (2007) carried out a study on women only and Arterburn et al. (2008) only looked at DHA supplementation over a two week period. Comparison of results from the different studies is difficult due to the different omega 3 fatty acid biomarkers used. This highlights the need to establish an international standard for assessing omega 3 fatty acid status.

The studies that reported fish to be more effective than fish oil capsules in increasing omega 3 fatty acid levels speculated that this was most likely due to capsules creating a lipidic bolus (Visioli et al., 2003; Elvevoll et al., 2006). The lipidic bolus is not created from the consumption of fish as omega 3 fatty acids are diluted by other nutrients present. A possible reason why no difference in response was seen in the present study is that subjects were asked to consume the capsules with food. Therefore the lipidic bolus may not have been created, also the capsules in this study were not as concentrated as those in other studies. This means the lipidic bolus may not have been created to the same extent as with capsules used in previous studies.

EPA and DHA in the salmon are esterified to the sn-2 position of TG, where as in fish oil, EPA and DHA are predominately found in the sn-1 and sn-3 positions (Visioli et al., 2003; Wijesundera & Abeywardena, 2004). At the sn-2 position EPA and DHA are, to a

large extent, preserved from hydrolysis during digestion and intestinal absorption of exogenous fat (Visioli et al., 2003).

The difference in response found by Visioli et al. (2003) may be explained by the use of ethyl ester capsules in their study. A study by Harris et al. (1988) that investigated the effect of consuming omega 3 fatty acids in the TG to the methyl ester reported no significant difference in the change in EPA plasma phospholipid, however DHA plasma phospholipid concentrations were higher from consumption of methyl esters. Yet other researchers concluded that EPA and DHA in either ester ethyl or TG form were equally well absorbed (Nordoy et al., 1991).

Compliance with treatment in similar studies was not reported by the researchers (Arterburn et al., 2008; Elvevoll et al., 2006; Harris et al., 2007; Visioli et al., 2003). Elvevoll et al. (2006) and Visioli et al. (2003) did not indicate how much of the treatment subjects consumed during the study. Harris et al. (2007) reported that they did not monitor intake of treatment, but stated that compliance of treatment in the capsule group was 97 ±8 % and 100 % compliance for the fish group. Arterburn et al. (2008) did not state if they monitored compliance, however they reported that subjects were required to consume over 90 % of their treatment to be eligible for inclusion. Compliance of subjects to the study protocol may also have resulted in the differences in conclusions drawn in the studies.

5.3.2 OMEGA 3 INDEX

An omega 3 index of 8 % or greater is required for the best cardio-protection (Harris & von Schacky, 2004). Supplementation of all the treatments caused a significant improvement in omega 3 index, in fact almost a third of the subjects had an omega 3 index of 8 % or greater at the end of the study. No difference was found in the increase in omega 3 index between consumption of salmon or salmon oil capsules. The consumption of 0.82 g per day of LC omega 3 fatty acids from either salmon or capsules for 8 weeks resulted in similar increases in the omega 3 index (capsule 2.25 [1.65 – 2.83] % and salmon 1.92 [1.46 – 2.38] %). However, the omega 3 index would most likely have increased further if supplementation was continued for a longer period as RBC omega 3 fatty acids do not reach a steady state until 6 months following regular

consumption (Katan et al., 1997). A study by Cao et al. (2006) reported that 2 g of EPA and DHA per day for 8 – 20 weeks was required to reach an omega 3 index of 8 %. The subjects in this present study consumed significantly less than 2g of omega 3 fatty acids from the treatment (highest amount 0.82 g EPA and DHA/day), however they almost reached recommended omega 3 index of 8 %.

5.4 PLASMA SELENIUM AND WHOLE BLOOD GLUTATHIONE PEROXIDASE

The subjects' baseline plasma selenium concentrations (salmon group 1.63 ±0.03 μmol/l and capsules group 1.57 ±0.26 μmol/l) were high compared to previous studies carried out on the New Zealand population (Thomson, 2004; Thomson & Robinson, 1980; Whanger et al., 1988). In 2004 Thomson reported plasma selenium concentrations ranging from 0.9 – 1.12 μmol/l in the New Zealand population from different regions of New Zealand. A possible reason for the subjects' high baseline selenium could be an increased intake of imported foods over recent years. Wheat from Australia has significantly more selenium than that from New Zealand and researchers have shown that people in the North Island of New Zealand consume more imported products which results in higher selenium status (Thomson et al., 2007). The subjects that participated in this study were from Auckland where imported foods are readily available. Despite the subjects high baseline concentrations the small amount of selenium (6.840 ±0.272 μg selenium/day) provided by the salmon created a significant increase in plasma selenium compared to the capsule treatment (0.16 ±0.13 versus 0.02 ±0.13 μmol/l, respectively).

A study by Thorngen & Akesson (1987) investigated the effect of fatty fish on selenium status. The authors found that consumption of 150 – 200 g of fatty fish per day (herring salmon or mackerel) providing 40 – 50 μg selenium per day for 6 weeks increased plasma selenium by 0.13 μmol/l. They stated that there was considerable individual human variation and concluded that selenium in fatty fish had relatively low bioavailability. In this present study there was an increase of 0.16 μmol/l from salmon intake over the 8 weeks from a food source providing much less selenium per day. Based on research carried out linking selenium status to adequate function of the body, the subject's baseline concentrations were high enough to protect against some cancers (>1.50 μmol/l) (Thomson, 2004). These researchers also reported that for full

expression of GPx, a plasma selenium concentration of 0.82 μmol/l or more is required. As the subjects in this study had a baseline selenium concentration greater than this, GPx was probably already fully expressed which may explain why no significant changes were seen in whole blood GPx concentrations. The findings of this study are of particular relevance to New Zealand as both dietary intakes of selenium and selenium status of the population have been shown to be low (Thomson, 2004). The consumption of two 120g servings of salmon a week would provide 7 μg of selenium per day and this study has demonstrated that this level of intake that it can increase plasma selenium by almost 10 % after 8 weeks of consumption.

5.5 LIPID PROFILE

Extensive evidence exists to demonstrate that omega 3 fatty acids can influence the blood lipid profile by increasing HDL-C and LDL-C, as well as lowering the TG concentrations (von Schacky, 2006). A meta-analysis by Harris (1997) reported that an intake of 3 – 4g per day of LC omega 3 fatty acids over 2 weeks or longer caused an increase of 5 % in LDL-C and decreased TG concentrations by 25 % in normolipidemic subjects. A more recent meta-analysis by Balk et al. (2006) found fish and fish oil only had a small effect on HDL-C and LDL-C, but was able to reduce TG by 15 % or more.

In the present study no significant change in the blood lipid profiles of the subjects was found. This result could be due to the treatments containing a maximum amount of omega 3 fatty acids of 0.816 ±0.055 g per day (3 – 4 g/day is required to see a change) and the average baseline blood lipid profile of the subject group being in the ideal range. There was a significant increase in HDL-C in the 6 capsule group causing HDL-C to be significantly different between the 6 capsule group and both the salmon and 4 capsule group at the end of the study. This increase in HDL-C in the 6 capsule group caused a significant lower level of TC:HDL-C between this group and the 4 capsule group. It is speculated that intake of DHA from the capsules may have increased HDL-C as DHA has been reported to increase HDL-C (von Schacky, 2004). However the 6 capsule group only consumed 0.7 g omega 3 fatty acids per day, while the effects of DHA have only been reported at intakes of 3 – 5 g of omega 3 fatty acids per day (Kris-Etherton et al., 2002). This effect was not seen in the salmon group that received similar amount of omega 3 fatty acids to the amount consumed by those in the 6 capsule group.

Harris et al. (2007) investigated the effect of fish and fish oil capsules on serum lipids. They reported a small increase in TG concentrations in the group consuming fish oil capsules compared to the group consuming fish. It was also reported that both TC and LDL-C increased in both groups. There were no significant changes in TG concentrations found in the present study.

5.6 BLOOD PRESSURE

Results of a meta-analysis by Morris et al. (1993) have shown that omega 3 fatty acids are able to decrease BP, however high dosages (3 g omega 3 fatty acids/day) are required. In this study no significant changes were found in BP. This result is possibly due to the low dosage of omega 3 fatty acids in treatment as the minimum intake required per day to obtain a reduction in BP is 3 g (Appel et al., 1993); the maximum consumed in this study was 0.816 ±0.055 g per day found in salmon. Also changes were reported as being more significant in those over 45 years of age and those with a BP of 140/90 mmHg or greater, none of the subjects matched this criteria (Geleijnse et al., 2002).

5.7 TOLERANCE OF TREATMENT

The first question in the tolerance questionnaire was optional and open-ended, “did you suffer any side effects?”, only nine subjects (42 possible) responded, all were from the capsule groups. However when asked specific questions on side effects there was significantly higher numbers of responses (21).

The tolerance questionnaire was based on a study investigating the effects of omega 3 fatty acid supplements on perinatal women (Freeman & Sinha, 2007). Burping and foul breath were the most frequent side effects reported in their study. Harris et al. (2007) compared the effects of fish and fish oil capsules on fishy aftertaste and reported that the frequency of fishy aftertaste was significantly higher in the capsule group than in the fish group (10/12 and 1/11, respectively). This is very similar to results found in this study. Based on the tolerance questionnaire burping was the most significant side effect;

reports of burping were significantly higher in the capsule groups as only one subject reported burping in the salmon group.

A question was also put to subjects about the best method of consuming the treatment. Forty three percent of subjects indicated consuming all the capsules "together" as the best consumption method. While only 19 % recommended consuming the capsules "throughout the day". Consuming all the capsules together might result in greater compliance as subjects may forget to consume the treatment if consumed throughout the day. Another study reported that compliance can be improved by consuming treatment at bedtime or with meals (Lee et al., 2008). The subjects in this present study were required to consume the treatment with a meal and capsules could not be consumed at bedtime.

5.8 FOOD CONSUMPTION HABITS

Subjects were requested to maintain their normal routine during the study period. This included maintenance of usual food consumption habits with the exception of adding the treatment. It was hypothesised that the addition of two servings of fatty fish per week to the diet would result in a reduction in intake of another protein source, such as red meat. The FFQ was used to determine if any changes in food sources containing omega 3 fatty acids and selenium occurred during the study or between groups.

Findings revealed that subjects' food consumption habits did not differ between the groups or baseline to end, except for baseline milk intake. The salmon group consumed significantly less milk than those in the 4 capsule group. The milk consumed was not fortified with omega 3 fatty acids (only one subject reported consuming omega 3 fatty acids fortified milk) and was not a significant source of omega 3 fatty acids or selenium. Therefore milk intake would not have influenced the status of the two nutrients measured.

Despite screening for subjects with a low fatty fish intake, fish intake was significantly higher amongst subjects than that reported in the National Nutrition Survey (Russell et al., 1999). Only 13 % of the population reported consuming fish (steamed, baked,

grilled or raw) at least once a week, however the average fish and seafood intake of the subjects in this study was 2 times a week.

As expected, a significant increase in the intake of fatty fish was seen in the salmon group (average of 2.5 servings/week). It appears that the salmon may have replaced lean, fresh and frozen fish as well as red meat and poultry in the diet, as these sources decreased from baseline to end (with 0.3, 0.5, 0.3 and 0.8 servings respectively/week) in the salmon group, although not significantly. A significant increase in fatty fish intake in the 2 capsule group was found, however this was only by 0.3 servings per week. The amount of PUFA spreads and oils may also influence omega 3 fatty acid status as these foods may contain small amounts of mainly ALA omega 3 fatty acids. It was found that PUFA spreads and oils decreased in the 2 capsule group by 1 serving per day (7 servings/week).

A correlation was found to exist between reported fatty fish intake and RBC EPA. Fatty fish intake also correlated with the omega 3 index at the end of the study, highlighting the significance of its intake on omega 3 fatty acid status.

Baseline plasma selenium positively correlated with processed fish consumption, this highlights the significance of fish consumption on selenium status of the subjects.

CHAPTER 6

6.1 SUMMARY

Salmon and fish oil are good sources of LC omega 3 fatty acids particularly EPA and DHA that are well recognised for their health benefits and protective effects against CVD and other chronic diseases. The omega 3 index is a recently introduced novel, physiological relevant, modifiable and independent marker of risk for sudden cardiac death (von Schacky & Harris, 2007).

Most health authorities and researchers recommend that healthy individuals consume 0.5 g of omega 3 fatty acids per day (Gebauer et al., 2006; Ministry of Healthy, 2006; Mozaffarian & Rimm, 2006). These recommendations assume equivalence in bioavailability between fatty fish and fish oil capsules. However there are few studies that have investigated the effect of different food matrices (fish or fish oil) on LC omega 3 fatty acid status.

Two earlier studies demonstrated that omega 3 fatty acids from fish were more effectively incorporated into plasma lipids than when administered as supplements (Elvevoll et al., 2006; Visioli et al., 2003). However two recent studies concluded that fish or fish oil supplements were equally effective at increasing blood lipids with omega 3 fatty acids (Arterburn et al., 2008; Harris et al., 2007).

Fatty fish such as salmon are not only rich in LC omega 3 fatty acids, they also contain other nutrients including selenium that is not available in fish oil supplements (Thorngren & Akesson, 1987). Selenium is an essential trace element used in many biological reactions via a number of different selenoproteins (Froslie et al., 1985). Selenium is associated with a reduction in the risk of the development of many chronic diseases (Brown & Arthur, 2001; Thomson et al., 2008). Amongst the New Zealand population selenium status was found to be sub-optimal due to low concentrations of selenium in the soil (Thomson & Robinson, 1986).

The aim of this research was to compare the effects of consuming LC omega 3 fatty acids from farmed salmon with salmon oil supplements. Furthermore, consideration was given to other contributing factors of consuming fatty fish such as selenium status.

Healthy volunteers (n=44) were randomly assigned to one of four groups consuming either two servings of 120 g farmed salmon per week or 2, 4 or 6 capsules of salmon oil per day for 8 weeks. Fasting blood samples, anthropometric measures (BMI and waist:hip ratio), BP and dietary habit information (via a FFQ) were collected at baseline and following 8 weeks of undergoing treatment regimes. The intake of LC omega 3 fatty acids and selenium by each subject was determined through analysing the fatty acid and selenium content of duplicate portions of cooked salmon and subtracting any unconsumed salmon. The fatty acid and selenium content of salmon oil capsules were analysed. The amount of LC omega 3 fatty acids and selenium consumed from capsules were calculated based on the percentage of compliance. The fatty acid content of salmon, capsules and RBC were analysed using GC-mass spectrometer. The omega 3 index was calculated as follows: omega 3 index = EPA + DHA in RBC/total fatty acids in RBC x 100 (Harris, 2008). Plasma selenium was analysed using a modified version of the method described by Jacobson & Lockitch (1988) and whole blood GPx was determined using the modified version of the method by Thomson et al. (1982). BP was measured using a digital automatic BP monitor. The blood lipid profiles were analysed by LABPLUS at Auckland City Hospital.

The oxidation values of the capsules were measured on capsules at 0, 4 and 8 weeks into the intervention. The anisidine value was determined using a UV visible spectrophotometer and a sodium thiosulphate titration was used to determine the peroxide value.

Differences within groups were analysed using the paired sample *t*-test or the Wilcoxon Ranked-Sum Test, for parametric variables and non-parametric variables respectively. The one-way ANOVA with post-hoc tests (Tukey's Honest Significance Difference Test) was used to determine the differences between groups for parametric variables, whereas the Kruskal-Wallis Test with post-hoc analysis and Bonferroni adjustments was used for non-parametric variables. The differences in plasma selenium between the salmon and capsule (three groups combined) groups were analysed while controlling for baseline selenium using the Analysis of Covariance (ANCOVA) Test. Spearmans Correlations Coefficients were used to determine any significant linear relationships. Linear regression analysis predictive models were fitted to the capsule data to predict

change in RBC LC omega 3 fatty acid levels and omega 3 index with intake of LC omega 3 fatty acids from capsules in amounts equivalent to that consumed from salmon.

LC omega 3 fatty acid intake from salmon and 2, 4 and 6 capsules were 0.82 ±0.055 (0.34 ±0.224 g EPA and 0.37 ±0.024 g DHA/day), 0.24 ±0.004 (0.09 ±0.001 g EPA and 0.12 ±0.002 g DHA/day), 0.47 ±0.004 (0.18 ±0.001 g EPA and 0.24 ±0.002 g DHA/day) and 0.68 ±0.022 (0.26 ±0.008 g EPA and 0.35 ±0.011 g DHA/day) g per day, respectively. Results from the predictive model showed that increases in RBC LC omega 3 fatty acid levels and omega 3 index were similar with intake of equal amounts of LC omega 3 fatty acids from salmon and capsules (RBC EPA: 0.80 [0.58 – 1.02] vs. 1.00 [0.71 – 1.27] %; RBC DHA: 0.93 [0.58 – 1.29] vs. 0.99 [0.68 – 1.31] %; omega 3 index: 1.92 [1.46 – 2.38] vs. 2.25 [1.65 – 2.83] %). The capsules used in this study did not contain any selenium (< 0.02 μg/day), whereas the average intake of selenium from salmon was 6.84 ±0.272 μg per day. Plasma selenium concentrations increased significantly in the salmon group compared to the capsule group, 0.16 ±0.13 vs. 0.02 ±0.13 μmol/l. No significant difference was found in the capsule group or in whole blood GPx for either group.

Throughout the study the salmon oil capsule oxidation values were within acceptable levels set for New Zealand (Ministry of Economic Development, 2009). BMI, waist:hip ratio and BP did not significantly differ between the groups of subjects or from baseline to end of the study. Neither did the blood lipid profile of subjects change significantly between the baseline and end of the study.

A significant increase in consumption of fatty fish over the study period was reported in the salmon group (2.5 (0.5, 2.8) servings per week). Although it was not significant, decreases in lean and processed fish, red meat and poultry were also found in the salmon group.

Salmon consumption appeared to have been better tolerated by the subjects than consuming salmon oil capsules. Subject suggestions for “best” method of consuming salmon oil capsules did not allow conclusions to be drawn as suggestions were often contradictory.

Consumption of similar amounts of LC omega 3 fatty acids from either salmon (2 weekly servings) or salmon oil (daily dosage) was equally effective in increasing RBC LC omega 3 fatty acid levels and omega 3 index. The omega 3 fatty acid status can therefore be improved by either method according to consumer preference. However consumption of salmon has the added benefit of significantly increasing selenium status.

6.2 CONCLUSION

Based on the results of this study the alternative hypothesis (H_1) is rejected. The consumption of equivalent amounts of LC omega 3 fatty acids from weekly intakes of FNZK salmon or daily intake of salmon oil capsules for 8 weeks in healthy subjects aged between 21 – 45 years was equally effective at increasing RBC levels of LC omega 3 fatty acids (EPA and DHA) and omega 3 index.

The alternative hypothesis (H_2) is accepted as the consumption of weekly intake of FNZK salmon resulted in a significant increase in selenium status when compared with daily intake of salmon oil capsules for 8 weeks in healthy subjects aged between 21 – 45 years.

Consumption of 0.82 g per day of omega 3 fatty acids from either salmon or salmon oil capsules over a period of 8 weeks resulted in an increase in omega 3 index of ±2 %. This increase might have been greater if the study was continued for a longer period.

In conclusion, omega 3 fatty acid status can be improved by either method according to the consumer's preference. However, consumption of salmon had the additional benefit of increasing plasma selenium concentrations as well as being a more readily tolerated form of ingestion than capsules. Eight weeks supplementation of omega 3 fatty acids from farmed salmon and salmon oil capsules did not cause a significant change in blood lipid profiles, BP, or food consumption habits.

6.3 STRENGTH OF THE PRESENT STUDY

The subjects in this study were matched by age and gender to ensure that the groups were similar as age and gender may influence the response to omega 3 fatty acids consumption. Once matched, subjects were then randomly assigned to the different treatment groups. Random allocation of subjects to treatment removes systematic differences between treatment groups that may influence the outcome of the study (Sibbald & Roland, 1998).

This present study used RBC omega 3 fatty acid levels as the biomarker for measuring the LC omega 3 fatty acids incorporation into the body from supplementation of LC omega 3 fatty acids. The RBC fatty acid levels can also be used to calculate the omega 3 index, a new biomarker for omega 3 fatty acids that is proven to have a high correlation with fish and omega 3 fatty acid intake, low biological variability and little day-to-day variations (Harris, 2007). Research supports the claim that RBC best reflects omega 3 fatty acid status (Arab, 2003; Di Marino et al., 2000; Harris et al., 2004). The duration of treatment used was based on previous studies that have shown 8 weeks to be sufficient to produce an increase in RBC LC omega 3 fatty acids (Katan et al., 1997). This study used a general linear response model fitted to the capsule data to compare LC omega 3 fatty acid intakes between salmon and salmon oil capsules. This also meant that EPA, DHA and total LC omega 3 fatty acid intakes could be reliably compared amongst the treatment regimes provided.

Treatments taken from the same raw source were used in this study (salmon versus salmon oil), to ensure that the differences produced in subjects were due to the food matrix. It ruled out possible effects of other variables such as the form of lipid consumed.

The treatment regimens adopted for this study were based on recommended intakes for fatty fish (2 servings/week), which is a manageable intake for most people (Roberts, 1999). The content of LC omega 3 fatty acids of salmon has been to shown to differ significantly depending on the cooking method used (Larsens et al., 2008). Cooking methods used by the subjects in the salmon group were similar as they were required to pan fry, oven bake or steam their salmon portions. These cooking methods were outlined by Larsens et al. (2008) as maintaining the highest omega 3 fatty acid levels.

Measurement of the omega 3 fatty acid intakes was very accurate as it was determined by analysing the duplicate portions of salmon cooked and returned by each subject in the salmon group.

It was a requirement that the capsules used in this study were stored in the fridge and kept out of the light. This was done to reduce the risk of oxidation which is associated with increased health implications (e.g. ageing, membrane damage, heart disease and cancer) and decreasing the omega 3 fatty acids content (Kanner, 2006; Kolakowska et al., 2006). The oxidation values of the capsules were measured over the study period to determine if at any point the oxidation levels were higher than acceptable.

This study monitored the compliance of the subjects to their allocated treatment. In addition to this, the exact amount of LC omega 3 fatty acids and selenium consumed from the treatment was determined based on information obtained from the subject's compliance diaries. The compliance of the subjects to their normal daily routines was also monitored using the diaries.

6.4 LIMITATIONS TO THE PRESENT STUDY

In order to accurately compare the effects of consuming LC omega 3 fatty acids from fish or fish oil capsules, the same amount of LC omega 3 fatty acids from the different treatments was required, this meant subjects needed to consume all their allocated treatment (or as close to 100 % as possible). To increase the likelihood of subjects consuming all their allocated treatment a study period of 8 weeks was used as it was proven to be the shortest duration possible to ensure a significant effect on RBC LC omega 3 fatty acids. However research has shown that a plateau in RBC EPA and DHA from supplementation is not reached until 6 months (Katan et al., 1997). Despite this, a significant change in RBC LC omega 3 fatty acids was shown in this study after 8 weeks of supplementation.

General linear regression equations cannot be used to make predictions when the values of the independent variables lie outside the observed range. In order to use the general linear regression equations the range of omega 3 fatty acids supplied by the capsules needed to be both lower and higher than the amount found in the salmon. The omega 3

fatty acids consumed from capsules ranged from 0.239 ±0.004 to 0.689 ±0.022 g per day, which was lower, than the amount consumed from salmon (0.816 ±0.055 g/day). However the linear predicted response model was still used after a statistician was consulted and it was determined that the capsule data could be extrapolated based on the linear relationship shown in previous studies between dosage of omega 3 fatty acids and RBC omega 3 fatty acid levels (Brown et al., 1991; Cao et al., 2006; Katan et al., 1997).

6.5 RECOMMENDATIONS FOR FUTURE STUDIES

Future studies should investigate the effect of supplementation of fish and fish oil capsules for 6 months to allow the RBC LC omega 3 fatty acid levels to reach a steady state, but all efforts should be made to ensure compliance and also to measure compliance during the course of the study.

This study only considered omega 3 fatty acids presented in the TG form from both the salmon and capsules. Further studies could compare the effects of consuming LC omega3 fatty acids in different lipid forms (e.g. ethyl esters, phospholipids) as well as from different sources (e.g. algal, mackerel). Further comparisons could be made between consuming fatty fish to foods enriched with omega 3 fatty acids.

This research investigated the response to treatment regimens from a healthy subject group. Additional studies may be done to determine the response in other target groups, such as those suffering from CVD or metabolic syndrome, who may benefit from LC omega 3 fatty acid supplementation.

No literature was found comparing incorporation of LC omega 3 fatty acids between men and women with supplementation of LC omega 3 fatty acids. Future research could be conducted to determine if any differences exist, as some researchers have indicated that there may be a difference (Burdge & Wootton, 2002; Crowe et al., 2008; Emken et al., 1994; Jeong & Yoon, 2007).

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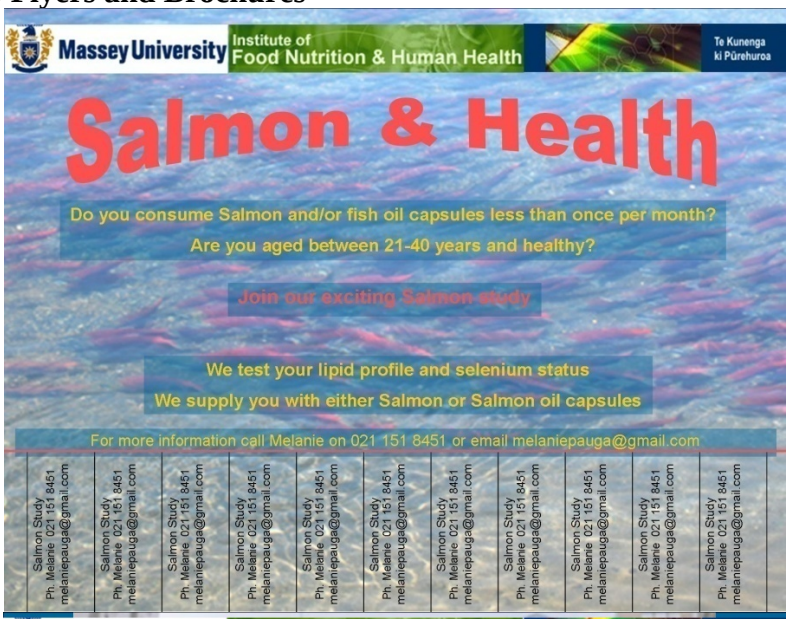
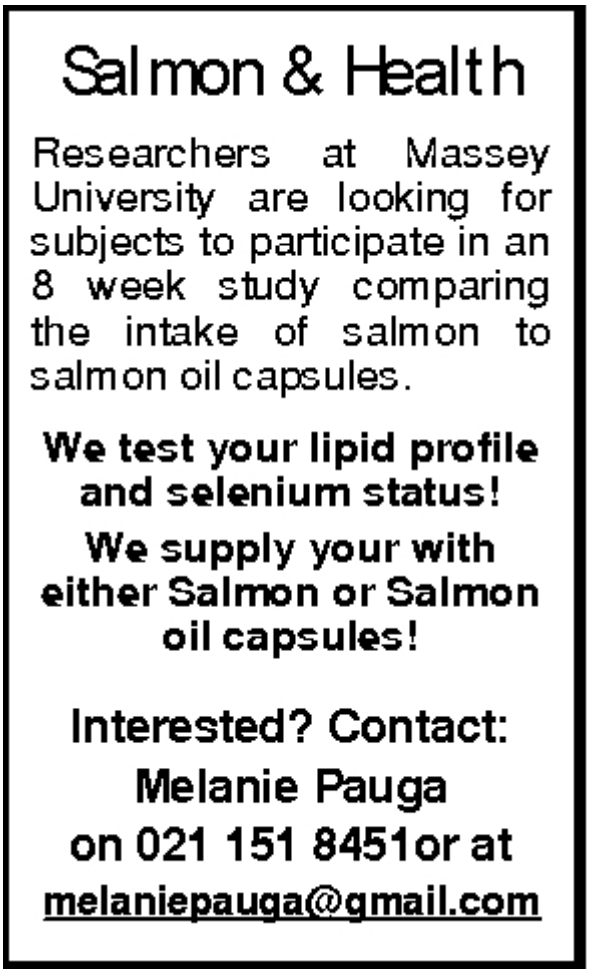
APPENDIX

Appendix A: Flyers and Brochures



Appendix B: North Shore Times Advertisement



Appendix C: Information Sheet

Institute of Food, Nutrition and Human Health
Massey University
Private Bag 102-904
North Shore Mail Centre
Auckland

Bioavailability of long chain fatty acids from farmed salmon compared to salmon oil capsules and the effect on selenium status

INFORMATION SHEET

We would like to invite you to take part in a university research project that will investigate how efficiently the intake of farmed salmon increases concentrations of omega-3 fats and selenium in the blood compared to intake of salmon oil capsules. Melanie Pauga (details below) is conducting this project as part of the requirements for her Masters in Human Nutrition at Massey University.

Project Contact:
Melanie Pauga
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Supervisor:
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Other co-workers involved in the study:

Dr Rozanne Kruger	(IFNHH, Massey University) (Co-supervisor)
Prof Marlena Kruger	(IFNHH, Massey University)
Dr Marie Wong	(IFNHH, Massey University)
Prof Christine Thompson	(University of Otago)
Dr Laurence Eyres	(University of Auckland)
A/Prof Hugh Morton	(Statistician, Massey University)

Why is this research important?

Salmon is a good source of omega-3 fats which has been shown to be important for several functions in the body, including protection from heart disease, inflammatory disease e.g. rheumatoid arthritis and it may also be of benefit for brain function and mood disorders, such as depression. For several reasons it may be more beneficial to consume these omega-3 fats from fish, such as Salmon, than from supplements. Salmon is also a rich source of selenium. Since soils in New Zealand are low in selenium this causes the selenium content of our food to be low, consequently the levels in our bodies are also affected. Greater consumption of salmon may therefore contribute towards improving our selenium status.

The aim of this research is to show the most beneficial method of increasing the intake of omega-3 fats by comparing the blood concentrations of omega-3 fats after intake of farmed salmon or salmon oil capsules for 8 weeks. Furthermore, the effects on selenium status in the blood will also be investigated. The omega-3 index level, a novel independent marker of risk for sudden cardiac death, will also be calculated from the concentrations of the omega-3 fats in the blood and compared.

Who are we looking for?

We are looking for forty four healthy volunteers to participate in this study. To take part in this study you should:

- Be between 21 - 40 years of age
- Have a low habitual intake of fatty fish (e.g. tuna, salmon, mackerel, sardines) of ***less*** than two times a month
- ***Not*** have taken fish oil supplements over the past 6 months
- ***Not*** have taken selenium supplements over the past 6 months
- ***Not*** have allergies to seafood
- ***Not*** smoke
- ***Not*** be pregnant, breastfeeding or planning to be in the near future
- Have no known condition or disease and not be using any medication for chronic disease (e.g. heart disease, diabetes, cancer etc)
- Be responsible and committed to the project.

What is going to happen?

If you decide to take part in this study you will be asked to complete a screening questionnaire to ensure that you fit the inclusion criteria of the study. The researcher will then make an appointment with you to visit the Massey University Albany campus early in the morning before you have eaten breakfast. We will reimburse you for your travel costs and give you a free breakfast. The following will happen during this visit:

- You will be given the opportunity to ask any questions and then asked to sign a consent form for participating in the study and to complete a form regarding personal details (e.g. contact details, Medical Practitioner's details, etc)
- Your blood pressure will be recorded
- Your height, weight, waist and hip circumference will be measured over light clothing
- A blood sample will be taken by a qualified phlebotomist (10ml which is equivalent to approximately 2 teaspoons). For this blood sample you need to have fasted overnight. You should not eat or drink anything (other than water) from 10pm the previous evening until after the blood sample has been taken. We will be measuring the omega-3 fat, selenium status (plasma selenium and whole blood glutathione peroxidase) and cholesterol concentrations of the blood sample. You have the right and can request to have any blood samples returned to you for blessing and return to the ground after they have been analysed.
- You will also be required to complete a 20 - 30 minute questionnaire regarding the food you eat at home. This will be explained by the researcher.

The above will take about 1 to 1 ½ hours in total.

You will then be randomly assigned by the researchers to one of four groups. This means that all participants that take part in the study will have an equal chance of being in any one of the 4 groups. One group will consume salmon for 8 weeks; the other 3 groups will consume different dosages of salmon oil capsules for 8 weeks. All the products will be supplied to you over the 8 weeks. During this period it is important that only the supplied salmon or salmon oil capsules are consumed and no other fish, fish oil capsules or supplements are consumed. It is also important that you maintain your normal daily routine, e.g. eating patterns, physical activity or alcohol consumption for the duration of the study. You will have to complete a weekly diary to confirm that you followed the study protocol.

After the 8 weeks, the researcher will again make an appointment with you for measurements and blood samples to be collected as during the first visit.

The data will only be used for the purposes of the study. Only the researchers will have access to personal information and this will be kept secure and confidential. Results of this study will be published and presented at conferences. No individual will be able to be identified.

Treatments

Salmon group:

Those allocated to the salmon group will be asked to consume 2 portions / week of salmon as part of their diet for the duration of the 8 weeks. The researchers will provide guidelines of how to include the salmon into your diet as well as instructions on how to prepare the salmon. The salmon will be supplied on a weekly basis through arrangements made with the researcher. It is important that we measure the amount of omega-3 fats and selenium in the salmon that you will be consuming so that we know exactly how much of these nutrients you have consumed. Cooking can affect the content of the nutrients in the salmon. We will therefore provide you with an additional portion of salmon and would like to request that you prepare both portions for the main meal at the same time. One portion should be served and eaten and the additional portion should be stored in a coded bag (that we will provide) in the freezer. We will collect these duplicate portions from you on a weekly basis at the same time when we deliver the salmon for the following week.

Additional portions of salmon will be available if needed for your spouse/partner to enjoy the salmon meal with you. This should be arranged with the researches.

Capsule group:

Those allocated to the capsule group will be asked to consume their capsules with meals over the 8 weeks. The researchers will provide clear instruction with regard to the dosage that should be consumed everyday and how the capsules should be consumed. These will be supplied on a monthly basis through arrangements made with the researcher. Capsules that are left over should be returned to the researcher on a monthly basis.

What are the benefits of taking part in this study?

You will receive information regarding your blood results and measurements (including cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, blood pressure and body mass index). You will also receive a brief report summarising the main findings of the project via mail or email and thereby become more aware of the health benefits of omega-3 fats.

The principal benefit of taking part in this study is that you will contribute to our better understanding of the effects of salmon and creating recommendations for the intake of salmon and salmon oil capsules for health benefits.

Risks & benefits

There will be no charges made for any of the tests that you undertake.

Some people may have a fear of having a blood sample taken or experience discomfort when the blood samples are taken. Occasionally a slight bruising will result. The bruising usually disappears within a day or two. Blood samples will be taken by a trained phlebotomist. There may be social or cultural discomfort from having a blood sample taken, however, privacy will be ensured, you will be treated with respect. You may also be accompanied by a support person if required. Every effort will be made to ensure your comfort and respect your participation.

There are no personal risks to your health, but the blood tests could potentially identify undiagnosed health problems. A Medical Practitioner will review your blood results. If they find that your blood results are outside normal parameters we will contact you to give feedback according to the advice from the Medical Practitioner. If further follow up is required from your own Medical Practitioner we will either provide you with a letter stating your blood results which you can give to your Medical Practitioner or, at your request, this letter can be directly sent to your Medical Practitioner.

Who is funding the research?

This research is funded by Massey University and The New Zealand King Salmon Company will sponsor the salmon for the study.

Participant's Rights

You are under no obligation to accept this invitation to take part in this research study. If you decide to participate, you have the right to:

- Decline to answer any particular question
- Withdraw from the study (at any time without having to give a reason)
- Ask any questions about the study at any time during participation
- Provide information on the understanding that your name will not be used
- Be given access to a summary of the project findings when it is concluded.

Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 07/72. If you have any concerns about the conduct of this research, please contact Professor John O'Neill, Chair, Massey University Human Ethics Committee: Southern A, telephone: (06) 350 5799 x 8771, email: humanethicsoutha@massey.ac.nz.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Injury Prevention, Rehabilitation and Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and / or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Thank you for participating in this study.

Appendix D: Screening Questionnaire

Institute of Food, Nutrition and Human Health
Massey University
Private Bag 102-904
North Shore Mail Centre
Auckland.

Bioavailability of long chain fatty acids from farmed salmon compared to salmon oil capsules and the effect on selenium status

Thank you for your interest in our research project. To ensure that you fit the inclusion criteria of the study, we would appreciate if you could answer the questions below. If you have any queries or concerns about the form, please feel free to contact Melanie Pauga or Welma Stonehouse during working hours on 021 151 8451 (Melanie) or ext 41207 (Welma).

When you have completed this form, please use the enclosed postage-paid envelope to return it to Melanie Pauga, Institute of Food, Nutrition and Human Health, Massey University, Albany, Auckland.

Name:_____

Gender (Please mark):

Male ☐

Female ☐

Date of birth:_____

Daytime telephone number:_____

Email address: _____

Have you ever been diagnosed with any of the following:
Indicate: √ Yes X No

Cancer	
Heart condition	
Gut disorder that interfere with the digestion and absorption of your food	
Diabetes or persistent sugar in the urine	
Endocrine disease (hormone trouble)	
Thyroid disease (e.g. goiter)	
Kidney problems	
Disorders of the liver	

Are you currently suffering from any other illness not listed above that could affect the fat and cholesterol processing in your body? (Please provide details)

_____

_____

_____

Are you taking any form of medication, including traditional or homeopathic medicine? (Please list) _____

_____

Do you smoke cigarettes?

Yes ☐

No ☐

Do you drink alcohol?

Yes ☐

No ☐

If yes, approximately how many standard drinks per week: _____

Do you have allergies to fish? _____

How often do you consume fish per month? _____

What type of fish do you usually consume?_____

Are you pregnant or breastfeeding? _____

Do you take any of the following?

Indicate: √ Yes x No

Fish oil supplements	
Selenium supplements	
Other supplements	

If you are taking fish oil, selenium or other supplements what is the name, brand an dosage of the supplements you are taking? _____

_____

If you have any other comments or questions please note below or contact Melani Pauga (021 1518451):

_____

_____

_____

_____

_____

_____

Appendix E. Appointment Sheet

Dear *(insert name)*,

Thank you for taking part in the Salmon study. Your time and input are much appreciated.

Your appointment is at ***(insert time)*** **am** on ***(insert day and month)*** **2008**

Before the study…

For the 10 hours prior to your appointment, please avoid eating or drinking (except for water) and avoid participating in exercise (walking is fine).

In addition to this for the two hours prior to your appointment, please avoid consuming anything (including water).

Please bring…

We will provide breakfast (breakfast cereal, toast, tea and coffee) but if you have any specific dietary requirements please bring your own food.

How to find the Salmon study…

The Salmon study is located in Building 27 (Gate 4) on the **Oteha Rohe** campus at Massey University (located off Albany Highway). Parking is available outside building 27.

If you can't attend your appointment or have any questions please phone Melanie Pauga on (021) 151 8451 or email melaniepauga@gmail.com.
Thank you very much. We look forward to meeting you.

Appendix F: Compliance Booklets

Appendix F1: Compliance Booklet - Salmon

Massey University | Institute of Food Nutrition & Human Health | Te Kunenga ki Pūrehuroa

Institute of Food, Nutrition and Human Health
Massey University
Private Bag 102-904
North Shore Mail Centre
Auckland

Subject Number:

Bioavailability of long chain fatty acids from farmed salmon compared to salmon oil capsules and the effect on selenium status

Dear Participant,

We very much appreciate your participation in this research project. By committing time and effort to this project we assume that it is just as important to you as it is to us that this project is a success. We need reliable data to be able to make recommendations for the intake of salmon and salmon oil capsules and to publish the findings of the study in a scientific journal. In order to do this it is extremely important that you consume the salmon we have provided to you according to the guidelines given and that you do not make major changes to your habitual daily routine for the duration of the study. Any changes can affect the results of the study and therefore the reliability of the results. We realise that changes are sometimes inevitable, for example illness. Therefore, we would appreciate it if you could supply information about your salmon intake and indicate any changes from your daily routine or with regard to the intake of the salmon by completing the included compliance diaries. Please complete one sheet for each week of the study.

Also included in this booklet is a calendar with important dates to remember, a short list of things to do each week, guidelines on how to store and cook the salmon and how to include it into your diet. Additional salmon samples are available for your spouse or partner to enjoy with you, arrangements for this should be made with Melanie.

If you have any further questions please feel free to contact Melanie Pauga on:

Mob: 021 151 8451
Email: melaniepauga@gmail.com

Or Welma Stonehouse on:
Tel: Tel: 414 0800 ext 41207
Email: W.Stonehouse@massey.ac.nz

Thank you again for taking part in this study.

Kind regards,

The Research Team

CALENDAR

Day	May		June		July	
Sunday	4		1			
Monday	5		2			
Tuesday	6		3		1	
Wednesday	7		4		2	
Thursday	8		5		3	
Friday	9		6		4	
Saturday	10		7		5	
Sunday	11		8		6	
Monday	12		9		7	
Tuesday	13		10		8	
Wednesday	14		11		9	
Thursday	15		12		10	
Friday	16		13		11	
Saturday	17		14		12	
Sunday	18		15		13	
Monday	19		16		14	
Tuesday	20		17		15	
Wednesday	21		18		16	
Thursday	22		19		17	
Friday	23		20		18	
Saturday	24		21		19	
Sunday	25		22		20	
Monday	26		23		21	
Tuesday	27		24		22	
Wednesday	28		25		23	
Thursday	29		26		24	
Friday	30		27		25	
Saturday	31		28		26	
Sunday			29		27	
Monday			30	BP 	28	
Tuesday			31			

KEY	
Blood sampling, measurements & blood pressure	BP 
Fish delivery	
Fill out compliance sheet	

WEEKLY TO DO LIST

	Week							
To do:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Cook 2 or 3 servings* of salmon twice per week and place one serving in one of the supplied plastic bags and freeze								
Fill in compliance diary								
Return compliance diary when salmon is delivered								

* One serving for you to consume, one to be placed in a plastic bag and frozen and the other to be consumed by your partner as arranged with the researcher.

Storing and Handling Salmon:

- Keep salmon refrigerated (at less than 4°C) in a plastic bag away from other raw meats;
- Eat within 3 to 4 days after receiving;
- Always clean hands thoroughly with hot water and soap before and after touching raw fish;
- Avoid cross-contamination by washing chopping boards and bench top with hot soapy water after handling raw salmon;
- Use a clean plate to bring cooked salmon to the table, not the plate used to carry raw salmon to the cooking surface.

Cooking Instructions:

Salmon is safe to use as you receive it, therefore the salmon does not require washing before you cook it, in fact, if you do a lot of the juices will be lost. Instead, pat the salmon all over with a damp paper towel.

A study conducted at the University of Auckland showed that the following preparation methods (steaming, oven baking and pan frying) produced the highest content of omega 3 levels in the salmon and was the most acceptable to consumers. Therefore for this study we would like you to use any of these methods for the preparation of the salmon portions.

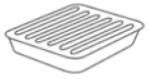
Oven Baking:

You will need:
5mL or 1 tsp of oil e.g. olive oil or cooking spray ***or*** tinfoil for lining
Baking dish
2 or 3 portions of provided salmon
Baking brush
Seasoning e.g. salt and pepper or herbs (e.g. fennel) or lemon juice
Oven

1. Preheat oven to 180°C.

2. Grease baking dish with oil (any kind of cooking oil is fine) or cooking spray or line with tinfoil.
3. Place 2 or 3 portions of salmon in the dish and brush with seasoning, for example oil, lemon juice or a mixture of both.
4. Once the oven is heated, place the baking dish with the salmon in and allow to cook for 10 to 15 minutes (it is not necessary to turn the salmon over).
5. Remove salmon and add other components of the meal e.g. baked potato and salad, rice and vegetables, flake and add to salad, or stir fried vegetables and noodles, *see recipe book for ideas*.

Pan Frying:



You will need**:**
5mL or 1 tsp of oil e.g. olive oil or cooking spray *and* a fry pan ***or*** a Teflon fry pan
2 or 3 portions of provided salmon

1. Place oil or cooking spray in the fry pan or use a Teflon fry pan and heat at a moderately high temperature (180°C).
2. Once heated place 2 or 3 salmon portions into the pan.
3. Allow the salmon to cook for approximately 3 minutes before turning it over and cooking on the other side for a further 3 minutes.
4. Remove salmon and add other components of the meal e.g. baked potato and salad, rice and vegetables or stir fried vegetables and noodles, *see recipe book for ideas*.

Steaming:

You will need**:**
Stainless steel steamer or place a steel container with holes in it on top of a pot and put a lid on that
500mL water
2 or 3 portions of provided salmon

1. Place 500mL of water in the bottom pot of the steamer, place on the heat and cover.
2. Allow this to boil before placing the salmon on the top pot of the steamer and cooking for approximately 6 minutes. Ensure that each piece of salmon is touching the bottom of the pot.
3. Remove salmon and add other components of the meal e.g. baked potato and salad, rice and vegetables or stir fried vegetables and noodles, *see recipe book for ideas*.

SALMON GROUP – COMPLIANCE DIARY
Weekly Compliance Diary

Subject Number:

Week 1 **Date:** _____

How many portions of salmon did you consume this week? _____

Please indicate which days and meals you consumed salmon by ticking the relevant boxes:

		Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Breakfast	Steaming							
	Oven baking							
	Pan frying							
	Other							
Lunch	Steaming							
	Oven baking							
	Pan frying							
	Other							
Dinner	Steaming							
	Oven baking							
	Pan frying							
	Other							

If other, please provide the details: _____

_____

Were you ill this week?
O Yes
O No
If yes, what was the nature of your illness? _____

Did you consume any medication for the illness?
O Yes
O No
If yes, please provide details of the medication used: _____

_____

Was there any change in your physical activity level this week?
O Yes
O No
If yes, please provide details of the change to your physical activity: _____

_____

Have there been any changes in your normal daily routine this week, e.g. eating habits, alcohol consumption, etc?
O Yes
O No
If yes, please provide more detail _____

_____

Do you have anything else you would like to report? _____

_____

Appendix F2: Compliance Booklet - Capsule

Institute of Food, Nutrition and Human Health
Massey University
Private Bag 102-904
North Shore Mail Centre
Auckland

Subject Number:

Bioavailability of long chain fatty acids from farmed salmon compared to salmon oil capsules and the effect on selenium status

Dear Participant,

We very much appreciate your participation in this research project. By committing time and effort to this project we assume that it is just as important to you as it is to us that this project is a success. We need reliable data to be able to make recommendations for the intake of salmon and salmon oil capsules and to publish the findings of the study in a scientific journal. In order to do this it is extremely important that you consume the salmon we have provided to you according to the guidelines given and that you do not make major changes to your habitual daily routine for the duration of the study. Any changes can affect the results of the study and therefore the reliability of the results. We realise that changes are sometimes inevitable, for example illness. Therefore, we would appreciate it if you could supply information about your salmon oil intake and indicate any changes from your daily routine or with regard to the intake of the salmon oil capsules by completing the included compliance diaries. Please complete one sheet for each week of the study.

Also included in this booklet is a calendar with important dates to remember and a short list of things to do each week.

If you have any further questions please feel free to contact Melanie Pauga on:

Mob: 021 151 8451
Email: melaniepauga@gmail.com

Or Welma Stonehouse on:
Tel: Tel: 414 0800 ext 41207
Email: W.Stonehouse@massey.ac.nz

Thank you again for taking part in this study.

Kind regards,

The Research Team.

CALENDAR

Day	May		June		July	
Sunday	4		1			
Monday	5		2			
Tuesday	6		3		1	
Wednesday	7		4		2	
Thursday	8		5		3	
Friday	9		6		4	
Saturday	10		7		5	
Sunday	11		8		6	
Monday	12		9		7	
Tuesday	13		10		8	
Wednesday	14		11		9	
Thursday	15		12		10	
Friday	16		13		11	
Saturday	17		14		12	
Sunday	18		15		13	
Monday	19		16		14	
Tuesday	20		17		15	
Wednesday	21		18		16	
Thursday	22		19		17	
Friday	23		20		18	
Saturday	24		21		19	
Sunday	25		22		20	
Monday	26		23		21	
Tuesday	27		24		22	
Wednesday	28		25		23	
Thursday	29		26		24	
Friday	30		27		25	
Saturday	31		28		26	
Sunday			29		27	
Monday			30	BP 	28	
Tuesday			31			

KEY	
Blood sampling, measurements & blood pressure	BP 
Tablet delivery/collection	
Fill out compliance sheet	

WEEKLY TO DO LIST

	Week							
To do:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Consume one vile of capsules during the day with meals								
Fill out compliance diary								
Return the vile and compliance diaries together								

Storing and Handling Salmon Oil Capsules:

To ensure the quality of the salmon oil capsules it is important that they are stored in adequate conditions.

- Keep the capsules refrigerated;
- Leave capsules in the container provided with the cap tightly on.

Please consume the capsules with meals daily. If you forget to consume the capsules one day, please consume it as soon as you remember or the following day.

The capsules can be consumed all at once or distributed during the day with meals.

SALMON OIL CAPSULE GROUP – COMPLIANCE DIARY

Subject Number:

Weekly Compliance Diary

Week 1 **Date:** _____

Did you consume your salmon oil capsules everyday this week?

O Yes
O No

If no, please provide more details:_____

Please indicate the number of capsules you consumed per day as well as whether you consumed the capsules with a meal and if so which meal by writing the number of capsules consumed in the relevant boxes:

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Breakfast							
Lunch							
Dinner							
No Meal							

Were you ill this week?

O Yes
O No

If yes, what was the nature of your illness?_____

Did you consume any medication for the illness?

O Yes
O No

If yes, please provide details of the medication used:_____

_____

Was there any change in your physical activity level this week?

O Yes
O No

If yes, please provide details of the change to your physical activity: _____

_____

Have there been any changes in your normal daily routine this week, e.g. eating habits, alcohol consumption, etc?

O Yes
O No

If yes, please provide more detail _____

_____

Do you have anything else you would like to report? _____

_____

Appendix G. Tolerance Questionnaire

Tolerance Questionnaire

Did you experience any side effects from the treatment given to you during the study?

- ☐ No
- ☐ Yes

Please explain the side effects or any other problems that you may have experienced when you consumed the capsules/salmon.

_____
_____
_____

Please consider the following side effects and indicate if you experienced any of them by ticking the box and also indicate the severity of the side effects experienced:

Dizziness:

- ☐ No
- ☐ Yes

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Insignificant Severe Very Severe

Diarrhea:

- ☐ No
- ☐ Yes

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Insignificant Severe Very Severe

Nausea:

- ☐ No
- ☐ Yes

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Insignificant Severe Very Severe

Bloating:

- ☐ No
- ☐ Yes

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Insignificant Severe Very Severe

Burping:

- ☐ No
- ☐ Yes

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Insignificant Severe Very Severe

Heart burn/reflux:

- ☐ No
- ☐ Yes

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Insignificant Severe Very Severe

Difficulty swallowing the treatment:

- ☐ No
- ☐ Yes

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Insignificant Severe Very Severe

Unpleasant breath:

- ☐ No
- ☐ Yes

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Insignificant Severe Very Severe

Tiredness:

- ☐ No
- ☐ Yes

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Insignificant Severe Very Severe

Other:

- ☐ No
- ☐ Yes, (*please specify*)_____

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Insignificant Severe Very Severe

How often did you suffer from the side effects over the period of the study?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Seldom Often Very Often

Did you consume capsules or salmon over the period of the study?

- ☐ Capsules
- ☐ Salmon

If you had to make recommendations for the most practical approach for consuming capsules what would it be? (*Please take into account the number of capsules, the time of day and the meals*)

_____

_____

If you had to make recommendations for the most practical approach for consuming salmon what would it be? (*Please take into account the size of the portion, the cooking method and the meal time used*)

_____

_____

Do you have anything else you would like to report? _____

_____

_____

Appendix H. Food Frequency Questionnaire

Qualitative Food Frequency Questionnaire

Please make sure when filling out this questionnaire that you:

- Tell us what **YOU** usually consume (not someone else in your household!).
- Fill out the form **YOURSELF**.
- Are accurate, but don't spend too much time on each food.
- Answer **EVERY** question.

Please answer by **ticking the box** which best describes **how often you consumed** a particular **food or beverage** in the **last month**.

Example:

Consider if you have sugar in all your drinks during the day as well as added to other food items and indicate how many times in the day you are consuming sugar. E.g. drinking 2 cups of coffee with sugar and 4 cups of tea with sugar, one bowl of cereal with sugar and sugar on pancakes at dinner resulting in choosing the category (4 Plus times per day)

In the past month I have eaten this food….									
Food items	I never eat this food	Less than once a month	1 to 3 times a month	Once per week	2 to 3 times per week	4 to 6 times per week	Once per day	2 to 3 times per day	4 plus times per day
Sugar									X

Consider if you have pineapple and indicate how many times you are consuming pineapple. E.g. eating pineapple slices with a hamburger meal once a week at lunch and eating carrot and pineapple salad once a week at dinner (2 to 3 times per week)

In the past month I have eaten this food….									
Food items	I never eat this food	Less than once a month	1 to 3 times	Once per week	2 to 3 times per week	4 to 6 times per week	Once per day	2 to 3 times per day	4 plus times per day
Pineapple					X				

questionnaire

1. How would you describe your eating pattern? (*Please mark one only*)
   - ☐ Consume a variety of all foods, including animal products
   - ☐ Consume eggs, dairy products, fish and chicken but avoid other meats
   - ☐ Consume eggs and dairy products, but avoid all meats and fish
   - ☐ Consume eggs, but avoid dairy products, all meats and fish
   - ☐ Consume dairy products, but avoid eggs, all meats and fish
   - ☐ Consume no animal products
   - ☐ Other (*please specify*)_____

Dairy Foods

2. Do you consume any type of milk?
   - ☐ No
   - ☐ Yes

   What type(s) do you consume most often? *(Please mark only those you usually have)*
   - ☐ Full cream milk (purple top): fresh, UHT or powdered
   - ☐ Standard milk (blue top): fresh, UHT or powdered
   - ☐ Skim milk (light blue top): fresh, UHT or powdered
   - ☐ Trim milk (green top): fresh, UHT or powdered
   - ☐ Super trim milk (light green top): fresh, UHT or powdered
   - ☐ Calcium enriched milk (yellow top) e.g. Xtra, Calci-Trim
   - ☐ Calcium and vitamin enriched milk e.g. Mega, Anlene
   - ☐ Calcium and protein enriched milk e.g. Sun Latte
   - ☐ Calcium and omega 3 enriched milk e.g. Vital
   - ☐ Standard soy milk (blue)
   - ☐ Light soy milk (light blue)
   - ☐ Calcium enriched soy milk (purple) e.g. Calci-Forte, Calci-Plus
   - ☐ Calcium and vitamin enriched soy milk e.g. Active
   - ☐ Calcium, vitamin and omega 3 enriched soy milk e.g. Essential
   - ☐ Calcium and high fibre enriched soy milk e.g. Calci-Plus High Fibre
   - ☐ Rice milk
   - ☐ I don't consume these types of food

□ Other *(please specify)* _____

3. On average, how many servings of milk do you consume per ***day***?

(*Please mark only one*)

(A 'serving' = 250 mL (1 cup))

E.g. 5 cups of coffee/tea using 50mL of milk + ½ cup of milk on cereal = 1 ½ servings per day

Per Day

□ None
□ < 1 serving
□ 1-2 servings
□ 3-4 servings
□ ≥ 5 servings

4. How often do you usually consume these foods or beverages?

Dairy Foods								
Please fill in one category for each food or beverage	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Flavoured milk (e.g. milkshake, iced coffee)								
Milk as a drink								
Milk on breakfast cereals								
Milk added to hot beverages made with water (e.g. coffee, tea, Milo)								
Hot beverages made with milk (e.g. café late)								
Cream or sour cream								
Ice cream								
Custard or dairy food								
Yoghurt, plain or flavoured (including fromage frais)								
Milk puddings (e.g. rice, semolina, instant)								
Cream cheese								
Cottage or ricotta cheese								

Dairy Foods								
Please fill in one category for each food or beverage	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Mozzarella, feta, or camembert								
Edam or gouda cheese								
Cheddar cheese (all varieties)								
Brie, blue and other specialty cheese								

Bread

5. Do you consume any type of bread?
   - ☐ No
   - ☐ Yes

   What type(s) of bread, rolls or toast do you consume most often? *(Please mark only those you usually have)*

   - ☐ White
   - ☐ White – high fibre
   - ☐ Wholemeal or wholegrain
   - ☐ Calcium enriched bread e.g. Vital
   - ☐ Omega 3 enriched bread e.g. Goodness Omega 3
   - ☐ I don't consume these types of food
   - ☐ Other (*please specify*) _____

6. On average, how many slices or rolls of bread (or toast) do you consume per ***day***?

   Per Day
   - ☐ None
   - ☐ < 1 serving
   - ☐ 1–2 servings
   - ☐ 3–4 servings

□ 5–6 servings
□ ≥ 7 servings

7. How often do you usually consume these foods?

Breads								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Plain white bread								
High fibre white bread								
Wholemeal or wholegrain bread								
Focaccia, bagel, pita or other speciality breads								
Paraoa Parai (fry bread)								
Rewena bread								
Doughboys or Maori bread								
Crumpet or croissant								
Waffle or doughnut								
Fruit or iced buns								
Savoury or dry biscuits, crisp bread, or crackers								

8. Do you consume butter, margarine or spreads on bread or crackers?
□ No
□ Yes

What type(s) do you consume most often? *(Please mark only those you usually use)*
□ Butter (all varieties)
□ Monounsaturated fat margarine e.g. Olive, Rice Bran, Canola Oil Spreads
□ Polyunsaturated fat margarine e.g. Sunflower Oil Spreads
□ Light monounsaturated fat margarine e.g. Olivio Spread Light
□ Light polyunsaturated fat margarine e.g. Flora Spread Light
□ Plant sterol enriched margarine e.g. Pro Active, Logical Spreads
□ Light plant sterol enriched margarine e.g. Pro Active Spread Light
□ Butter and margarine blend e.g. Country Soft, Butter Lea

☐ I don't consume these types of foods
☐ Other *(please specify)* _____

9. On average, how many servings of butter, margarine or spreads do you consume per ***week***? (*Please **mark one** only*)
(A 'serving' = 1 level teaspoon or 5 ml)
E.g. 1 sandwich with butter thinly spread on two pieces of bread = 2 servings

Per Week

☐ None
☐ < 4 servings
☐ 4–6 servings
☐ 7–9 servings
☐ 10–12 servings
☐ 13–15 servings
☐ ≥ 16 servings

Breakfast Cereals

10. Do you usually consume breakfast cereal?
☐ No
☐ Yes
What breakfast cereal(s) do you consume most often? (*Please mark only those you usually have.*)
☐ Refined cereals e.g. Cornflakes or Rice Bubbles
☐ Bran based cereals including fruity varieties e.g. Special K, Muesli, All Bran
☐ Sweetened e.g. Nutrigrain, Cocoa Pops
☐ I don't consume these types of food
☐ Other (*please specify*)_____

11. On average, how many servings of breakfast cereal do you consume per ***week***? (*Please **mark one** only*)
(A 'serving' = 1 cup porridge or cornflakes or ½ cup muesli or 2 weetbix)
E.g. 1 cup of porridge 3 times per week + 2 weetbix 4 times a week = 7 servings per week

Per Week

☐ None
☐ < 4 servings
☐ 4–6 servings
☐ 7–9 servings

□ 10–12 servings
□ 13–15 servings
□ ≥ 16 servings

12. How often do you usually consume these foods?

Breakfast Cereals								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Porridge, rolled oats, oat bran, oat meal								
Muesli (all varieties)								
Weetbix (all varieties)								
Cornflakes or rice bubbles								
Bran based cereals (all varieties e.g. All Bran, Sultana Bran)								
Light and fruity cereals (e.g. Special K, Light and Tasty)								
Chocolate based cereals (e.g. Milo cereal, Coco Pops)								
Sweetened cereals (e.g. Nutrigrain, Fruit Loops, Honey Puffs, Frosties)								
Breakfast drinks (e.g. Up and Go)								

Starches

13. Do you consume any type of starchy foods such as rice and pasta?
□ No
□ Yes

14. On average, how many servings of foods such as pasta, and rice do you consume per ***week***? (*Please **mark one** only*)
(A 'serving' = 1 cup cooked rice/pasta)
E.g. 1 cup of rice + ½ cup of pasta included in a lasagne pasta dish = 1 ½ servings

Per Week

- □ None
- □ < 4 servings
- □ 4–6 servings
- □ 7–9 servings
- □ 10–12 servings
- □ 13–15 servings
- □ ≥ 16 servings

15. How often do you usually consume these foods?

Starches								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Rice, white								
Rice, brown								
Rice, wild								
Couscous								
Pasta, white (e.g. spaghetti, vermicelli, instant noodles)								
Pasta, whole grain								

Meat

16. Do you consume pork, beef, mutton, hogget or lamb?
    - □ No
    - □ Yes

Do you trim any excess fat off these meats? *(Please **mark one** only)*

- □ Always
- □ Often
- □ Occasionally
- □ Never cut the fat off meat

□ I don't consume these types of food

17. On average, how many servings of meat do you consume per ***week***? (*Please **mark one** only*)
(A 'serving' = 90 - 100g or ½ a cup of meat without bone)
E.g. ½ cup of savoury mince + 2 small lamb chops = 2 servings

Per Week

□ None
□ < 1 serving
□ 1-3 servings
□ 4-6 servings
□ ≥ 7 servings

18. How often do you consume the following?

Meat								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Beef mince dishes (e.g. rissoles, meatloaf, hamburger pattie)								
Beef or veal mixed dishes								
Beef or veal - roast, chop, steak, schnitzel								
Corned beef (including canned) or brisket								
Hogget or mutton mixed dishes (e.g. stews)								
Hogget or mutton - roast, chops								
Lamb mixed dishes (e.g. casserole, stir-fry)								
Lamb - roast, chop, steak								
Pork - roast, chop, steak								
Boiled bones – all varieties								
Sausage, frankfurter or saveloy								

Meat								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Bacon or ham								
Luncheon meats, salami or brawn								
Liver (including pate)								
Other offal (e.g. kidneys)								
Venison/game								

Poultry

19. Do you consume poultry e.g. chicken, turkey or duck?
    - ☐ No
    - ☐ Yes

    Do you remove the skin from poultry? (*Please **mark one** only*)
    - ☐ Always
    - ☐ Often
    - ☐ Occasionally
    - ☐ Never remove the skin from chicken
    - ☐ I don't consume these types of food

20. On average, how many servings of poultry do you consume per ***week***? (*Please **mark one** only*)
    (A 'serving' = 100 - 120g of poultry with bones or ½ cup of flaked chicken)
    E.g. 1 chicken breast + 3 chicken drumsticks + 1 chicken thigh = 4 servings per week

    Per Week
    - ☐ None
    - ☐ <1 serving
    - ☐ 1-3 servings
    - ☐ 4-6 servings
    - ☐ ≥ 7 servings

21. How often do you consume the following?

Poultry								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Chicken mixed dishes (e.g. casserole, stir-fry)								
Chicken - roast, fried, stewed, grilled								
Turkey or duck								
Mutton bird								

Fish and Seafood

22. Do you consume any type of fish or seafood?

- ☐ No
- ☐ Yes

23. On average, how many servings of fish and seafood do you consume per ***week,*** consider all types e.g. fresh, frozen or tinned?

(*Please **mark one** only*)

(A 'serving' = 80 – 120g of fish or seafood)

E.g. 1 small tin of fish = 1 serving per week

Per Week

- ☐ None
- ☐ < 1 serving
- ☐ 1-3 servings
- ☐ 4-6 servings
- ☐ ≥ 7 servings

24. How do you normally cook fish (you can mark more than one box)?

- ☐ I don't cook it
- ☐ Raw
- ☐ Oven baked
- ☐ Deep fried
- ☐ Grilled
- ☐ Micro waved
- ☐ Steamed
- ☐ Poached

25. How often do you consume the following?

Fresh/Frozen/Smoked Fish								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Barracouta								
Bass								
Baxter's Dogfish								
Blue Maomao								
Bluenose								
Brill								
Butterfish (Profret)								
Cardinal								
Carp Grass								
Carp Kio								
Carp Silver								
Cod								
Dogfish								
Dory John								
Dory Lookdown								
Eel								

Fresh/Frozen/Smoked Fish								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Elephant fish								
Flat fish								
Flounder								
Frostfish								
Garfish (Piper)								
Gemfish								
Grey Mullet								
Groper								
Gurnard								
Hake								
Hapuku								
Hoki								
Javelin								
Kahawai								
Kina								
Kingfish								
Leatherjacket								
Ling								
Mackerel Blue								
Mackerel Jack								
Mackerel Peruvian Jack								
Marlin								
Moki								
Monkfish								
Moonfish								
Mullet								
Nuiean fish								
Orange Roughy								

Fresh/Frozen/Smoked Fish								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Oreo Dory								
Parore								
Perch								
Pilchard								
Pink Scorpion fish flesh								
Porae								
Rattail								
Rays Bream								
Ribaldo								
Rig								
Ruby								
Rudderfish								
Salmon								
Sanma								
Sardines								
Sea Perch								
Shark/lemon fish								
Silver Dory								
Skate								
Slick Head								
Snapper								
Sole								
Southern Blue Whiting								
Spiny dogfish								
Sprat								
Stargazer								
Swordfish								
Tarakihi								

Fresh/Frozen/Smoked Fish								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Tench								
Toadfish								
Toothfish								
Trevally								
Trout								
Trumpeter								
Tuna (Albacore, Butterfly, Southern Bluefin)								
Tuna Skipjack								
Tuna Slender								
Warehou Blue								
Warehou (Silver/White)								

26. How often do you consume the following?

Processed fish and seafood								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Canned Kipper								
Canned Smoked Herring								
Canned Anchovies								
Canned Herring								
Canned Mackerel								
Canned Rollmops								
Canned Salmon								
Canned Sardines								
Canned Tuna								

Processed fish and seafood								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Frozen crumbed fish Patties /Cakes / Fingers/ Nuggets/ Portions								
Frozen uncrumbed fish Patties /Cakes / Fingers/ Nuggets/ Portions								
Other (state):								

27. How often do you consume the following?

Other Seafood								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Shrimp/prawn								
Lobster/cray								
Crab								
Scallops								
Mussels								
Oysters								
Clams								
Whitebait								
Cockle								
Paua								
Pipi								
Roe								
Squid/octopus/calamari/ cuttlefish								
Other (*state*):								

Other Seafood								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day

28. Do you cook meat, poultry, fish and/or eggs with fat or oil?
    - ☐ No
    - ☐ Yes

    What type(s) do you use most often? *(Please mark only those you usually have)*
    - ☐ Butter (all varieties)
    - ☐ Monounsaturated fat margarine e.g. Olive, Rice Bran, Canola Oil Spreads
    - ☐ Polyunsaturated fat margarine e.g. Sunflower Oil Spreads
    - ☐ Light monounsaturated fat margarine e.g. Olivio Spread Light
    - ☐ Light polyunsaturated fat margarine e.g. Flora Spread Light
    - ☐ Plant sterol enriched margarine e.g. Pro Active, Logical Spreads
    - ☐ Light plant sterol enriched margarine e.g. Pro Active Spread Light
    - ☐ Butter and margarine blend e.g. Country Soft, Butter Lea
    - ☐ Saturated fat oils e.g. Lard, Dripping, Coconut oil
    - ☐ Monounsaturated fat oils e.g. Olive, Canola, Avocado, Soybean, Peanut, Rice Bran oil
    - ☐ Polyunsaturated fat oil e.g. Sunflower, Corn, Safflower, Cottonseed, Sesame seed, Grape seed oil
    - ☐ Cooking spray
    - ☐ Don't know
    - ☐ I don't consume these types of food
    - ☐ Other *(please specify)* _____

29. On average, how many times do you use fat or oil to cook per ***week***?

    Per Week
    - ☐ None
    - ☐ < 1 serving
    - ☐ 1-3 servings
    - ☐ 4-7 servings
    - ☐ 8-10 servings

□ 11-14 servings
□ ≥ 15 servings

30. When you use fat or oil to cook how many servings of fat or oil do you use per ***dish***? (*Please **mark one** only*)
(A 'serving' = 1 level teaspoon or 5 ml)

Per Dish
□ None
□ < 1 serving
□ 1 serving
□ 2 servings
□ 3 servings
□ 4 servings
□ ≥ 5 servings

31. Do you consume dressings or sauces with meat, poultry and/or fish?
□ No
□ Yes

What type(s) do you use most often?
(Please mark only those you usually have)
□ Butter (all varieties)
□ Monounsaturated fat margarine e.g. Olive, Rice Bran, Canola Oil Spreads
□ Polyunsaturated fat margarine e.g. Sunflower Oil Spreads
□ Light monounsaturated fat margarine e.g. Olivio Spread Light
□ Light polyunsaturated fat margarine e.g. Flora Spread Light
□ Plant sterol enriched margarine e.g. Pro Active, Logical Spreads
□ Light plant sterol enriched margarine e.g. Pro Active Spread Light
□ Butter and margarine blend e.g. Country Soft, Butter Lea
□ Cream/sour cream
□ Dressings e.g. Tartare, Mayonnaise, Thousand Island, Ranch
□ Light dressings
□ Yoghurt dressing
□ Mustard

□ Saturated fat oils e.g. Lard, Dripping, Coconut oil
□ Monounsaturated fat oils e.g. Olive, Canola, Avocado, Soybean, Peanut, Rice Bran oil
□ Polyunsaturated fat oil e.g. Sunflower, Corn, Safflower, Cottonseed, Sesame seed, Grape seed oil
□ I don't consume dressing or sauce
□ Don't know
□ Other *(please specify)* _____

32. On average, how many times do you consume dressings and sauces with meat, poultry and/or fish per ***week***?

Per Week

□ None
□ < 1 serving
□ 1-3 servings
□ 4-7 servings
□ 8-10 servings
□ 11-14 servings
□ ≥ 15 servings

33. When you consume dressings and sauces with meat, poultry and/or fish how many servings do you consume per ***dish***?
(*Please **mark one** only*)
(A 'serving' = 1 level teaspoon or 5 ml)

Per Dish

□ None
□ < 1 serving
□ 1 serving
□ 2 servings
□ 3 servings
□ 4 servings
□ ≥ 5 servings

Eggs

34. Do you consume eggs?

□ No
□ Yes

What type(s) do you consume most often? *(Please mark only those you usually have)*
□ Eggs
□ Free Range eggs
□ Organic eggs
□ Barn mixed graded eggs
□ I don't consume these types of food
□ Other *(please specify)* _____

35. On average, not counting eggs used in baking/cooking, how many eggs do you usually eat per ***week***? (*Please **mark one** only*)
Per Week
□ None
□ < 1egg
□ 1 egg
□ 2 eggs
□ 3 eggs
□ 4 eggs
□ ≥ 5 eggs

Vegetables

36. Do you consume vegetables?
□ No
□ Yes

37. On average, how many servings of vegetables (fresh, frozen, canned) do you consume a ***day***?
Do not include vegetable juices. (*Please **mark one** only*)
(A 'serving' = 1 medium potato/kumara or ½ cup cooked vegetables or 1 cup of salad vegetables)
E.g. 2 medium potatoes + ½ cup of peas = 3 servings
Per Day
□ None
□ < 1 serving
□ 1 serving
□ 2 servings

□ 3 servings
□ ≥ 4 servings

38. How often do you usually consume these foods?

Vegetables								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Potato - boiled, mashed, baked or roasted								
Hot potato chips or kumara chips/French fries/wedges								
Pumpkin - boiled, roasted or mashed								
Kumara - boiled, roasted or mashed								
Mixed frozen vegetables								
Green beans								
Silver beet, spinach								
Carrots								
Cabbage, coleslaw								
Sweet corn								
Mushrooms								
Tomatoes								
Beetroot								
Taro								
Taro leaf (e.g. palusami)								
Green bananas								
Watercress								
Puha								
Sprouts (e.g. alfalfa, mung)								
Turnips or Swedes								
Parsnip								
Karengo (seaweed)								

Vegetables								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Onions or leeks								
Cauliflower								
Broccoli or broccoflower								
Brussel sprouts								
Courgette/zucchini, marrow, eggplant, squash								
Kamo kamo								
Pacific Island yams								
Yams								
Capsicum (or peppers)								
Celery								
Cassava								
Asparagus								
Breadfruit								
Cucumber								
Avocado								
Lettuce								
Other green leafy vegetables (e.g. Whitloof)								

39. Do you cook vegetables with fat or oil e.g. deep fry, butter on carrots, or stir fry?
- ☐ No
- ☐ Yes

What type(s) do you use most often?
(Please mark only those you usually have)
- ☐ Butter (all varieties)
- ☐ Monounsaturated fat margarine e.g. Olive, Rice Bran, Canola Oil Spreads
- ☐ Polyunsaturated fat margarine e.g. Canola, Sunflower Oil Spreads
- ☐ Light monounsaturated fat margarine e.g. Olivio Spread Light

□ Light polyunsaturated fat margarine e.g. Flora Spread Light
□ Plant sterol enriched margarine e.g. Pro Active, Logical Spreads
□ Light plant sterol enriched margarine e.g. Pro Active Spread Light
□ Butter and margarine blend e.g. Country Soft, Butter Lea
□ Saturated fat oils e.g. Lard, Dripping, Coconut oil
□ Monounsaturated fat oils e.g. Olive, Canola, Avocado, Soybean, Peanut, Rice Bran oil
□ Polyunsaturated fat oil e.g. Sunflower, Corn, Safflower, Cottonseed, Sesame seed, Grape seed oil
□ Cooking Spray
□ Don't know
□ I don't consume these types of food
□ Other *(please specify)* _____

Legumes

40. Do you consume legumes e.g. chickpeas/dried peas, soybeans, dried beans, or Dahl?
□ No
□ Yes

41. On average, how many servings of beans (fresh, frozen, canned) do you consume a ***day***? (*Please **mark one** only*)
(A 'serving' = ½ cup or 125mL of cooked legumes)

Per Day
□ None
□ < 1 serving
□ 1 serving
□ 2 servings
□ 3 servings
□ ≥ 4 servings

42. How often do you usually consume these foods?

Legumes								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day

Legumes								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Soybeans								
Tofu								
Beans (including baked beans), lentils								
Chickpeas/peas								
Dahl								

Fresh Fruit

43. Do you consume fruit?
    - ☐ No
    - ☐ Yes

44. On average, how many servings of fruit (fresh, frozen or stewed) do you consume per ***day***?
    Do not include fruit juice or dried fruit. (*Please **mark one** only*)
    (A 'serving' = 1 medium piece or 2 small pieces of fruit or ½ cup of stewed fruit)
    E.g. 1 apple + 2 small apricots = 2 servings

    Per Day
    - ☐ None
    - ☐ < 1 serving
    - ☐ 1 serving
    - ☐ 2 servings
    - ☐ ≥ 3 servings

45. How often do you usually consume these foods?

Fresh Fruit								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Apple								

Fresh Fruit								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Pear								
Banana								
Orange, mandarin or tangelo								
Grapefruit								
Peach, nectarine, plum or apricot								
Mango, paw-paw or persimmons								
Pineapple								
Grapes								
Strawberries and other berries or cherries								
Melon (e.g. watermelon, rockmelon etc.)								
Kiwifruit								
Feijoas								
Tamarillos								

Preserved Fruit (dried, canned)

46. Do you consume preserved fruit?

- ☐ No
- ☐ Yes

47. On average, how many servings of preserved fruit (dried or canned) do you consume per ***day***? (*Please **mark one** only*)

(A 'serving' = 50g or ½ cup of dried fruit)

E.g. 5 prunes + ½ cup of raisins = 2 servings

Per Day

- ☐ None
- ☐ < 1 serving
- ☐ 1 serving

□ 2 servings
□ ≥ 3 servings

48. How often do you usually consume these foods?

Preserved Fruit								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Sultanas, raisins or currants								
Other dried fruit (e.g. apricots, prunes, dates)								
Preserved or canned fruit in syrup								
Preserved or canned fruit in water or juice								
Stewed dried fruit								

Beverages

49. Do you consume any other beverages other than milk or water?
□ No
□ Yes

50. On average, how many glasses of beverages other than milk or water do you consume per ***day***?
(A 'serving' = 250mL)

Per Day
□ None
□ < 1 serving
□ 1-3 servings
□ 4-6 servings

□ ≥ 7 servings

51. How often do you usually consume these beverages?

Beverages								
Please fill in one category for each beverages	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Fruit juice (e.g. Just Juice, Fresh-up, Robinson's or Rio Gold etc.)								
Vegetable juice (e.g. tomato juice)								
Fruit drink e.g. Choice, Rio Splice etc.								
Powdered drinks (e.g. Raro, Vita-fresh etc.)								
Low-calorie cordial								
Cordial								
Diet carbonated drink (e.g. diet sprite)								
Carbonated drinks (e.g. coke, lemonade etc.)								
Sport's drinks (e.g. Gatorade, Powerade etc.)								
Water (including unflavoured mineral water, soda water, tap water)								
Coffee								
Coffee – decaffeinated								
Coffee substitute (e.g. Inka)								
Koko								
Tea								
Herbal tea								
Soy beverages								
Beer – low alcohol								
Beer – ordinary								

Beverages								
Please fill in one category for each beverages	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Red wine								
White wine or champagne / sparkling wine								
Wine cooler								
Sparkling grape juice								
Sherry or port								
Spirits, liqueurs								

Condiments (dressings & sauces)

52. Do you consume any condiments?
    - ☐ No
    - ☐ Yes

53. How often do you usually consume these foods?

Dressings and Sauces								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Mayonnaise								
Low-calorie salad dressing								
Salad dressing								
Tomato sauce								
Chutney								
Mustard								
Gravy								
White sauce/cheese								

Dressings and Sauces								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
sauce etc.								
Coconut cream								

Miscellaneous

54. Do you consume homemade baked products e.g. cakes, puddings and muffins?
    - ☐ No
    - ☐ Yes

55. How often do you usually consume these foods?

Homemade Miscellaneous Foods								
Please fill in one category for each food	Never	Less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2 or more times per day
Homemade cakes, scones or pikelets								
Homemade muffins - all types								
Homemade sweet pies or sweet pastries								
Homemade other puddings or desserts (not including milk-based puddings)								
Homemade biscuits								

56. When baking these foods do you use fat or oil?
    - ☐ No
    - ☐ Yes

    What type(s) of fat or oil do you use most often?
    (Please mark only those you usually have)

□ Butter (all varieties)
□ Monounsaturated fat margarine e.g. Olive, Rice Bran, Canola Oil Spreads
□ Polyunsaturated fat margarine e.g. Sunflower Oil Spreads
□ Light monounsaturated fat margarine e.g. Olivio Spread Light
□ Light polyunsaturated fat margarine e.g. Flora Spread Light
□ Plant sterol enriched margarine e.g. Pro Active, Logical Spreads
□ Light plant sterol enriched margarine e.g. Pro Active Spread Light
□ Butter and margarine blend e.g. Country Soft, Butter Lea
□ Saturated fat oils e.g. Lard, Dripping, Coconut oil
□ Monounsaturated fat oils e.g. Olive, Canola, Avocado, Soybean, Peanut, Rice Bran oil
□ Polyunsaturated fat oil e.g. Sunflower, Corn, Safflower, Cottonseed, Sesame seed, Grape seed oil
□ Don't know
□ I don't consume these types of food
□ Other *(please specify)* _____

57. Do you consume premade baked products from retail outlets such as cakes, muffins?
□ No
□ Yes

58. How often do you usually consume these foods?

Purchased Miscellaneous Foods								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Purchased cakes, scones or pikelets								
Purchased muffins - all types								
Purchased sweet pies or sweet pastries								
Purchased other puddings or desserts (not including milk-based puddings)								
Purchased plain sweet biscuits								
Cream filled and/or chocolate biscuits								

59. How often do you usually consume these foods?

Miscellaneous Foods								
Please fill in one category for each food	**Never**	**Less than once a month**	**1-3 times per month**	**Once per week**	**2-4 times per week**	**5-6 times per week**	**Once per day**	**2 or more times per day**
Sugar added to food/drinks								
Jam, honey, marmalade or syrup								
Vegemite or marmite								
Peanut butter, other nut spread								
Brazil nuts								
Walnuts								
Other nuts e.g. peanuts, almonds, cashew, pistachio, macadamia								
Muesli bars								
Chocolate (including chocolate bars e.g. Moro bars)								
Potato crisps, corn chips, Twisties etc.								
Meat pie, sausage roll or other savoury								
Pizza								
Canned or packet soup								
Home-made soup								

60. On average, how often do you consume 'takeaways' ***per week***?

Per Week

- □ Never
- □ < 1 serving
- □ 1-2 servings

□ 3-4 servings
□ 4-6 servings
□ ≥ 7 servings

61. Which takeaway food is your favourite which you consume the most regularly?

□ Fish and chips
□ Pizza
□ Burgers
□ Fried chicken
□ Chinese takeaways
□ Other (*please specify*): _____

Thank you!