

**STRATEGIES FOR INCREASING CONSUMPTION OF
N-3 POLYUNSATURATED FATTY ACIDS AND THEIR
EFFECTS ON CARDIAC ARRHYTHMIAS IN HUMANS**



by

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This thesis is dedicated to my wife, Kerrie and to my children, Sarah and Alex

ABSTRACT

Evidence from epidemiological and randomized dietary intervention studies suggests that n-3 PUFA, which are present in fish oils, are protective for coronary artery disease mortality. Animal studies suggest that protection may be via an anti-arrhythmic effect. Western societies consume very little fish. This thesis explores (a) a strategy to increase consumption of n-3 PUFA through incorporation of n-3 rich foods into the diet and (b) the effects of n-3 PUFA supplementation on ventricular arrhythmias in humans.

Sixteen healthy volunteers were provided with a range of food items, either naturally high in n-3 PUFA or fortified with fish oil, for incorporation into their diet. Significant increases in the proportion of n-3 PUFAs were observed in plasma, mononuclear cell and platelet phospholipid fractions.

Forty one patients with an ICD in situ for at least 12 months were supplemented with foods high in LC n-3 PUFA and capsules to achieve a target intake of the n-3 fatty acids eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) of at least 900 mg/d. Subjects served as their own controls, with arrhythmias recorded by the ICD between implant and commencement of the dietary intervention constituting the baseline data. The planned intervention period is 18 months. Paired annualized arrhythmia rates for the baseline and intervention periods were compared using Wilcoxon's Sign Rank Test. Interim analysis conducted on (a) 20 subjects with at least 12 months intervention data, and (b) 33 subjects with at least 6 months intervention data, found that 70% of subjects in each analysis had a reduction in annualized arrhythmia rate, compared to 20% who had an increase in their annualized arrhythmia rate.

Eleven subjects with inducible, sustained ventricular tachycardia (VT) at electrophysiology (EP) testing (including 9 subjects inducible on anti-arrhythmic drug therapy) were supplemented with 900 mg/d LC n-3 PUFA for at least 3 weeks. On repeat EP study, 6 subjects (55%) no longer had inducible VT, 3 subjects required an extra stimulus to induce the arrhythmia, with no change in the remaining 2 subjects.

These studies are the first to provide direct evidence that increasing consumption of long chain n-3 PUFA may be effective in reducing the risk of serious ventricular arrhythmias in humans. However, the uncontrolled design of the studies requires that further work be undertaken in this area, and these studies provide important preliminary data for proposed further studies.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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.....31/10/03.....

ABBREVIATIONS

AA	Arachidonic Acid (20:4 n-6)
AF	Atrial Fibrillation
AHA	American Heart Association
ALA	α -Linolenic Acid (18:3 n-3)
AMI	Acute Myocardial Infarction
ANOVA	Analysis of Variance
ATP	Anti-Tachycardia Pacing
BMI	Body Mass Index
BSA	Bovine Serum Albumin
CCO	Coconut Oil
CHD	Coronary Heart Disease
CI	Confidence Intervals
CICR	Calcium Induced Calcium Release
CO	Corn Oil
CVD	Cardiovascular Disease
d	Day
DART	Diet and Reinfarction Trial
DHA	Docosahexaenoic Acid (22:6 n-3)
dl	decilitre
DPA	Docosapentaenoic Acid (22:5 n-3)
DXR	Doxorubicin
EGM	Electrogram
EP	Electrophysiology
EPA	Eicosapentaenoic Acid (20:5 n-3)
ER	Endoplasmic Reticulum
FFA	Free Fatty Acid
FO	Fish Oil
g	Gram
GLA	γ -Linolenic Acid (18:3 n-6)
ICD	Implantable Cardioverter-Defibrillator
ISO	Isoproterenol

kg	kilogram
LA	Linoleic Acid
LC	Long Chain
LF(ref)	Low Fat Reference Diet
LO	Linseed Oil
LVEF	Left Ventricular Ejection Fraction
m	metre
mM	millimolar
mg	milligram
MI	Myocardial Infarction
mJ	Megajoule
mm	Millimetre
MO	Menhaden Oil
MRFIT	Multiple Risk Factor Intervention Trial
nd	Not Detected
NHANES 1	First National Health and Nutrition Examination Survey
NSR	Normal Sinus Rhythm
NSVT	Non-Sustained Ventricular Tachycardia
OA	Oleic Acid (18:1 n-9)
OO	Olive Oil
OR	Odds Ratio
PES	Programmed Electrical Stimulation
PC	Phosphatidylcholine
PUFA	Polyunsaturated Fatty Acids
PVC	Premature Ventricular Contraction
P/S	Polyunsaturated Fat / Saturated Fat
QTc	Heart Rate Adjusted QT Interval
RR	Relative Risk
RVA	Right Ventricular Apex
RVOT	Right Ventricular Outflow Tract
SAFO	Safflower Oil
SBO	Soybean Oil
SCD	Sudden Cardiac Death
SD	Standard Deviation

SDA	Stearidonic Acid (18:4 n-3)
secs	Seconds
SF	Saturated Fat
SR	Sarcoplasmic Reticulum
SSO	Sunflower Seed Oil
SVT	Supra-Ventricular Tachycardia
TAM	Total Arrhythmic Mortality
TFO	Tuna Fish Oil
VERP	Ventricular Effective Refractory Period
VF	Ventricular Fibrillation
VFT	Ventricular Fibrillation Threshold
VT	Ventricular Tachycardia
wk	Week
μ	Micro

PUBLICATIONS & ABSTRACTS ARISING FROM THIS THESIS

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Metcalf RG, Mantzioris E, Cleland LG and James MJ. A practical approach to increasing intake of n-3 fats: Use of novel foods enriched with n-3 fats. 17th International Congress of Nutrition, Vienna, August, 2001.

Metcalf RG, Cowie R, Young GD, James MJ and Cleland LG. A pilot study to investigate the effects of omega-3 fatty acids on inducible, sustained ventricular tachycardia in patients undergoing electrophysiology testing. Annual Scientific Meeting, The Australian Society for Medical Research - South Australian Division, Adelaide, June 2002.

Metcalf RG, Young GD, James MJ and Cleland LG: The effects of n-3 fatty acids on the incidence of arrhythmias in patients with implanted cardioverter-defibrillators (ICDs). Annual Scientific Meeting, The Cardiac Society of Australia and New Zealand, Adelaide, August 2003 (submitted).

Metcalf RG, Cowie R, Young GD, James MJ and Cleland LG: A pilot study to investigate the effects of omega-3 fatty acids on inducible, sustained ventricular tachycardia in patients undergoing electrophysiology testing. Annual Scientific

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Role of the Student in each of the Studies

Diet Study (Chapter 2)

My role in this study was

- Participate in study design
- Initial screening of subjects and enrolment
- Liaison with manufacturers of fish oil fortified foods
- Supply and resupply of foods
- Diet diary analysis
- Biochemical analysis (erythrocyte, plasma and food fatty acids)
- Data analysis
- Write-up

ICD Study (Chapter 3)

My role in this study was

- Participate in study design
- Identification of potential subjects from appointment lists and initial contact with patients re the study
- Enrolment of subjects in Adelaide (subjects who have their ICD follow-up visits in country areas were enrolled by Dr Young)
- Management of dietary items including acquisition and resupply to subjects
- Development of simplified omega-3 diet diary
- All data acquisition including searching for, and collating records of arrhythmic events for all subjects

- Collating arrhythmic events for independent analysis by 2 electrophysiologists
- Data analysis
- Write-up

ICD Study (Chapter 3)

My role in this study was

- Participate in study design
- Data acquisition
- Data analysis
- Write-up

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

The notion that certain fatty acids were an essential part of the diet was first proposed in the late 1920s by Burr and Burr, after it was noted that rats fed a fat-free diet developed dermatitis, had retarded growth and impaired fertility (Burr & Burr, 1929). The addition of 10 drops/d of lard, containing about 10% linoleic acid (LA) and traces of α -linolenic acid (ALA), was sufficient to prevent or reverse the deficiency syndromes. These fatty acids were considered to be new vitamins, and assigned as vitamin F. However, these observations languished because it was not possible to reproduce the deficiency syndromes in humans fed extremely low-fat diets for 6 months. Therefore the essentiality of these fatty acids in humans remained in question.

The first description of essential fatty-acid deficiency in humans was described in 1958 when a number of infants fed a fat-free milk-based formula as a part of a feeding study developed a severe dermatitis which was corrected by the addition of LA, but not by saturated fatty acids (Hansen *et al*, 1958). It was not until 1982 that the first case of ALA deficiency was described in a 6 year-old girl who had been maintained on total parenteral nutrition with a safflower oil (SAFO) fat source. SAFO is almost devoid of ALA, and after about 5 months the girl developed evidence of neuropathy, including numbness, tingling, weakness, blurred vision, inability to walk and psychological disturbances. These symptoms resolved completely when her fat supplement was changed to soybean oil which contains about 7-10% ALA (Holman *et al*, 1982).

The long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) were largely ignored until a 25 year epidemiological study of Greenland Inuit covering the period 1950 - 1974, found a virtual absence of coronary heart disease (CHD), and greatly reduced psoriasis, asthma and diabetes mellitus compared to comparable Danish populations (Kromann & Green, 1980). Detailed analysis of the Greenland Inuit diet by Bang and Dyerberg found a diet comprised mainly of seal and fish, along with some whale and sea birds (Bang *et al*, 1980). The diet was high in total fat (about 40% of total energy), but with about half the saturated fat, double the monounsaturated fat and 5 times the n-3 PUFA content of the typical Danish diet. The consumption of n-6 PUFAs was also low, with a resultant very low n-6:n-3 PUFA dietary ratio. The authors attributed the low incidence of ischaemic heart disease in this population in part to the anti-thrombotic effects of the LC n-3 PUFAs in the diet.

At about the same time, studies from Japan noted that fishing villages and coastal communities with extremely low incidences of coronary disease had high fish consumption and correspondingly high levels of plasma n-3 PUFAs compared to inland farming communities where fish consumption was lower and CHD rates were higher (Kagawa *et al*, 1982; Yamori *et al*, 1985).

During the mid to late 1980s, increased fish consumption was found to be associated with a reduced risk of cardiovascular disease (CVD) in western populations (Kromhout *et al*, 1985; Burr *et al*, 1989; Dolecek & Grandits, 1991), and these studies have generated great interest in elucidating the possible mechanisms by which n-3 PUFA protect against CVD. Of particular interest is the dramatic protection against sudden cardiac death (SCD) that is provided by n-3 PUFA, and the associated anti-arrhythmic effects of this class of fatty acid.

The remainder of this chapter will:

- review the current knowledge on the biochemistry and dietary intake of n-3 PUFAs,
- examine the evidence for their cardio-protective properties, and finally
- examine the possible mechanisms of protection with particular emphasis on their anti-arrhythmic properties.

1.2 BIOCHEMISTRY OF FATTY ACIDS

1.2.1 Chemical Structure of Fatty Acids

Fatty acids are hydrocarbon chains that contain a terminal carboxyl group and have the general formula $\text{CH}_3(\text{CH}_2)_n\text{COOH}$. They are classified as either saturated (ie there are no double bonds in the carbon chain, and the fatty acid molecule contains the maximal number of hydrogen atoms) or as unsaturated (ie, there is at least 1 double bond in the carbon chain, and the molecule contains less than the maximal number of hydrogens).

Unsaturated fatty acids are further classified by the number of double bonds, (monounsaturates have 1 double bond, while polyunsaturates have 2 or more double bonds), and the position of the first double bond relative to the terminal methyl group, ie, either as n-3, n-6, or n-9. The nomenclature of fatty acids is generally in the form $x:y$ n-z, where x refers to the number of carbon atoms, y refers to the number of double bonds, and z refers to the position of the first double bond relative to the terminal methyl group. This system may also use omega (ie, ω -3, ω -6 and ω -9) notation in place of the n- notation.

Fatty acids may also be referred to in the form $a,b,c...-x:y$, where $a, b, c...$ refers to the positions of the double bonds relative to the carboxy terminus of the molecule, eg 9,12,15-18:3 is the full designation for ALA. In plants and higher eukaryotes, double bonds in

unsaturated fatty acids are in the *cis* configuration, with double bonds normally occurring at 3 carbon intervals.

All plants and animals are able to synthesise fatty acids *de novo* from acetyl co-enzyme A via fatty acid synthase, to form palmitic acid (16:0) and stearic acid (18:0). To maintain their structure, function and fluidity, cell membranes require unsaturated fatty acids, and all plants and animals possess the mechanism for inserting a single double bond (Δ^9 desaturase) between the 9th and 10th carbon atoms (counting from the carboxy terminal end of the chain), converting stearic acid to oleic acid (OA, 18:1 n-9). Plants and some microorganisms are also able to insert additional double bonds into OA, a Δ^{12} desaturase converts OA to LA (18:2 n-6), and a Δ^{15} desaturase converts LA to ALA (18:3 n-3). Some plants also possess a Δ^6 desaturase which converts LA and ALA to γ -linolenic acid (GLA, 18:3 n-6) and stearidonic acid (SDA, 18:4 n-3) respectively. However, animals lack the Δ^{12} and Δ^{15} desaturases, and must therefore obtain all of their n-6 and n-3 requirements from their food supply. The simplified structures of some of the important fatty acids are depicted in Figure 1.1

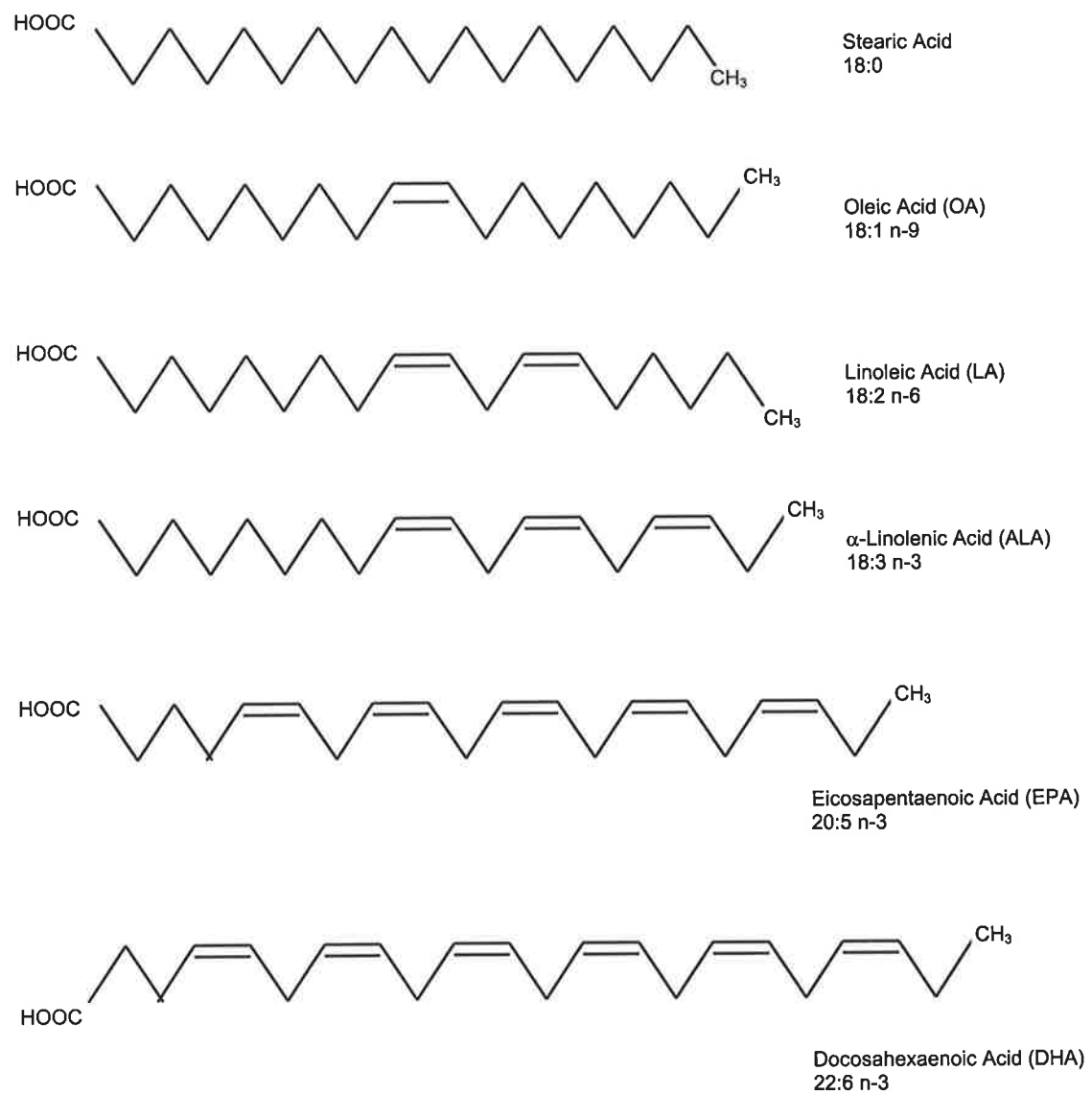


Figure 1.1 The chemical structure of some of the major fatty acids

1.2.2 Metabolic Conversion of ALA to EPA and DHA

Animal cells can convert dietary 18-carbon fatty acids to longer chain metabolites via a series of desaturation and elongation steps in the endoplasmic reticulum (ER) (Figure 1.2).

The efficiency with which the conversion of ALA to the LC n-3 PUFAs occurs in humans remains controversial (Gerster, 1998). Metabolic studies suggest that up to 60% of ingested ALA is utilized for energy production via β -oxidation to CO_2 , with less than 5% converted to DHA, and the remaining used as a source of acetate for the synthesis of saturated and monounsaturated fatty acids (Brenna, 2002). When healthy volunteers were fed diets which gave an approximate 9-fold increase in ALA intake through the use of flaxseed oil and a flaxseed oil based spread, there was only a 2.5 fold increases in the EPA content of plasma phospholipids and neutrophil phospholipids (Mantzioris *et al*, 1995).

Until recently, it was presumed that the 22-carbon fatty acids 22:6 n-3 and 22:5 n-6 were synthesised via a Δ^4 desaturase from 22:5 n-3 and 22:4 n-6 respectively. However, it has been demonstrated that DHA is formed by retroconversion from 24:6 n-3 (and therefore, presumably also 24:5 n-6 to 22:5 n-6) and proceeds via straight chain β -oxidation in the peroxisomes (Luthria *et al*, 1996; Mohammed *et al*, 1997; Su *et al*, 2001).

Fatty Acid Class

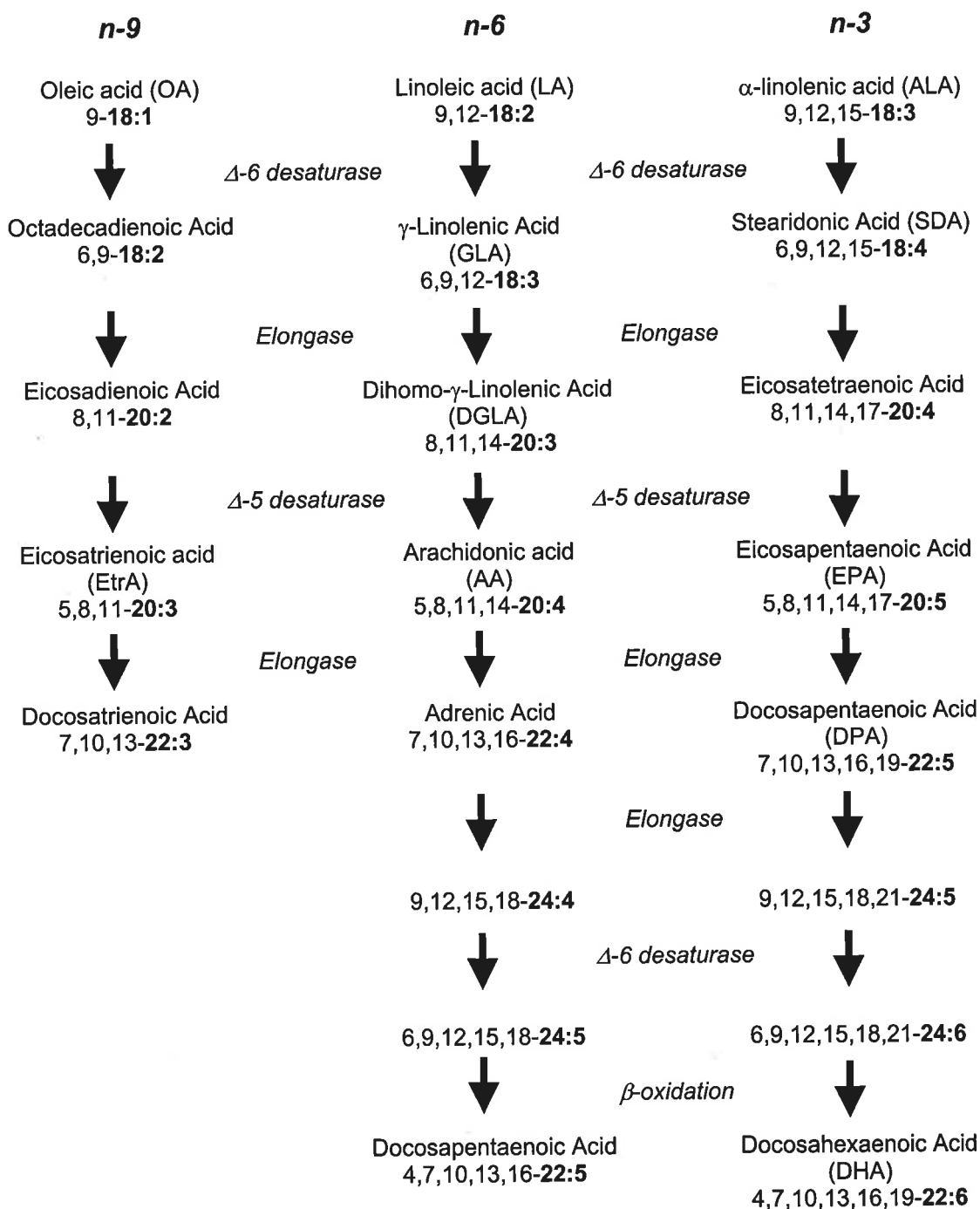


Figure 1.2 The metabolic conversion of 18-carbon *n*-9, *n*-6 and *n*-3 fatty acids to long chain polyunsaturated fatty acids via the desaturase/elongase pathway

Thus, for endogenously produced 22:6 n-3 to be incorporated into membrane, EPA is metabolised in the ER to 24:6 n-3 via two rounds of elongation followed by desaturation via Δ^6 desaturase. The resultant 24:6 n-3 then translocates to the peroxisomes, undergoes β -oxidation, and the resultant DHA translocates back to the ER for incorporation into membrane phospholipids. The Δ^6 desaturase involved in this process is the same enzyme which desaturates LA and ALA, and therefore, desaturation of 24:5 n-3 is probably subject to considerable competitive inhibition from the more abundant 18-carbon fatty acids. While the analogous n-6 fatty acids are produced via the same pathway, very little 24-C n-6 fatty acids are produced as the n-3 fats are the preferred substrate for the desaturation and elongation enzymes.

1.3 DIETARY INTAKE OF N-3 PUFA

1.3.1 Dietary sources of polyunsaturated fatty acids

The dietary polyunsaturated fatty acids consumed by humans are obtained from both vegetable and animal sources. The n-6 PUFA, LA, occurs much more abundantly than its n-3 homologue ALA, and is especially predominant as a storage lipid in the seeds of commercial oil-seed plants. With improvements in agricultural practice and food technology over the past century, the development of cheap seed oils rich in n-6 PUFA such as sunflower, safflower and corn oils, have largely replaced animal fats in food production. On the other hand, the significant dietary sources of ALA are limited to canola, flaxseed, soybeans and to a lesser extent, walnuts. The naturally occurring dietary source of EPA and DHA is limited mainly to fish, although it does occur in much smaller amounts in red meat, and populations which consume large quantities of red meat such as in Australia, may obtain a significant portion of their LC n-3 PUFA from this source, with one study finding red meat contributed 29% of the total LC n-3 PUFA intake (Ollis *et al*,

1999). It is estimated that the imbalance between the consumption of n-6 PUFA, as LA, and n-3 PUFA has increased from a ratio of about 1:1 throughout the major period of human evolution to a ratio more likely somewhere between 10:1 and 25:1 (Simopoulos, 1991).

As the n-6 and n-3 classes of PUFAs have profoundly differing biological effects, it is possible that this dramatic alteration in their relative intakes, particularly the decrease in n-3 PUFAs, has had a number of unforeseen health consequences in communities consuming low amounts of n-3 PUFA. The remainder of this chapter will examine the effects of n-3 PUFA on one area of health which is a major cause of morbidity and mortality in Western communities, cardiovascular disease.

1.4 THE EFFECTS OF n-3 FATTY ACIDS ON CARDIOVASCULAR DISEASE IN HUMANS

Cardiovascular disease is a broad term which encompasses all of the diseases which affect the heart and blood vessels, and includes coronary artery disease, peripheral vascular disease and cerebrovascular disease. CVD is the leading cause of death in Western countries, accounting for about 40% of all deaths (Australian Bureau of Statistics, 1999; Hoyert *et al*, 1999).

A 25 year survey of the disease patterns of a traditional population of Greenland Inuit found remarkably low incidences of acute myocardial infarction (AMI) compared to typical western European populations (Kromann & Green, 1980). In 1972, Bang *et al* found that the Inuit had lower plasma levels of total cholesterol (~ 18% reduction) and triglycerides (~ 55% reduction) than a group of typical Danes, despite consuming a diet very high in animal fats, cholesterol and protein, and low in carbohydrates. The diet

consisted almost exclusively of fish, marine mammals and sea birds, and as a consequence, the Inuit had a greatly increased intake of the LC n-3 PUFAs EPA and DHA (Bang *et al*, 1976). Collectively, these seminal studies suggested a negative relationship between EPA & DHA intake and MI.

1.4.1 Effects of fish or n-3 PUFA consumption on cardiovascular disease

In the ensuing quarter century since the observations of Bang & Dyerberg and Kromann & Green, a large number of epidemiological and dietary intervention studies, together with animal studies, have examined fish consumption, dietary n-3 PUFA intake and bio-markers of n-3 PUFA consumption in relation to cardiovascular disease. However, there is considerable heterogeneity in the methods used and outcomes examined. This heterogeneity is problematic for presentation of the results. Therefore, complementary approaches have been used. In sections 1.4.1.1 to 1.4.1.7, the information has been grouped according to the outcome measures (total mortality, CVD mortality, CHD mortality, etc.). Following this, the information is presented in tabular form (Tables 1.1 to 1.4), grouped according to the "dependent variable" (fish intake, LC n-3 PUFA intake, biomarkers of n-3 PUFA intake) and type of study.

1.4.1.1 Total Mortality

Due to difficulties sometimes encountered in determining an exact cause of death, total mortality is often used as a primary endpoint in epidemiological and intervention studies.

1.4.1.1.1 Cohort studies

1.4.1.1.1.1 Studies showing a benefit

The Multiple Risk Factor Intervention Trial (MRFIT) was a multi-centre clinical intervention trial in the primary prevention of CHD in a population of men identified as being at high risk of developing CHD based on smoking status, diastolic blood pressure and serum cholesterol levels, but without clinical symptoms of CHD. Subjects were randomly assigned to either the active intervention arm (interventions to reduce cholesterol and blood pressure and smoking cessation), or a 'usual-care' arm where subjects were referred to their usual source of medical care. Analysis of diet and mortality among the cohort of participants allocated to the 'usual care' group found a 24% reduction in the risk of total mortality for those subjects whose consumption of LC n-3 PUFA (EPA + DPA+DHA) was in the highest quintile (mean intake of 664 mg/d) compared to those in the lowest quintile (0 mg/d) with a significant inverse dose response from the lowest to highest quintiles ($p=0.01$) (Dolecek & Grandits, 1991).

The US Physicians Health Study is a large cohort study of about 22,000 US male physicians who were free of diagnosed cardiovascular disease in 1982. After 11 years of follow-up, and when fish intake was stratified by the frequency of consumption, there were significant reductions in the adjusted relative risk (RR) for total mortality associated with consumption of 1-2 servings/week (RR, 0.71, 95% Confidence Intervals (CI), 0.55-0.91), 2-5 servings/week (RR, 0.70, 95% CI, 0.54-0.89), and >5 servings/week (RR, 0.73, 95% CI, 0.55-0.96) compared with <1 serving/month. The overall RR for consumption of ≥ 1 serving/week was 0.70 (95% CI, 0.55-0.89) suggesting that there was no additional benefit in terms of total mortality in consuming more than 1-2 meals/week (Albert *et al*, 1998).

In the First National Health and Nutrition Examination Survey (NHANES 1) (Gillum *et al*, 2000), a cohort study of almost 9000 men and women in which the cohort was stratified by race and gender, a significant reduction in the risk for total mortality was found after 18 years of follow-up only for white men consuming 1 fish meal/week compared to no fish consumption (RR, 0.76; 95% CI, 0.63-0.91). Again, there was no additional benefit from consuming fish more frequently, with an adjusted RR of 0.85 (95% CI, 0.68-1.06) for consumption of more than 1 meal/week being not significantly different from no fish consumption. Similar, but non significant associations between fish consumption and total mortality were seen in black women (RR, 0.82; 95% CI, 0.52-1.28) and white women (RR, 0.90; 95% CI, 0.71-1.15) for consumption of greater than 1 meal/week compared to no fish consumption. No association was seen in black men (RR, 1.11; 95% CI, 0.68-1.81) for consumption of greater than 1 meal/week compared to no fish consumption.

1.4.1.1.1.2 Studies showing no benefit

Not all cohort studies have found an association between fish consumption and total mortality. Mann *et al* (1997) examined a range of dietary factors influencing total mortality and CHD mortality in a cohort comprised generally of health conscious subjects with a low risk for CVD, being about 20% smokers, of high socioeconomic status, about 40% vegetarian/vegan, with only about 13% of subjects classified as overweight, with a body mass index (BMI, defined as weight (kg) / height (m)²) ≥ 25 , and in whom the total mortality rate was about half of that of the general population. The RR for total mortality was 0.96 (95% (CI) 0.76-1.21) for consumption of at least 1 fish meal/week compared with fish never eaten.

In contrast to the low risk population in the study of Mann *et al* above, subjects in the Western Electric Study (Davignus *et al*, 1997) were at high risk of both CHD and total mortality, being mainly blue collar workers, >50% smokers, mean BMI >25, and with mean systolic blood pressure of 132 mm Hg. After 30 years of follow-up during which 57% of the cohort died, the RR for total mortality for fish consumption of at least 35 g/d was 0.85 (95% CI 0.64-1.10) compared to no fish consumption.

In a Dutch cohort study of 272 men and women aged over 63 years from a general practice in Rotterdam, there was no association after 17 years of follow-up between fish consumption and total mortality, with a RR for subjects who consumed fish (mean intake of 24 g/d) of 0.96 (95% CI 0.72-1.30) compared to those who ate no fish (Kromhout *et al*, 1995).

1.4.1.1.2 Dietary Intervention Studies

1.4.1.1.2.1 Studies showing a benefit

In the Diet and Reinfarction Trial (DART) (Burr *et al*, 1989), a secondary prevention study among men who had survived an AMI, the subjects were randomised to be given advice on three dietary regimens;

- 1) to reduce fat intake to 30% of energy and to increase the ratio of polyunsaturated fatty acids:saturated fatty acids (P/S ratio) to 1,
- 2) to increase their intake of fatty fish to at least 2 meals/week, and
- 3) to increase their intake of fibre to 18 g/d.

Each dietary regimen was randomised independently, and therefore, there were 8 possible combinations of advice, including a group that received advice on all regimens, and a group that received no advice on any of the regimens. When each of the dietary regimens

were analysed independently of the others, those men who were advised to increase their consumption of fatty fish had a RR of 0.71 (95% CI, 0.54-0.93) for all cause mortality over 2 years compared to those not receiving advice to increase fish consumption. The estimated mean daily intake of EPA in the fish advice group was 330mg compared with 100 mg in those not in the fish advice group. There was a significant separation of the survival curves of the fish advice group and the no fish advice group after about 2 months. There were no significant differences in the RR of total mortality for subjects in the groups advised to reduce their total fat intake and increase the P/S ratio in their diet (RR, 1.00; 95% CI, 0.77-1.30), or to increase their intake of cereal fibre (RR, 1.27; 95% CI, 0.99-1.65), compared to those receiving no advice.

The GISSI-Prevenzione Study (GISSI Investigators, 1999) was a secondary prevention trial of 11,324 patients who had survived an MI, in which subjects were randomly allocated to;

- encapsulated fish oil (0.85g/d n-3 PUFA as ethyl esters, EPA:DHA 1:2),
- vitamin E (300 mg/d),
- fish oil plus vitamin E, or
- placebo.

After a mean follow-up period of 3.5 years, n-3 PUFA supplementation was associated with a RR for total mortality of 0.80 (95% CI, 0.67-0.94) when the fish oil only group was compared to the placebo group. This benefit was observed against a background diet in which about 73% of subjects were consuming at least 1 fish meal/week at baseline, which increased to about 88% of subjects by 42 months. Time course analysis showed that the reduction in risk for total mortality became significant after 90 days of n-3 PUFA supplementation (Marchioli *et al*, 2002).

1.4.1.1.2.2 Studies showing no benefit

No dietary intervention studies which have examined the effects of fish or n-3 PUFA consumption on total mortality have failed to show a significant benefit of the intervention.

1.4.1.1.3 Other studies

An ecological study examining the relationship between total mortality (obtained from the World Health Organisation) and apparent fish consumption (obtained from the Food and Agriculture Organisation of the United Nations) over 3 time periods between 2 and 30 years prior to the mortality data, and across 36 countries, found significant adjusted inverse relationships ($p < 0.001$) between log all-cause mortality and log fish consumption in all 3 time periods and in both men and women (Zhang *et al*, 1999).

1.4.1.2 Cardiovascular Disease Mortality

Cardiovascular disease mortality includes fatal MI, SCD and stroke.

1.4.1.2.1 Cohort studies

1.4.1.2.1.1 Studies showing a benefit

In the MRFIT study there was an adjusted 41% reduction in the risk of CVD mortality in the highest quintile of LC n-3 PUFA intake (664 mg/d) compared to lowest quintile (0 mg/d) and a significant inverse dose response from the lowest to highest quintiles of LC n-3 PUFA intake (Dolecek & Grandits, 1991). In the Chicago Western Electric Study there was a significant inverse dose response in CVD mortality across the 4 strata of fish consumption ranging from 0 intake to ≥ 35 g/d (Daviglus *et al*, 1997).

1.4.1.2.1.2 Studies showing no benefit

On the other hand, the 2 separate analyses of the US Physicians Health Study after 4 years (Morris *et al*, 1995) and 11 years (Albert *et al*, 1998) of follow-up, failed to show any benefit of fish consumption on CVD mortality. Indeed, in the 4 year analysis the multivariate RR for CVD mortality for consumption of 1 fish meal/week compared with <1 meal/week was 2.2 (95% CI, 1.4-4.8), while higher levels of consumption had adjusted RRs of 1.7 (95% CI, 0.9-3.4) for 2-4 meals/week and 2.2 (95% CI, 0.8-5.9) for ≥ 5 meals/week (Morris *et al*, 1995). By the 11 year follow-up, the adjusted RRs for CVD mortality was <1 for all categories of fish consumption greater than at least 1 fish meal/month compared with less than 1 meal/month, although the decrease in risk was not statistically significant in any category (RR, 0.79; 95% CI, 0.51-1.23 for 1-2 meals/week, RR, 0.84; 95% CI, 0.54-1.30 for 2-5 meals/week and RR, 0.81; 95% CI 0.49-1.33 for ≥ 5 meals/week (Albert *et al*, 1998). In the NHANES 1 study there was also no association between fish consumption and CVD mortality, with RRs of CVD mortality associated with consumption of more than 1 fish meal/week of 0.95 (95% CI, 0.68-1.35) for white men, 1.11 (95% CI, 0.68-1.81) for black men, 1.06 (95% CI, 0.75-1.50) for white women and 0.99 (95% CI, 0.51-1.93) for black women (Gillum *et al*, 2000).

1.4.1.2.2 Dietary intervention studies

The GISSI-Prevenzione study reported a RR of CVD mortality of 0.70 (95% CI, 0.56-0.87) in the group allocated to n-3 PUFA compared to placebo (GISSI Investigators, 1999), with time course analysis indicating that the benefits of n-3 PUFAs for CVD mortality compared were significant after 240 days (Marchioli *et al*, 2002).

1.4.1.3 Coronary Heart Disease Mortality

Deaths due to CHD are generally defined as those certified as due to International Classification of Diseases codes 410 (AMI), 411 (other acute & subacute forms of ischemic heart disease), 412 (old myocardial infarction), 413 (angina pectoris) and 414 (other forms of chronic ischemic heart disease, including coronary atherosclerosis, aneurysm of heart, other specified forms of chronic ischemic heart disease and chronic ischemic heart disease, unspecified)

Death from coronary heart disease is by far the most reported endpoint in studies of the effects of fish consumption or n-3 PUFA in heart disease, and like CVD mortality, the reported benefit have been mixed.

1.4.1.3.1 Cohort studies

1.4.1.3.1.1 Studies reporting a benefit

The Zutphen study (Kromhout *et al*, 1985), which was originally the Dutch contribution to the Seven Countries Study (Keys *et al*, 1966), was a landmark study in that it was the first longitudinal cohort study to examine the relationship between fish consumption and CHD. After 20 years of follow-up (1960-1980), consumption of ≥ 30 g of fish/d (about 2 fish meals /week) was associated with a 64% reduction in the risk of death from CHD. However, a more recent analysis of the Zutphen study for the period 1970-1990 failed to show any relationship between fish consumption and CHD mortality in this cohort (Oomen *et al*, 2000). When combined with other cohorts from Finland and Italy (from the original Seven Countries Study), a significant protective effect of fatty fish consumption on CHD mortality was found (34% reduction in risk compared to those that consumed no fatty fish), whereas no protective effect was found with consumption of lean fish or total fish. It was

postulated that the lack of a protective effect of total fish consumption may have been due to lean fish being consumed by a far larger proportion of the cohort population.

In the Western Electric Study (Davignus *et al*, 1997), there was 38% reduction in the risk of death from CHD associated with the consumption of ≥ 35 g of fish/d when compared to no intake as well as an inverse dose response of CHD mortality to increasing fish consumption. In the Rotterdam study, there was an adjusted reduction in risk of 49% for subjects who consumed fish compared to those who ate no fish (Kromhout *et al*, 1995).

In the Nurses Health Study (Hu *et al*, 2002), a long-term cohort study examining the health of about 120,000 females, there was a 34% reduction in risk of CHD mortality associated with consumption of 1 fish meal/week compared to less than 1 meal/month, while in those who consumed at least 5 meals/week, the reduction in risk was a 45%. When LC n-3 PUFA consumption was estimated from the fish intake, an intake of 0.08% of energy (the median of the 3rd quintile and equivalent to about 150 mg/d LC n-3 PUFA in a 7 megajoule (mJ) diet) was associated with a 31% reduction in risk compared to an intake of 0.03% of energy (the median of the lowest quintile and equivalent to 60 mg/d LC n-3 PUFA in a 7 mJ diet). Increasing consumption of LC n-3 PUFA to 0.24% of energy (median of the 5th quintile and equivalent to a daily intake of 460 mg LC n-3 PUFA in a 7 mJ diet), reduced the risk of CHD mortality by only a further 6% to a 37% reduction in risk compared to the lowest quintile. Overall, these results suggest that for CHD mortality, small increases in fish or LC n-3 PUFA consumption from a low base may have significant protective effects. Consumption of LC n-3 PUFA has also been associated with reductions in the risk of CHD mortality in the MRFIT study (Dolecek & Grandits, 1991), where there was a 40% decrease from the highest to lowest quintiles of intake.

Fish consumption may be particularly beneficial in groups at high risk of CHD as demonstrated by results from the Honolulu Heart Program (Rodriguez *et al*, 1996), a cohort study amongst Japanese men living in Hawaii. Consumption of at least 2 fish meals/week was associated with a 50% decrease in risk for death from CHD compared to less than 2 meals/week, but only in men smoking more than 30 cigarettes/d. There was no association with men smoking <30 cigarettes/d.

1.4.1.3.1.2 Studies reporting no benefit

As with total mortality, the study of healthy individuals by Mann *et al* (1997) failed to find an association between fish consumption and CHD. This lack of effect may again relate to the generally healthy lifestyle of the subjects. A similar trend was found in the Health Professionals Follow-Up Study (Ascherio *et al*, 1995), a cohort study involving about 45,000 male health professionals, where the mean intake of n-3 PUFA in the lowest quintile was 70 mg/d and ranged up to 110 mg/d, or equivalent to about 0.7 and 1 fish meals/week respectively. As highlighted by the investigators, even subjects in the lowest quintile of fish intake were possibly already consuming quantities sufficient to offer protection from CHD. In addition, members of this cohort had generally healthy lifestyles, with a mean BMI <25 and only about 10% of the cohort being current smokers.

1.4.1.3.2 Dietary intervention studies

In the only published study of fish oil supplementation in the acute post MI phase, the Indian Infarct Survival Study (Singh *et al*, 1997), 360 patients with suspected AMI were randomised within 24 hours of the onset of symptoms to receive 1.8 g/d EPA+DHA, 2.9 g/d ALA or placebo. During the first 10 days of treatment, there were 4 deaths in the fish oil group, and 6 deaths in the placebo group, and by 28 days, there were significantly less

cardiac events in the fish oil group. After 1 year of follow-up, total CHD mortality was significantly lower in the fish oil group than the placebo group (11.4% vs. 22%). The effects of ALA will be discussed later.

1.4.1.3.3 Case-control studies

In a case-control study nested in the Cardiovascular Health Study, 54 cases of fatal CHD were matched with the same number of controls in a cohort of elderly men and women (≥ 65 yrs) and without evidence of CHD at entry. The proportion of EPA + DHA in plasma phospholipids was significantly lower in cases ($3.3 \pm 0.8\%$ of total fatty acids) than in controls ($3.8 \pm 1.3\%$ of total fatty acids) and a 1 standard deviation (SD) increase in plasma phospholipid EPA + DHA was associated with an adjusted odds ratio (OR) of fatal CHD of 0.30 (95% CI, 0.12-0.76) (Lemaitre *et al*, 2003).

1.4.1.3.4 Other studies

The ecological study outlined in section 1.4.1.1.3 also found significant adjusted inverse relationships ($p < 0.01$) between log CHD mortality and log fish consumption in all 3 time periods and in both men and women (Zhang *et al*, 1999).

1.4.1.4 All CHD (fatal plus non-fatal MI plus SCD)

A case-control analysis of a subgroup of the MRFIT study (Simon *et al*, 1995), using subjects allocated to the 'usual care' (control) arm of the study, examined the relationship between serum fatty acids and the development of CHD. Men in this group who went on to develop CHD (fatal or non-fatal MI or sudden cardiac death) were selected as cases, and matched with an identical number of controls who did not develop CHD during the average follow-up period of 6.9 years. In the serum phospholipid fraction, a 1 SD increase

in the n-3 PUFAs DPA and DHA (increases of 0.25% and 1.22 % of total fatty acids respectively) was associated with a 33 % decrease in the risk of CHD.

In the Nurses Health Study after 16 years of follow-up, fish consumption of 1-3 meals/month was associated with a reduced risk of total CHD (fatal CHD plus non-fatal MI) (RR, 0.79; 95% CI, 0.64-0.97) compared to <1 meal/month, while consumption of fish at the rate of 1/week, 2-4/week and ≥ 5 /week were associated with RRs of 0.72 (95% CI, 0.59-0.88), 0.72 (95% CI, 0.57-0.91) and 0.69 (95% CI, 0.52-0.93) respectively, with a significant inverse dose response. When dietary LC n-3 PUFA intake was estimated from fish consumption, increases in LC n-3 PUFA intake from 0.03% of dietary energy (median of the lowest quintile) to 0.08 % of dietary energy (median of the third quintile), 0.14% of dietary energy (median of the fourth quintile) and 0.24% of energy (median of the highest quintile) were associated with RRs of 0.79 (95% CI, 0.66-0.94), 0.69 (95% CI, 0.57-0.84) and 0.69 (95% CI, 0.57-0.84) respectively, again with a significant inverse dose response relationship observed (Hu *et al*, 2002).

1.4.1.5 Sudden Cardiac Death (SCD)

Sudden cardiac death (SCD) is defined as any death from cardiac causes that occurs within 1 hour of the onset of symptoms, implying that in most cases, the underlying cause is a ventricular arrhythmia. It is the most common cause of cardiovascular death, accounting for about 50% of all CVD deaths, is often the first indication of heart disease, and it may also occur in individuals with no underlying structural heart disease. Reduction in the RR of SCD has been associated with fish consumption, n-3 PUFA intake and blood levels of n-3 PUFAs.

1.4.1.5.1 Cohort Studies

1.4.1.5.1.1 Studies showing a benefit

Consumption of at least 1 fish meal/week was associated with an adjusted RR for SCD of 0.48 (95% CI, 0.24-0.96) compared to less than 1 meal/month in the Physicians Health Study (Albert *et al*, 1998). There appeared to be no additional advantage with higher consumption levels as the RR associated with 1-2 meals/week was 0.47 (95% CI, 0.23-0.98) and for ≥ 5 meals/week was 0.39 (95% CI, 0.15-0.96) compared to less than 1 meal/month. Using estimates of LC n-3 PUFA intake from the dietary fish data, those men that ate fish were grouped into quartiles of LC n-3 PUFA intake and compared to those that rarely or never ate fish and had a LC n-3 PUFA intake of less than 0.3 g/month (<10 mg/d). The adjusted RR of SCD associated with intakes of LC n-3 PUFA of 2.7-4.9 g/month (the 2nd quartile and equivalent to 90-160 mg/d) was 0.34 (95% CI, 0.15-0.75), while for an intake of at least 7.4 g/month (the highest quartile and equivalent to ≥ 243 mg/d) the RR was 0.43 (95% CI, 0.20-0.93).

In a nested case-control analysis of the Physicians Health Study comparing cases of SCD with matched controls, cases had significantly lower baseline whole-blood levels of EPA, DHA and total LC n-3 PUFA than controls. The RRs of SCD associated with baseline whole blood LC n-3 PUFA levels (EPA + DPA + DHA) compared to the lowest quartile were 0.19 (95% CI, 0.05-0.69) in the 3rd quartile and 0.10 (95% CI, 0.02-0.48) in the highest quartile (Albert *et al*, 2002).

1.4.1.5.1.2 Studies showing no benefit

The Western Electric Study (Daviglius *et al*, 1997) found a significant association of fish consumption with non-sudden death, but no association with sudden death. The exact

definition of sudden cardiac death in this study is unclear, and it appears to be different to the common definition of death within 1 hour of the onset of symptoms.

1.4.1.5.2 Case-Control Studies

Very similar intakes of LC n-3 PUFA to those found to be protective of SCD in the Physicians Health study outlined above (Albert *et al*, 2002) were reported by Siscovick *et al* (1995) in the Seattle case-control study. A monthly intake of LC n-3 PUFA of about 2-4 g/month (the 2nd quartile and equivalent to about 70-130 mg/d) was associated with an adjusted OR for primary cardiac arrest of 0.7 (95% CI 0.6-0.9) compared to no intake. Consumption of LC n-3 PUFA in the 3rd quartile (4.1 - 7.4 g/month or 130 - 245 mg/d) and in the 4th quartile (> 7.4 g/month or > 245 mg/d) were associated with odds ratios for primary cardiac arrest of 0.5 (95% CI 0.4-0.8) and 0.4 (95% CI 0.2-0.7) respectively. Analysis of erythrocyte phospholipid fatty acids found an inverse association between the risk of primary cardiac arrest and the proportion of EPA + DHA in the membranes, with an odds ratio of 0.5 (95% CI 0.4-0.8) in the 2nd quartile (mean of quartile 4.3% of total fatty acids) compared to the lowest quartile (mean of quartile 3.3% of total fatty acids). Further increases in membrane EPA + DHA to 5% and 6.5% of total fatty acids (mean of the 3rd and 4th quartiles respectively) were associated with odds ratios for primary cardiac arrest of 0.3 (95% CI 0.2-0.6) and 0.1 (95% CI 0.1-0.4). With further multivariate analysis of n-3 PUFA intake while controlling for erythrocyte n-3 PUFA levels, the decrease in RR was no longer significant, whereas when erythrocyte n-3 PUFA levels were analyzed after adding n-3 PUFA intake into the model, the association was still significant. These results suggest that tissue levels of n-3 PUFA are a more sensitive indicator than dietary intake in modulating risk for SCD, and further suggest that other dietary effects may be influencing tissue levels of n-3 PUFAs (Siscovick *et al*, 1995).

1.4.1.5.3 Dietary Intervention Studies

In the GISSI study, an n-3 intake of 850 mg/d in the form of ethyl esters was associated with a 45% reduction in risk of SCD compared to placebo after 3.5 years of follow-up (GISSI Investigators, 1999). A subsequent time-course analysis of this study showed that the divergence of the survival curves for SCD between n-3 PUFA and placebo became significantly different after 4 months, with a reduction in RR of 53% in favour of n-3 PUFAs (Marchioli *et al*, 2002).

In the Indian Infarct Survival Study (Singh *et al*, 1997), the small number of cases of SCD after 1 year of follow-up (2 in the fish oil group and 8 in the placebo group) prevented the reduction in the risk of SCD in the fish oil supplemented group from reaching statistical significance (RR, 0.24; 95% CI 0.03-2.0).

1.4.1.6 Stroke

1.4.1.6.1 Cohort Studies

1.4.1.6.1.1 Studies showing a benefit

In the NHANES 1 study, in which the cohort was stratified by race and gender, white women who consumed more than 1 fish meal/week had a RR for incident stroke of 0.55 (95% CI, 0.32-0.93) compared to those who never ate fish. There was no significant association between fish intake and stroke incidence in white men (RR, 0.85; 95% CI, 0.49-1.32) for consumption of more than 1 meal/week. Black men and women combined who ate any fish had RRs of 0.53 (95% CI, 0.32-0.90) for stroke incidence and 0.37 (95% CI, 0.16-0.85) for stroke death compared to those who never ate fish (Gillum *et al*, 1996).

In a 15 year follow-up of the Zutphen Study, there was a significant unadjusted reduction in risk of total stroke incidence among men who consumed at least 20 g/d of fish compared to those who ate less than 20 g/d (RR, 0.49; 95% CI, 0.24-0.99), whereas following adjustment for potential confounders, the RR was unaltered, but borderline significant (Keli *et al*, 1994).

An analysis of the Health Professionals Follow-up Study after 12 years of follow-up found a significant reduction in the risk of ischaemic stroke associated with the consumption of at least 1 fish meal/month compared to less than 1/month (RR, 0.56; 95% CI, 0.38-0.83) (He *et al*, 2002). There was no additional benefit in consuming more than 1-3 meals/month (RR 0.56; 95% CI, 0.37-0.84), and the RR associated with the consumption of more than 5 meals/week was 0.54 (95% CI, 0.31-0.94). There was no association between fish consumption and total stroke or haemorrhagic stroke. When the intake of LC n-3 PUFA was estimated from fish intake, there was benefit in terms of ischaemic stroke in consuming 50-200 mg/d (RR, 0.56; 95% CI, 0.35-0.88). There was no further benefit in consuming greater amounts with the RR for those consuming 400-600 mg/d being 0.54 (95% CI, 0.32-0.91), while for those consuming more than 600 mg/d, the benefit was no longer significant (RR, 0.73; 95% CI, 0.43-1.25) (He *et al*, 2002).

An analysis of the Nurses Health Study after 14 years of follow-up found a significant reduction in risk of thrombotic stroke associated with consumption of 2-4 fish meals/week compared to those consuming less than 1 meal/month (adjusted RR, 0.52; 95% CI, 0.27-0.99) and an inverse dose response for increasing consumption of fish (Iso *et al*, 2001). When the intake of LC n-3 PUFA was estimated from fish intake, women in the highest quintile of LC n-3 PUFA intake (median intake of quintile, 481 mg/d) had a significant

reduction in risk of total stroke compared to those with intakes in the lowest quintile whose median intake was 77 mg/d (RR, 0.72; 95% CI, 0.53-0.99). There was no significant dose response relationship for this endpoint. For women whose LC n-3 PUFA intake was in the 3rd quintile (median intake 171 mg/d), there were significant reductions in risk of ischaemic stroke (RR, 0.67, 95% CI, 0.47-0.98) and thrombotic infarction (RR, 0.64, 95% CI, 0.43-0.9) compared to those with intakes in the lowest quintile. For women in the highest quintile, although the RRs were similar to those in the 3rd quintile they were no longer statistically significant (RR, 0.71, 95% CI, 0.46-1.1 and RR, 0.67, 95% CI, 0.42-1.07 respectively).

1.4.1.6.1.2 Studies showing no benefit

There was no association between fish consumption and stroke mortality in the Gothenburg study (Lapidus *et al*, 1986).

1.4.1.7 Myocardial Infarction

Myocardial infarction, both fatal and non-fatal, has been a commonly reported endpoint in studies examining the effects of fish oil and/or n-3 PUFA in cardiovascular disease. Like total mortality and CHD mortality, studies have shown either a protective effect or no benefit.

1.4.1.7.1 Cohort studies

1.4.1.7.1.1 Studies showing a benefit

Following 6 years of follow-up in the Health Professionals Follow-up Study (Ascherio *et al*, 1995), there were significant reductions in risk for both non-fatal MI and any MI associated with 2-3 fish meals/week (mean intake of 37g/d) of 33% (95% CI 0.46-0.97)

and 31% (95% CI 0.51-0.94) respectively, compared with <1 fish meal/month. For any MI, there was also a significant risk reduction of 35% (95% CI 0.46-0.92) associated with consumption of 4-5 fish meals/week. However, consumption of ≥ 6 meals/week conferred no protection from MI, with RRs for non-fatal MI and any MI of 0.96 (95% CI 0.63-1.47) and 0.90 (95% CI 0.63-1.28) respectively. There was also no dose response relationship between increasing fish consumption and either endpoint.

Similarly, in the Kuopio Ischemic Heart Disease Risk Factor Study (Rissanen *et al*, 2000), a prospective cohort study of 1871 men conducted in Finland, serum levels of DHA+DPA in the highest quintile were associated with a RR of 0.44 (95% CI, 0.11-0.65) of acute coronary events (defined as MI plus acute chest pain) compared to the lowest quintile after 10 years of follow-up. There was also a significant inverse dose response association between serum DHA+DPA and acute coronary events, whereas there was no association between serum EPA and the risk of acute coronary events.

1.4.1.7.1.2 Studies showing no benefit

Two analyses of the Physicians' Health Study examining the relationship between MI and fish or n-3 PUFA consumption have been reported. In the first analysis of the cohort after 4 years of follow-up, no relationship was found between total or non-fatal MI and any level of fish consumption when compared to <1 meal/week, or level of n-3 PUFA intake compared to <70 mg/d (Morris *et al*, 1995), nor were there dose response associations between increasing fish consumption or n-3 PUFA intake and either MI endpoint. In the second analysis, a nested case-control study among 222 participants who suffered an MI during the first 5 years of follow-up together with matched controls, there was also no relationship found between the incidence of total MI (fatal or non-fatal) and plasma

phospholipid or cholesterol ester levels of EPA or DHA or both n-3 PUFAs combined (Guallar *et al*, 1995).

1.4.1.7.2 Case-Control Studies

1.4.1.7.2.1 Studies showing a benefit

In contrast to the Physicians' Health Study above, a nested case-control analysis of 78 cases of first ever MI in the Västerbotten Intervention Programme (a community intervention program on cardiovascular disease prevention, which was a part of the WHO Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) study) found adjusted reductions in risk for first ever acute MI of 51% and 57% associated with a plasma phospholipid EPA + DHA level >5.5% and >6.5% of total fatty acids, compared to those with levels below 5.5% (Hallgren *et al*, 2001).

In a case-control study from Oslo which examined the association between adipose tissue fatty acids, which reflects long-term dietary intake, and the risk of a first MI, the adipose tissue content of EPA, DPA and DHA were all significantly lower in cases than in controls. The adjusted odds ratio for a first MI in the highest quintile of adipose tissue LC n-3 PUFA was 0.18 (95% CI, 0.04-0.80) compared to the lowest quintile (Pedersen *et al*, 2000).

A case-control study from Milan among 507 survivors of MI and 478 hospital controls, found a reduction in risk of non-fatal MI associated with consumption of at least 2 fish meals/week (OR, 0.68; 95% CI, 0.47-0.98), and with LC n-3 PUFA intakes in the middle tertile (OR, 0.67; 95% CI, 0.47-0.96) and highest (OR, 0.67; 95% CI, 0.47-0.95) tertile (Tavani *et al*, 2001). The upper cutoff point for the lowest and middle tertiles were 0.81

g/wk (116 mg/d) and 12.8 g/wk (183 mg/d) respectively. Stronger inverse associations were found between LC n-3 PUFA intakes and high risk patients. The ORs for current smokers, patients with total cholesterol >200 mg/dl, and those with a first degree relative with MI, in the highest tertile of LC n-3 PUFA intake were 0.39 (95% CI, 0.21-0.75), 0.42 (95% CI, 0.24-0.74) and 0.40 (95% CI, 0.19-0.85) respectively, compared with those in the lowest tertile. There were also significant dose response relationships across tertiles for each subgroup. There were no significant associations between LC n-3 PUFA intake and non-smokers, patients with total cholesterol $\leq$ 200 mg/dl, or those without a first degree relative with MI.

A nested case-control analysis of the Honolulu Heart Program, consisting of subjects who died during follow-up, had a protocol autopsy of the heart and coronary arteries and were free from moderate to severe coronary atherosclerosis, found significant reductions in the risk of ischaemic myocardial lesions associated with consumption of fish at least twice/week compared to less than twice/week (OR, 0.35, 95% CI, 0.15-0.86 for any ischaemic lesion and OR, 0.35; 95% CI 0.15-0.83 for small (<0.5 cm) lesions) (Burchfiel *et al*, 1996).

1.4.1.7.2.2 *Studies showing no benefit*

In contrast to the Oslo study above, the EURAMIC study (a multi-centre European case-control study with 639 men with a first MI) failed to find any difference in adipose tissue DHA between cases and controls. There was also no association between adipose tissue DHA and the risk of first MI in the highest quintile compared to the lowest quintile (OR, 0.87; 95% CI, 0.53-1.43) (Guallar *et al*, 1999). The nested case-control analysis of the Cardiovascular Health Study which matched 125 cases of non-fatal MI with an equal

number of controls, found no difference in the plasma phospholipid proportions of EPA + DHA in cases and controls, or of risk of non-fatal MI associated with a 1 SD increase in plasma phospholipid EPA + DHA (OR, 0.97; 95% CI, 0.71-1.33) (Lemaitre *et al*, 2003).

1.4.1.8 Studies Presented According to Dependent Variable

The sections 1.4.1.1 to 1.4.1.7 have outlined the evidence provided from cohort, case-control and randomized dietary intervention studies for a protective effect of fish and/or LC n-3 PUFA intake, or biomarkers of LC n-3 PUFA intake on the risk of cardiovascular disease in humans. For a review of this information which is complementary, the studies are presented in tabular form (Tables 1.1 to 1.3).

Table 1.1 Studies examining the effects of fish consumption on cardiovascular disease

Study	Study Type	Population	Follow-up (years)	Endpoints	Comparisons	Major Outcomes:
Chicago Western Electric (Daviglius <i>et al</i> , 1997)	Cohort	1,822 men 40-55 years & free of cardiovascular disease at enrolment	30	MI, CHD, CVD & total mortality.	fish consumption stratified by daily intake (0, 1-17, 18-34, ≥35 g/d)	Increasing fish consumption inversely related with mortality from MI, CHD, and CVD (no association with total mortality) MI mortality: 44% ↓ in risk for ≥35 g/d compared to no fish. CHD mortality: 38% ↓ in risk for ≥35 g/d compared to no fish Non-sudden MI mortality: 67% ↓ in risk for ≥35 g/d compared to no fish
Diet & Reinfarction Trial (DART) (Burr <i>et al</i> , 1989)	Randomized dietary intervention	2033 men post MI	2	CHD & total mortality, Non-fatal MI, Total CHD events	dietary advice to consume ≥2 fatty fish meals/wk vs. no advice	Total mortality: 29% ↓ in risk in fish advice gp No significant ↓ in risk for any other endpoint
Gothenburg Study (Lapidus <i>et al</i> , 1986)	Cohort	1,462 women aged 38-60 years	12	Total, stroke & MI mortality	Fish consumption	No association between fish consumption and any endpoint
Health Professionals Follow-Up (Ascherio <i>et al</i> , 1995)	Cohort	44,895 male health professionals aged 40-75 years at enrolment	6	CHD mortality, any CHD, non-fatal MI, any MI, CABG	fish consumption stratified by daily intake (means of quintiles; 0, 7, 18, 37, 69, 119 g/d) and endpoints	65% ↑ in risk of CABG in highest quintile 33% ↓ in risk of non-fatal MI in 3 rd quintile. 31% ↓ in risk of any MI in 3 rd quintile No association between fish consumption and any other endpoint
Health Professionals Follow-Up (He <i>et al</i> , 2002)	Cohort	43,671 male health professionals aged 40-75 years at enrolment	12	Stroke	Fish meals stratified by frequency (<1/mo, 1-3/mo, 1/wk, 2-4/wk, ≥5/wk)	Significant ↓ in risk of ischaemic stroke of 43%, 44%, 45% & 46 in stratas 2, 3, 4 & 5 respectively. When consumption dichotomized, 44% ↓ in risk of ischaemic stroke associated with ≥1 meal/mo compared to < 1 meal/mo Significant ↓ in risk of total stroke of 43% associated with 2-4 meals/wk No association with haemorrhagic stroke
Honolulu Heart Program (Rodriguez <i>et al</i> , 1996)	Cohort	3,310 men, current smokers, aged 45-68 years at enrolment	23	CHD mortality, CHD incidence	fish consumption dichotomised by intake (< 2 meals/wk vs. ≥ 2 meals/wk), and number cigarettes/d (<20, 20-30, >30)	In heavy smokers (>30 cig/d), 50% & 28% ↓ in risk of CHD mortality & CHD incidence in high fish consumers
Honolulu Heart Program (Burchfiel <i>et al</i> , 1996)	Nested case-control	120 men from HHP who had autopsy & were without moderate-severe coronary atherosclerosis	-	Any myocardial lesion, small myocardial lesion at autopsy	Fish consumption dichotomized by intake (<2 meals/wk vs. ≥2 meals/wk)	≥2 meals/wk associated with 65% ↓ in risk

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Table 1.1 continued

Study	Study Type	Population	Follow-up (years)	Endpoints	Comparisons	Major Outcomes:
Mann <i>et al</i> 1997 (Mann <i>et al</i> , 1997)	Cohort	10,802 men & women 16-79 years	13	CHD & total mortality	Fish consumption stratified by frequency (never, <1/week, ≥1/week)	No association between fish consumption and IHD or all-cause mortality
Milan (Tavani <i>et al</i> , 2001)	Case-control	507 cases 478 controls (men & women, age 25-79 yrs)		1 st ever non-fatal MI	Total fish & fresh fish intake stratified by no. portions/wk (<1, 1-2 or ≥2)	32% ↓ in risk for those eating ≥2 fish portions/wk compared to <1 portion/wk no significant association between fresh fish and endpoint
National Health and Nutrition Examination Survey 1 (NHANES 1) (Gillum <i>et al</i> , 1996)	Cohort	5,192 men & women 45-74 years	12	Stroke	Fish meals stratified by frequency (never, <1/wk, 1/wk, ≥1/wk)	45% ↓ in risk of stroke in white women aged 45-74 consuming fish ≥1/wk 49% ↓ in risk of incidence of stroke, and 74% ↓ in risk of stroke death in black men & women aged 45-74 consuming any fish
National Health and Nutrition Examination Survey (NHANES 1) (Gillum <i>et al</i> , 2000)	Cohort	8825 men & women (25-74y) free of CVD	19	CVD & total mortality. IHD incidence (analysis by gender & race)	Fish meals stratified by frequency (never, <1/wk, 1/wk, ≥1/wk)	24% ↓ in risk of total mortality in white men consuming 1 meal/week (Sig inverse trend for fish consumption and all-cause mortality in white men. Non-significant trend in white and black women. No association in black men)
Norway (Vollset <i>et al</i> , 1985)	Cohort	11,000 men	14	MI, CHD & all-cause mortality	Fish index (frequency of consumption)	No association between fish consumption and death from CHD
Nurses Health (Iso <i>et al</i> , 2001)	Cohort	79,839 women aged 34-59 & free of CVD at baseline	14	Stroke	Fish meals stratified by frequency (<1/mo, 1-3/mo, 1/wk, 2-4/wk, ≥5/wk) quintiles of LC n-3 PUFA intake	48% ↓ in risk of thrombotic infarction for women eating 2-4 servings/wk 28% ↓ in risk of total stroke in highest quintile 33% ↓ in risk of ischaemic stroke in 3rd quintile
Nurses Health (Hu <i>et al</i> , 2002)	Cohort	84,688 women aged 34-59 yrs & free of CVD at baseline	16	Total CHD, Fatal CHD, Non-fatal MI	Fish meals stratified by frequency (<1/mo, 1-3/mo, 1/wk, 2-4/wk, ≥5/wk)	Inverse relationship between fish consumption and risk of each endpoint. Total CHD: 21% ↓ in risk for 1-3/mo, 34% ↓ in risk for ≥5/wk. Fatal CHD: 34% ↓ in risk for 1/wk, 45% ↓ in risk for ≥5/wk. Non-fatal MI: 26% ↓ in risk for 1/wk, 27% ↓ in risk for ≥5/wk.
Rotterdam (Kromhout <i>et al</i> , 1995)	Cohort	272 men & women >63 years	17	CHD & total mortality	fish intake (median 24 g/d) vs. no fish intake	49% ↓ in risk of CHD mortality for fish-eaters (men and women) 59% ↓ in risk for men eating fish (no association with women). 66% ↓ in risk of MI in fish eating men. No association with total mortality

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Table 1.1 continued

Study	Study Type	Population	Follow-up (years)	Endpoints	Comparisons	Major Outcomes:
Swedish Twins (Norell <i>et al</i> , 1986)	Cohort	10,966 men & women aged 42-80	14	MI & CHD mortality	Fish consumption stratified as high, moderate & low	30% ↓ in risk of MI mortality in high fish consumption gp compared to low fish consumption
US Physicians Health (Morris <i>et al</i> , 1995)	Cohort	21,185 men aged 40-84 yrs	4	CVD mortality, Total CVD events, Total MI, Non-fatal MI Stroke,	Fish meals stratified by frequency (<1/wk, 1/wk, 2-4/wk, ≥5/wk)	No association with fish consumption and any endpoint
US Physicians Health (Albert <i>et al</i> , 1998)	Cohort	20,551 men aged 40-84 yrs	11	SCD, Non-sudden cardiac death, CHD, CVD, & total mortality	Fish meals stratified by frequency (<1/mo, 1-3/mo, 1/wk, 2-4/wk, ≥5/wk)	SCD: 53% ↓ in risk for 1-2/wk, 61% ↓ in risk for ≥5/wk (48% ↓ in risk for ≥1/wk) Total mortality: 29% ↓ in risk for 1-2/wk, 27% ↓ in risk for ≥5/wk (30% ↓ in risk for ≥1/wk) No association with MI, non-sudden cardiac death, CHD, or CVD mortality
Västerbotten Intervention Programme (Hallgren <i>et al</i> , 2001)	Case-control	78 cases (80% men) 156 controls (80% men) without previous AMI or stroke		1 st ever AMI	Fish consumption dichotomised by intake (<1 or ≥1 meal/wk)	No association with fish consumption (fatty fish or lean fish)
Zutphen (Kromhout <i>et al</i> , 1985)	Cohort	852 men aged 40-59 years	20	CHD mortality	Fish consumption stratified by daily intake (0, 1-14, 15-29, 30-44, ≥45 g/d)	64% ↓ in risk for consumption ≥30g fish/d
Zutphen (Keli <i>et al</i> , 1994)	Cohort	552	15	stroke	Fish consumption dichotomized by daily intake (<20 g/d vs. ≥20 g/d)	51% ↓ in risk of total stroke
Zutphen & 3 Cohorts from Seven Countries Study (Oomen <i>et al</i> , 2000)	Cohort	2738 men	20	CHD mortality	Fish consumption by type (fatty, lean, total) 0 consumption vs. any consumption	34% ↓ in risk for fatty fish No association between lean fish or total fish consumption and CHD mortality

Table 1.2 Studies examining the effects of LC n-3 PUFA intake on cardiovascular disease

Study	Study Type	Population	Follow-up (years)	Endpoints	Comparisons	Major Outcomes:
Multiple Risk Factor Intervention Trial (MRFIT) (Dolecek & Grandits, 1991)	Cohort	6,258 men aged 35-57 at high risk for CHD assigned to usual care arm of MRFIT	6-8	CHD, CVD & total mortality	Regression analysis of total LC n-3 PUFA (EPA+DPA+DHA) intake and endpoints (quintiles; 0.00, 0.01, 0.05, 0.15, 0.66 g/d)	40%, 41%, & 24% ↓ in risk of CHD mortality, CVD mortality & total mortality respectively from highest to lowest quintile.
Health Professionals Follow-Up (Ascherio <i>et al</i> , 1995)	Cohort	44,895 male health professionals aged 40-75 years	6	CABG, non-fatal MI, any MI, CHD mortality, any CHD	Regression analysis of LC n-3 PUFA intake and endpoints (quintiles; 70, 150, 240, 340 & 580 mg/d)	No association between fish consumption and any endpoint.
Health Professionals Follow-Up (He <i>et al</i> , 2002)	Cohort	43,671 male health professionals aged 40-75 years at enrolment	12	Stroke	Stratified LC n-3 PUFA intake (<50, 50-200, 200-400, 400-600, >600 mg/d)	Significant ↓ in risk of ischaemic stroke of 44%, 37%, 45% in strata 2, 3 & 4 respectively. 27% ↓ risk in strata 5 (>600 mg/d) was not significant (95% CI 0.43-1.25) No association with total stroke or haemorrhagic stroke
Seattle (Siscovick <i>et al</i> , 1995)	Case-control	334 cases 493 controls Aged 25-74, & all without prior evidence of CVD	n/a	Primary cardiac arrest	EPA+DHA intake (0 intake & quartiles) Mean of quartiles; 32, 98, 182 & 450 mg/d	30%, 50% & 60% ↓ in risk associated with 2 nd , 3 rd & highest quartiles respectively of EPA+DHA intake compared to zero intake
US Physicians Health (Albert <i>et al</i> , 1998)	Cohort	20,551 men aged 40-84 yrs	11	SCD	LC n-3 PUFA intake (0 intake & quartiles: 0.3-<2.7 g/mo, 2.7-<4.9 g/mo, 4.9-<7.4 g/mo, ≥7.4 g/mo)	SCD: 66% ↓ in risk for 2 nd quartile, 57% ↓ in risk for highest quartile vs. 0 intake
Indian Experiment of Infarct Survival-4 (Singh <i>et al</i> , 1997)	Randomised dietary intervention	360 patients with suspected AMI	1	SCD CHD mortality NF MI Total CHD	MaxEPA (1.8 g EPA+DHA/d vs. placebo (100 mg/d aluminum hydroxide)	30% ↓ in risk of Total Cardiac Events in fish oil group 48% ↓ in CHD mortality 49% ↓ in non-fatal reinfarction
GISSI-Prevenzione Trial (GISSI Investigators, 1999)	Randomised dietary intervention	11324 men with recent (≤3 mo) MI	42 mo	total, CVD & CHD mortality, SCD	860 mg EPA + DHA / d vs. placebo	Consumption of n-3 PUFA resulted in: 20% ↓ in all cause mortality 30% ↓ in cardiovascular deaths (incl. 35% ↓ in cardiac death, 35% ↓ in coronary death, 45% ↓ in sudden death)

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Table 1.2 continued

Study	Study Type	Population	Follow-up (years)	Endpoints	Comparisons	Major Outcomes:
Milan (Tavani <i>et al</i> , 2001)	Case-control	507 cases 478 controls (men & women, age 25-79 yrs)	n/a	1 st ever non-fatal MI	intake of n-3 PUFA stratified by tertile (lowest, intermediate, highest)	33% ↓ in risk from intermediate & highest to lowest tertiles of n-3 PUFA intake sub-group analysis: ↓ in risk from highest to lowest tertile for n-3 PUFA intake; 61% in current smokers; 58% for subjects with total cholesterol >200 mg/dl; 60% in subjects with a 1 st degree relative with AMI
Nurses Health (Iso <i>et al</i> , 2001)	Cohort	79,839 women aged 34-59 & free of CVD at baseline	14	Stroke	Quintiles of n-3 PUFA intake (0.077, 0.118, 0.171, 0.221, 0.481 g/d)	Total stroke; 31% & 28% ↓ in risk in 3 rd & 5 th quintiles respectively, Thrombotic infarction ; 36% ↓ in risk in 3 rd quintile , Ischaemic stroke; 33% ↓ in risk in 3 rd quintile
Nurses Health (Hu <i>et al</i> , 2002)	Cohort	84,688 women aged 34-59 yrs in 1980	16	Total CHD, Fatal CHD, Non-fatal MI	Quintiles of n-3 PUFA intake (0.03, 0.05, 0.08, 0.14, 0.24 % of dietary energy from lowest to highest quintiles respectively)	Inverse relationship between average n-3 intake and risk of each endpoints: Total CHD: 22% ↓ in risk in 3 rd quintile, 33% ↓ in risk in 5th quintile. Fatal CHD: 31% ↓ in risk in 3 rd quintile, 37% ↓ in risk in 5th quintile . Non-fatal MI: 25% ↓ in risk in 4th quintile, 31% ↓ in risk in 5th quintile.

Table 1.3 Studies examining biomarkers of fish or n-3 PUFA consumption on cardiovascular disease

Study	Study Type	Population	Follow-up (years)	Endpoints	Comparisons	Major Outcomes:
Cardiovascular Health Study (Lemaitre <i>et al</i> , 2003)	Nested Case-Control	179 cases & controls, men & women ≥ 65 yrs free of CHD	n/a	Fatal CHD, Non-fatal MI	Plasma Phospholipid EPA + DHA,	70%↓ in risk of fatal CHD associated with 1 SD increase in plasma phospholipid EPA + DHA. Lower plasma phospholipid EPA+DHA in cases of fatal CHD than controls No association with non-fatal MI
EURAMIC Study (Guallar <i>et al</i> , 1999)	Case-control	639 cases 700 controls all men ≤ 70 yrs	n/a	1 st ever AMI	Adipose tissue DHA	No association
Kuopio Ischaemic Heart Disease Risk Factor Study (Rissanen <i>et al</i> , 2000)	Cohort	1871 men aged 42-60 yrs with no CVD at baseline	10	Acute coronary events (MI + acute chest pain)	Quintiles of serum DPA + DHA	44%↓ in risk associated with DPA + DHA in highest quintile compared to lowest.
MRFIT (Simon <i>et al</i> , 1995)	Case-control	94 cases & 94 controls from Usual care group of MRFIT	6.9	CHD (Total MI or SCD)	Serum phospholipid DPA (22:5 n-3) & DHA (22:6 n-3)	Approx 40% ↓ in risk associated with a 1SD increase in serum phospholipid DPA and DHA
Oslo (Pedersen <i>et al</i> , 2000)	Case-control	100 cases (72% men) 98 controls (72% men) aged 45-75 yrs	n/a	1 st ever non-fatal MI	Quintiles of adipose tissue LC n-3 PUFA	82% ↓ in RR from highest to lowest quintile
Physicians' Health (Guallar <i>et al</i> , 1995)	Nested Case-control	222 case-control pairs from within Physicians Health study cohort	5	Total MI	Quintiles of plasma phospholipid & cholesterol ester n-3 PUFAs	No relationship between plasma phospholipid or cholesterol ester n-3 PUFA and incidence of MI
Physicians' Health (Albert <i>et al</i> , 2002)	Case-control	94 cases & 184 controls, all without prior evidence of CVD. All men aged 40-84 yrs at baseline	17	SCD	Baseline whole blood LC n-3 PUFA into quartiles	n-3 PUFA content inversely related to risk of SCD 72% & 81% ↓ in risk from 3 rd and highest quartiles to lowest quartile.
Seattle (Siscovick <i>et al</i> , 1995)	Case-control	334 cases 493 controls Aged 25-74, & all without prior evidence of CVD	n/a	SCD	Erythrocyte LC n-3 PUFA stratified by quartiles (means of quartiles; 3.3%, 4.3%, 5.5%, 6.5% of total fatty acids)	50%, 70% & 90% ↓ in risk associated with erythrocyte LC n-3 PUFA in 2 nd , 3 rd and highest quartiles respectively compared to lowest quartile
Västerbotten Intervention Programme (Hallgren <i>et al</i> , 2001)	Nested Case-control	78 cases & 156 matched controls	n/a	1 st ever AMI	Plasma phospholipid EPA+DHA (tertiles)	57%↓ in risk associated with PP EPA+DHA >6.5% of total fatty acids compared to 5.5% of total fatty acids

1.4.1.9 Concluding Remarks on the Effects of LC n-3 PUFA on Cardiovascular Disease

There is now a large literature of studies examining the association between cardiovascular disease and fish/fish oil consumption or n-3 PUFA status. While many of these studies have found positive associations, there are also some that have found no association. The majority of these studies are observational, i.e. cohort or case-control studies, and therefore, only associations can be determined from these studies rather than addressing causality. Comparisons between individual studies are often difficult due to the large number of individual endpoints examined, e.g. total mortality, CVD mortality, CHD mortality, sudden death, CHD events, stroke, and MI (fatal and/or non fatal), and the number of markers employed, e.g. total fish consumption, fatty fish consumption, n-3 PUFA intake, and plasma, erythrocyte, whole blood and adipose tissue n-3 PUFA levels. Differences in the definitions used to classify the various endpoints (e.g. sudden death) and calculate the markers (e.g. calculations of fish consumption and n-3 PUFA intake) make direct comparisons between studies difficult.. There are also vast differences in the demographics of the populations studied, ranging from blue collar workers at high risk of cardiovascular disease through to health professionals, physicians and vegetarians with few risk factors for CVD. Combinations of these differences may account for the variation in the outcomes reported in these observational studies, and highlight the dangers of over-interpreting the findings from observational studies.

The results of the randomised intervention studies have all demonstrated a benefit of increased fish consumption or fish oil on cardiovascular health.

1.4.2 The Effects of α -Linolenic Acid Intake on Cardiovascular Disease Risk in Humans

In addition to the benefits of LC n-3 PUFA of marine origin, there have also been reports of consumption of the plant-derived n-3 PUFA, ALA providing benefit by reducing the risk of cardiovascular disease.

1.4.2.1 Studies Showing a Benefit of ALA Consumption

1.4.2.1.1 Cohort Studies

In the 'usual care' arm of the MRFIT study, there was a significant inverse relationship between total mortality and ALA consumption from the lowest quintile (mean intake 0.87 g/d) to the highest quintile (mean intake 2.80 g/d) with an adjusted risk reduction of 31% (Dolecek & Grandits, 1991). The inverse associations between ALA consumption and both CHD mortality and CVD mortality, with adjusted RRs of 0.68 and 0.63 respectively for ALA consumption in the highest quintile compared to the lowest, did not reach statistical significance with p values of 0.15 and 0.07 respectively for the trends.

A difference in ALA consumption equivalent to a 1% increase in dietary energy was associated with an adjusted RR for MI of 0.41 (95% CI, 0.21-0.80) in the Health Professionals Follow-Up Study (Ascherio *et al*, 1996). In a 10 Mj diet, 1% of dietary energy equates to 2.7 g ALA. Higher dietary intake of ALA was also found to be associated with a decreased risk of CHD mortality amongst women in the Nurses' Health Study (Hu *et al*, 1999), with participants in the highest quintile of ALA intake (with a mean intake of 1.36 g/d) having an adjusted RR of 0.55 (95% CI, 0.32-0.94) compared to the quintile with the lowest intake (with a mean intake of 0.71 g/d). A significant inverse dose response was observed between ALA intake and CHD mortality. There was no

association between ALA intake and non-fatal MI (adjusted RR, 0.85; 95% CI, 0.61-1.19) for intakes in the highest quintile compared to the lowest.

In a cross-sectional analysis of 4406 men and women in the National Heart, Lung and Blood Institute Family Heart Study (Djousse *et al*, 2001), there were significant reductions in the prevalence of CHD (history of MI, percutaneous transluminal coronary angioplasty or coronary artery bypass graft, or abnormal Q-waves on 12 lead ECG) associated with ALA intake. The adjusted prevalence ORs for men with intakes in the 3rd (mean, 0.78 g/d), 4th (mean, 0.90 g/d) and 5th (mean, 1.14 g/d) quintiles were 0.61 (95% CI, 0.39-0.96), 0.58 (95% CI, 0.38-0.87) and 0.60 (95% CI, 0.39-0.92) respectively, while for women with ALA intakes in the 4th (mean, 0.76 g/d) and 5th (mean, 0.96 g/d) quintiles were 0.30 (95% CI, 0.13-0.68) and 0.42 (95% CI, 0.22-0.84) respectively. (95% CI,)

1.4.2.1.2 Case-Control Studies

In the Cardiovascular Health Study, a 1 SD higher plasma phospholipid ALA was associated with an OR of fatal CHD of 0.48 (95% CI, 0.24-0.96) (Lemaitre *et al*, 2003). There was no association between a 1 SD higher plasma phospholipid ALA and non-fatal MI (OR, 1.07; 95% CI, 0.71-1.41).

1.4.2.1.3 Randomized Dietary Intervention Studies

Mustard seed oil was used as the source of ALA in the Indian Infarct Survival Study-4 (Singh *et al*, 1997), a randomized secondary prevention study, where supplementation with 2.9 g/d of ALA was associated with a 40% decrease in the incidence of total cardiac deaths, a 41% decrease in non-fatal reinfarctions and a 19% decrease in total cardiac events compared to placebo after 1 year of follow-up.

Increased ALA consumption, in conjunction with the adoption of a Mediterranean-style diet, incorporating increased consumption of fruits, vegetables, legumes and nuts, has been successful in reducing the risk of CVD mortality in secondary prevention of patients who had survived an MI, compared to those patients given standard dietary advice. In the Lyon Diet Heart Study (de Lorgeril *et al*, 1994), advice to adopt a Mediterranean- type diet, along with the provision of a rapeseed oil based margarine (experimental group), was associated with significant reductions in risk of CVD mortality (RR, 0.27; 95% CI, 0.12-0.59) and total mortality (RR, 0.30; 95% CI, 0.11-0.82) compared to patients provided with usual dietary advice from hospital dietitians or physicians (control group). The effects of the intervention were so strong that the study was stopped after mean follow-up of 27 months into a planned 5 year project. Although ALA consumption was 3-fold higher in the intervention group (mean, 0.81 g/d) than the control group (mean, 0.27 g/d), and plasma ALA and EPA levels were significantly higher in the experimental group, analysis of the relationship between ALA intake and the primary endpoints was not presented. There were 8 sudden deaths in the control group compared with none in the experimental group, suggesting an anti-arrhythmic effect of some portion of the diet. The final report of the Lyon Diet Heart Study which extended the follow-up period to a mean of 46 month/patient found significant reductions in risk associated with consumption of the experimental diet for CVD mortality (RR, 0.35; 95% CI, 0.15-0.83), total primary endpoints (CVD mortality + non-fatal MI; RR, 0.28; 95% CI, 0.15-0.53), and total mortality (RR, 0.44; 95% CI, 0.21-0.94). At the 46 month analysis, there was a significant association between plasma ALA and total primary endpoints (RR, 0.20, 95% CI, 0.05-0.84) (de Lorgeril *et al*, 1999).

The Indo-Mediterranean Diet Heart Study (Singh *et al*, 2002) was a randomized dietary intervention study comparing an Indian adaptation of the Mediterranean diet with the NCEP Step 1 diet among 1000 patients at high risk of CHD, having either angina pectoris, MI or surrogate risk factors of CHD. The mean ALA consumption in the intervention arm was 1.8 g/d, predominantly from mustard oil, soybean oil and walnuts, compared with a mean ALA consumption of 0.8g/d in the control arm. After 2 years of follow-up, the intervention diet was associated with significant reductions in risk for non-fatal MI (rate ratio, 0.47; 95% CI, 0.28-0.79), SCD (rate ratio, 0.33; 95% CI, 0.13-0.86) and total cardiac endpoints (rate ratio, 0.48; 95% CI, 0.33-0.71).

1.4.2.2 Studies Showing no Benefit of ALA Consumption

Not all studies have reported inverse associations between ALA and CVD risk. The Zutphen Study reported an increased risk of CHD events associated with ALA intake (Oomen *et al*, 2001) and the Norwegian Study (Pedersen *et al*, 2000) reported an increased risk of CHD events associated with adipose tissue ALA content (Pedersen *et al*, 2000). However, both studies also found positive associations between ALA and *trans*-fatty acids, with the investigators concluding in each case, that the association probably occurred through margarine consumption, and that the increased CHD risk was possibly due to increased *trans*-fatty acids. The Zutphen study also undertook separate analysis of cases where ALA consumption came from sources not containing *trans*-fatty acids, and found no association with CHD risk (Oomen *et al*, 2001). The ALA consumption in this group was 10-fold lower than in the group consuming *trans*-fatty acids, with mean intake around 0.05% of energy, or 100 – 150 mg/d, and was probably not sufficient to have an effect.

An examination of adipose tissue ALA failed to find an association with the incidence of first MI in the multicentre EURAMIC study, with a multivariate OR for adipose tissue ALA in the highest quintile of 0.70 (95% CI, 0.32-1.54) compared to the lowest quintile. There was a significant association between adipose tissue ALA in the highest quintile and MI for age and centre adjusted data only (OR, 0.42; 95% CI, 0.22-0.81) (Guallar *et al*, 1999)

The studies presented above have been summarized in tabular form (Table 1.4).

Table 1.4 Studies examining the effects of α -linolenic acid consumption on cardiovascular disease

Study	Study Type	Population	Follow-up (years)	Endpoints	Comparisons	Major Outcomes:
Cardiovascular Health Study (Lemaitre <i>et al</i> , 2003)	Nested Case-Control	179 cases & controls, men & women ≥ 65 yrs free of CHD	n/a	Fatal CHD, Non-fatal MI	Plasma Phospholipid ALA	52%↓ in risk of fatal CHD associated with 1 SD increase in plasma phospholipid ALA. No association with non-fatal MI
EURAMIC Study (Guallar <i>et al</i> , 1999)	Case-Control	639 cases 700 controls all men ≤ 70 yrs	n/a	1 st ever AMI	Adipose tissue ALA (quintiles)	31% ↓ in risk associated with ALA in highest quintile compared to lowest (age and centre adjusted only). No significant ↓ in risk in multivariate analysis
Health Professionals Follow-Up Study (Ascherio <i>et al</i> , 1996)	Cohort	43,757 male health professionals aged 40-75 years	6	Total MI, CHD mortality	Regression analysis of ALA intake and CVD	59% ↓ in risk of MI associated with increase in ALA intake by 1% of energy
Indian Experiment of Infarct Survival-4 (Singh <i>et al</i> , 1997)	Randomised dietary intervention	360 patients with suspected AMI	1	SCD CHD mortality Non-fatal MI Total CHD	Mustard seed oil (2.9 g ALA/d) vs. placebo (100 mg/d aluminum hydroxide)	41%↓ in non-fatal reinfarction 40%↓ in CHD mortality 19% ↓ in total CHD No association with SCD
Indo-Mediterranean Diet Heart Study (Singh <i>et al</i> , 2002)	Randomized Dietary Intervention	1000 men & women with angina, MI or CHD risk factors	2	MI (fatal & non-fatal), SCD, total cardiac endpoint	Mediterranean diet + mustard or soybean oil vs. Step 1 NCEP prudent diet	53% ↓ in non-fatal MI 67% ↓ in SCD 52% ↓ in total cardiac events
Lyon Diet Heart Study (de Lorgeril <i>et al</i> , 1994)	Randomized Dietary Intervention	584 men & women post MI	27 mo	CVD mortality Non-fatal MI	Randomized advice 1) usual hospital advice 2)Mediterranean diet	Experimental diet associated with: 76%↓ in risk for CVD mortality 73% ↓ in risk for total primary endpoints 81% ↓ in risk for cardiac death
Lyon Diet Heart Study (de Lorgeril <i>et al</i> , 1999)	Randomized Dietary Intervention	584 men & women post MI	46 mo	CVD mortality Non-fatal MI	Randomized advice 1) usual hospital advice 2)Mediterranean diet	80% ↓ in risk of primary endpoints associated with plasma ALA Experimental diet associated with: 65%↓ in risk for CVD mortality 72% ↓ in risk for total primary endpoints 56% ↓ in risk for total mortality
Multiple Risk Factor Intervention Trial (MRFIT) (Dolecek & Grandits, 1991)	Cohort	6,258 men aged 35-57 at high risk for CHD assigned to usual care arm of MRFIT	6-8	CHD, CVD & all-cause mortality	Regression analysis of ALA intake & endpoints (Quintiles of ALA consumption; 0.87, 1.27, 1.58, 1.93 & 2.80 g/d)	31% ↓ in risk for all cause mortality from highest to lowest quintiles of ALA intake No association with CHD or CVD mortality

continued next page

Table 1.4 continued

Study	Study Type	Population	Follow-up (years)	Endpoints	Comparisons	Major Outcomes:
National Heart, Lung, & Blood Institute Family Heart Study (Djousse <i>et al</i> , 2001)	Cross-sectional	2024 men & 2382 women	n/a	Any CHD	Quintiles of ALA intake (0.53, 0.67, 0.78, 0.90 & 1.14 g/d for men; 0.46, 0.58, 0.65, 0.76 & 0.96 g/d for women)	Inverse association of ALA consumption and CHD. 40% ↓ in risk in men from lowest to highest quintile 58% ↓ in risk in women from lowest to highest quintile
Nurses Health Study (Hu <i>et al</i> , 1999)	Cohort	76,283 women aged 30-55 at baseline	10	CHD mortality, Total CHD, Non-fatal MI	Quintiles of ALA consumption (0.71, 0.86, 0.98, 1.12 & 1.36 g/d) and endpoints.	45% & 25% ↓ in risk for CHD mortality and total CHD respectively in highest quintile. No association with non-fatal MI
Oslo (Pedersen <i>et al</i> , 2000)	Case-Control	100 cases (72% men) 98 controls (72% men) aged 45-75 yrs	n/a	1 st ever non-fatal MI	Quintiles of adipose tissue LC n-3 PUFA	ALA positively associated with risk of MI (ALA associated with <i>trans</i> -fatty acids)
Zutphen Study (Oomen <i>et al</i> , 2001)	Cohort	667 men free of CHD at baseline	10	CHD mortality, Non-fatal MI	Regression analysis of ALA consumption and endpoints	No association between ALA and CHD risk. (ALA associated with <i>trans</i> -fatty acid intake). When examined without sources containing <i>trans</i> -fatty acids, still no association with CHD risk (intakes 10 fold lower, 0.05% of energy, 0.1 – 0.15 g/d)

1.4.3 Possible Mechanisms of Cardiovascular Protection

A number of possible mechanisms have been proposed for the cardio-protective effects of n-3 PUFAs, including the prevention of arrhythmias, triglyceride lowering, anti-inflammatory properties, stimulation of endothelial nitric oxide, and anti-thrombotic actions (Kris-Etherton *et al*, 2002). This thesis focuses on arrhythmias and is not concerned with the other potentially protective mechanisms. The remainder of this chapter will examine the experimental evidence for anti-arrhythmic effects of n-3 PUFAs.

1.4.3.1 Anti-Arrhythmic Properties of n-3 PUFA

A ventricular arrhythmia is a potentially fatal abnormal heart rhythm that results from electrical impulses arising from the ventricles instead of the heart's natural pacemaker (the sino-atrial node).

The evidence for the anti-arrhythmic properties of n-3 PUFA come primarily from animal and cell culture experiments conducted over the past 15 years.

1.4.3.1.1 Animal models

Animal models used to investigate the effect of n-3 fatty acids on cardiac arrhythmias include, pigs, rats, marmoset monkeys and dogs, with a range of experimental methods including coronary artery occlusion and reperfusion, isolated perfused working hearts utilizing Langendorff techniques, and programmed electrical stimulation (PES). A description of the studies follows and an overview is provided in Table 1.5.

1.4.3.1.1.1 *Surgical occlusion and reperfusion*

Despite a number of conflicting early reports on the effects of dietary fish oil on ventricular arrhythmias (Hartog *et al*, 1986; Hartog *et al*, 1987), McLennan and co-workers conducted a number of experiments in rats and later in marmoset monkeys, that demonstrated conclusively the anti-arrhythmic effects of n-3 PUFAs in these animal models. Using a technique of surgical ligation of a coronary artery to produce ischaemia and then releasing the tension on the ligature to produce a period of reperfusion, they were able to demonstrate significant reductions in the incidence of both ventricular tachycardia (VT) and ventricular fibrillation (VF) of the order of 30% - 80% during ischaemia and reperfusion compared to either a low fat or saturated fat diet (McLennan *et al*, 1988; McLennan *et al*, 1989; McLennan *et al*, 1990; McLennan, 1993). They were also able to demonstrate reductions in the mean duration of VT and VF in some (McLennan *et al*, 1988; McLennan *et al*, 1989) but not all studies (McLennan *et al*, 1990; McLennan, 1993).

A number of these studies, but not all, have also demonstrated a protective effect of n-6 PUFA during ischaemia (McLennan *et al*, 1988; McLennan *et al*, 1989; McLennan *et al*, 1990; Charnock *et al*, 1991; McLennan, 1993). However, during reperfusion n-3 PUFA appears to have a clear advantage over n-6 PUFA in reducing arrhythmias, with only 2 studies showing n-6 PUFA as effective as n-3 PUFA (McLennan *et al*, 1990; McLennan *et al*, 1993).

For n-6 PUFA to be effective in reducing arrhythmias, they must be present in the diet in high levels. In particular, Charnock *et al* (1991) demonstrated that fish oil can significantly reduce arrhythmias during ischaemia against a background diet high in saturated fat (13% n-3 PUFA, 41% saturates), whereas the n-6 PUFAs are only effective in high proportions

against a low saturated fat background (57% n-6 PUFA, 15% saturates), and totally without effect against background diet high in saturated fat.

Billman and co-workers (1994) prepared a canine model of VF by surgically occluding the left anterior descending coronary artery to produce a myocardial infarction, and placing an inflatable cuff around the circumflex artery, thereby enabling VF to be reliably induced by inflating the cuff during treadmill exercise. Dogs which are susceptible to VF using this model are always inducible under these conditions in subsequent tests. They were infused with an emulsion of EPA and DHA in the free fatty acid (FFA) form about 1 hour prior to repeating the protocol, and 10 of 13 dogs were protected from developing VF. The protocol was repeated again 1 week later with an infusion of either saline or Intralipid, with all animals developing VF. Infusion of the n-3 PUFA emulsion was associated with significant reductions in heart rate whilst resting and during exercise, a shortening of the QTc interval, and a prolongation of the P-R interval (Billman *et al*, 1997). In a later paper, they reported that purified EPA and DHA, when infused bound to albumin, were equally efficacious in protecting dogs from fatal VF (Billman *et al*, 1999).

1.4.3.1.1.2 Isolated perfused working hearts

The use of the isolated perfused working heart model allows the anti-arrhythmic properties of the dietary fatty acids which have been incorporated into cardiac membranes to be examined without nervous, hormonal or circulating cellular influences. Utilising this methodology, Sargent and Riemersma (1990), Yang *et al* (1993a), Isensee and Jacob (1994), Pepe and McLennan (1996) and Anderson *et al* (1996) all found decreases in the incidence and severity of arrhythmias in rats fed fish oil compared to control diets consisting of saturated fat, n-6 PUFA or low fat reference diets.

1.4.3.1.1.3 Programmed Electrical Stimulation

Programmed electrical stimulation (PES) is an electrophysiological technique where catheter electrodes are placed directly onto the myocardium, which allows pacing of the heart by the application of an electrical impulse to the myocardium via an external stimulator. The electrodes can be placed either externally via open heart surgery in experimental animal models, or internally via percutaneous catheterization in animals and humans. This system allows the assessment of the conduction properties of the heart, and also allows the induction and termination of cardiac arrhythmias using various electrical stimulation protocols.

Experiments utilizing PES with marmoset monkeys showed that long term (30 months) dietary LC n-3 PUFAs in the form of fish oil (23% of total fat) and n-6 PUFAs in the form of sunflower seed oil (54% of total fat) have equivalent efficacy in increasing the VF threshold (VFT, the amount of current required to induce a VF using a standardized stimulation protocol), under both control and ischaemic conditions, compared to saturated fat. During ischaemia, the incidence of sustained VF (> 30 secs) was significantly lower in fish oil fed animals than in those fed a saturated fat diet (McLennan *et al*, 1992b). Under the influence of the β -adrenergic receptor agonist, isoproterenol (ISO), there was a larger proportion of animals fed fish oil that were non-inducible (56% compared to 33% for those fed saturated fat and 23% for those fed LA rich sunflower seed oil) (McLennan *et al*, 1992b).

A similar protective effect of fish oil over saturated fat was seen with PES in isolated, perfused, working rat hearts where the VFT was about 3 fold higher under control

conditions and twice a high under ischaemic conditions in rats fed fish oil compared to saturated fat (Pepe & McLennan, 1996).

When two groups of marmosets were fed diets with the same level of saturated fat but mixed with fish oil or sunflower seed oil, those fed a fish oil/saturated fat diet had a VFT more than double that of animals fed the n-6/saturated fat diet under both control and ischaemic conditions (McLennan *et al*, 1993), demonstrating greater anti-arrhythmic potency of the LC n-3 PUFAs over n-6 PUFAs .

1.4.3.1.1.4 Induction of arrhythmias by pro-arrhythmic stressors

Administration of a fatal dose of digitalis immediately following coronary artery ligation resulted in the onset of VT/VF within 15 minutes in all 5 dogs fed a control diet. In 5 animals supplemented with 100 mg/kg/d of EPA for 8 weeks, the minimum time for the development of an arrhythmia following digitalis administration was 25 minutes, with 1 animal not developing an arrhythmia at all (Kinoshita *et al*, 1994). In marmoset monkeys undergoing PES, infusion of ISO resulted in a significant increase in the proportion of animals having sustained episodes of VF. Animals fed a diet supplemented with tuna oil for 30 months suffered significantly fewer episodes of sustained VF than animals fed a low fat reference diet or an iso-caloric diet high in saturated fat (McLennan *et al*, 1992b).

1.4.3.1.2 Anti-arrhythmic effects of ALA in experimental animals

McLennan and Dallimore (1995) demonstrated a protective effect in rats of ALA on the incidence of arrhythmias in reperfusion (but not in ischaemia) through the addition of canola oil to the diet. However, when a similar amount of ALA was added via soybean oil (7.3% vs. 8% in canola), which has a much higher proportion of LA (50% vs. 20% in

canola), and therefore a much higher n-6/n-3 ratio (7.0 vs. 2.7), the protective effect of the n-3 fatty acid was no longer apparent. The myocardial phospholipids of the animals fed the canola supplement had significantly higher levels of n-3 PUFA, and significantly lower levels of n-6 PUFA than those fed the soybean supplement.

An ALA supplement in the form of linseed oil also provided a protective effect against VF during reperfusion compared to a low fat reference diet, but not against VF in ischaemia or against VT (Isensee & Jacob, 1994). Purified FFAs of ALA were also found to be equally as efficacious as EPA and DHA in protecting dogs from fatal VF in the model prepared by Billman *et al* (1999).

Table 1.5 Studies examining the effect of n-3 PUFA supplementation on arrhythmia in experimental animals

Reference	Animal Model	Experimental Model	Supplement	Reference Group(s)	Amount of Supplementation	Duration	Major Outcomes
Culp <i>et al</i> (1980)	dogs	Electrically induced coronary artery thrombosis	MO	commercial dog chow	25% of energy	36 – 45 d	VF (sudden death): no diff between gps. PVC: incidence – MO 30% vs. control 80% (stats not included) Infarct size: MO < control (3% vs. 25%)
Hartog <i>et al</i> (1986)	pigs	Surgical occlusion (5 mins) & reperfusion (10 mins) repeated 5 times	Mackerel oil (26% EPA+DHA)	lard	9.1% (w/w)	8 wks	VT/VF: No diff between control & experimental gps
Hartog <i>et al</i> (1987)	pigs	Surgical occlusion (5 mins) & reperfusion (10 mins) repeated 5 times	Mackerel oil/lard	lard	4.5% (w/w)	16 wks	VT/VF: incidence – reperfusion; mackerel/lard < lard PVC: incidence – reperfusion; mackerel/lard < lard during (9/animal vs. 26/animal)
McLennan <i>et al</i> (1988)	rats	Surgical occlusion (15 mins) & reperfusion (10 mins)	TFO (23% EPA + DHA)	LF(ref) SF SSO	12% (w/w)	12 mo	(values presented for TFO, SSO, LF, SF respectively) VT: incidence - TFO < SSO, LF & SF during ischaemia (20%, 80%, 91%, 91%) & reperfusion (50%, 80%, 92%, 100%); mean duration - TFO < SSO, LF, SF (~3s, ~16.5s, ~14.5s, ~14s) during reperfusion VF: incidence - ischaemia; TFO & SSO < LF & SF (nil, 10%, 36%, 64%), reperfusion; TFO < SSO, LF, SF during (nil, 30%, 36%, 50%). No VF occurred in TFO gp, so unable to compare duration Arrhythmia score¹: ischaemia – TFO < LF & SF (1.8, 2.0, 3.4, 4.9), reperfusion – TFO < SSO, LF, SF (1.5, 2.4, 3.1, 4.3)
McLennan <i>et al</i> (1989)	rats	Surgical occlusion (15 mins) & reperfusion (10 mins)	TFO (23% EPA + DHA)	LF(ref) SF SSO	12% (w/w)	6, 12, 18 mo	Only reported comparisons against LF gp (values presented for TFO, SSO, LF, SF respectively) VT: incidence - ischaemia; TFO & SSO < LF (41%, 68%, 93%, 97%), reperfusion TFO < LF (59%, 82%, 94%, 100%). mean duration - ischaemia ;TFO & SSO < LF < SF (15s, 15s, 25s, 38s) reperfusion ; TFO < LF (7s, 15s, 18s, 15s). VF: incidence - ischaemia; TFO & SSO < LF – (6%, 21%, 46%, 68%), reperfusion ; TFO < LF (12%, 30%, 50%, 70%); mean duration - ischaemia; TFO & SSO < LF < SF (2s, 21s, 36s, 42s), reperfusion ; TFO < LF (11s, 30s, 48s, 60s)
Hock <i>et al</i> (1990)	rats	Surgical occlusion (15 mins) & reperfusion (6 or 24 hrs)	MO	CO	12% (energy)	4 wks	Combined incidence VT/VF: ischaemia & reperfusion: MO < CO (14% vs. 91%) Arrhythmia score¹: MO < CO (1.3 vs. 5.6) % Survival following 24hrs reperfusion: MO > CO (76% vs. 41%)

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Table 1.5 continued

Reference	Animal Model	Experimental Model	Supplement	Reference Group(s)	Amount of Supplementation	Duration	Major Outcomes
McLennan <i>et al</i> (1990)	rats	Surgical occlusion (15 mins) & reperfusion (10 mins)	TFO (23% EPA + DHA)	SF SSO	12% (w/w)	9 mo following 9 mo on SF diet	(values presented for TFO, SSO, SF respectively) Log₁₀ PVC: reperfusion ; TFO < SSO & SF (1.79, 2.34, 2.24) VT: incidence - ischaemia ; TFO & SSO < SF (57%, 57%, 100%), reperfusion ; no diff (71%, 86%, 100%); mean duration; no diff during either ischaemia (47.7s, 74.4s, 35.0s) or reperfusion (10.7s, 10.7s, 12.1s). VF: incidence - ischaemia; TFO & SSO < SF (14%, 29%, 71%) reperfusion ; TFO & SSO < SF (29%, 29%, 83%) mean duration – insufficient numbers to calculate p values Arrhythmia score¹: ischaemia; TFO and SSO < SF (2.2, 2.0, 4.1); reperfusion; TFO < SSO < SF (2.1, 3.0, 6.3)
Sargent & Riemersma (1990)	rats	Isolated working heart – ischaemia (20 mins)	FO (CPL, Lipid Teknik, Stockholm)	P/S ratio 0.3	7% (energy)	Not stated	VF: incidence - FO < control (25% vs. 59%)
Charnock <i>et al</i> (1991)	rats	Surgical occlusion (15 mins)	SF/FO (MaxEPA)	SF SF/SSO SSO	6% FO w/w, (n-3 PUFA = 13% tot FA)	44 wks	(values presented for SF/FO, SSO, SF, SF/SSO respectively) PVC (beats/15 mins): SF/FO & SSO < SF & SF/SSO (353, 541, 1338, 1246) VT: incidence - no diff (70%, 70%, 100%, 100%); mean duration - SF/FO & SSO < SF & SF/SSO (5.1s, 14.8s, 98.1s, 84.5) VF: incidence - SF/FO < SF & SF/SSO. (SSO not diff to any other gp) (0%, 20%, 60%, 60%); mean duration - SF/FO & SSO < SF & SF/SSO (0s, 2.4s, 24.2s, 48.9s) Arrhythmia score¹: SF/FO & SSO < SF & SF/SSO (1.8, 2.3, 3.8, 4.4)
McLennan <i>et al</i> (1992b)	marmoset monkey	Programmed electrical stimulation	TFO (23% n-3 PUFA)	LF(ref) SF SSO	8% (w/w)	30 mo	values presented for TFO, SSO, SF, LF respectively VFT: control period - TFO & SSO > SF. LF not diff to any gp (15.1mA, 15.2mA, 8.4mA, 11.1mA). ischaemic period - TFO & SSO > LF & SF (13.5mA, 13.4mA, 6.8mA, 7.6mA) VF: incidence - ischaemia ; TFO < SF (nil, 13%, 44%, 27%) - ISO; 37%, 54%, 58%, 57% (stats not provided)

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Table 1.5 continued

Reference	Animal Model	Experimental Model	Supplement	Reference Group(s)	Amount of Supplementation	Duration	Major Outcomes
McLennan (1993)	rats	Surgical occlusion (15 mins) & reperfusion (10 mins)	FO (ShakleeEPA)	OO SF SSO	12% (w/w)	12 wks	Statistical comparisons only shown with SF (all values shown for FO, SSO, OO, SF respectively) Log₁₀ PVC: ischaemia - FO < SF (1.88, 2.09, 2.42, 2.41), reperfusion - FO < SF (1.08, 1.56, 1.93, 2.18) VT: incidence - ischaemia; FO < SF (35%, 92%, 87%, 86%), reperfusion; FO < SF (21%, 67%, 88%, 86%); mean duration - ischaemia; no diff (17s, 21s, 30s, 27s); reperfusion; no diff (1.8s, 9.1s, 16.2s, 87.9s) VF: incidence - ischaemia; FO < SF (nil, 38%, 47%, 57%), reperfusion ; no diff (nil, 8%, 63%, 29%) Arrhythmia score¹: ischaemia - FO & SSO < SF (1.9, 3.2, 4.3, 4.7) reperfusion - FO & SSO < SF (0.6, 1.6, 3.2, 3.0) Total time in sinus rhythm: ischaemia; FO & SSO > SF (879s, 784s, 638s, 574s) reperfusion; FO & SSO > SF (295s, 288s, 249s, 272s) Mortality: ischaemia ;FO & SSO < SF (nil, 8%, 40%, 50%); reperfusion; no diff (nil, nil, 13%, nil)
McLennan <i>et al</i> (1993)	marmoset monkey	Programmed electrical stimulation	SF/FO (ShakleeEPA)	SF/SSO	3% FO (w/w)	16 wks	VFT: control conditions - SF/FO > SF/SSO 33.3mA vs. 14.3mA); ischemic conditions (19.6mA vs. 5.5mA)
Yang <i>et al</i> (1993a)	rats	Isolated working heart - ischaemia (15 mins) & reperfusion (10 mins)	FO (Eskimo-3, 32% EPA + DHA)	butter	12% (w/w)	5 d	VT/ VF incidence : reperfusion - FO < control (37%, 78%)
Billman <i>et al</i> (1994)	VF susceptible mongrel dogs	Post MI Surgical occlusion	FO concentrate (70% n-3s as FFAs)	saline Intralipid	10 ml	IV infusion 50-60 mins prior	VF: incidence - FO < saline and Intralipid (13%, 100%, 100%)
Isensee & Jacob (1994)	rats	Isolated working heart - ischaemia (20 mins) & reperfusion (20 mins)	Linseed oil FO (33% n-3)	LF(ref) CCO CO	10% (w/w)	10 wks	all values shown for FO, LO, CO, CCO, LF respectively VT: incidence ; ischaemia (nil, 60%, 44%, 56%, 60%) no stats presented VF: incidence ; ischaemia - FO < all others (10%, 40%, 45%, 67%, 75%); reperfusion - FO & LO < LF (40%, 60%, 67%, 80%, 100%)

Continued next page

Table 1.5 continued

Reference	Animal Model	Experimental Model	Supplement	Reference Group(s)	Amount of Supplementation	Duration	Major Outcomes
Kinoshita <i>et al</i> (1994)	mongrel dogs	Surgical occlusion - ischaemia (3 hrs)	EPA ester	Standard chow	100mg/kg/d	8 wks	PVC: EPA < control (221/3h vs. 393/3h) VF: incidence - no diff (20% for both gps) Arrhythmia score ² : EPA < control (2.2 vs. 3.6)
McLennan & Dallimore, (1995)	rats	Surgical occlusion (5 or 15 mins) & reperfusion (10 mins)	Canola oil	OO SBO SSO	11% (w/w)	12wks	all values shown for CAN, OO, SBO, SSO, respectively (all gps compared against OO only) ischaemia - no diff in any arrhythmia parameter VF: incidence - reperfusion: CAN < OO (4%, 45%, 38%, 35%) Arrhythmia score ¹ : CAN < OO (0.9, 2.4, 2.2, 2.5) TAM: CAN < OO (8%, 33%, 22%, 17%)
Anderson <i>et al</i> (1996)	rats	Isolated working heart – reperfusion only (foll 20 mins ischaemia)	FO (Maxepa)	LF (ref) CCO SAFO	1ml/d	8 wks	all values shown for FO, SAFO, CCO, LF respectively: reperfusion only PVC: FO < CCO & LF (9, 13, 13, 16) VT: incidence - FO < CCO & LF (37.5%, 50%, 100%, 92.3%); mean duration - FO < CCO & LF (4s, 9s, 14s, 23s) respectively VF: incidence - FO < CCO & LF (12.5%, 33.3%, 85.7%, 84.6%); mean duration - FO < CCO & LF (34s, 95s, 236s, 178s) Arrhythmia score ¹ : FO < CCO & LF (1.6, 3.5, 7.4, 6.2)
McLennan <i>et al</i> (1996)	rats	Surgical occlusion & ischaemia (15 mins)	purified ethyl esters: EPA DHA EPA (26%) + DHA(37%)	OO	0.5% (w/w), 1.11% energy (0.73%en EPA + DHA mixture)	5 wks	VF: incidence - DHA & EPA/DHA < OO & EPA (20%, 10%, 80%, 70% respectively) Arrhythmia score ¹ : DHA & EPA/DHA < OO & EPA (2.1, 2.3, 4.8, 4.9)
Pepe & McLennan (1996)	rats	Isolated working heart - ischaemia (15 mins) & reperfusion (10 mins); programmed electrical stimulation	FO (ShakleeEPA)	LF(ref) SF	12% (w/w)	16 wks	PVC: ischaemia - FO < LF & SF (3, 7, 11 respectively) VT: reperfusion - FO < LF & SF (10%, 60%, 70%) VF: reperfusion - FO < LF & SF (nil, 50%, 80%) VFT: control - FO > LF & SAT (15.1mA, 6.6mA, 5.6mA); ischaemia - FO > LF & SAT (8.8mA, 5.8mA, 3.7mA) VF: Mean duration - control; FO < LF & SAT (3.3s, 20.7s, 22.7s); Ischaemia; FO < LF & SAT (7.0s, 25.0s, 27.3s)
Billman <i>et al</i> (1997)	VF susceptible mongrel dogs	surgical occlusion	FFA (70% n-3: 34% EPA, 25.5% DHA)	Intralipid or Saline (each dog served as own control)	1 - 10 g	IV	VT/VF: incidence - FO < saline or Intralipid (23% vs. 100%)

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Table 1.5 continued

Reference	Animal Model	Experimental Model	Supplement	Reference Group(s)	Amount of Supplementation	Duration	Major Outcomes
Billman <i>et al</i> (1999)	VF susceptible mongrel dogs	surgical occlusion	Purified FFA EPA DHA ALA	Intralipid or Saline (each dog served as own control)	1 ml	IV	VF: incidence - EPA , DHA, ALA < saline or Intralipid (29%, 25%, 25%, 100%)

¹ (McLennan *et al*, 1988)

² (Curtis *et al*, 1987)

Abbreviations:

Diets: CCO, coconut oil; CO, corn oil; FFA, free fatty acid; FO, fish oil; LF(ref), low fat reference diet; LO, linseed oil; MO, Menhaden oil; OO, olive oil, SAFO, safflower oil; SBO, soybean oil; SF, sheep fat; SSO, sunflower seed oil; TFO, tuna fish oil;

Outcomes: NSR, normal sinus rhythm; PVC, premature ventricular contractions; TAM, total arrhythmic mortality; VF, ventricular fibrillation; VFT, ventricular fibrillation threshold; VT, ventricular tachycardia

1.4.3.1.3 In vitro experiments

Following the initial epidemiological evidence of a decrease in fatal cardiac events, and the animal work of McLennan, Alexander Leaf and co-workers at Harvard undertook a series of *in vitro* experiments, using cultured neonatal rat cardiomyocytes to elucidate the mechanisms by which n-3 PUFAs stabilize the electrical activity of myocardial membrane and the cardiac rhythm.

1.4.3.1.3.1 Effects of n-3 PUFA on Ca^{++} channels

In their initial experiments, they demonstrated a protective effect of 5 μ M EPA in the tissue culture medium on the arrhythmogenic effects of ouabain. In the presence of ouabain, control cardiomyocytes developed toxic cytosolic Ca^{++} concentrations whereas cardiomyocytes incubated in EPA maintained physiologic Ca^{++} concentrations (Hallaq *et al*, 1990) indicating a possible effect of EPA on Ca^{++} channels. In further work, a 30% decrease in Ca^{++} influx in response to ouabain was observed in neonatal rat cardiomyocytes incubated in EPA or DHA compared to those incubated in AA or control cells (Hallaq *et al*, 1992). Nitrendipine (a dihydropyridine L-type Ca^{++} channel inhibitor) also protects against ouabain toxicity by inhibiting Ca^{++} influx, and additionally, it affects contractility of the cell. When DHA was added to nitrendipine, ouabain toxicity was prevented without inhibition of contractility observed with nitrendipine alone. EPA and DHA inhibited the binding of nitrendipine to its receptor in a non-competitive manner suggesting that the PUFA binds at a site remote from the receptor, but one which modulates the binding of nitrendipine. EPA and DHA had no effect on the other L-type Ca^{++} channel blockers verapamil and diltiazem, which have similar effects to nitrendipine in preventing ouabain toxicity and inhibiting contractility. EPA and DHA thus appear to affect a different site to these drugs (Hallaq *et al*, 1992). A similar protective effect of

DHA was demonstrated in cardiomyocytes treated with Bay K8644, an L-type Ca^{++} channel agonist with 5 μM DHA preventing the decrease in the amplitude of contractions and increase in the spontaneous beating rate associated with administration of this drug (Hallaq *et al*, 1992). Similar protective effects of DHA were subsequently demonstrated in adult rat cardiomyocytes (Pepe *et al*, 1994).

The FFA form of the n-3 PUFAs, when added acutely to the cell culture medium, was found to be as effective in preventing ouabain toxicity as n-3 PUFAs which had been esterified into membranes over 3-5 days. These modulating effects on L-type Ca^{++} channels can be reversed by removal of the n-3 PUFA from the cell membrane by the addition of delipidated bovine serum albumin (BSA).

Other agents or conditions known to increase cytosolic $[\text{Ca}^{++}]$ resulting ultimately in a rigor contraction and cell death include KCl and anoxia. In adult rat cardiomyocytes, 10 μM DHA added either acutely (20 minutes) or chronically (72 hours) significantly attenuated the increase in cytosolic $[\text{Ca}^{++}]$ associated with both 50 mM KCl (3.4 fold increase with KCl vs. 2.2 fold increase with KCl + acute DHA and 1.9 fold increase KCl + chronic DHA) and anoxia (10 fold increase vs. 4.2 fold increase for anoxia + acute DHA and 1.7 fold increase for anoxia + chronic DHA). In cells treated with anoxia + 50 mM KCl, the increase in cytosolic $[\text{Ca}^{++}]$ was unmeasurable and cell death occurred rapidly. Pretreatment with 10 μM DHA reduced significantly the increase in cytosolic $[\text{Ca}^{++}]$ and doubled the survival time of the cells (Rinaldi *et al*, 2002).

1.4.3.1.3.2 Effects of n-3 PUFA on the sarcoplasmic reticulum

LC n-3 PUFAs may also have anti-arrhythmic effects at the level of the sarcoplasmic reticulum (SR). It is known that in post ischaemia damaged cardiomyocytes, cellular Ca^{++} regulation is affected, such that the SR becomes overloaded with Ca^{++} and there is spontaneous release of Ca^{++} from the SR which activates the $\text{Na}^+/\text{Ca}^{++}$ exchanger generating an inward depolarizing current that may be large enough to initiate an arrhythmogenic action potential (Stern *et al*, 1988). EPA and DHA have been found to inhibit the spontaneous release of Ca^{++} from the SR, possibly through effects on the $\text{Na}^+/\text{Ca}^{++}$ exchanger, and also reduce the availability of Ca^{++} for uptake by the SR by lowering the intracellular $[\text{Ca}^{++}]$, thereby requiring a greater length of time for the SR to acquire the amount of calcium needed to induce a spontaneous release event. Both of these effects combine to decrease the frequency of spontaneous Ca^{++} release events, and therefore reducing the risk of development of an arrhythmic event (Negretti *et al*, 2000).

Doxorubicin (DXR) is an anti-neoplastic antibiotic used mainly in the treatment of solid and haematological tumours. Its use is restricted by the high incidence of cardiac toxicity and it has been shown to have direct effects on Ca^{++} release channels of the SR by binding at a site on the ryanodine-sensitive Ca^{++} -induced Ca^{++} release (CICR) channel that overlaps the binding site for caffeine (a known CICR agonist) (Pessah *et al*, 1990; Bowling *et al*, 1994). In isolated adult rat ventricular cardiomyocytes, Vitelli *et al* (2002) demonstrated complete inhibition of both the DXR and caffeine induced increase in cytoplasmic $[\text{Ca}^{++}]$ by 10 μM DHA added to the perfusate.

1.4.3.1.3.3 Effects of n-3 PUFA on Na⁺ channels

Various studies have demonstrated modulating effects of LC n-3 PUFA on voltage-dependent Na⁺ channels. The FFA form of EPA has been shown to inhibit Na⁺ current through fast Na⁺ channels responsible for the phase 0 of the action potential in neonatal rat cardiomyocytes (Xiao *et al*, 1995), and it also had a similar effect when the α -subunit of the human cardiac Na⁺ channel was transfected into a human embryonic kidney cell line which lack a voltage-dependent Na⁺ channel (Xiao *et al*, 1998).

Specific effects of the FFA form of n-3 PUFAs on voltage-gated Na⁺ channels was demonstrated by the inhibition of a radioligand binding ([benzoyl-2,5-³H] batrachotoxinin A 20 α -benzoate ([³H]BTXB), an alkaloid neurotoxin derivative which binds specifically to the voltage-gated Na⁺ channel), by the administration of the FFA forms of n-3 PUFAs. The inhibition was dose-dependent and reversible upon removal of the n-3 PUFA by addition of delipidated BSA (Kang & Leaf, 1996).

Class I anti-arrhythmics exert their effects by inhibition of voltage-gated Na⁺ channels, and are effective in suppressing PVCs in the short term. However, in the Cardiac Arrhythmia Suppression Trial, long term use of class I anti-arrhythmics was associated with a poorer outcome and higher mortality than placebo (The Cardiac Arrhythmia Suppression Trial (CAST) Investigators, 1989). A possible explanation for this finding may be the upregulation of Na⁺ channel expression in rats chronically treated with the class I drug mexiletine (Taouis *et al*, 1991; Duff *et al*, 1992). Work by Kang *et al* (1997) confirmed the 4 fold upregulation of Na⁺ channel expression in cultured neonatal rat cardiomyocytes exposed to mexiletine. However, when cells were exposed to 20 μ M EPA in addition to mexilitine, the upregulation of Na⁺ channel expression was reduced to 2.5 fold. When EPA

alone was incubated with the cells, there was no effect on Na⁺ channel expression compared to placebo, indicating that although n-3 PUFA have a blocking effect on Na⁺ channels, they do not mimic class I anti-arrhythmics in upregulating Na⁺ channel expression during chronic administration.

1.4.3.1.3.4 Effects of n-3 PUFA on electrophysiological parameters of cardiomyocytes

Addition of 5-10 µM EPA or DHA to isolated neonatal rat ventricular cardiomyocytes resulted in 50-80% reduction in the spontaneous contraction rate of the cells without significant change in the amplitude of contractions (Kang & Leaf, 1994), and resulted in a 49% increase in the strength of the depolarising current required to initiate an action potential due to increases in both the action-potential threshold and the resting membrane potential (Kang *et al*, 1995).

1.4.3.1.3.5 Effects of n-3 PUFA on drug induced arrhythmic activity in cardiomyocytes

The addition of ISO to rat cardiomyocytes results in an increased rate of contraction and intermittent spasmodic contractures, and in groups of confluent cells produces asynchronous contraction activity. Both EPA and DHA (5-10 µM) when added to the perfusate as FFA is able to terminate ISO-induced arrhythmic activity if added after ISO, or prevent the onset of arrhythmic activity when added together with ISO (Kang & Leaf, 1995). Adult rat ventricular cardiomyocytes from rats fed fish oil for 3 weeks demonstrated significantly greater resistance to ISO-induced asynchronous contractile activity compared to rats fed a saturated fat diet, with the EC₅₀ for fish oil fed animals more than double that of the SF fed animals (Leifert *et al*, 2000; Leifert *et al*, 2001). However, acutely administered DHA does not prevent ISO induced increases in peak Ca⁺⁺ current or cytosolic free [Ca⁺⁺] (Pepe *et al*, 1994). The addition of 10 µM DHA or EPA as

FFA reduced the proportion of adult rat atrial myocytes contacting asynchronously in response to 10 μ M ISO to about 25-30%, whereas the methyl ester of DHA had no effect (Jahangiri *et al*, 2000).

The studies presented above are summarized in Table 1.6.

Table 1.6 Effects of n-3 PUFAs on arrhythmias in vitro

Reference	Experimental model	Comparisons	Major Outcomes
Hallaq <i>et al</i> (1990)	Cultured neonatal rat cardiomyocytes	5 μ M EPA, 5 μ M AA, control	EPA prevented development of toxic cytosolic Ca ⁺⁺ in response to ouabain. AA had no effect
Hallaq <i>et al</i> (1992)	Cultured neonatal rat cardiomyocytes	5 μ M EPA or DHA	30% $\downarrow$ in Ca ⁺⁺ influx modulating effect of L-type Ca ⁺⁺ channels
Kang & Leaf (1994)	Cultured neonatal rat cardiomyocytes	5-10 μ M EPA, DHA, α -LNA (FFA):	<i>Myocyte contraction rate</i> : $\downarrow$ 50-80% with EPA, DHA within 3 minutes. $\downarrow$ 30-50% with α -LNA within 7 minutes
Pepe <i>et al</i> (1994)	Single isolated adult rat ventricular cardiomyocytes	5 μ M DHA	Inhibit BAY K 8644 (L-type Ca ⁺⁺ channel agonist) Inhibit nitrendipine (L-type Ca ⁺⁺ channel antagonist)
Kang <i>et al</i> (1995)	Cultured neonatal rat cardiomyocytes	10 μ M EPA (FFA)	<i>Resting membrane potential</i> : 10% $\uparrow$ <i>Action potential threshold</i> : 17% $\uparrow$ <i>Cycle length of excitability</i> : 133% $\uparrow$ (DHA, α -LNA – mentioned in paper that similar results obtained but no results presented)
Kang & Leaf (1995)	Cultured neonatal rat cardiomyocytes	5-10 μ M EPA, DHA, α -LNA (FFA):	complete inhibition of tachyarrhythmia induced by; μ M ISO , 2 μ g/ml cholera toxin 250 μ M membrane permeable dibutyl cAMP
Xiao <i>et al</i> (1995)	Cultured neonatal rat cardiomyocytes	5-10 μ M EPA, DHA	<i>Voltage-activated Na⁺ current (I_{Na})</i> : peak I _{Na} $\downarrow$ 51% (5 μ M) and 64% (10 μ M) Free carboxyl group required to block Na ⁺ channel
Reithmann <i>et al</i> (1996)	Cultured neonatal rat cardiomyocytes	60 μ M DHA for 72 hrs	α_1 - & β -adrenoceptor-stimulated increase in: - <i>contraction velocity</i> ; sig lower (2% & 6% vs. 42% & 43% for controls) - <i>induction of arrhythmias</i> (α_1 - only); lower (13% vs. 82% for control)
Weylandt <i>et al</i> (1996)	Cultured neonatal rat cardiomyocytes	15 μ M EPA or DHA for 48 hrs - FFAs removed by addition of BSA	<i>ISO & high extracellular Ca⁺⁺ (7mM) induced arrhythmias</i> : cells with FFAs removed - no diff between control & those incubated with EPA or DHA. Perfusion with free EPA or DHA terminated most arrhythmias in all groups.
Kang & Leaf (1996)	Cultured neonatal rat cardiomyocytes	EPA, DHA, ALA (FFA)	Dose-dependent inhibition of [³ H]BTXB (alkaloid toxin derivative) binding to voltage sensitive Na ⁺ channel at 28, 29 & 33 μ M respectively FFA required for effect (methyl esters ineffective)
Kang <i>et al</i> (1997)	Cultured neonatal rat cardiomyocytes	20 μ M EPA; 20 μ M mexiletine; 20 μ M EPA + 20 μ M mexiletine; placebo	20 μ M EPA: $\leftrightarrow$ [³ H]BTXB binding 20 μ M mexiletine: 406% $\uparrow$ in [³ H]BTXB 20 μ M EPA + 20 μ M mexiletine: 406% $\uparrow$ in [³ H]BTXB
Xiao <i>et al</i> (1997)	Single isolated rat ventricular cardiomyocytes	Various [EPA] (FFA)	Dose dependent inhibition of L-type Ca ⁺⁺ channels (50% inhibition achieved with 0.8 μ M in neonatal and 2.1 μ M in adult cardiomyocytes)
Bogdanov <i>et al</i> (1998)	Single isolated rat ventricular cardiomyocytes	5-10 μ M EPA, DHA (FFA)	$\downarrow$ transient outward K ⁺ current $\uparrow$ action potential duration

Continued next page

Table 1.6 continued

Reference	Experimental model	Comparisons	Major Outcomes
Xiao <i>et al</i> (1998)	Human embryonic kidney cells (HEK293t) transfected with α -subunit of human cardiac Na^+ channel	5 μM EPA (FFA)	Dose and voltage dependent inhibition of voltage gated Na^+ channel Accelerated transition from resting to inactivated state Slowed recovery from inactivated state
Leifert <i>et al</i> (1999)	Single isolated adult rat ventricular cardiomyocytes	25 μM EPA, DHA or ALA	$\uparrow$ activation potentials of Na^+ channel by 9.2, 10.1 & 8.3 mV respectively $\downarrow$ inactivation potentials of Na^+ channel by 22.3, 17.1 & 20.5 mV respectively
Leifert <i>et al</i> (2000)	Cultured adult rat cardiomyocytes	Rats fed FO ($\Sigma\text{n-3}$ = 800 mg/d) or SF diet for 3 weeks	<i>ISO induced asynchronous contractile activity:</i> cardiomyocytes from FO fed rats less susceptible than those from SF fed rats
Negretti <i>et al</i> (2000)	Single isolated rat ventricular cardiomyocytes	10 μM EPA or DHA	$\downarrow$ frequency of spontaneous Ca^{++} release $\downarrow$ intracellular $[\text{Ca}^{++}]$
Leifert <i>et al</i> (2001)	Cultured adult rat cardiomyocytes	Rats fed FO ($\Sigma\text{n-3}$ = 5% of energy) or SF diet for 3 weeks	<i>ISO induced asynchronous contractile activity:</i> cardiomyocytes from FO fed rats less susceptible than those from SF fed rats

Abbreviations: FO, fish oil; ISO, isoproterenol; SF, saturated fat; SR, sarcoplasmic reticulum

1.4.3.1.4 Protection from cardiac dysfunction by fish-oil

Using isolated perfused working heart models, assessment of cardiac dysfunction during global ischaemia and reperfusion have demonstrated clear protective effects of fish oil. Yang *et al* (1993a; 1993b) demonstrated smaller reduction in the force of cardiac contraction and a smaller increase in coronary perfusion pressure under these conditions in fish-oil fed rats compared to butter fed rats. In one of these reports, the benefit was seen after just 5 days of fish-oil supplementation (Yang *et al*, 1993a). Paulson *et al* (1992) reported that rats fed fish oil had greater recovery of cardiac contractile performance as indicated by aortic flow, cardiac output, cardiac work and rate of pressure change per unit of time ($+dP/dt$, $-dP/dt$), following global ischaemia and reperfusion than rats fed corn oil. Rats fed fish oil have been reported to have a smaller loss of creatine kinase from ischaemic myocardium compared to rats fed butter (Yang *et al*, 1993a) or corn oil (Hock *et al*, 1990). Long-term feeding of a fish oil based diet to marmoset monkeys resulted in higher left ventricular ejection fractions under both control conditions and during adrenaline induced stress test compared to animals fed either a low fat reference diet or saturated fat diet (McLennan *et al*, 1992a)

Under normoxic conditions, utilising an isolated working heart model, Pepe and McLennan (2002) demonstrated that rats fed fish-oil had a lower myocardial oxygen consumption (MVO_2), a much more efficient cardiac oxygen use and a higher maximum cardiac output than rats fed either a saturated fat or low-fat reference diet. Following 15 minutes of global low-flow ischaemia and reperfusion, recovery of cardiac output and efficiency of oxygen use were highest, and MVO_2 was lowest, in the fish-oil fed rats. During ischaemia, the decrease in coronary venous pH, and the coronary venous washout of lactate and creatine kinase was significantly lower in the fish-oil fed rats.

A potential mechanism for this effect may be the inhibition of the cardiac sarcolemmal Na^+/H^+ transporter. During hypoxia, the intracellular pH increases due to the production of lactic acid. The Na^+/H^+ transporter removes H^+ from the cell in exchange for extracellular Na^+ . During hypoxia, there is a reduction in available ATP, and therefore the capacity to remove Na^+ via the Na^+/K^+ ATPase is reduced. The elevated sarcolemmal $[\text{Na}^+]$ activates the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, resulting in Ca^{++} overload (Figure 1.3). Ca^{++} overload is a well recognised factor in cardiac arrhythmias. Both EPA (at $\geq 50 \mu\text{M}$) and DHA (at $\geq 25 \mu\text{M}$) have been shown to inhibit the H^+ -dependent Na^+ uptake in preincubated porcine cardiac sarcolemmal vesicles by 30-50% compared to control vesicles (Goel *et al*, 2002). This mechanism would also provide a potential explanation for the protective effect of EPA and DHA observed during reperfusion, where a sudden restoration of blood flow reduces the extracellular $[\text{H}^+]$ thereby increasing activity of the Na^+/H^+ transporter and consequently the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, resulting in even further increases in intracellular $[\text{Ca}^{++}]$ and additional injury.

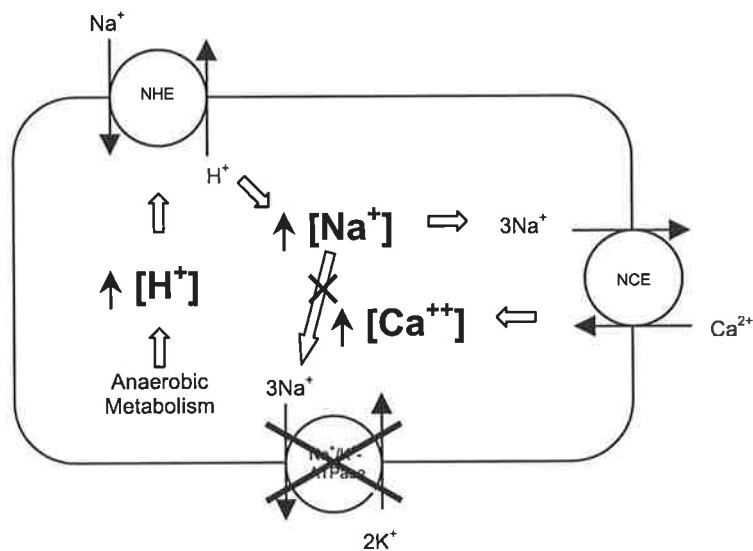


Figure 1.3 Mechanism by which Na^+ and Ca^{2+} accumulate in cardiomyocytes during ischaemia. Hypoxia prompts intracellular acidosis which activates the sarcolemmal Na^+/H^+ exchanger (NHE) which exchanges extracellular Na^+ for intracellular H^+ . Due to a decrease in available ATP, intracellular Na^+ is unable to be pumped out of the cell via the Na^+/K^+ -ATPase and accumulates in the cell. The increased intracellular $[\text{Na}^+]$ inhibits the efflux of Ca^{2+} through the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCE), and may also promote Ca^{2+} entry into the cell via the NCE in reverse mode. (Adapted from Gazmuri *et al* (2002)).

1.5 INCORPORATION OF LC N-3 PUFA INTO CARDIAC

MEMBRANES

Feeding experiments with both rats (Charnock *et al*, 1986; Charnock *et al*, 1992a) and marmosets (Charnock *et al*, 1992b) have shown that animals fed diets containing very low amounts of ALA as the only source of n-3 PUFA (< 1% of total fatty acids), and even despite high levels of LA with n-6/n-3 ratios >40, are able to accumulate large amounts (up to 15% of total fatty acids) of DHA into cardiac membrane phospholipids. In mice fed a diet containing soybean oil (SBO) as the only source of n-3 PUFA, (7.4% ALA with no LC n-3 PUFA) or crocodile oil (CO, 4% LC n-3 PUFA), the DHA content of cardiac membrane phospholipids was about 23% of total fatty acids in the SBO fed animals

compared with about 32% in the CO fed animals, while the DHA content of liver phospholipids was about 10% and 13% respectively (Watkins *et al*, 2001). In general, the proportions of ALA, EPA and DPA in cardiac membrane phospholipids are extremely low (<0.5% of total fatty acids) and often undetectable, indicating that either the cardiomyocyte is highly selective in incorporating DHA into cardiac phospholipids, or the cardiomyocyte is highly efficient in metabolizing the available n-3 precursors to DHA.

Watkins *et al* (2001) demonstrated that when mice are fed DHA, there is preferential incorporation into the cardiolipin phospholipid fraction. However, when DHA is preferentially incorporated into the phosphatidylcholine (PC) fraction when diets containing the metabolic precursors are fed. Preformed DHA entering a cell is available for acylation into phosphatidic acid or into a lyso-phospholipid, thereby becoming available for the biosynthesis or remodelling of all phospholipid classes. Metabolic precursors of DHA can undergo either acylation into phospholipid or elongation/desaturation to DHA. The final step in the formation of DHA requires translocation to peroxisomes, a single round of β -oxidation with the resulting fatty acid preferentially moved to the ER for acylation into lyso-phospholipid (Sprecher & Chen, 1999). If this pathway is present in cardiomyocytes it could explain the absence of DHA formed from precursors in cardiolipin as cardiolipin synthesis and remodeling occurs only on the inner-mitochondrial membrane (Schlame & Haldar, 1993). The proportion of DHA in cardiac membrane phospholipid was positively correlated with the proportion of PC in the heart without an increase in total phospholipids (Watkins *et al*, 2001), suggesting that DHA induced the conversion of phosphatidylethanolamine (PE) to PC. It is possible that the cardioprotective effects of n-3 PUFAs result not only from the effects on ion channels of increased proportion n-3 PUFA in cardiac membrane phospholipids, but also from

DHA-induced changes in the proportions of individual phospholipid classes present in membranes.

1.6 AIMS

Overall, there is considerable evidence from cohort, case-control, and intervention studies which indicate a preventive cardiovascular effect from dietary n-3 PUFAs. However, the existence of this evidence has not translated into widespread uptake of n-3 enriched diets in Western communities, which are generally low consumers of fish, with a large proportion of the population never consuming fish. Capturing better the health benefits which can derive from dietary n-3 fats requires (a) a greater understanding of their mechanism of action in order to improve their cardio-protective effects, and (b) practical improvements in the design of n-3 PUFA rich diets to provide practical food choices which are suitable for daily use on a lifetime basis, thereby providing a chronic increase in tissue levels of n-3 fatty acids.

Epidemiological evidence suggests that increasing consumption of dietary n-3 PUFA has a protective effect against sudden cardiac death, and therefore, against potentially fatal ventricular arrhythmias. While there is conclusive evidence that LC n-3 PUFAs have anti-arrhythmic properties in animals, the levels of dietary fish oil used in experimental animal models of arrhythmia were typically in the order of 15-25% of fat, making them considerably higher than would be practical for free living humans in the Western context. In comparison, in the GISSI-Prevenzione study where about 1 g/d of LC n-3 PUFA was given, a subject on a 10 MJ/d diet with 36% of energy coming from fat would have consumed LC n-3 PUFA at the of only 1% of dietary fat.

In addition to the dangers of interpreting experimental evidence across species, translating the animal results to human populations is limited by the large differences in the intake of n-3 PUFAs.

This project consists of 3 studies concerned with increasing the health benefits of n-3 PUFA. The first part of the project is a diet study which examines the potential of using a variety of novel foods to increase the consumption of n-3 PUFA. This dietary approach utilizes a number of everyday food items fortified with fish oil, as well as some items naturally high in n-3 PUFA, both ALA and the long chain fats EPA and DHA.

The remaining parts of the project examine effects of n-3 PUFA on potentially fatal ventricular arrhythmias in two separate and diverse studies. The first study is a long-term dietary intervention study in patients with implantable cardioverter-defibrillators (ICDs), in which the effect of increasing consumption of fish oil on the incidence of ventricular arrhythmias over a 12-18 month period is examined.

The second study is a pilot study to determine whether short-term supplementation with fish oil has any effect in suppressing previously inducible ventricular arrhythmias during electrophysiology (EP) testing. Any observed effect will then be used for sample size calculations in a more rigorous examination of the effects of fish oil on the suppression of previously inducible arrhythmias in a dose response study.

The results of these studies should contribute to the knowledge of the cardio-protective properties of n-3 PUFA as well as providing dietary approaches to implementation of their use for both cardiac patients and the general community

CHAPTER 2

A PRACTICAL APPROACH TO INCREASING INTAKES OF N-3 POLYUNSATURATED FATTY ACIDS: USE OF NOVEL FOODS ENRICHED WITH N-3 FATS

2.1 INTRODUCTION

As noted previously, there are considerable health benefits in increasing the tissue levels of n-3 PUFAs, particularly the long-chain fatty acids of marine origin, EPA and DHA. The vast majority of the population in most Western countries have very low levels of these fatty acids, principally due to two major factors.

Firstly, the only natural dietary source of EPA and DHA is fish, and the consumption of fish in most western countries is low, as described previously in chapter 1.

Secondly, the conversion rate of the plant derived n-3 PUFA, ALA to the long chain metabolites is poor. A large proportion (50-60%) of ingested ALA is used in β -oxidation for energy production (Brenna, 2002).

Metabolic studies indicate that the conversion of ALA to EPA and DHA is compromised by high levels of dietary LA. High intakes of LA result in increased competition for the Δ^6 -desaturase enzyme, with less enzyme available for the conversion of ALA to stearidonic acid (18:4n-3). High intakes of LA also inhibit the incorporation of ingested preformed LC n-3 PUFAs into cellular membranes (Cleland *et al*, 1992).

Alternative strategies are needed to deliver increased dietary LC n-3 PUFAs to the wider community without radical changes to normal dietary habits. One such strategy is to fortify common food items, such as long-life milk, sausages, dip and margarine spread with fish oil. The aim of this study, therefore, was to determine the intake of n-3 PUFA achievable through the use of foods high in n-3 PUFA, and to assess the popularity and contribution to the total n-3 PUFA intake, of the individual food items.

2.2 MATERIALS AND METHODS

2.2.1 Ethical Approval

Ethical approval for the dietary intervention study was obtained from the Royal Adelaide Hospital Research Ethics Committee. All volunteers were fully informed of the nature of the study, and received written information regarding the study. Each volunteer was provided with the opportunity to discuss their participation in the study with a member of their family or a friend. Written consent was received from each participant prior to the commencement of the study. All personal information and results were kept confidential.

2.2.2 Subjects

Healthy male subjects were recruited from a list of volunteers held by the Rheumatology Unit, Royal Adelaide Hospital. Subjects completed a 2 week run-in period when they consumed their normal diet, followed by a 4 week dietary intervention period. Subjects attended the clinic for 4 visits during the study, at -2, 0, 2 and 4 weeks. At each visit, fasting venous blood samples were taken by venepuncture, and the subject's weight was recorded using standard bathroom scales. Clinic visits for each

subject were scheduled at approximately the same time of day, and subjects were asked to fast for 12 hours prior to blood collection.

2.2.3 Diets

Subjects were provided with a range of products designed to increase their intake of n-3 PUFA, and decrease their intake of n-6 PUFA. This was achieved by providing a range of products as follows;

- Products rich in ALA were: canola oil (Meadow Lea Foods Pty Ltd, Sydney, Australia); flaxseed based products (stabilized flaxseed meal, and Alena Energy Drink, both provided by ENRECO, Manitowoc, WI, USA)
- Products rich in EPA and DHA were: fresh fish (mullet and deep sea bream); canned fish (pink salmon and sardines; Safcol Holdings, Adelaide, Australia); items fortified with fish oil (Ropufa-30; Roche Vitamins, Sydney, Australia) during manufacture; namely, sausages (manufactured by a local butcher with supplied fish oil), luncheon meat (Conroys Smallgoods, Adelaide, Australia), long-life milk (Pauls Ltd, Brisbane, Australia), margarine spread (Meadow Lea Foods Pty Ltd, Sydney, Australia), and french onion dip (manufactured in the hospital kitchen),
- Products high in monounsaturated fatty acids to replace n-6 PUFA in the background diet were: salad dressing and mayonnaise (Meadow Lea Foods Pty Ltd, Sydney, Australia).

Subjects were also provided with muffin mix (White Wings Foods, Sydney, Australia) from which shortening had been omitted, so that canola oil could be added during preparation. The fish oil was fortified with 1000ppm dl- α -tocopherol.

Subjects were not asked to consume set quantities of each food, but rather were instructed to incorporate these foods into their normal diet in desired quantities, and advice was provided on using the novel foodstuffs. Foods were provided in quantities sufficient for the whole family, to avoid separate meal preparation being required for participants.

Dietary advice was also provided on foods rich in n-6 PUFA which were to be avoided as far as practicable, e.g. nuts, cooking oils, and commercial food products high in n-6 rich vegetable oils (Appendix A) .

2.2.4 Diet Diaries

Subjects were instructed to complete weighed food records for 3 days/week (2 days during the week, and 1 day on the weekend) for 2 weeks prior to the dietary intervention period to provide data on baseline nutrient intakes, and for the 4 week dietary intervention period. Subjects were to record dietary intakes on the same days of each week. Instruction was provided on the maintenance of the diet diary, and on the use of weighed measurements and standard household metric measures. Subjects were provided with digital domestic kitchen scales with 2 gm increments (Salter Selectronic 2200, or Salter Electronic Trimscale, Salter Housewares, Tonbridge, UK) for use throughout the study. Diet diaries were checked at each of the visits to verify entries.

Diet diaries were analysed using the "Diet-1" computer software package (Xyris Software Pty. Ltd., Highgate Hill, Brisbane, Australia) which is based on the Australian Nutrient Data Table NUTTAB95 (Australian Government Publishing Service, Canberra, Australia) which had been modified to include the ALA, EPA, DHA, LA, AA

and OA content of Australian foods and ingredients, as well as the novel fish oil fortified foods. The fatty acid composition of n-3 enriched foods was determined by GLC.

2.2.4.1 Fatty Acid Extraction of Foods

Total fat was extracted from food items, ground or homogenized when required, by mixing 1 gm of food with 2 ml methanol, and then mixing vigorously with 4 ml chloroform. Following centrifugation, the chloroform layer was removed into pre-weighed flasks and evaporated to dryness under a stream of nitrogen using a rotary evaporator. The flasks were then re-weighed to obtain the total fat content. All extractions were performed in triplicate. A small sample of the total lipid extract was transferred to a vial for trans-esterification and GLC analysis as described in section

2.2.5.4. The fatty acid composition of the provided foods is shown in table 2.1.

Table 2.1 Selected fatty acid content of the food items provided to the subjects during the trial

FOOD	18:1n-9	18:2n-6	18:3n-3	20:5n-3	22:5n-3	22:6n-3	Total n-3
Canola Oil	59.3 [†]	19.2	7.9	0	0	0	7.9
Margarine spread	50.6	5.2	0.34	0.36	0.03	0.52	1.34
Salad Dressing	10.0	1.1	0.1	0	0	0	0.1
Mayonnaise	12.5	11.2	0.6	0	0	0	0.6
Milk	0.4	0.05	0.01	0.03	0.00	0.04	0.10
Flaxseed mixture (Alena)	5.7	5.35	16.7	0	0	0	16.7
Stabilised flaxseed meal	4.9	3.3	11.6	0	0	0	11.6
Luncheon meat	4.9	0.8	0.12	0.10	0.03	0.15	0.43
Sardines	0.1	0.05	0.02	0.12	0.03	0.57	0.72
Pink Salmon	2.3	0.22	0.13	0.67	0.23	1.96	3.00
Deep Sea Bream	0.2	0.01	0.00	0.04	0.01	0.23	0.29
Mullet	0.3	0.03	0.02	0.40	0.09	0.38	0.89
Sausages	6.0	0.38	0.08	0.21	0.03	0.33	0.73
French Onion Dip	6.2	0.49	0.22	0.33	0.06	0.46	1.17

[†](gm/100 gm edible portion)

2.2.5 Cell Separations For Fatty Acid Analysis

At each visit, 20 ml of fasting blood was taken from the subjects by venepuncture using a 21 gauge needle and mixed with 4 ml of 4.5% EDTA in water, and 4 ml of 6% dextran in normal saline (pH 7.0), and incubated in a water bath at 37°C for 30 minutes

to allow sedimentation of the erythrocytes under gravity. The plasma layer was removed and loaded onto a Ficoll-Paque density gradient (1.077 gm/ml, Lymphoprep, Nycomed Pharma, Oslo), and centrifuged at 110 x g for 10 minutes at 25°C.

2.2.5.1 Platelets

The resulting top layer containing the platelet-rich plasma was removed into a separate tube, centrifuged at 200 x g for 25 minutes. The plasma fraction was removed from the platelet pellet and 3 ml was stored at -20°C for lipid extraction. The platelet pellet was resuspended in 4 ml of 0.2% saline for 30 seconds to lyse any remaining erythrocytes. To return the saline solution to 0.9%, 4 ml of 1.6% saline was added. Following centrifugation at 320 x g for 10 minutes, the supernatant was discarded, and the pellet resuspended in 1.5 ml of normal saline.

2.2.5.2 Mononuclear Cells

The remaining density gradient which was now platelet depleted was centrifuged at 200 x g for 25 minutes. The mononuclear cell layer was removed from the gradient interface, washed once with PBS, and centrifuged at 320 x g for 10 minutes. The supernatant was discarded, and the pellet resuspended in 1.5 ml of normal saline. The remainder of the density gradient containing the neutrophils was discarded.

2.2.5.3 Fatty Acid Extraction

Fatty acids were extracted from the platelet and mononuclear cell suspensions with 2 ml of methanol, followed by 4 ml of chloroform, and following centrifugation at 320 g for 10 minutes, the chloroform layer was removed and evaporated to dryness using a Speed Vac Concentrator (Savant Instruments Inc, Farmingdale, NY, USA). The lipids were

then re-dissolved in 2 ml of chloroform/methanol (9:1 v/v) and stored at -20°C until analysis.

Fatty acids were extracted from plasma as describe above except for a doubling of the solvents, i.e. 4 ml of methanol and 8 ml of chloroform.

2.2.5.4 Fatty Acid Analysis

The previously stored total lipid samples were evaporated to dryness under a stream of nitrogen, and re-dissolved in 150 µl of chloroform/methanol (9:1 v/v). Phospholipids were separated from triglycerides and cholesteryl esters by activated silica thin layer chromatography, developed in petroleum spirit/acetone (3:1 v/v). The phospholipid fraction was scraped directly into vials containing 2 ml (5 ml for plasma) of 1% (v/v) H₂SO₄ in methanol, and transesterified at 70°C for 3 hours. The resultant methyl esters were extracted into heptane and stored in an autosampler vial with a minimal amount of sodium sulphate.

The fatty acid methyl esters were separated by GLC (HP 6890 gas chromatograph, Hewlett-Packard, Palo Alto, CA, USA) equipped with a 50m capillary column (0.33mm internal diameter) coated with BPX-70 (0.25-µm film thickness; SGE Pty Ltd, Victoria, Australia). The injector temperature was set at 250°C and the detector (flame ionization) temperature was set at 300°C. The initial oven temperature was 140°C and was programmed to rise to 220°C at 5°C/min. Helium was used as the carrier gas at a velocity of 35 cm/s. The fatty acid methyl esters were identified against pure lipid standards (Nucheck Prep Inc, Elysian, MN, USA). All organic solvents contained butylated hydroxy anisole (0.005%) as an antioxidant.

2.2.6 Statistical Analysis

Comparisons between time points were done using Repeated Measures Analysis of Variance. Where statistically significant results were found, post-hoc analysis was carried out using Newman-Keuls Multiple Comparisons Test. Results are presented as mean $\pm$ SD. All statistical analyses were completed using Winks version 4.5 Professional Edition (TexaSoft, Cedar Hill, TX, USA).

2.3 RESULTS

2.3.1 Subjects

Sixteen participants completed the study, and there were no withdrawals. The participants had a mean age of 39.2 ± 4.4 years, were 178 ± 6 cm tall and weighed 82.1 ± 9.6 kg at the time of enrolment. There was a small but significant increase in BMI during the dietary intervention period (Table 2.2).

Table 2.2 BMI recordings of the study participants at each visit

	Baseline		Dietary Intervention Period	
	-2 weeks	0 weeks	2 weeks	4 weeks
BMI (kg/m ²)	$25.8 \pm 2.4^{1,2,a}$ (22.0-29.7) ¹	25.8 ± 2.6^a (21.4-29.7)	26.0 ± 2.6^b (21.4-29.7)	26.2 ± 2.6^b (21.7-30.4)

¹ $\bar{x} \pm SD$; range in parentheses.

² values within a row with different superscript letters indicate significant differences. $p < 0.001$ (Repeated Measures ANOVA, post-hoc analysis by Newman-Keuls multiple comparisons method).

2.3.2 Dietary Intakes

2.3.2.1 Macronutrient Intakes

Macronutrient intakes were estimated from the diet diaries, and are provided in Table 2.3. There was a significant increase in the amount of protein in the diet during the first dietary intervention period compared to baseline, both in terms of g/day, and as % of dietary energy. There was no further increase during the 2nd dietary intervention period. There were no changes in total fat or carbohydrate intake during the study.

Table 2.3 Estimated daily macronutrient intakes

Macronutrient	Baseline	Dietary Intervention	
		Week 2	Week 4
Energy			
(Mj/d)	10.0±1.6 (8.3-13.1) ¹	10.2±1.8 (7.7-13.5)	10.5±2.1 (8.0-14.7)
(Kcal/d)	2390±383 (1982-3129)	2449±425 (1846-3226)	2509±507 (1914-3509)
Protein			
(g/d)	102±18 ^a (79-144)	116±19 ^b (83-157)	121±17 ^b (92-164)
(% of energy)	17.5±2.3 ^{2,a} (14.6-22.6)	19.4±2.4 ^b (15.0-23.5)	19.9±2.2 ^b (15.7-24.8)
Fat			
(g/d)	91±20 (61-118)	95±20 (60-129)	98±29 (53-151)
(% of energy)	33.7±5.5 (26.3-44.9)	34.1±4.1 (27.0-39.9)	34.1±5.6 (24.5-42.7)
Carbohydrate			
(g/d)	283±67 (199-425)	280±69 (171-417)	281±67 (200-406)
(% of energy)	45.2±6.6 (34.5-54.2)	43.5±6.0 (34.6-53.4)	42.9±5.8 (33.3-52.0)
Alcohol			
(g/d)	12.1±13.4 (0-40)	10.2±12.4 (0-39)	11.1±12.3 (0-43.3)
(% of energy)	3.5±4.0 (0.0-12.7)	3.0±3.9 (0.0-11.8)	3.1±3.8 (0.0-15.1)

¹ $\bar{x} \pm \text{SD}$; range in parentheses

² values within a row with different superscript letters indicate significant differences $p < 0.001$ (repeated measures ANOVA, post-hoc analysis by Newman-Keuls multiple comparisons method)

2.3.2.2 Fatty Acid Intakes

During the 4 weeks of the dietary intervention, there were significant increases in the intake of oleic acid (20%), and all of the n-3 PUFAs, whereas the intake of linoleic acid decreased by 30%. There were no changes in the estimated intakes of any of the individual fatty acids between the two dietary intervention periods (Table 2.4). There was an 81% decrease in the total n-6/total n-3 ratio during the dietary intervention period compared to the baseline period.

Table 2.4 The estimated daily intake of selected fatty acids

Fatty Acids	Baseline	Dietary Intervention	
		2 weeks	4 weeks
18:1 n-9 (g/d)	29.6±6.9 ^{a,1,2} (18.3-37.9)	35.8 ±7.5 ^b (23.6-55.2)	35.5 ±10.4 ^{b*} (18.5-55.4)
(% of energy)	10.8±2.4 ^a (7.6-14.7)	12.9±2.2 ^b (9.1-17.4)	12.4±2.6 ^{b*} (8.1-18.0)
18:2 n-6 (g/d)	13.1±5.4 ^a (6.7-28.8)	9.3±2.1 ^b (5.8-14.1)	9.0±2.8 ^{bt} (4.7-17.0)
(% of energy)	4.8±2.1 ^a (2.7-11.3)	3.3±0.5 ^b (2.7-4.3)	3.1±0.6 ^{bt} (2.1-4.4)
18:3 n-3 (g/d)	1.4±0.6 ^a (0.5-2.6)	4.2 ±1.8 ^b (2.1-8.6)	4.0±2.3 ^{bt} (0.9-10.2)
(% of energy)	0.51±0.24 ^a (0.22-1.05)	1.50±0.60 ^b (0.84-3.22)	1.3±0.7 ^{bt} (0.4-3.4)
20:5 n-3 (g/d)	0.03±0.03 ^a (0.00-0.08)	0.52±0.19 ^b (0.21-0.86)	0.50±0.25 ^{bt} (0.11-1.07)
(% of energy)	0.01±0.01 ^a (0.00-0.03)	0.19±0.07 ^b (0.06-0.32)	0.17±0.07 ^{bt} (0.04-0.35)
22:5 n-3 (g/d)	0.00±0.00 ^a (0.00-0.00)	0.12±0.05 ^b (0.05-0.19)	0.11±0.06 ^{bt} (0.01-0.25)
(% of energy)	0.00±0.00 ^a (0.00-0.00)	0.04±0.02 ^b (0.01-0.07)	0.04±0.02 ^{bt} (0.00-0.06)
22:6 n-3 (g/d)	0.09±0.07 ^a (0.00-0.23)	1.01±0.38 ^b (0.41-1.88)	1.01±0.42 ^{bt} (0.27-1.79)
(% of energy)	0.04±0.03 ^a (0.00-0.10)	0.33±0.14 ^b (0.12-0.68)	0.35±0.13 ^{bt} (0.10-0.54)
Total n-3 (g/d)	1.5±0.76 ^a (0.7-2.8)	5.9±2.1 ^b (3.3-11.2)	5.7±2.9 ^{bt} (2.1-12.6)
(% of energy)	0.56±0.26 ^a (0.28-1.19)	2.1±0.7 ^b (1.3-4.2)	1.9±0.8 ^{bt} (0.9-4.1)

¹ $\bar{x} \pm \text{SD}$; range in parentheses² values within a row with different superscript letters indicate significant differences.*p<0.01, [†]p<0.001 (repeated measures ANOVA, post-hoc analysis by Newman-Keuls multiple comparisons method)

2.3.3 Contribution of Provided Foods to Overall n-3 PUFA Intake

The contribution to total n-3 PUFA intake of each of the provided food items is shown in table 2.5. This was calculated only for those subjects consuming each item on the diet recording days. All subjects consumed the cooking oil, flaxseed products and margarine spread at some time during the study period, while only 50% of subjects ate sardines on

at least 1 of the recording days. Fish (canned and fresh) provided the largest contribution to the LC n-3 PUFA intake, with canned salmon providing about 24% of the EPA and 34% of the DHA intakes of those eating salmon. Fresh fish (deep sea bream and mullet) together provided about 19% each of the EPA and DHA intake of those subjects who ate fresh fish.

Flaxseed products provided about 57% of the total ALA intake, with canola cooking oil providing a further 19%. The margarine spread was primarily olive oil based, and therefore contributed only a very small proportion of the ALA intake.

Of the novel fish oil enriched products, the margarine spread proved the most popular, with all subjects consuming it on at least 1 recording day. The mean intake was 18 gm/day, and provided about 160 mg/day of EPA + DHA, which represented about 10% of the total EPA + DHA in the diet. Milk was the next most popular food, being consumed by 15/16 subjects, with the mean intake amongst consumers of about 275 ml/day. This contributed about 200 mg/day (12.8% of total intake) of EPA + DHA. The sausages provided a mean daily intake of about 230 mg/day of EPA + DHA to the sausage consumers, a level similar to that of fresh fish. The least popular of the novel foods were the luncheon meat and the dip, with only 9/16 subjects consuming these items on any recording day. Among consumers, the luncheon meat and dip contributed a mean daily intake of EPA + DHA of 122 and 82 mg respectively

Table 2.5 Subject consumption of the provided foods, and estimates of the contribution of those foods to the overall intake of n-3 PUFA's.

Food	No. of subjects consuming	Daily Intake (gm/day)	ALA		EPA		DHA	
			gm/day	% of total ALA	mg/day	% of total EPA	mg/day	% of total DHA
Canola Oil	16	8.1±3.7 ¹	0.64±0.29 ²	19.1±11.0	-	-	-	-
Flaxseed products	16	18.1±13.4	2.6±2.0	56.6±19.7	-	-	-	-
Margarine spread	16	18.1±11.5	0.06±0.04	1.6±0.9	63±40	12.3±4.5	93±59	9.2±4.0
Milk	15	285±175		<1%	83±51	16.0±6.4	117±72	11.3±4.7
Fresh Fish	14	65.5±35.3		<1%	101±80	19.4±13.0	178±102	18.9±11.1
Sausages	14	42.8±32.1		<1%	92±70	16.7±7.6	138±104	12.6±6.5
Pink Salmon	13	26.9±16.6		<1%	115±71	23.8±13.9	338±208	33.8±17.5
Luncheon meat	9	32.8±31.4		<1%	32±31	5.6±4.7	50±47	4.5±3.4
French Onion Dip	9	15.3±10.2		<1%	51±34	11.6±10.5	71±47	8.3±8.3
Sardines	8	36.6±23.9		<1%	54±35	9.7±6.4	260±170	21.9±12.3

¹ $\bar{x} \pm SD$

² the mean quantity and percentage of the n-3 PUFA contributed by the food in only those subjects consuming that particular food. ie the number of subjects consuming each item is the number of subjects who indicated that they consumed that item on at least 1 of the recording days. The intakes are the estimated daily intakes of each item amongst those subjects who consumed that item

2.3.4 Plasma and Cellular Fatty Acids

2.3.4.1 Plasma Phospholipid Fatty Acids

Changes in selected plasma phospholipid fatty acids are shown in Table 2.6 and Figure 2.1.

There were significant decreases in the proportions of n-6 PUFAs in plasma phospholipids following 2 weeks of dietary intervention. LA decreased by 14.8%, DGLA by 27.5%, AA by 9.1%, while total n-6 PUFAs decreased by 14.6% from baseline. There were no significant changes in any n-6 PUFA between week 2 and week 4.

There were significant increases in the proportion of ALA (56%), EPA (174%), DHA (80%) and total n-3 (79%) in the plasma phospholipid fraction following 2 weeks of dietary intervention. There were no significant changes in any n-3 PUFA between week 2 and week 4.

Table 2.6 The fatty acid composition of plasma phospholipids

Fatty Acid	Baseline		Dietary Intervention Period	
	-2 weeks	0 weeks	2 weeks	4 weeks
Total Sats	42.7±0.8 ^{a1,2,3}	42.2±0.9 ^b	42.0±1.2 ^b	42.3±0.9 ^{bt}
18:1 n-9	9.9±1.0	9.8±0.9	10.1±0.9	9.9±0.7
Total Monos	13.4±1.1	13.4±1.1	14.0±0.9	13.7±0.7
n-6				
18:2	20.9±3.3 ^a	20.9±3.2 ^a	17.8±3.6 ^b	17.6±3.4 ^{bt}
20:3	3.80±0.85 ^a	3.82±0.78 ^a	2.77±0.63 ^b	2.76±0.76 ^{bt}
20:4	10.6±2.1 ^{ab}	11.0±2.3 ^a	10.0±1.8 ^{bc}	9.6±1.7 ^{ct}
Total	36.5±1.5 ^a	36.9±1.4 ^a	31.5±1.9 ^b	30.8±2.1 ^{bt}
n-3				
18:3	0.20±0.07 ^a	0.23±0.09 ^a	0.36±0.19 ^b	0.29±0.14 ^{ab*}
20:5	1.04±0.34 ^a	1.13±0.34 ^a	3.10±0.86 ^b	3.38±0.88 ^{bt}
22:5	1.41±0.29	1.39±0.21	1.43±0.28	1.38±0.23
22:6	3.64±0.70 ^a	3.76±0.81 ^a	6.76±1.04 ^b	7.23±1.16 ^{bt}
Total	6.3±0.9 ^a	6.5±1.0 ^a	11.6±1.6 ^b	12.3±2.0 ^{bt}

¹ $\bar{x} \pm SD$

² values represent % of total fatty acids

³ values within a row with different superscript letters indicate significant differences $p < 0.01$, $^{\dagger} p < 0.001$ (repeated measures ANOVA, post-hoc analysis by Newman-Keuls multiple comparisons method)

2.3.4.2 Mononuclear Cell Phospholipids Fatty Acids

Changes in selected mononuclear cell phospholipid fatty acids are shown in Table 2.7 and Figure 2.1.

During the first 2 week period of dietary intervention, there were small but significant increases in the mononuclear cell OA and total monounsaturated fatty acid levels, each of 5.7%. There were no further changes after 4 weeks of dietary intervention (Table 2.7).

There were significant decreases in the proportions of n-6 PUFAs in mononuclear cell phospholipids after 2 weeks of diet modification, with falls of 4.7% for LA, 10.4% for DGLA, 10.7% for AA and 11.3% for total n-6 PUFA. During the final 2 weeks of dietary modification, the LA content increased to levels similar to those recorded at baseline, while DGLA increased by 5.2% to a level intermediate between baseline and week 2. There were no other significant changes in n-6 PUFA during the final 2 weeks of dietary modification.

Unlike plasma phospholipids, the EPA, DHA and total n-3 PUFA levels in mononuclear cell phospholipids increased significantly during both periods of dietary intervention. The EPA levels increased by 182% during the first 2 weeks of dietary intervention, and then by a further 16% during the second 2 week period to a final level 226% above the baseline value. DHA levels increased by 59% at 2 weeks, and by a further 18% at 4 weeks, taking the final level to 87% above baseline, and total n-3 PUFA increased by 38% at 2 weeks and a further 8% at 4 weeks, to a final increase of 50% over baseline.

There were no significant changes in the DPA levels during the dietary intervention periods.

Table 2.7 The fatty acid composition of mononuclear cell phospholipids

Fatty Acid	Baseline		Dietary Intervention Period	
	-2 weeks	0 weeks	2 weeks	4 weeks
Total Sats	43.0±1.3 ^{a1,2,3}	42.2±0.6 ^b	43.5±0.8 ^a	43.1±0.6 ^{a†}
18:1 n-9	12.0±0.7 ^a	12.2±0.6 ^a	12.9±0.8 ^b	12.9±0.9 ^{b†}
Total Monos	15.7±0.9 ^a	15.8±0.8 ^a	16.7±0.9 ^b	16.7±0.9 ^{b†}
n-6				
18:2	5.39±0.99 ^{ab}	5.60±0.87 ^a	5.33±0.89 ^b	5.61±0.94 ^{a*}
20:3	1.91±0.41 ^a	1.93±0.38 ^a	1.73±0.34 ^b	1.82±0.43 ^{ct†}
20:4	23.4±1.0 ^a	23.9±1.1 ^b	21.3±1.0 ^c	21.2±1.0 ^{ct†}
Total	34.7±1.1 ^a	35.4±0.8 ^b	31.2±0.7 ^c	31.0±0.7 ^{ct†}
n-3				
18:3	nd ⁴	nd	nd	nd
20:5	0.40±0.09 ^a	0.40±0.10 ^a	1.09±0.30 ^b	1.25±0.34 ^{ct†}
22:5	2.79±0.38	2.79±0.25	2.88±0.32	2.91±0.37
22:6	2.22±0.33 ^a	2.33±0.36 ^a	3.65±0.38 ^b	4.08±0.28 ^{ct†}
Total	5.48±0.48 ^a	5.52±0.39 ^a	7.62±0.70 ^b	8.26±0.70 ^{ct†}

[†] $\bar{x} \pm \text{SD}$

² values represent % of total fatty acids

³ values within a row with different superscript letters indicate significant differences $p < 0.05$, $^{\dagger}p < 0.001$ (repeated measures ANOVA, post-hoc analysis by Newman-Keuls multiple comparisons method)

⁴ not detected

2.3.4.3 Platelet Phospholipid Fatty Acids

Changes in selected mononuclear cell phospholipid fatty acids are shown in Table 2.8 and Figure 2.1.

During the first 2 weeks of dietary intervention, there was a small but significant decrease of 2.3% in the proportion of saturated fat in platelet phospholipid, and a small but significant increase in the proportion of OA of 6.2%. During the second period of dietary intervention, the proportion of OA increased significantly by a further 2% (Table 2.8).

There were significant decreases in the proportions of n-6 PUFAs in platelet phospholipids after 2 weeks of diet modification, with falls of 10.1% for LA, 9.4% for

DGLA, 2.6% for AA and 5.7% for total n-6 PUFA. During the second 2 weeks of dietary modification, the LA content increased by 5.1% and the DGLA content increased by 6.3% to levels intermediate between baseline and week 2. The proportion of AA decreased by a further 3.5% and total n-6 PUFA decreased by a further 1.9% during the second 2 weeks of dietary modification.

The proportion of ALA in platelet phospholipids increased by 63% during the first 2 weeks of dietary intervention, with no further increase during the second 2 week period. EPA levels increased by 166% at 2 weeks, and by a further 10% at 4 weeks, taking the final level to 193% above baseline. DPA levels increased by 9% at 2 weeks, with no further increase during the second 2 week period. DHA levels increased by 70% at 2 weeks, and by a further 10% at 4 weeks, taking the final level to 87% above baseline, and total n-3 PUFA increased by 49% at 2 weeks and a further 7% at 4 weeks, to a final increase of 59% over baseline.

Table 2.8 The fatty acid composition of platelet phospholipids

Fatty Acid	Baseline		Dietary Intervention Period	
	-2 weeks	0 weeks	2 weeks	4 weeks
Total Sats	43.1±1.1 ^{a1,2}	43.4±0.6 ^a	42.4±0.8 ^b	42.3±0.6 ^{b†}
18:1 n-9	14.5±0.7 ^a	14.6±0.7 ^a	15.5±0.7 ^b	15.8±0.8 ^{ct}
Total Monos	18.2±1.0 ^a	18.2±0.9 ^a	19.2±0.9 ^b	19.6±0.9 ^{ct}
n-6				
18:2	5.06±0.75 ^a	5.04±0.69 ^a	4.53±0.81 ^b	4.76±0.72 ^{ct}
20:3	1.64±0.35 ^a	1.59±0.33 ^b	1.44±0.31 ^c	1.53±0.32 ^{dt}
20:4	23.4±1.0 ^a	23.5±1.0 ^a	22.9±1.3 ^b	22.1±1.0 ^{ct}
Total	33.3±1.0 ^a	33.2±0.8 ^a	31.3±0.9 ^b	30.7±0.9 ^{ct}
n-3				
18:3	0.056±0.019 ^a	0.059±0.021 ^a	0.096±0.036 ^b	0.083±0.030 ^{b†}
20:5	0.42±0.09 ^a	0.41±0.10 ^a	1.09±0.26 ^b	1.20±0.31 ^{ct}
22:5	2.13±0.32 ^a	1.97±0.22 ^b	2.14±0.22 ^a	2.15±0.29 ^{a*}
22:6	1.69±0.32 ^{a*}	1.64±0.29 ^a	2.78±0.35 ^b	3.07±0.45 ^{ct}
Total	4.33±0.45 ^a	4.09±0.39 ^a	6.11±0.56 ^b	6.51±0.77 ^{ct}

¹ $\bar{x} \pm SD$

² values represent % of total fatty acids

³ values within a row with different superscript letters indicate significant differences *p<0.05, †p<0.001 (repeated measures ANOVA, post-hoc analysis by Newman-Keuls multiple comparisons method)

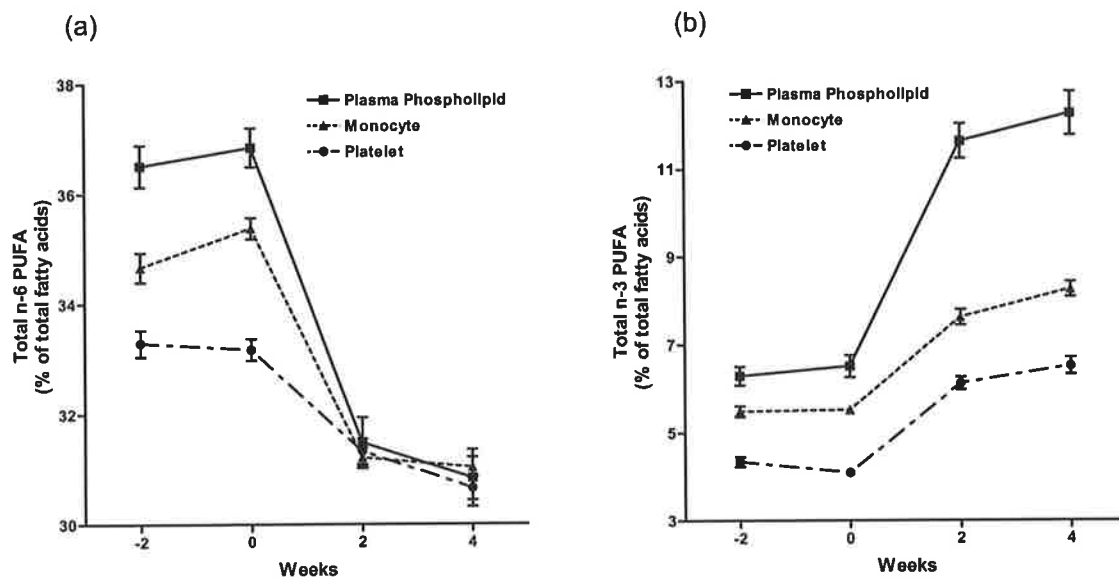


Figure 2.1 Changes in total n-6 PUFA (a) and total n-3 PUFA (b) throughout the study in each of the phospholipid sources examined

2.4 DISCUSSION

LC n-3 PUFA intakes of the order of 200–400 mg/day have been recommended for community-wide intakes (Scientific Review Committee, 1990; The British Nutrition Foundation, 1992; de Deckere *et al*, 1998; Simopoulos *et al*, 2000) and intakes of about 1 gm/day as recommended for people with documented CHD (Kris-Etherton *et al*, 2002). In this study, we have demonstrated that by providing subjects with everyday food items fortified with fish oil, these levels are readily achievable.

Lovegrove *et al* (1997) fortified some everyday items with fish oil such as bread and spread, along with a number of sweet foods, including biscuits, cake, ice cream and milkshake powder, and used a portion exchange system with the aim of achieving a daily intake of 1.8 gm EPA + DHA. Likewise, Mantzioris *et al* (2000) used a similar dietary approach with fish-oil fortified savoury products like sausages and dip, and had a large emphasis on ingestion of fresh fish, and set a target EPA content in mononuclear cell phospholipids of 1.5% of total fatty acids. Subjects achieved mean daily intakes of EPA + DHA of 1.4 g and 1.8 g respectively. However, in both studies, the investigators gave no indication of the popularity of each food item, or the contribution of each food item to the total LC n-3 PUFA intake.

In the present study, we have used similar foods to those used by Mantzioris *et al* (2000) with the addition of fish oil fortified margarine spread and long-life milk. This is the first study to examine the food preferences of the subjects, and the contribution of the individual food items to the overall intakes of EPA and DHA.

A combination of the mean daily intakes of margarine spread (18 g/day providing 156 mg of EPA+DHA), milk (285 ml/day providing 200 mg of EPA+DHA), sausages (4 /week providing an average daily intake of about 250mg EPA+DHA), dip (15 g/day providing about 120mg of EPA+DHA) and luncheon meat (32 g/day providing about 80 mg of EPA + DHA) gives a total daily intake of about 800 mg. This intake is achieved without canned or fresh fish and it approaches the mean daily intake of 1 g of EPA + DHA as recommended for people with documented CHD (Kris-Etherton *et al*, 2002).

A number of other studies have examined the effects of incorporating fish-oil enriched products in the diet. Saldeen and colleagues (1998) added fish oil to bread and instructed subjects to consume 125 g/day, which provided a daily LC n-3 PUFA intake of 300mg. They observed increases in plasma phospholipid EPA and DHA of 32% and 18% respectively after 2 weeks. When 500 ml/day of fish oil fortified, partially fat reduced milk, also providing 300 mg EPA + DHA daily, was consumed for 6 weeks, plasma phospholipid EPA and DHA both increased by 31% (Visioli *et al*, 2000).

Wallace *et al* (2000) used microencapsulated fish oil incorporated into bread, biscuits and soup to achieve a daily intake of 900 mg of LC n-3 PUFA/day, and reported that subjects expressed a preference for the bread as it was easier to incorporate into the normal diet. Increases in platelet EPA and DHA of 82% and 44% respectively were observed after 4 weeks. In the current study, both mononuclear cell and platelet EPA increased by about 170% in the first 2 weeks, with further small but significant increases during the next 2 weeks, however, only 2 subjects achieved mononuclear cell EPA levels of 1.5% of total fatty as proposed by Mantzioris *et al* (2000) as a potential target for anti-inflammatory benefits. However, it must be noted that a considerable portion of the total EPA and DHA in this study came from the consumption of fresh or

canned fish, and achieving mononuclear cell EPA levels approaching 1.5% of total fatty acids is unlikely through the consumption of novel fish oil fortified foods alone.

A major hurdle to the generalized worldwide enrichment of foodstuffs with fish oil is the limited availability of fish stocks, and the possibility of decreased supply and increased cost of fish oil (Naylor *et al*, 2000). The use of renewable terrestrial sources of n-3 PUFA therefore becomes important. While the efficiency of the conversion of ALA to the longer chain n-3 metabolites remains controversial (Gerster, 1998), there is clearly an indication for promoting an increase in ALA consumption. Increasing ALA consumption to about 9 gm/day through the addition of flaxseed to the diet as margarine spread (Mantzioris *et al*, 1994) or baked in muffins (Cunnane *et al*, 1995) significantly increased plasma phospholipid proportions of EPA and DPA. Previous research suggests that minimising the background intake of LA (the predominant n-6 PUFA) is important in maximising the uptake of LC n-3 PUFA (Cleland *et al*, 1992) and the conversion of ALA to EPA (Emken *et al*, 1994).

Irrespective of elevation in tissue LC n-3 PUFA, ALA consumption itself has been associated with reduced risk of cardiovascular disease in some, but not all studies. The Health Professionals Follow-up Study (Ascherio *et al*, 1996) found a 59% reduction in the risk of MI associated with an increase in ALA consumption of 1% of dietary energy. In a 10 Mj diet, a 1% increase in energy equates to 2.7 gm ALA, an amount found in about 2 tablespoons of ground flaxseed meal. A similar quantity of ALA supplementation (2.9 gm/day in the form of mustard oil) was given in the Indian Infarct Survival study (Singh *et al*, 1997), a secondary prevention study among survivors of suspected acute MI, which found a significant reduction in the risk of total cardiac

events and non-fatal MI compared to placebo (28% vs. 34.7% and 15.0% vs. 25.4% respectively) after 12 months of follow-up. In both the Oslo Study (Pedersen *et al*, 2000) and the Zutphen Study (Oomen *et al*, 2001), ALA consumption was not associated with cardiovascular benefits.

A future alternative to the use of ALA or fish oil may be the use terrestrially derived plant oils rich in stearidonic acid (SDA 18:4n-3). The use of this oil by-passes the rate-limiting Δ^6 -desaturase in the metabolic pathway to the LC n-3 PUFAs. It has recently been shown that 1.5 gm/day SDA is about 1/3 as effective as the equivalent amount of EPA at elevating plasma phospholipid EPA (James *et al*, 2003). SDA occurs naturally in a number of plants, eg in species of *Echium*, and *Asperugo* (Guil-Guerrero *et al*, 2001). Prospective sources of SDA include purification from algal sources (Ishihara *et al*, 2000), and genetically modified Canola (*Brassica napus*), in which the introduction of a Δ^6 -desaturase, and increased expression of Δ^{12} and Δ^{15} -desaturases resulted in an oil containing 25% SDA and 35 % ALA (Knutzon, 1999). An intake of 1 tablespoon of this oil would provide 5 gm of SDA, or the equivalent of about 1.2 gm EPA, an amount that would be easily achievable through substitution of existing dietary fat sources.

Significant reductions in risk of all-cause mortality (31%) and cardiovascular disease mortality (37%) were associated with the highest quintile of ALA consumption compared to the lowest quintiles (mean intakes, 2.80gm/day and 0.87gm/day respectively) in the MRFIT Study (Dolecek & Grandits, 1991), while in the Nurse's Health Study (Hu *et al*, 1999), there was a 45% reduction in coronary heart disease mortality from the lowest (0.71gm/day) to highest (1.36 gm/day) quintiles of ALA consumption. The Lyon Diet Heart Study (de Lorgeril *et al*, 1994) found a significant

reduction in risk of both cardiac death and non-fatal myocardial infarction (MI) associated with increased consumption of ALA.

In the study presented here, subjects consumed a mean intake of 4.1 gm ALA/day, of which over 50% was derived from flaxseed meal. In this study, the margarine spread was olive oil based, whereas had a canola oil based margarine spread been used, the mean ALA intake would have increased by about 1 gm/day.

Having a wide range of n-3 PUFA enriched products enables those people who do not eat fish habitually or who never eat fish, to benefit from the increased intake of n-3 PUFA without radical modification of their normal dietary habits. Increased daily consumption of n-3 PUFA from an early age may help prevent the onset of a variety of so-called "lifestyle" diseases, such as atherosclerosis, diabetes, essential hypertension and obesity (Das, 2000) thereby reducing the need for high therapeutic doses of n-3 PUFA which have been the hallmark of most intervention studies to date.

CHAPTER 3

EFFECTS OF N-3 FATTY ACIDS ON THE INCIDENCE OF ARRHYTHMIAS IN PATIENTS WITH IMPLANTED CARDIOVERTER-DEFIBRILLATORS (ICDS)

3.1 BACKGROUND

As discussed in chapter 1, both increased dietary consumption of n-3 PUFA, and elevated biomarkers of n-3 PUFA consumption are associated with significant reductions in the incidence of sudden cardiac death (Siscovick *et al*, 1995; Singh *et al*, 1997; GISSI Investigators, 1999; Albert *et al*, 2002), presumably as a result of a decrease in fatal ventricular arrhythmias (Huikuri *et al*, 2001). This suggests that n-3 PUFA may have anti-arrhythmic properties and both animal (McLennan, 2001) and cell culture (Leaf *et al*, 1999a) studies have supported this view.

To date, the only direct evidence of an anti-arrhythmic effect of n-3 PUFA in humans has been a small study examining the effect on patients with frequent ventricular premature complexes. Following 16 weeks of supplementation with cod liver oil, there was a non-significant 48% reduction in total PVCs, and a significant reduction in the proportion of patients experiencing $\geq 70\%$ reduction in PVCs (Sellmayer *et al*, 1995).

Over the last 2 decades, the development of the ICD has provided an effective therapy for people with recurrent ventricular arrhythmias. The ICD is an implanted device that is able to continuously monitor the cardiac rhythm. According to predetermined and programmable criteria the ICD can respond to a cardiac arrhythmias and apply therapies that include anti-tachycardia pacing, cardioversion or defibrillation.

For any detected arrhythmia the ICD is able to classify the arrhythmia, record the date and time of occurrence, the treatment applied and the outcome of that therapy. In some cases an electrocardiographic snapshot of the arrhythmia is also stored in the device memory. This information can be downloaded for interpretation at a later date by telemetry linkage. Long-term storage of arrhythmic events on computer disk allows a comparison of the retrospective history of arrhythmic events for individual patients against a prospective intervention.

The ICD has provided a non-pharmacological therapy for patients at high risk of ventricular arrhythmias. Thus, novel pharmacological therapies could be tested in this group without compromising the protection provided to the patient should any arrhythmia occur. This population has been used in this study to investigate the anti-arrhythmic effects of n-3 PUFA, where the test agent was provided within encapsulated fish oil, fish oil fortified food items (long-life milk and margarine spread) and canned fish. The general design involved an examination of the pattern of ventricular arrhythmias prior to, and following the introduction of a high n-3 PUFA diet.

3.2 MATERIALS AND METHODS

3.2.1 Ethical Approval

Ethical approval for the dietary intervention study was obtained from the Royal Adelaide Hospital (RAH) Research Ethics Committee. All volunteers were fully informed of the nature of the study, and received written information regarding the study. Each subject was provided with the opportunity to discuss their participation in the study with a member of their family or a friend. Written consent was received from each participant prior to the commencement of the study. All personal information and results were kept confidential.

3.2.2 Subjects

Subjects were recruited from the ICD follow-up clinics of a cardiology practice in Adelaide, South Australia. A number of these subjects live in country areas up to 800 Km from Adelaide and travel to Adelaide for their regular ICD follow-ups. The cardiologist also conducts regular consulting sessions in a number of towns throughout South Australia and a number of subjects were recruited from these clinics, eg at Gawler (approx. 50 Km north of Adelaide) and at Murray Bridge (approx 80 Km east of Adelaide). Subjects who had had their ICD *in situ* for at least 12 months were identified. Patients with ICDs generally have regular clinical follow-up at approximately 6 monthly intervals. Prospective subjects were contacted by telephone during the week prior to a scheduled follow-up visit to explain the study and gauge their interest in participation. Information sheets were forwarded by mail to those interested to allow time to read through the study information and discuss the study with family or friends. Informed consent was obtained at the time of their follow-up visit, and baseline data were collected.

3.2.3 Implantable Cardioverter/Defibrillators

All subjects had ICDs capable of storing electrograms (EGM), either Medtronic model 7219 (in 1 subject and had been replaced prior to enrolment in the study), 7220, 7221, 7223, 7227, 7229 or 7271 (Medtronic Inc, Minneapolis, MN, USA), or Guidant model 1748, 1762, 1774, 1782, 1783, 1790, 1793, 1821, 1831, 1850, or 1851 (Guidant Corp, Indianapolis, IN, USA). Three subjects had had their original ICD replaced prior to the commencement of the study, and where event data was available from the original device, it was included in the baseline data. Two subjects had their original ICD replaced during the follow-up period.

3.2.4 Data Collection

3.2.4.1 Ventricular Arrhythmias

Baseline data on arrhythmic events was obtained from 3 sources:

- 1) Events that had been printed at the time of telemetric interrogation of the ICD and stored with the subject's case notes. Data on some of these events were obtained from the RAH where subjects had been admitted to hospital and their ICD interrogated.
- 2) the cardiology practice had maintained a system of regularly backing-up information from ICD interrogations onto disk, and many of the events were successfully retrieved from this system. Not all sessions, however, were stored to disk. Data from Medtronic models 7220, 7221 and 7223 is able to be stored to disk via the Medtronic programmer. However, there is currently no function available on the programmer to enable data from these devices stored on disk to be read. Medtronic Technical Support have a translation program which enables these files to be read on standard PC, but which was never released. The Medtronic representative in

Adelaide was able to obtain a copy of the program that facilitated obtaining event information from those subjects. The resultant output from the file translation is a text file containing all of the data and parameters stored in the ICD at the time of saving the file. It does not provide a visual form of the EGM, however it does provide R-R interval data and the marker tags. An example of the output obtained from the translation program for an arrhythmic event is provided in Appendix 2.

- 3) Where data on individual arrhythmic events was missing, reference to specific events were, in some instances, identified from patient casenotes by matching dates of clinic visits with periods of time during which missing events occurred.

Where ICD output on individual events were available, they were reviewed independently by two experienced electrophysiologists to assess whether they were ventricular arrhythmias. Where the independent assessment differed, agreement was reached by consensus.

Not all events had complete sets of summary text and EGM. The major reasons for this were:

- 1) Some subjects had been admitted to a hospital via the emergency department, the ICD had been interrogated and either events were not printed, or data not saved to disk prior to clearing the data.
- 2) Limitations on the storage capacity of the devices where a subject had had a large number of events. This occurred primarily on Guidant devices where only a small number of EGMs are stored with more recent events overwriting older events, resulting in older events then having interval reports and episode detail reports, or

only episode detail reports available. In the case of some events, all information had been overwritten by more recent events.

- 3) Printouts of events had been misplaced, or
- 4) Data had been cleared without printing all episodes, or saving data to disk.

All ICD models except for the Medtronic 7219 and 7220 serially number all events which meet the detection criteria for arrhythmic events programmed into the device at the time, and therefore the total number of events recorded for each subject was known and any missing events could be identified. For subjects with Medtronic model 7219 and 7220 devices, which do not serially number events, only those events which had been printed and stored with casenotes, or referred to specifically in casenotes, were able to be included in the analysis. Guidant devices serially number non-sustained events and atrial tachycardia response mode switches (some models) in the same sequence as ventricular arrhythmias, and these events were excluded from the analysis.

Retrospective data was collected on arrhythmias recorded by the ICD from the time of implant to the commencement of n-3 PUFA supplementation, except where anti-arrhythmic drug therapy was introduced or ceased between the time of implant and enrollment. In these cases, baseline data collection was recorded from the time of introduction (or cessation) of anti-arrhythmic drug therapy to the commencement of n-3 PUFA supplementation. Similarly, data collection was ceased during the n-3 PUFA supplementation period when anti-arrhythmic drug therapy was altered. Therefore, arrhythmic events were only recorded where anti-arrhythmic drug therapy was unchanged during the baseline and intervention periods. For example, a patient who was not taking anti-arrhythmic therapy at the time of implant, but subsequently commenced

drug therapy due to recurrent arrhythmia were considered to have begun their baseline data collection period at the time they commenced the anti-arrhythmic drug therapy. A patient taking anti-arrhythmic drug therapy from the time of implant, and who ceased taking the drug following enrollment, had follow-up data recorded up to the time of the cessation of the drug therapy.

An arrhythmic event was defined as any event meeting the detection criteria programmed into the device at the time of the event, and was recorded by the ICD. This included episodes detected in the VF zone where therapy was initiated (ie, charging commenced), but was subsequently aborted due to failure to reconfirm the tachyarrhythmia, and episodes detected in the VT zone for which no therapy was programmed. A VF was defined as any event detected by the device in the VF zone, and will therefore include some episodes of rapid VT.

All events recorded by the ICD were therefore classified as;

- a) ventricular arrhythmia confirmed from individual event data (with therapy as; anti-tachycardia pacing (ATP), cardioversion following ATP, no therapy programmed, cardioversion as 1st therapy or therapy aborted failing reconfirmation),
- b) ventricular arrhythmia confirmed from casenotes (with above therapies plus therapy unknown),
- c) not a ventricular arrhythmia,
- d) unknown arrhythmic event where no data on the event is available.

The planned period of n-3 PUFA supplementation is 18 months, with a minimum of 12 months of dietary intervention required to qualify for inclusion in the final analysis.

This thesis reports interim results based on 3 separate analyses;

- 1) those subjects who have completed ≥ 12 months of follow-up with baseline periods calculated as outlined above,
- 2) those subjects who have completed ≥ 6 months of follow-up with baseline periods calculated as outlined above,
- 3) the subjects from part 2) with arrhythmia rates calculated on identical baseline and follow-up periods, either by adjusting their baseline data to an identical number of days as their follow-up by counting back from the day of enrollment into the study, or in the case of 3 subjects who have had longer follow-up periods than baseline periods, their follow-up periods were adjusted to an identical number of days as their baseline periods, with arrhythmic events occurring within these periods only being examined.

3.2.4.2 Premature Ventricular Complexes (PVCs)

Subjects with devices equipped with PVC counters (Medtronic Gem series model numbers 7227 and 7271, and Guidant Ventak Prizm model 1851) were included in this analysis. Single PVCs (singlets and doublets on the Guidant device) and runs of PVCs (2, 3 or 4 consecutive PVCs on the Medtronic device, and >2 consecutive PVCs on the Guidant device) were calculated as the daily mean in both the baseline and follow-up periods from the total number of PVCs and runs of PVCs in each period.

3.2.4.3 Ventricular Effective Refractory Period (VERP)

Due to the possibility that measuring VERP may induce a tachyarrhythmia, all subjects were fully informed of the potential risks associated with conducting the test and given a choice as to whether they wished to participate in that part of the study. VERP was measured at each visit by utilising the Electrophysiology Study (EPS) function of the ICD. An 8 beat drive chain was initiated with an S1-S1 cycle length of 600ms, and with a single extra stimulus introduced at an S1-S2 cycle length of 300ms at the baseline visit, and 20ms longer than the previous VERP at subsequent visits. The S1-S2 cycle length was then reduced in 10ms decrements until refractoriness was reached.

This protocol was used successfully for 17 months. However, following the induction of a fast VT resulting in an ICD discharge during the VERP measurement in one subject, the protocol was altered to reduce the risk of inducing further arrhythmias. The stimulation protocol commenced with the S1-S2 cycle length at about 220 ms at the baseline test, and at 20ms below the previous VERP for subsequent tests, and was increased in 10ms increments until the S2 extra stimulus captured, thereby ensuring that the S2 extra stimulus captured during only 1 cycle of the stimulation protocol. The VERP was defined as the longest cycle length at which S2 did not capture.

Subjects who were taking Class III anti-arrhythmics were excluded from the analysis as these drugs are known to prolong the VERP.

3.2.5 Natural History of Ventricular Arrhythmias

To examine the natural history of ventricular arrhythmias, the time between implant of the ICD and enrolment into the study was divided into 6 monthly periods and the

number of appropriate events in each period was determined. All 41 subjects currently involved in the study were included in this analysis, except for (1) those subjects who had alterations to anti-arrhythmic drug therapy during the baseline period, and (2) those subjects with unknown events.

3.2.6 Diets

Subjects living within the Adelaide metropolitan area were initially provided with dietary sources of LC n-3 PUFA; namely, margarine spread (MeadowLea Foods, Sydney, Australia), long-life milk (Pauls Ltd, Brisbane, Australia), and canned pink salmon and canned sardines (Safcol Pty Ltd, Adelaide, Australia). Subjects were also encouraged to consume additional fresh fish, and dietary advice to reduce n-6 PUFA intake was provided. Estimates of the n-3 PUFA content of fresh fish were obtained from the Australian Seafood Handbook (Yearsley *et al*, 1999). Dietary advice was provided to obtain a target intake of at least 900 mg EPA + DHA per day in accordance with their dietary preferences. Initial dietary advice was to consume 1 tablespoon of margarine spread/day + 500 ml of milk/day + 2-3 cans of fish/week as this was estimated to provide about 800-1000 mg n-3 PUFA/day. As most of the subjects in this study were likely to be elderly, and with a number of associated health conditions, it was decided to keep the recording of dietary information as simple as possible. Therefore, a simple diet diary was devised to enable estimation of EPA and DHA intake (Appendix C). No attempt was made to record n-6 PUFA intake. Subjects returned for initial follow-up visits after 1 month and again after 3 months to assess dietary intake. Subjects who were not able to achieve estimated intakes of around the target level were provided with enteric-coated fish oil capsules (Natures Own Reflux Free Omega-3 Fish Oil, Bulivants Natural Health Products Pty Ltd, Brisbane, Australia), which contain 180

mg EPA plus 120 mg DHA per capsule, to bring their estimated intake up to the target. Compliance was assessed by calculation of the estimated EPA + DHA intake, and by changes in plasma and red blood cell phospholipid fatty acids.

Subjects telephoned when they required more supplies, and these were sent direct to their homes via courier.

Due to the impracticalities of providing n-3 PUFA enriched milk and margarine to subjects living outside of the Adelaide metropolitan area, they were provided with fish oil capsules (Natures Own Reflux Free Omega-3 Fish Oil, Bulivants Natural Health Products Pty Ltd, Brisbane, Australia) and instructed to take 3 per day. Additional capsules were sent as required by mail. Subjects consuming only capsules were not required to complete the n-3 diet diary, and compliance was assessed by changes in plasma and red blood cell phospholipid fatty acids.

3.2.7 Fatty acid Analysis

All fatty acid analyses were performed by the Child Nutrition Research Centre (CNRC), located at Flinders Medical Centre, Adelaide, Australia.

At each visit, a 4 ml non-fasting venous blood sample was collected into lithium heparin tubes by venepuncture, for fatty acid analysis. Samples were forwarded to CNRC via the South Australian State Pathology Service courier. Depending on the time of day the blood was collected, it may have been refrigerated overnight prior to processing.

Plasma and erythrocytes were separated by centrifugation at 2000 x g for 5 minutes at room temperature. Erythrocytes were washed three times with cold 0.9% saline, and approximately 1ml of erythrocytes were added to 0.9% saline to make a final volume of 1.5ml. The total lipids were extracted with propanol (2ml) and chloroform (4ml). Plasma was centrifuged at 15110g for 2 minutes, and approximately 1ml of supernatant was added to 0.9% saline to make a final volume of 1.5ml. Total lipid was extracted with methanol (2ml) and chloroform (4ml). Following centrifugation at 1560g for 10 minutes, the chloroform layer was removed and evaporated to dryness using a vacuum concentrator (Heto Maxidry Plus, Denmark). The total lipid extract was re-dissolved in 150µl of chloroform:methanol (9:1), and the phospholipid fractions was separated by activated silica thin-layer chromatography using a petroleum spirit:acetone (3:1) mobile phase.

The phospholipid fraction was scraped directly into vials containing 2 ml of 1% (v/v) H₂SO₄ in methanol, and transesterified at 70°C for 3 hours. The resultant methyl esters were extracted into heptane and stored in an autosampler vial with a minimal amount of sodium sulphate.

The fatty acid methyl esters were separated by GLC (HP 6890 gas chromatograph, Hewlett-Packard, Palo Alto, CA, USA) equipped with a 50m capillary column (0.33mm internal diameter) coated with BPX-70 (0.25-µm film thickness; SGE Pty Ltd, Victoria, Australia). The injector temperature was set at 250°C and the detector (flame ionization) temperature was set at 300°C. The initial oven temperature was 140°C and was programmed to rise to 220°C at 5°C/min where it was held for 1 min followed by a 20°C/min ramp to 260°C. Helium was used as the carrier gas at a flow rate of 34 cm/s.

The fatty acid methyl esters were identified against pure lipid standards (Nucheck Prep Inc, (Elysian, MN, USA). All organic solvents were analytical grade and contained butylated hydroxyanisole (0.005%) as an antioxidant.

3.2.8 Statistical Methods

Statistical analysis was conducted using Winks v 4.5 Professional Edition (TexaSoft, Cedar Hill, TX, USA). Statistical significance was determined at $p < 0.05$, and all analysis was conducted using 2-tailed tests.

3.2.8.1 Incidence of Arrhythmias

Because this is an interim analysis, most subjects had different periods of baseline and follow-up data. Therefore, arrhythmic events for each subject were normalized to the number of events/year in each period. The annualized arrhythmia rates were not normally distributed, and therefore, comparisons between the baseline and intervention periods were conducted using non-parametric, paired data analysis (Wilcoxon's Signed Rank Test).

3.2.8.2 PVCs

Daily PVC rates were also not normally distributed, and therefore comparisons were also conducted by Wilcoxon's Signed Rank Test. Due to the large variation in PVC rates, the change in daily PVC rates were normalized to percentage change.

3.2.8.3 VERP

As not all subjects had follow-up at the same time points, VERP at approximately 3 months, 6 months and 12-18 months were compared separately to baseline by paired *t*-test.

3.2.8.4 Natural History of Ventricular Arrhythmias

As there were progressively fewer subjects with baseline data for each 6 month period beyond the 12 months minimum baseline data required to qualify for inclusion into this study, separate paired and repeated measures analyses were conducted for the time periods 1-2, 1-3, 1-4, 1-5 and 1-6 (time period 1 = the 1st 6 months post implant, period 2 = 6-12 months post implant, etc up to period 6 = 30-36 months post implant). Separate analyses were undertaken for subjects with and without anti-arrhythmic drug therapy, as well as the combined cohort. Each analysis utilized the total number of subjects who had complete data in each time series, ie for the combined cohort, there were 24 subjects included in the paired analysis of time periods 1-2, 22 subjects in the repeated measures analysis for time periods 1-3 etc down to 8 subjects for time periods 1-6. All statistical analyses were conducted using non-parametric tests, Wilcoxon's Sign Rank Test for periods 1-2 and Friedmans's Test for Repeated Measures with post-hoc analysis by Tukey's Multiple Comparisons Test for the remaining periods.

3.2.8.5 Fatty Acids

Blood collection and fatty acid analysis was planned for 1, 3, 6, 12 and 18 months from enrollment. However, blood collection did not occur at every time point for every subject. Also, due to the practical difficulties of conducting a clinical trial without interfering with standard treatment, bloods collected at each designated time point were

not exactly the same length of time from enrollment. Using a published method to deal with these problems, summary measures were calculated for each subject to allow comparison with baseline values (Matthews *et al*, 1990). Estimated mean fatty acid levels (as a proportion of total fatty acids) throughout the intervention period were obtained for each subject by calculating the total area under the curve using the trapezoid rule (Prism v3.02, GraphPad Software Inc, San Diego, CA, USA) and dividing by the total follow-up time to normalize the values due to differing total follow-up periods. This value was then compared to the baseline value using paired *t*-test.

3.3 RESULTS

3.3.1 Clinical Characteristics of Subjects

A total of 47 subjects with at least 12 months of baseline data have been enrolled in the study. The study will continue until all subjects have completed 18 months of the dietary intervention. This interim analysis is provided for the purposes of this thesis. At the end of August 2002, 20 subjects had completed at least 12 months of n-3 PUFA supplementation and a further 13 subjects (33 in total) had completed at least 6 months. Analysis of both groups will be presented here. In addition, 4 subjects had withdrawn from the study, 3 due to gastrointestinal side effects of the fish oil and one subject with hypertrophic cardiomyopathy underwent a heart transplant for incessant, intractable VT; however, she failed to qualify for inclusion in the final analysis due to having less than 6 months follow-up without a change in anti-arrhythmic therapy. One subject died 82 days following enrollment. The remaining 9 subjects had completed less than 6 months of follow-up at the end of August 2002, and are continuing in the study. The clinical characteristics of the 20 subjects who had completed at least 12 months follow-up and the 33 subjects who had completed at least 6 months follow-up are provided in table 3.1.

Table 3.1 Baseline clinical characteristics of the subjects who had completed ≥ 12 months or ≥ 6 months of follow-up

	cohort	
	≥ 12 months	≥ 6 months
<i>n</i>	20	33
Age	68.0 $\pm$ 9.0 (40.1–76.5)	66.4 $\pm$ 9.0 (40.1–84.1) ¹
Sex (% male)	90	90
Baseline days	848 $\pm$ 366 (367–1671)	862 $\pm$ 388 (367–1952)
Study days	474 $\pm$ 76 (364–595)	384 $\pm$ 132 (182–595)
Underlying heart disease. <i>n</i> (%)		
Coronary Artery Disease	18 (90)	30 (91)
Dilated Cardiomyopathy	1 (5)	1 (3)
Hypertrophic Cardiomyopathy	1 (5)	2 (6)
Drug therapy. <i>n</i> (%)		
amiodarone	5 (25)	7 (21)
aspirin	8 (40)	14 (42)
β -blockers	11 (55)	18 (55)
ACE inhibitors	16 (80)	23 (70)
Angiotensin II receptor antagonist	2 (10)	3 (9)
Ca ⁺⁺ channel blockers	0 (0)	1 (3)
Statin	8 (40)	18 (55)
Diuretics	8 (40)	12 (36)
Warfarin	5 (25)	6 (18)
Digoxin	5 (25)	6 (18)

¹ $\bar{x}\pm$ SD (range in parentheses)

3.3.2 Cohort with ≥ 12 Months Follow-up

The 20 subjects who had completed at least 12 months of follow-up had a combined total of 16952 days (46.6 years) of baseline data, and 9478 days (25.9 years) of follow-up data.

3.3.2.1 Incidence of Arrhythmias

3.3.2.1.1 Ventricular arrhythmias

In the baseline period there were 151 ventricular arrhythmic events distributed amongst 19 subjects at a mean rate of 3.25/person year (Table 3.2). Of these, 60 events distributed amongst 15 subjects were confirmed by independent analysis of event data, with the remaining 91 events distributed amongst 9 subjects identified from the examination of casenote records.

During the intervention period, there were 27 ventricular arrhythmic events distributed amongst 7 subjects at a mean rate of 1.04/person year. Of these, 25 events distributed amongst 7 subjects were confirmed from individual event data, with the remaining 2 events from 1 subject confirmed from casenote records.

The median and maximum annualized arrhythmia rates for an individual in the baseline period were 1.4 and 17.4 events/year respectively, compared with 0.0 and 5.8 events/year respectively in the intervention period (Table 3.3 and Figure 3.1).

Fourteen subjects had a reduction in their annualized arrhythmia rate, with 5 subjects having an increased rate (Figure 3.2), and no change in the rate in the remaining subject (subject #12), who had no events in either period. There were 13 subjects who had no events in the intervention period, compared with 1 subject in the baseline period. There was a statistically significant reduction in the annualized arrhythmia rate when analyzed on a paired basis ($p=0.028$, Wilcoxon's Signed Rank Test, Figure 3.2).

Table 3.2 Arrhythmias recorded in 20 subjects completing ≥ 12 months follow-up

	Baseline			Intervention		
	No. of events	Rate / person year	No. patients with events	No. of events	Rate / person year	No. patients with events
<i>Confirmed from episode data</i>						
VT - ATP	32	0.69 ¹	4	7	0.27	3
VT - ATP/CV	3	0.06	2	1	0.04	1
VT - no therapy	0	0	0	1	0.04	1
VT - total	35	0.75	5	9	0.35	4
VF - CV	18	0.39	8	12	0.46	4
VF - therapy aborted	7	0.15	4	4	0.15	3
VF - total	25	0.54	10	16	0.62	5
Total arrhythmias	60	1.29	15	25	0.96	7
<i>Confirmed from case notes</i>						
VT - ATP	53	1.14	3	0	0	0
VT - ATP/CV	1	0.02	1	0	0	0
VT - no therapy	0	0.00	0	0	0	0
VT - total	54	1.16	4	0	0	0
VF - CV	12	0.26	6	2	0.08	1
VF - therapy aborted	1	0.02	1	0	0	0
VF - total	13	0.28	6	2	0.08	1
VTVF unknown therapy	24	0.52	2	0	0	0
Total arrhythmias	91	1.96	9	2	0.08	1
<i>Total ventricular events</i>						
VT - ATP	85	1.83	6	7	0.27	3
VT - ATP/CV	4	0.09	3	1	0.04	1
VT - no therapy	0	0	0	1	0.04	1
VT - total	89	1.92	7	9	0.35	4
VF - CV	30	0.65	12	14	0.54	4
VF - therapy aborted	8	0.17	5	4	0.15	3
VF - total	38	0.82	13	18	0.69	5
VTVF unknown therapy	24	0.52	2	0	0	0
Total	151	3.25	19	27	1.04	7
<i>Non-ventricular events</i>						
VT - ATP	34	0.71	4	0	0	0
VT - ATP/CV	11	0.24	3	2	0.08	2
VT - no therapy	37	0.80	2	12	0.46	1
VT - total	82	1.75	5	14	0.54	3
VF - CV	11	0.24	2	1	0.04	1
VF - therapy aborted	0	0	0	0	0	0
VF - total	11	0.24	2	1	0.04	1
Total Non-ventricular events	93	1.98	6	15	0.58	3
<i>Unknown events</i>	208	4.59	5	0	0.00	0

¹ total number of events / total number of years of follow-up for all subjects combined

Abbreviations: VT - ATP, ventricular tachycardia with anti-tachycardia pacing; VT - ATP/CV, ventricular tachycardia with anti-tachycardia pacing followed by cardioversion; VF - CV, ventricular fibrillation with cardioversion

Table 3.3 Annualized arrhythmia rates in 20 subjects completing ≥ 12 months follow-up

	Baseline					Intervention				
	Percentiles					Percentiles				
	0	25	50	75	100	0	25	50	75	100
<i>Confirmed from episode data</i>										
VT - ATP	0 ¹	0	0	0	17.44	0	0	0	0	3.66
VT - ATP/CV	0	0	0	0	1.41	0	0	0	0	0.97
VT - no therapy	0	0	0	0	0	0	0	0	0	0.97
VT - total	0	0	0	0.22	17.44	0	0	0	0	3.66
VF - CV	0	0	0	0.39	4.98	0	0	0	0	3.89
VF - therapy aborted	0	0	0	0	3.98	0	0	0	0	1.24
VF - total	0	0	0.11	0.60	8.96	0	0	0	0.53	3.89
Total arrhythmias	0	0.05	0.46	1.12	17.44	0	0	0	1.70	5.80
<i>Confirmed from case notes</i>										
VT - ATP	0	0	0	0	13.93	0	0	0	0	0
VT - ATP/CV	0	0	0	0	0.67	0	0	0	0	0
VT - no therapy	0	0	0	0	0	0	0	0	0	0
VT - total	0	0	0	0	13.93	0	0	0	0	0
VF - CV	0	0	0	0.29	2.01	0	0	0	0	1.24
VF - therapy aborted	0	0	0	0	0.28	0	0	0	0	0
VF - total	0	0	0	0.29	2.01	0	0	0	0	1.24
VTVF unknown therapy	0	0	0	0	15.19	0	0	0	0	0
Total arrhythmias	0	0	0	1.45	15.19	0	0	0	0	1.24
<i>Total ventricular events</i>										
VT - ATP	0	0	0	0.64	17.44	0	0	0	0	3.66
VT - ATP/CV	0	0	0	0	1.14	0	0	0	0	0.97
VT - no therapy	0	0	0	0	0	0	0	0	0	0.97
VT - total	0	0	0	1.17	17.44	0	0	0	0	3.66
VF - CV	0	0	0.29	0.89	4.98	0	0	0	0	3.89
VF - therapy aborted	0	0	0	0.21	3.98	0	0	0	0	1.24
VF - total	0	0	0.33	1.25	8.96	0	0	0	0.53	4.97
VTVF unknown therapy	0	0	0	0	15.19	0	0	0	0	0
Total	0	0.44	1.43	6.41	17.44	0	0	0	1.70	5.80*
<i>Non-ventricular events</i>										
VT - ATP	0	0	0	0	14.84	0	0	0	0	0
VT - ATP/CV	0	0	0	0	2.75	0	0	0	0	0.80
VT - no therapy	0	0	0	0	18.26	0	0	0	0	7.37
VT - total	0	0	0	0.25	35.38	0	0	0	0	7.37
VF - CV	0	0	0	0	3.67	0	0	0	0	0.61
VF - therapy aborted	0	0	0	0	0	0	0	0	0	0
VF - total	0	0	0	0	3.67	0	0	0	0	0.61
Total Non-ventricular events	0	0	0	0.61	35.38	0	0	0	0	7.98
<i>Unknown events</i>	0	0	0	0.50	50.25	0	0	0	0	0

¹ individual annualized arrhythmia rate calculated for each subject

* $p < 0.05$ Wilcoxon's Signed Rank Test (paired analysis) comparing individual annualized event rates (see Fig 3.2 and text)

Abbreviations: VT - ATP, ventricular tachycardia with anti-tachycardia pacing; VT - ATP/CV, ventricular tachycardia with anti-tachycardia pacing followed by cardioversion; VF - CV, ventricular fibrillation with cardioversion

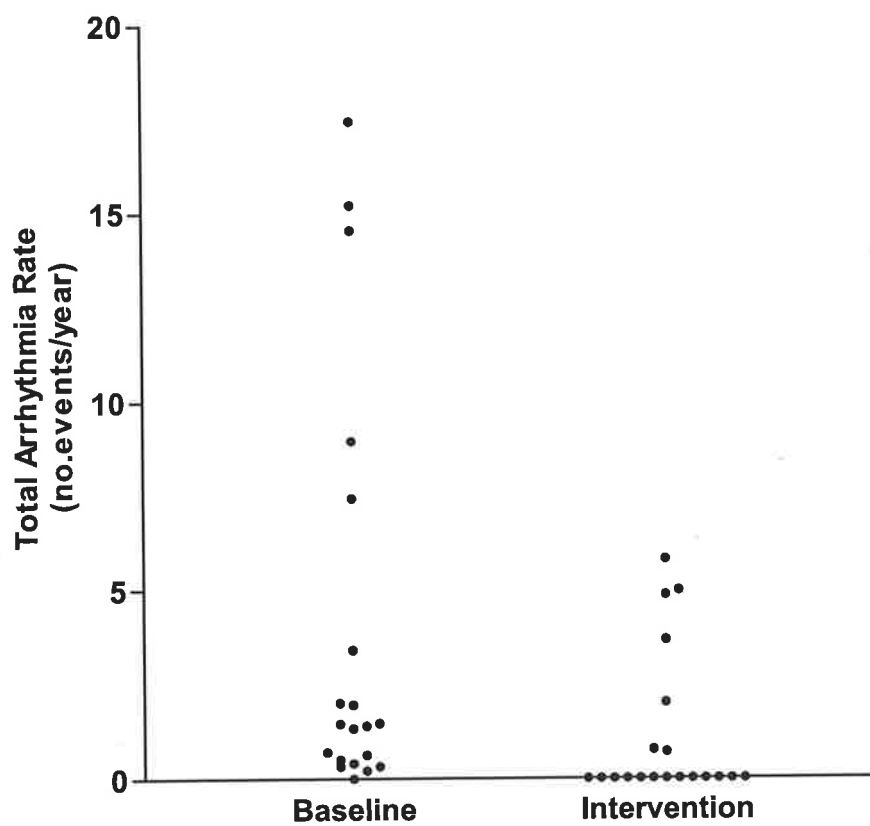


Figure 3.1 The total arrhythmia rate (no. of events/year) for baseline and intervention periods for the cohort with 20 subjects with ≥ 12 months follow-up

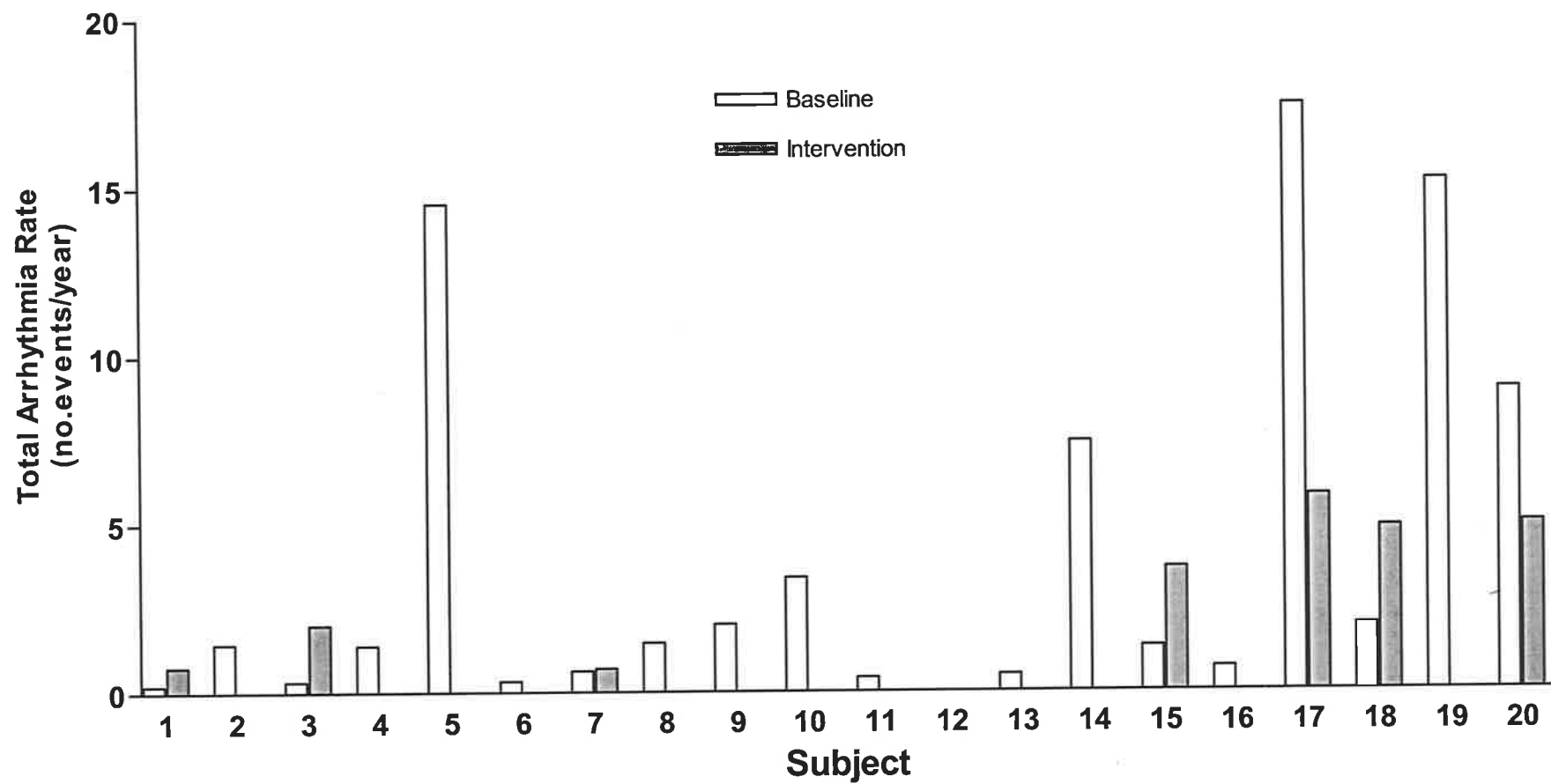


Figure 3.2 Comparison of the annualized arrhythmia rates for each of the 20 subjects who had completed at least 12 months of follow-up. The subjects are sorted in order of the ratio of baseline/intervention time, with the highest ratio on the left. The absence of a bar indicates that no arrhythmias were recorded

3.3.2.1.2 Non-ventricular events

There were 92 events distributed among 6 subjects recorded by the ICDs in the baseline period which were considered to be not ventricular arrhythmias, and 15 events among 3 subjects in the intervention period. Of these, a total of 13 events resulted in cardioversion following unsuccessful ATP therapy, and 12 resulted in cardioversion as a first therapy, resulting in a total of 25 events throughout the baseline and intervention periods for which inappropriate ICD discharges were recorded.

3.3.2.1.3 Unknown events

There were 208 unknown events recorded by ICDs in this group for which no data were available, distributed amongst 4 subjects:

- Subject VC was admitted to hospital 9 days post ICD implant following 4 episodes of defibrillator discharge. The hospital summary indicates that the shocks were appropriate treatments, however, the EGM traces were not available for review, and the data was cleared from the ICD following interrogation in hospital. These events have been included in the analysis as appropriate events. The device counters indicate that there were another 5 episodes recorded by the device during the period following implant until the data was cleared. It is possible that these events were ventricular arrhythmias which occurred during the 'electrical storm' and were either aborted cardioversions or VTs treated with ATP that were not reported in the patient case notes.
- Subject SD had a total of 179 events that were unable to be identified. He was admitted to a country hospital following a series of discharges from the ICD, and following interrogation of the device, the physician reported that some were triggered by atrial fibrillation (AF), while some EGMs suggested a rapid VT which were treated

appropriately. It was also mentioned that the patient had been having a lot of episodes of VT which had been detected by the device, however, VT therapies were not activated. It is therefore likely that a large number of these events were ventricular in origin, while some resulted in clearly inappropriate discharges. It is also probable that information on a large number of these events would not be available even if all events were printed out at the time of interrogation due to the storage capacity of the device (Medtronic Microjewel model 7223) being limited to about 50 events.

- Subject AJ had 22 unknown arrhythmic events. He had been admitted to hospital following an 'electrical storm' during which he had a number of episodes of VT, the last of which exhausted all therapies, and he remained in conscious VT until it was reverted in hospital via IV lignocaine. All of these episodes have the EGMs available. Mr AJ was next seen about 3 months later following recurrent discharge from the ICD when it was determined that there was a problem with the sensing lead, which was subsequently replaced. There were 62 events stored on the device which were deemed to be inappropriate. It is probable that the 22 unknown events were a part of the sensing lead problem, and the details were not recorded on the ICD as the maximum data storage capacity of the device had been reached, and these earlier events had been overwritten by more recent events.
- Subject EG has had 6 unknown events. He was admitted to hospital following multiple discharges from his ICD, and interrogation of the events revealed that the sensing lead was 'double counting', ie counting the R wave and the T wave as separate ventricular events, effectively doubling the heart rate. His device was reprogrammed and he was discharged, only to be readmitted about 1 month later with a similar problem. There were 4 events recorded by the device between the 2 admissions which were presumably cleared from the device following interrogation after his second admission.

He eventually had the lead replaced, and 2 days later his device recorded a further 2 episodes of VT, however, no other details of these 2 events are available, although it is probable that these events were ventricular arrhythmias as the lead problem had been corrected, and all of the patient's other events had been ventricular arrhythmias.

As it is highly likely that some of the unknown arrhythmic events were ventricular in origin, a modeling exercise has been undertaken to include those unknown events that are considered as probable ventricular events into the total number of ventricular arrhythmias. For this exercise, there are a total of 57 events amongst 3 subjects that are included; subject VC with 5 events, EG with 2 events (the 2 following the lead replacement) and for subject SD, 50 events have been included although the actual number could be considerably higher or lower than this. With these events included, the total number of ventricular arrhythmic events in the baseline period increased to 208 at a rate of 4.48/person year, with the annualized within subject rate remaining significant with an even lower p value ($p=0.01$, Wilcoxon's Signed Rank Test).

In this analysis, 2 subjects had Medtronic model 7220 devices (which do not serially number each event), with each subject recording 1 event in the baseline period. It is possible that these 2 subjects may have had additional arrhythmic events in the baseline period which have not been identified.

3.3.2.2 Diets

Of the 20 subjects who completed ≥ 12 months of follow-up, 9 lived in country areas and therefore consumed only fish oil capsules for this study. The remaining 11 subjects commenced the study consuming the n-3 PUFA rich diet, although 1 subject subsequently

transferred to capsules only after 1 month due to a dislike of the provided food items. One subject consuming the n-3 PUFA diet failed to adequately complete the n-3 diet diary, and therefore the analysis of the dietary contribution of each source of n-3 PUFA is based on 9 subjects.

The mean daily intake of EPA + DHA was 941 ± 299 mg/day. The highest mean daily intake was 1353 mg and the lowest was 543 mg in a woman who attempted to make up the shortfall with capsules but reported that she had difficulty in swallowing them and therefore stopped taking them after 1 capsule.

One subject went to live with one of his children in Canberra for a 6 month period during the study and consumed only capsules and a small amount of fresh fish whilst there. His contribution from capsules to the total EPA + DHA intake was therefore considerably higher, at 72% of total intake, than the other subjects. During the period that he was in Adelaide, the contribution of capsules to his total EPA + DHA intake was 56%, with margarine spread contributing 8.5%, milk contributing 30%, and all fish combined contributing 5.5%. Only 2 subjects consumed no capsules during the course of the study.

Of the food items provided to subjects, milk and canned pink salmon both provided about 25% of the daily intakes of EPA + DHA among those consuming those items. Amongst the 9 subjects consuming margarine spread, the mean intake of EPA + DHA from that source was 101 mg/day, or about 11% of the daily total. The contribution of each n-3 PUFA source to the total intake is provided in table 3.4.

Table 3.4 Subject consumption of LC n-3 PUFA sources, and estimates of the contribution of those foods to the overall intake of EPA + DHA

n-3 PUFA source	Number of consumers	Estimated daily intake of EPA + DHA from each source (mg/day)	% of total EPA + DHA among consumers
Margarine spread	9	101±75 (10-272) ^{1,2}	10.7±5.9 (1-20) ^{1,3}
Milk	8	247±115 (21-392)	26.3±12.7 (2-42)
Pink salmon	9	230±148 (14-545)	25.5±17.0 (2-60)
Sardines	7	141±93 (4-306)	13.7±10.0 (1-34)
Fresh fish	8	57±39 (17-125)	6.1±4.4 (2-13)
Capsules	7	295±154 (139-622)	31.3±19.0 (19-72)

¹ $\bar{x}$ ±SD (range in parentheses)

² the intake of EPA + DHA contributed by the food in only those subjects consuming that source

³ the contribution of each source to the total intake of EPA + DHA among those subjects consuming that source

3.3.2.3 Fatty acids

There were significant increases in the proportion of n-3 PUFAs in both plasma phospholipids and red blood cell phospholipids compared with baseline (Table 3.5 and Figures 3.3 & 3.4). EPA increased by 94% in plasma phospholipids and by 76% in red blood cells. DHA increased by 31% in plasma phospholipids and by 22% in red blood cells. While DPA also increased significantly in red blood cells, the rise was marginal at 7%, whereas a 6% increase in plasma phospholipids was not statistically significant. Total n-3 PUFA increased by 38% in plasma phospholipids and by 23% in red blood cells.

All n-6 PUFAs examined decreased significantly, although the fall in LA was small at 3% in both plasma and red blood cells, whereas AA fell by 12% in plasma phospholipids, and by 10% in red blood cells. Total n-6 PUFA fell by 7% in plasma phospholipids and by 9% in red blood cells.

There were small increases in the proportion of total saturated fats of 1% and 2% in plasma and red blood cells respectively, which were statistically significant on paired analysis.

Likewise, there were small but significant decreases of 5% and 4% in the proportions of OA and total monounsaturates respectively in plasma phospholipids.

Table 3.5 The fatty acid composition of plasma and red blood cell phospholipids in 20 subjects with ≥ 12 months follow-up

Fatty Acid	baseline	Follow-up					Mean ¹
		1 month	3 months	6 months	12 months	18 months	
Time (months)	0	1.1 $\pm$ 0.3 ²	3.0 $\pm$ 0.5	6.5 $\pm$ 0.9	11.9 $\pm$ 1.6	16.8 $\pm$ 1.7	
<i>Plasma Phospholipids</i>							
<i>n</i>	19 ^{3,4}	10	12	17	17	12	19
Total Sats	42.8 $\pm$ 1.2	42.8 $\pm$ 1.1	43.6 $\pm$ 1.3	43.3 $\pm$ 0.7	43.3 $\pm$ 1.0	43.6 $\pm$ 1.0	43.3 $\pm$ 0.7*
18:1 n-9	10.7 $\pm$ 1.4	9.8 $\pm$ 1.0	9.9 $\pm$ 0.9	9.9 $\pm$ 1.0	10.3 $\pm$ 1.3	9.8 $\pm$ 1.0	10.2 $\pm$ 0.8*
Total Monos	14.5 $\pm$ 1.6	13.5 $\pm$ 1.0	13.7 $\pm$ 1.0	13.5 $\pm$ 1.3	14.1 $\pm$ 1.4	13.5 $\pm$ 1.3	13.9 $\pm$ 1.1*
n-6							
18:2	19.2 $\pm$ 2.1	18.3 $\pm$ 1.9	18.7 $\pm$ 2.4	18.9 $\pm$ 2.8	18.1 $\pm$ 1.7	19.2 $\pm$ 2.7	18.6 $\pm$ 2.0*
20:4	11.0 $\pm$ 2.7	10.6 $\pm$ 2.3	9.3 $\pm$ 2.1	9.6 $\pm$ 1.9	9.4 $\pm$ 2.0	9.6 $\pm$ 2.3	9.7 $\pm$ 2.0 [‡]
Total	34.8 $\pm$ 1.9	33.1 $\pm$ 1.2	32.2 $\pm$ 1.4	32.6 $\pm$ 1.7	31.4 $\pm$ 1.5	32.8 $\pm$ 2.3	32.4 $\pm$ 1.3 [‡]
n-3							
20:5	1.19 $\pm$ 0.47	2.39 $\pm$ 0.69	2.43 $\pm$ 1.11	2.37 $\pm$ 0.72	2.74 $\pm$ 1.08	1.98 $\pm$ 0.66	2.31 $\pm$ 0.76 [‡]
22:5	1.20 $\pm$ 0.32	1.35 $\pm$ 0.33	1.32 $\pm$ 0.13	1.33 $\pm$ 0.24	1.22 $\pm$ 0.18	1.19 $\pm$ 0.27	1.27 $\pm$ 0.20
22:6	3.90 $\pm$ 0.94	5.38 $\pm$ 0.78	4.98 $\pm$ 0.88	5.07 $\pm$ 1.18	5.52 $\pm$ 1.10	5.27 $\pm$ 1.18	5.12 $\pm$ 0.93 [‡]
Total	6.61 $\pm$ 1.13	9.43 $\pm$ 0.95	9.17 $\pm$ 1.54	9.13 $\pm$ 1.93	9.90 $\pm$ 1.81	8.87 $\pm$ 1.63	9.12 $\pm$ 1.40 [‡]
<i>Red Blood Cell Phospholipids</i>							
<i>n</i>	20 ³	10	12	17	17	12	20
Total Sats	41.6 $\pm$ 0.9	41.5 $\pm$ 0.7	42.8 $\pm$ 1.3	42.7 $\pm$ 0.8	42.2 $\pm$ 1.1	42.1 $\pm$ 0.9	42.3 $\pm$ 0.9 [†]
18:1 n-9	13.9 $\pm$ 0.8	14.3 $\pm$ 0.6	14.0 $\pm$ 0.5	13.8 $\pm$ 1.0	14.0 $\pm$ 0.7	13.9 $\pm$ 0.7	13.9 $\pm$ 0.7
Total Monos	18.4 $\pm$ 1.0	18.9 $\pm$ 0.8	18.4 $\pm$ 0.6	18.2 $\pm$ 1.3	18.4 $\pm$ 0.9	18.2 $\pm$ 1.0	18.4 $\pm$ 0.9
n-6							
18:2	9.3 $\pm$ 1.7	8.8 $\pm$ 1.3	9.0 $\pm$ 1.5	9.2 $\pm$ 1.7	8.8 $\pm$ 1.6	9.0 $\pm$ 1.1	9.0 $\pm$ 1.5*
20:4	14.4 $\pm$ 1.2	13.9 $\pm$ 1.3	12.7 $\pm$ 0.9	12.9 $\pm$ 1.3	12.6 $\pm$ 1.3	13.0 $\pm$ 1.1	13.0 $\pm$ 1.2 [‡]
Total	29.7 $\pm$ 1.5	28.4 $\pm$ 1.3	26.6 $\pm$ 1.0	26.8 $\pm$ 1.8	26.0 $\pm$ 2.0	26.7 $\pm$ 2.0	26.9 $\pm$ 1.6 [‡]
n-3							
20:5	0.95 $\pm$ 0.33	1.39 $\pm$ 0.38	1.64 $\pm$ 0.43	1.73 $\pm$ 0.54	1.93 $\pm$ 0.45	1.69 $\pm$ 0.49	1.67 $\pm$ 0.39 [‡]
22:5	3.01 $\pm$ 0.52	3.24 $\pm$ 0.59	3.20 $\pm$ 0.43	3.22 $\pm$ 0.48	3.37 $\pm$ 0.37	3.34 $\pm$ 0.49	3.23 $\pm$ 0.38 [†]
22:6	5.05 $\pm$ 1.11	5.57 $\pm$ 0.98	6.04 $\pm$ 0.89	6.10 $\pm$ 0.87	6.67 $\pm$ 0.81	6.60 $\pm$ 1.17	6.16 $\pm$ 0.76 [‡]
Total	9.3 $\pm$ 1.2	10.4 $\pm$ 1.0	11.2 $\pm$ 1.1	11.3 $\pm$ 1.6	12.3 $\pm$ 1.1	11.9 $\pm$ 1.7	11.3 $\pm$ 1.1*

¹ $\bar{x}$ $\pm$ SD of individual subject's values calculated from summary measures (area under curve/total follow-up time) as outlined in section 3.2.9.1 (* p <0.05, [†] p <0.01, [‡] p <0.001 paired t -test comparing baseline and follow-up mean)

² $\bar{x}$ $\pm$ SD months from enrolment

³ number of subjects who had blood samples at each nominated time point

⁴ the plasma phospholipid sample of 1 subject was contaminated and not included in the analysis

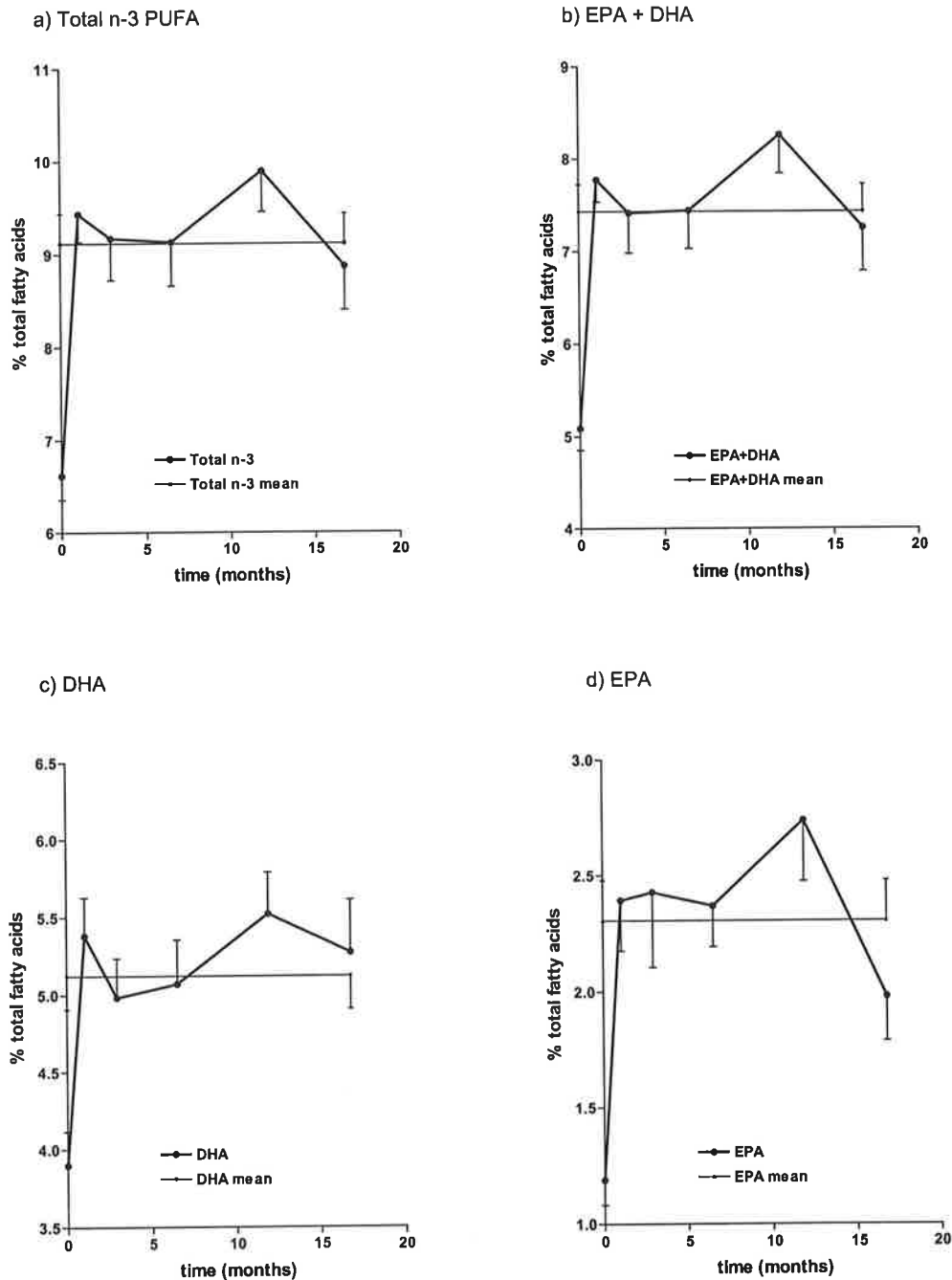


Figure 3.3 Changes in plasma phospholipid n-3 fatty acids during the course of the study in 20 subjects with ≥ 12 months follow-up. The values are $\bar{x} \pm SD$ at each time point as presented in table 3.2 (thick lines). The straight lines represent the mean fatty acid level attained during the study period, with the error bars representing the SD of the individual mean values (calculated from the area under the curve divided by the length of follow-up time for each subject as detailed in section 3.2.10.5). Note the differences in the scale of the Y-axis

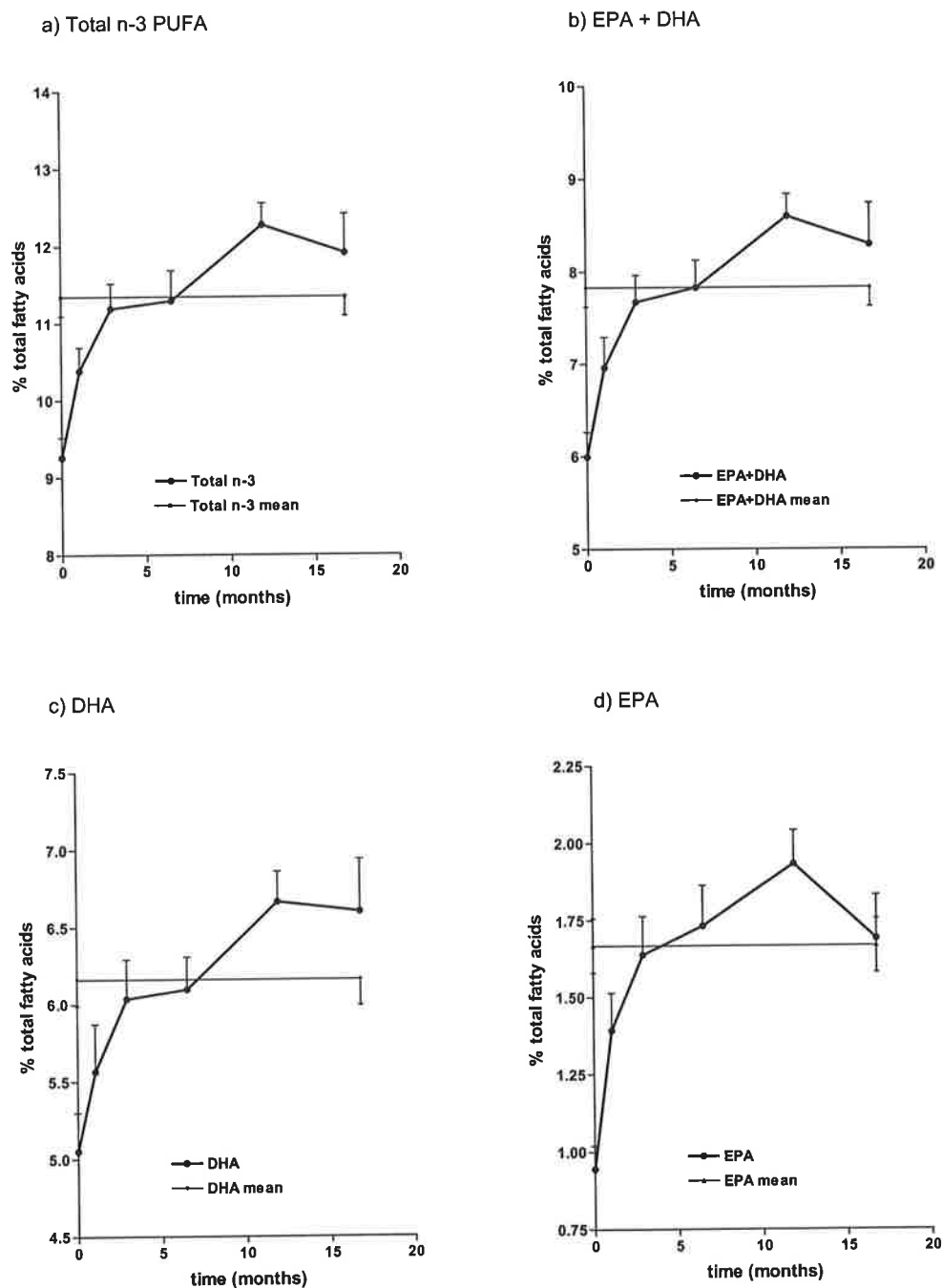


Figure 3.4 Changes in red blood cell n-3 fatty acids during the course of the study in 20 subjects with ≥ 12 months follow-up. The values are $\bar{x} \pm SD$ at each time point as presented in table 3.2 (thick lines). The straight lines represent the mean fatty acid level attained during the study period, with the error bars representing the SD of the individual mean values (calculated from the area under the curve divided by the length of follow-up time for each subject as detailed in section 3.2.10.5). Note the differences in the scale of the Y-axis

3.3.3 Cohort with ≥ 6 Months Follow-up

Section 3.3.2 provides an analysis of the cohort with ≥ 12 months follow-up. Being an interim analysis, and for the purposes of inclusion in this thesis, a similar analysis of the cohort with ≥ 6 months follow-up is provided. The 33 subjects who had completed at least 6 months of follow-up had a combined total of 28452 days (77.9 years) of baseline data, and 12674 days (34.7 years) of follow-up data.

3.3.3.1 Incidence of Arrhythmias

3.3.3.1.1 Ventricular arrhythmias

In the baseline period there were 280 ventricular arrhythmic events distributed amongst 29 subjects at a mean rate of 3.59/person year (Table 3.6). Of these, 163 events distributed amongst 25 subjects were confirmed by independent analysis of event data, with the remaining 117 events distributed amongst 13 subjects identified from the examination of casenote records.

During the intervention period, there were 41 ventricular arrhythmic events distributed amongst 14 subjects at a mean rate of 1.18/person year. Of these, 39 events distributed amongst 14 subjects were confirmed from individual event data, with the remaining 2 events from 1 subject confirmed from casenote records.

The median and maximum annualized arrhythmia rates for an individual in the baseline period were 1.4 and 23.9 events/year respectively, compared with 0.0 and 7.5 events/year respectively in the intervention period (Table 3.7 and Figure 3.5). Twenty three subjects had a reduction in their annualized arrhythmia rate, with 7 subjects having an increased rate (Figure 3.6), and no change in the rate in the remaining 3 subjects (subjects #5, #15,

#25), who had no events in either period. There were 19 subjects who had no events in the intervention period, compared with 4 subject in the baseline period.

There was a statistically significant reduction in the annualized arrhythmia rate when analyzed on a paired basis ($p=0.003$, Wilcoxon's Signed Rank Test, Figure 3.6). This reduction was largely due to a significant decrease in arrhythmias detected in the VT zone and treated with anti-tachycardia pacing therapy, where there were 181 events amongst 13 subjects in the baseline period and 11 events amongst 6 subjects in the follow-up period ($p=0.03$, Wilcoxon's Signed Rank Test), and the number of events/person year fell from 2.3 to 0.3.

Table 3.6 Total arrhythmias recorded in 33 subjects completing ≥ 6 months follow-up

	Baseline			Intervention		
	No. of events	Rate / person year	No. patients with events	No. of events	Rate / person year	No. patients with events
<i>Confirmed from episode data</i>						
VT - ATP	115	1.48 ¹	11	11	0.32	6
VT - ATP/CV	5	0.06	3	4	0.12	2
VT - no therapy	0	0	0	1	0.03	1
VT - total	120	1.54	12	16	0.46	7
VF - CV	29	0.37	12	16	0.46	6
VF - therapy aborted	14	0.18	9	7	0.20	6
VF - total	43	0.55	17	23	0.66	10
Total arrhythmias	163	2.09	25	39	1.12	14
<i>Confirmed from case notes</i>						
VT - ATP	66	0.85	5	0	0	0
VT - ATP/CV	1	0.01	1	0	0	0
VT - no therapy	1	0.01	1	0	0	0
VT - total	68	0.87	6	0	0	0
VF - CV	14	0.18	8	2	0.06	1
VF - therapy aborted	11	0.14	3	0	0	0
VF - total	25	0.32	10	2	0.06	1
VTVF unknown therapy	24	0.31	2	0	0	0
Total arrhythmias	117	1.50	13	2	0.06	1
<i>Total ventricular events</i>						
VT - ATP	181	2.32	13	11	0.32	6
VT - ATP/CV	6	0.08	4	4	0.12	2
VT - no therapy	1	0.01	1	1	0.01	1
VT - total	188	2.41	14	16	0.46	7
VF - CV	43	0.55	16	18	0.52	6
VF - therapy aborted	25	0.32	11	7	0.20	6
VF - total	68	0.87	21	25	0.72	10
VTVF unknown therapy	24	0.31	2	0	0	0
Total	280	3.59	29	41	1.18	14
<i>Non-ventricular events</i>						
VT - ATP	41	0.51	5	2	0.06	2
VT - ATP/CV	12	0.15	4	2	0.06	2
VT - no therapy	39	0.50	3	12	0.50	1
VT - total	92	1.17	8	16	0.46	5
VF - CV	11	0.14	2	1	0.03	1
VF - therapy aborted	1	0.01	1	0	0	0
VF - total	12	0.15	3	1	0.03	1
Total Non-ventricular events	104	1.37	9	17	0.49	5
<i>Unknown events</i>	211	2.72	6	0	0	0

¹ total number of events / total number of years of follow-up for all subjects combined

Abbreviations: VT - ATP, ventricular tachycardia with anti-tachycardia pacing; VT - ATP/CV, ventricular tachycardia with anti-tachycardia pacing followed by cardioversion; VF - CV, ventricular fibrillation with cardioversion

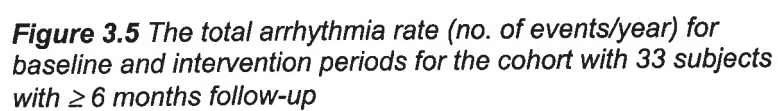
Table 3.7 Annualized arrhythmia rates in 33 subjects completing ≥ 6 months follow-up

	Baseline					Intervention				
	Percentiles					Percentiles				
	0	25	50	75	100	0	25	50	75	100
<i>Confirmed from episode data</i>										
VT - ATP	0 [†]	0	0	0.46	23.90	0	0	0	0	3.73
VT - ATP/CV	0	0	0	0	1.14	0	0	0	0	3.21
VT - no therapy	0	0	0	0	0	0	0	0	0	0.97
VT - total	0	0	0	0.64	23.90	0	0	0	0	4.28
VF - CV	0	0	0	0.45	4.98	0	0	0	0	3.89
VF - therapy aborted	0	0	0	0.28	3.98	0	0	0	0	1.80
VF - total	0	0	0.22	0.83	8.96	0	0	0	1.04	3.89
Total arrhythmias	0	0.11	0.67	1.54	23.90	0	0	0	1.76	7.50
<i>Confirmed from case notes</i>										
VT - ATP	0	0	0	0	13.93	0	0	0	0	0
VT - ATP/CV	0	0	0	0	0.67	0	0	0	0	0
VT - no therapy	0	0	0	0	0.28	0	0	0	0	0
VT - total	0	0	0	0	13.93	0	0	0	0	0
VF - CV	0	0	0	0.14	2.01	0	0	0	0	1.24
VF - therapy aborted	0	0	0	0	2.20	0	0	0	0	0
VF - total	0	0	0	0.34	2.20	0	0	0	0	1.24
VTVF unknown therapy	0	0	0	0	15.19	0	0	0	0	0
Total arrhythmias	0	0	0	1.05	15.19	0	0	0	0	1.24
<i>Total ventricular events</i>										
VT - ATP	0	0	0	1.14	23.90	0	0	0	0	3.73*
VT - ATP/CV	0	0	0	0	1.14	0	0	0	0	3.21
VT - no therapy	0	0	0	0	0.28	0	0	0	0	0.97
VT - total	0	0	0	1.25	23.90	0	0	0	0	4.28*
VF - CV	0	0	0	0.79	4.98	0	0	0	0	3.89
VF - therapy aborted	0	0	0	0.43	3.98	0	0	0	0	1.80
VF - total	0	0	0.37	1.34	8.96	0	0	0	1.04	4.97
VTVF unknown therapy	0	0	0	0	15.19	0	0	0	0	0
Total	0	0.46	1.43	6.35	23.90	0	0	0	1.76	7.50 [†]
<i>Non-ventricular events</i>										
VT - ATP	0	0	0	0	14.84	0	0	0	0	1.68
VT - ATP/CV	0	0	0	0	2.75	0	0	0	0	0.80
VT - no therapy	0	0	0	0	18.26	0	0	0	0	7.37
VT - total	0	0	0	0.16	35.34	0	0	0	0	7.37
VF - CV	0	0	0	0	3.67	0	0	0	0	0.61
VF - therapy aborted	0	0	0	0	0.45	0	0	0	0	0
VF - total	0	0	0	0	3.67	0	0	0	0	0.61
Total Non-ventricular events	0	0	0	0.41	35.38	0	0	0	0	7.98
<i>Unknown events</i>										
	0	0	0	0	50.25	0	0	0	0	0

[†] Individual annualized arrhythmia rate calculated for each subject

* $p < 0.05$; [†] $p < 0.01$ Wilcoxon's Signed Rank Test (paired analysis) comparing individual annualized event rates (see Fig 3.6 and text)

Abbreviations: VT - ATP, ventricular tachycardia with anti-tachycardia pacing; VT - ATP/CV, ventricular tachycardia with anti-tachycardia pacing followed by cardioversion; VF - CV, ventricular fibrillation with cardioversion



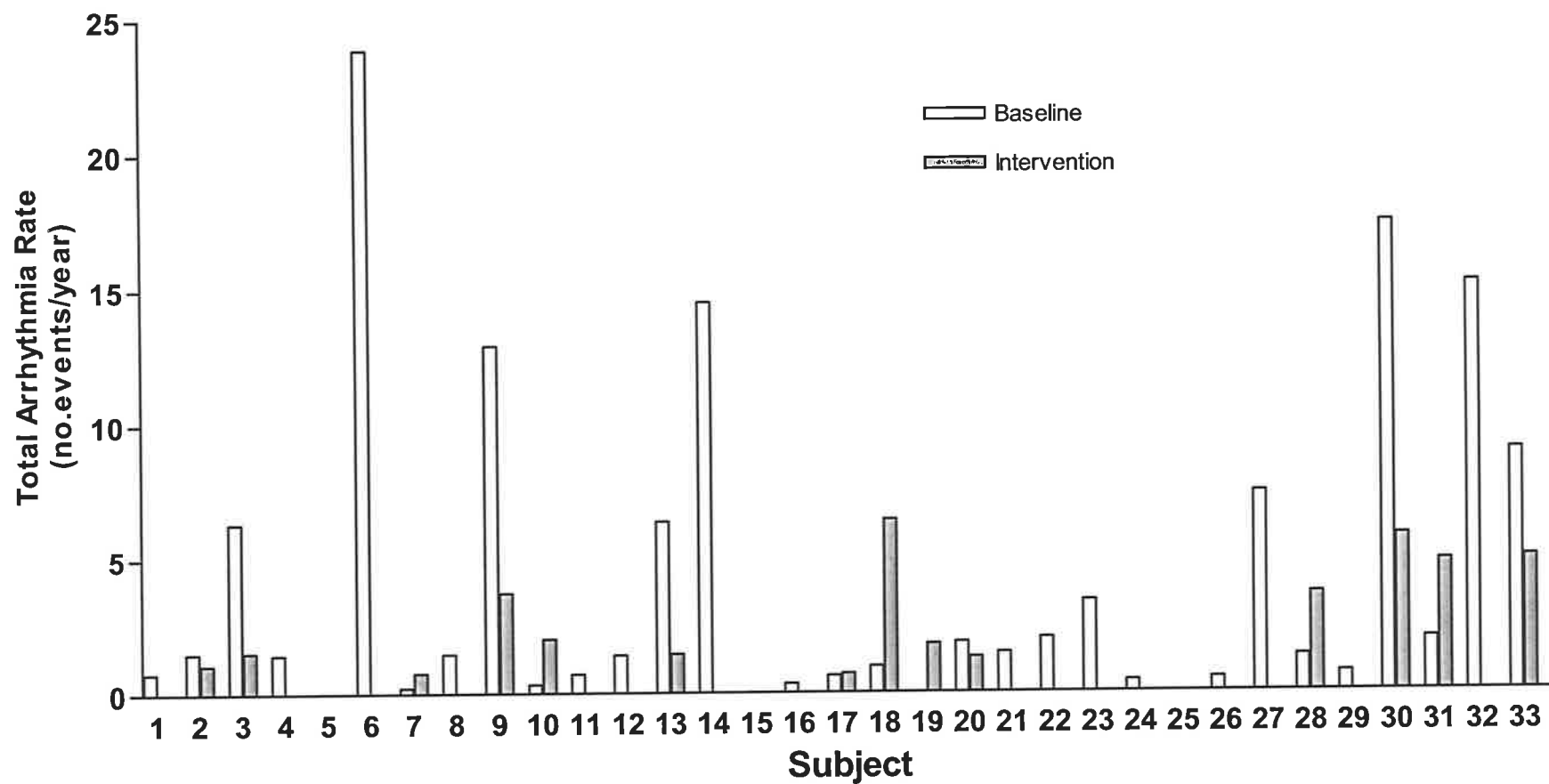


Figure 3.6 Comparison of the annualized arrhythmia rates for each of the 33 subjects who had completed at least 6 months of follow-up. The subjects are sorted in order of the ratio of baseline/intervention time, with the highest ratio on the left. The absence of a bar indicates that no arrhythmias were recorded

3.3.3.1.2 Non-ventricular events

There were 103 events distributed among 9 subjects recorded by the ICDs in the baseline period which were considered to be not ventricular arrhythmias, and 17 events in 5 subjects in the follow-up period. Of these, a total of 14 events resulted in cardioversion following unsuccessful ATP therapy, and 12 resulted in cardioversion as a first therapy, resulting in a total of 26 events throughout the baseline and intervention periods for which inappropriate ICD discharges were recorded.

3.3.3.1.3 Unknown events

There were 211 unknown arrhythmic events in the baseline period, distributed amongst 6 subjects, ie the 208 events outlined in section 3.3.2.1.3 plus:

- Subject GS has had 1 unknown event. He had 4 events recorded in the first 8 weeks following implant, however, only 3 of the events (1 x VT and 2 in the VF zone) were printed out. Device counters indicate that the missing event was registered in the VT zone, however, no other information is available.
- Subject GG has 2 unknown events in the first 6 weeks following implant. Device counters indicate that both events were detected in the VT zone. He had a number of subsequent events in the next year which were assessed as being SVTs inappropriately treated with ATP, and he then had a large number of appropriately treated VTs during an ‘electrical storm’.

There were no unknown events in the follow-up period. When the same modeling exercise is undertaken as described in section 3.3.2.1.3, with the addition of subject GS with 1 event, the total number of arrhythmias in the baseline period increased to 338, distributed amongst 29 subjects equivalent to 4.34 events /person year, with the annualized within

subject rate remaining significant with an even lower p value ($p=0.0071$, Wilcoxon's Signed Rank Test).

In this analysis, 1 subject had a Medtronic model 7219 device in addition to the 2 subjects identified in section 3.3.2.1.1 that had Medtronic model 7220 devices, with this subject having 6 events recorded in the baseline period on this device prior to it being replaced. It is possible that these 3 subjects may have had additional events in the baseline period which have not been identified.

3.3.3.2 Diets

Of the 33 subjects who completed ≥ 6 months of follow-up, 19 lived in country areas and therefore consumed only capsules. The remaining 14 subjects commenced the study consuming the n-3 PUFA rich diet, however, 2 subjects subsequently transferred to capsules only due to a dislike of the provided food items. Two subjects consuming the n-3 PUFA diet failed to adequately complete the n-3 diet diary, and therefore the analysis of the dietary contribution of each source of n-3 PUFA is based on 10 subjects.

The mean daily intake of EPA + DHA was 930 ± 284 mg/day. The highest mean daily intake was 1353 mg and the lowest was 543 mg in a woman who attempted to make up the shortfall with capsules but reported that she had difficulty in swallowing them and therefore stopped taking them after 1 capsule. The contribution of each n-3 PUFA source to the total intake is provided in table 3.8. One subject went to live with one of his children in Canberra for a 6 month period during the study and consumed only capsules and a small amount of fresh fish whilst there. His contribution from capsules to the total EPA + DHA

intake was therefore considerably higher, at 71% of total intake, than the other subjects.

Only 2 subjects consumed no capsules during the course of the study.

Table 3.8 Subject consumption of LC n-3 PUFA sources, and estimates of the contribution of those foods to the overall intake of EPA + DHA

n-3 PUFA source	Number of consumers	Estimated daily intake of EPA + DHA from each source (mg/day)	% of total EPA + DHA among consumers
Margarine spread	10	104±72 (10-272) ^{1,2}	11.2±5.8 (1-20) ^{1,3}
Milk	9	248±108 (21-392)	26.7±12.0 (2-42)
Pink salmon	10	231±140 (14-545)	25.7±16.0 (2-60)
Sardines	7	141±93 (4-306)	13.7±10.0 (1-34)
Fresh fish	9	62±39 (17-125)	6.8±4.6 (2-13)
Capsules	8	272±157 (112-622)	29.1±18.7 (13-72)

¹ $\bar{x} \pm SD$ (range in parentheses)

² the intake of EPA + DHA contributed by the food in only those subjects consuming that source

³ the contribution of each source to the total intake of EPA + DHA among those subjects consuming that source

3.3.3.3 Fatty Acids

Changes in plasma and red blood cell phospholipid fatty acids were similar to those observed in the in analysis of the subgroup of subjects completing more than 12 months of intervention. There were significant increases in the proportion of n-3 PUFAs in both plasma phospholipids and red blood cell phospholipids compared with baseline (Table 3.9 and Figures 3.7 & 3.8). EPA increased by 72% in plasma phospholipids and by 70% in red blood cells. DHA increased by 28% in plasma phospholipids and by 21% in red blood cells. DPA increased by 8% in plasma phospholipids and by 6% in red blood cells. Total n-3 PUFA increased by 32% in plasma phospholipids and by 22% in red blood cells.

All n-6 PUFAs examined decreased significantly, although the fall in LA was small at 6% in plasma phospholipids and 2% in red blood cells, whereas AA fell by 12% in plasma phospholipids and by 8% in red blood cells. Total n-6 PUFA fell by 9% in plasma phospholipids and by 8% in red blood cells.

Again, there were small statistically significant increases in the proportion of total saturated fats in plasma and red blood cells of 1% in each. Likewise, there were small but significant decreases of 4% and 3% in the proportions of OA and total monounsaturates respectively in plasma phospholipids, but no changes in red blood cells.

Table 3.9 The fatty acid composition of plasma and red blood cell phospholipids in 33 subjects with ≥ 6 months follow-up

Fatty Acid	baseline	Follow-up					Mean ¹
		1 month	3 months	6 months	12 months	18 months	
Time (months)	0	1.1 $\pm$ 0.3 ²	3.0 $\pm$ 0.4	6.6 $\pm$ 0.9	11.7 $\pm$ 1.6	16.8 $\pm$ 1.7	
<i>Plasma Phospholipids</i>							
<i>n</i>	30 ^{3,4}	12	15	27	20	12	30
Total Sats	42.9 $\pm$ 1.0 ¹	42.8 $\pm$ 1.0	43.5 $\pm$ 1.2	43.4 $\pm$ 0.8	43.4 $\pm$ 1.0	43.6 $\pm$ 1.0	43.3 $\pm$ 0.7 [†]
18:1 n-9	10.8 $\pm$ 1.3	10.0 $\pm$ 1.1	10.0 $\pm$ 0.8	10.3 $\pm$ 1.1	10.3 $\pm$ 1.3	9.7 $\pm$ 1.0	10.4 $\pm$ 0.9*
Total Monos	14.5 $\pm$ 1.4	13.7 $\pm$ 1.1	13.7 $\pm$ 1.0	13.8 $\pm$ 1.3	14.1 $\pm$ 1.4	13.5 $\pm$ 1.3	14.1 $\pm$ 1.1*
n-6							
18:2	18.8 $\pm$ 2.5	18.1 $\pm$ 1.8	18.0 $\pm$ 2.6	18.3 $\pm$ 2.8	18.1 $\pm$ 1.9	19.2 $\pm$ 2.7	18.3 $\pm$ 2.2*
20:4	10.8 $\pm$ 2.4	10.7 $\pm$ 2.1	9.7 $\pm$ 2.2	9.5 $\pm$ 1.7	9.4 $\pm$ 1.9	9.6 $\pm$ 2.3	9.8 $\pm$ 1.8 [‡]
Total	34.5 $\pm$ 2.3	33.1 $\pm$ 1.2	32.0 $\pm$ 1.4	32.0 $\pm$ 1.9	31.4 $\pm$ 1.6	32.8 $\pm$ 2.3	32.3 $\pm$ 1.6 [‡]
n-3							
20:5	1.28 $\pm$ 0.51	2.32 $\pm$ 0.65	2.49 $\pm$ 1.01	2.37 $\pm$ 0.62	2.70 $\pm$ 1.00	1.98 $\pm$ 0.66	2.20 $\pm$ 0.67 [‡]
22:5	1.25 $\pm$ 0.28	1.36 $\pm$ 0.30	1.37 $\pm$ 0.16	1.38 $\pm$ 0.22	1.23 $\pm$ 0.18	1.19 $\pm$ 0.27	1.32 $\pm$ 0.19*
22:6	3.91 $\pm$ 0.99	5.35 $\pm$ 0.73	5.16 $\pm$ 0.88	5.21 $\pm$ 1.07	5.46 $\pm$ 1.03	5.27 $\pm$ 1.18	5.02 $\pm$ 0.91 [‡]
Total	6.75 $\pm$ 1.36	9.34 $\pm$ 0.90	9.45 $\pm$ 1.53	9.33 $\pm$ 1.67	9.79 $\pm$ 1.69	8.87 $\pm$ 1.63	8.93 $\pm$ 1.35 [‡]
<i>Red Blood Cell Phospholipids</i>							
<i>n</i>	31 ³	12	15	27	20	12	
Total Sats	41.8 $\pm$ 0.8	41.7 $\pm$ 0.7	42.6 $\pm$ 1.3	42.5 $\pm$ 0.8	42.1 $\pm$ 1.0	42.2 $\pm$ 0.9	42.2 $\pm$ 0.6 [†]
18:1 n-9	14.2 $\pm$ 0.9	14.4 $\pm$ 0.6	14.2 $\pm$ 0.7	14.1 $\pm$ 0.9	14.1 $\pm$ 0.7	13.9 $\pm$ 0.7	14.2 $\pm$ 0.7
Total Monos	18.8 $\pm$ 1.0	18.9 $\pm$ 0.8	18.5 $\pm$ 0.6	18.4 $\pm$ 1.1	18.6 $\pm$ 0.9	18.2 $\pm$ 1.0	18.6 $\pm$ 0.9
n-6							
18:2	9.2 $\pm$ 1.5	8.6 $\pm$ 1.2	8.7 $\pm$ 1.5	9.0 $\pm$ 1.5	8.8 $\pm$ 1.5	9.0 $\pm$ 1.1	8.9 $\pm$ 1.4 [†]
20:4	14.2 $\pm$ 1.0	14.0 $\pm$ 1.2	12.9 $\pm$ 1.0	12.8 $\pm$ 1.2	12.7 $\pm$ 1.2	13.1 $\pm$ 1.2	13.1 $\pm$ 1.0 [‡]
Total	29.2 $\pm$ 1.6	28.2 $\pm$ 1.4	26.4 $\pm$ 1.0	26.5 $\pm$ 1.7	26.0 $\pm$ 1.8	26.8 $\pm$ 2.0	26.9 $\pm$ 1.5 [‡]
n-3							
20:5	0.95 $\pm$ 0.31	1.39 $\pm$ 0.35	1.70 $\pm$ 0.45	1.76 $\pm$ 0.47	1.93 $\pm$ 0.47	1.71 $\pm$ 0.52	1.60 $\pm$ 0.38 [‡]
22:5	3.04 $\pm$ 0.44	3.25 $\pm$ 0.53	3.25 $\pm$ 0.41	3.35 $\pm$ 0.47	3.38 $\pm$ 0.34	3.31 $\pm$ 0.49	3.28 $\pm$ 0.34 [‡]
22:6	4.89 $\pm$ 1.03	5.58 $\pm$ 0.89	6.08 $\pm$ 0.81	6.22 $\pm$ 0.87	6.61 $\pm$ 0.76	6.55 $\pm$ 1.13	5.98 $\pm$ 0.81 [‡]
Total	9.1 $\pm$ 1.2	10.4 $\pm$ 0.9	11.3 $\pm$ 1.1	11.6 $\pm$ 1.5	12.2 $\pm$ 1.1	11.9 $\pm$ 1.7	11.1 $\pm$ 1.1 [‡]

¹ $\bar{x}$ $\pm$ SD of individual subject's values calculated from summary measures (area under curve/total follow-up time) as outlined in section 3.2.9.1 (* p <0.05, [†] p <0.01, [‡] p <0.001 paired t -test comparing baseline and follow-up mean)

² $\bar{x}$ $\pm$ SD months from enrollment

³ number of subjects who had blood samples at each nominated time point

⁴ the plasma phospholipid sample of 1 subject was contaminated and not included in the analysis

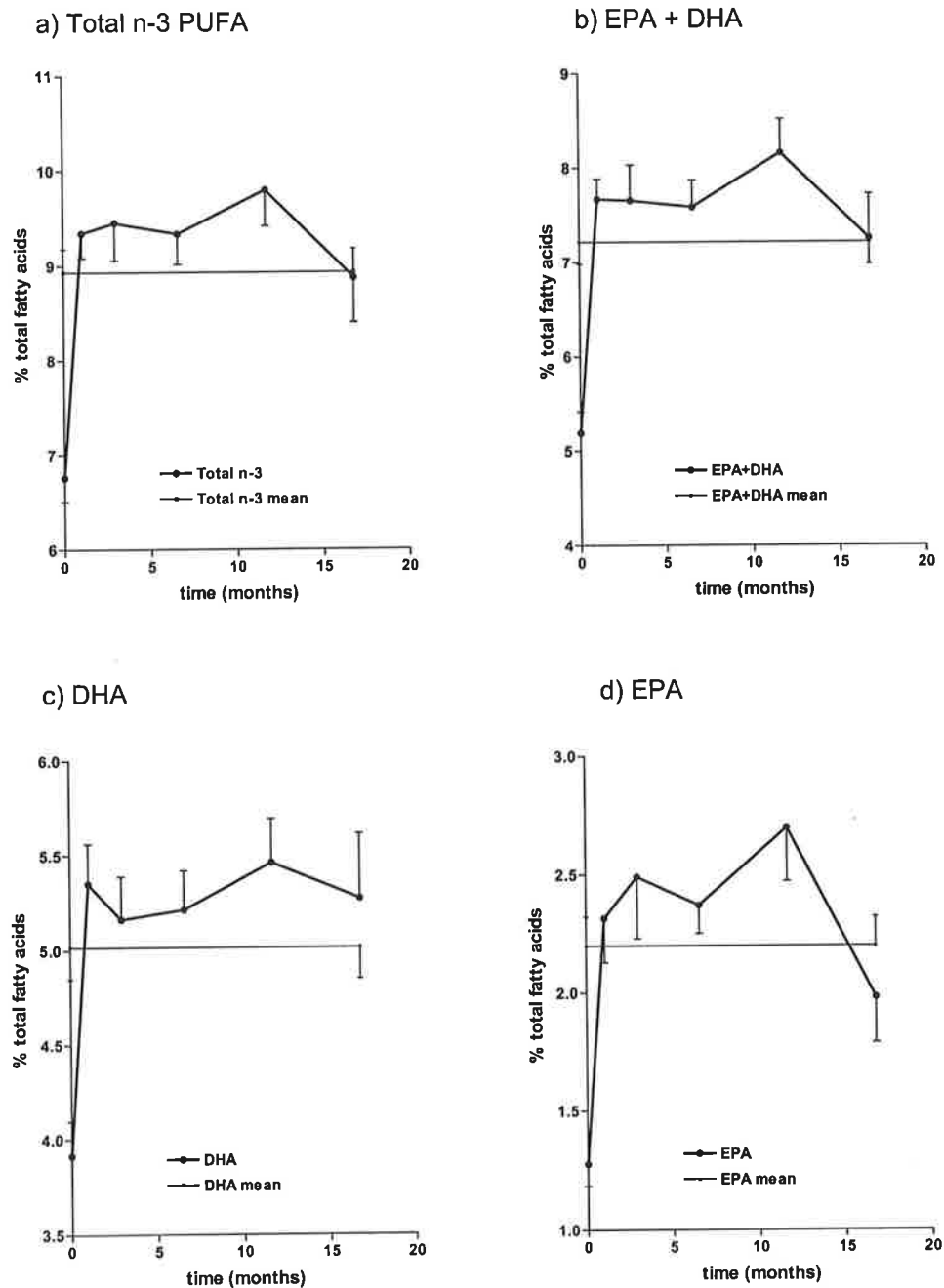


Figure 3.7 Changes in plasma phospholipid n-3 fatty acids during the course of the study in 33 subjects with ≥ 6 months follow-up. The values are $\bar{x} \pm SD$ at each time point as presented in table 3. (thick lines). The straight lines represent the mean fatty acid level attained during the study period, with the error bars representing the SD of the individual mean values (calculated from the area under the curve divided by the length of follow-up time for each subject as detailed in section 3.2.10.5). Note the differences in the scale of the Y-axis

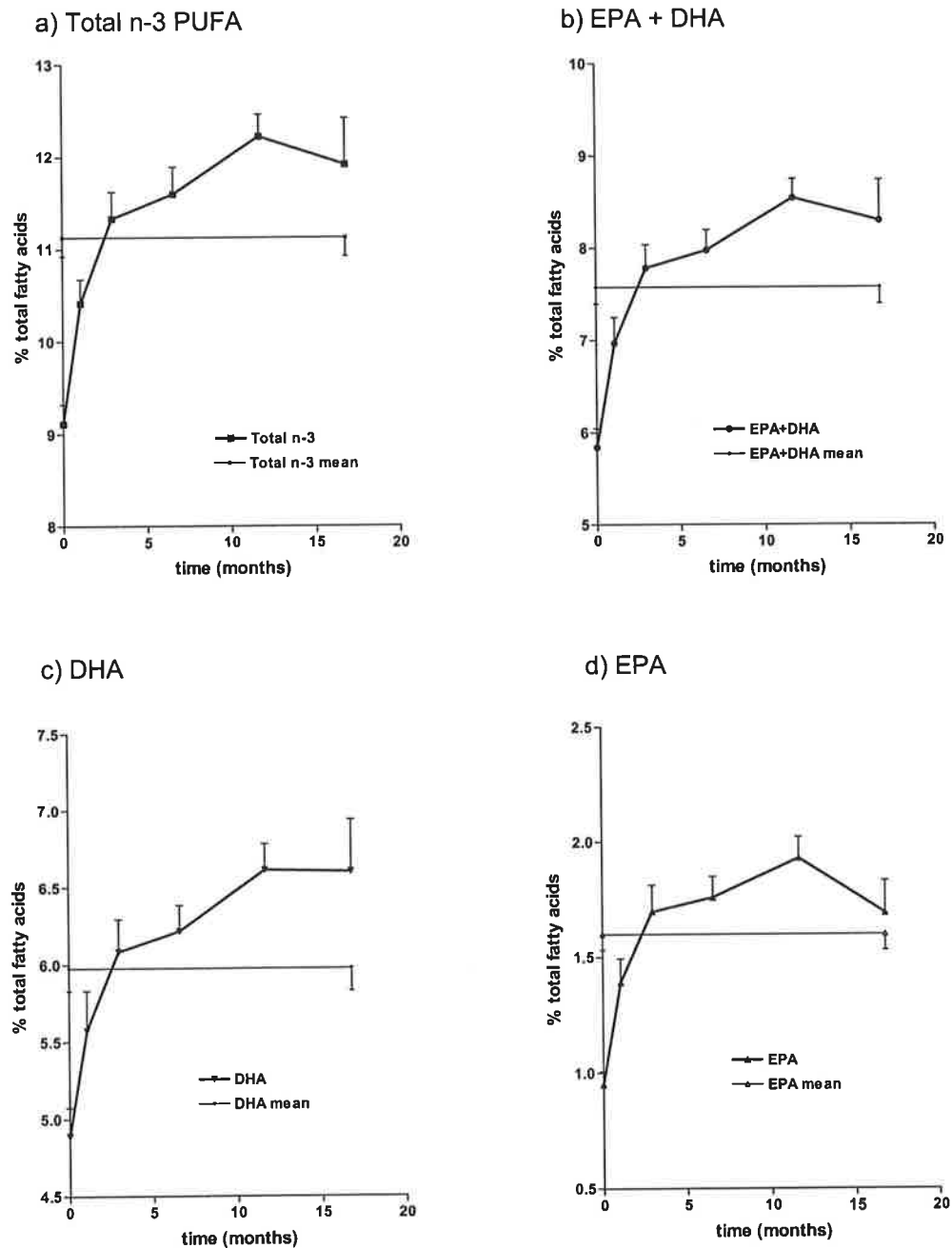


Figure 3.8 Changes in red blood cell n-3 fatty acids during the course of the study in 33 subjects with ≥ 6 months follow-up. The values are $\bar{x} \pm SD$ at each time point as presented in table 3.2 (thick lines). The straight lines represent the mean fatty acid level attained during the study period, with the error bars representing the SD of the individual mean values (calculated from the area under the curve divided by the length of follow-up time for each subject as detailed in section 3.2.10.5). Note the differences in the scale of the Y-axis

3.3.4 Cohort Analysis Using Equalized Baseline and Follow-up Periods

In the analyses presented in sections 3.3.2 and 3.3.3, the intervention periods of ≥ 12 months or ≥ 6 months are compared with baseline periods which are variable and generally are longer than the intervention period. Because this could bias the analysis towards a favourable dietary effect, the data were re-organized so that there were equivalent baseline and intervention periods for each individual. This was achieved either by adjusting the individual baseline data to an identical number of days as the intervention period by counting back from the day of enrollment into the study, or in the case of 3 subjects who have had longer intervention periods than baseline periods, their intervention periods were adjusted to an identical number of days as their baseline periods. Only arrhythmic events occurring within these periods were included in this analysis.

When the baseline and follow-up periods were equalized, the mean time in each period was 361 ± 124 days, with a total of 12821 days (35.1 years) in each of the baseline and follow-up periods.

3.3.4.1 Incidence of Arrhythmias

3.3.4.1.1 Ventricular arrhythmias

In the baseline period there were 157 ventricular arrhythmic events distributed amongst 20 subjects at a mean rate of 4.5 events/person year (Table 3.10). Of these, 103 events distributed amongst 17 subjects were confirmed by independent analysis of event data, with the remaining 54 events distributed amongst 6 subjects identified from the examination of casenote records.

During the intervention period, there were 40 ventricular arrhythmic events distributed amongst 147 subjects at a mean rate of 1.1 events/person year. Of these, 38 events distributed amongst 14 subjects were confirmed from individual event data, with the remaining 2 events from 1 subject confirmed from casenote records.

The median and maximum annualized arrhythmia rates for an individual in the baseline period were 0.7 and 45.3 events/year respectively, compared with 0.0 and 7.5 respectively in the intervention period (Table 3.11 and Figure 3.9).

Sixteen subjects had a reduction in their annualized arrhythmia rate during the intervention period compared to the baseline period, with 8 subjects having an increased rate, and no change in the rate in the remaining 9 subjects (8 of whom had no events in either period, with the remaining subject (# 5) having 1 event in each period) (Figure 3.10). There were 13 subjects who had no events in the baseline period, compared with 19 subject in the intervention period. There were no statistically significant differences between the baseline and follow-up periods in terms of annualized arrhythmia event rates ($p=0.10$, Wilcoxon's Signed Rank Test,), or when analyzed using the total number of events ($p=0.07$, Wilcoxon's Signed Rank Test).

Table 3.10 Total arrhythmias recorded in 33 subjects completing ≥ 6 months follow-up with equal baseline and follow-up periods

	Baseline			Intervention		
	No. of events	Rate / person year	No. patients with events	No. of events	Rate / person year	No. patients with events
<i>Confirmed from episode data</i>						
VT - ATP	71	2.02 [†]	7	11	0.31	6
VT - ATP/CV	3	0.09	2	4	0.11	2
VT - no therapy	0	0	0	1	0.03	1
VT - total	74	2.11	8	16	0.46	7
VF - CV	18	0.51	8	15	0.43	6
VF - therapy aborted	11	0.31	6	7	0.20	6
VF - total	29	0.83	12	22	0.63	10
Total arrhythmias	103	2.93	17	38	1.08	14
<i>Confirmed from case notes</i>						
VT - ATP	27	0.77	1	0	0	0
VT - ATP/CV	1	0.03	1	0	0	0
VT - no therapy	0	0	0	0	0	0
VT - total	28	0.80	2	0	0	0
VF - CV	2	0.06	2	2	0.06	1
VF - therapy aborted	1	0.03	1	0	0	0
VF - total	3	0.09	3	2	0.06	1
VTVF unknown therapy	23	0.66	1	0	0	0
Total arrhythmias	54	1.54	6	2	0.06	1
<i>Total ventricular events</i>						
VT - ATP	98	2.79	8	11	0.31	6
VT - ATP/CV	4	0.11	3	4	0.11	2
VT - no therapy	0	0	0	1	0.03	1
VT - total	102	2.91	9	16	0.46	7
VF - CV	20	0.57	9	17	0.48	6
VF - therapy aborted	12	0.34	7	7	0.20	6
VF - total	32	0.91	13	24	0.68	10
VTVF unknown therapy	23	0.66	1	0	0	0
Total	157	4.47	20	40	1.14	14
<i>Non-ventricular events</i>						
VT - ATP	37	1.05	5	2	0.06	2
VT - ATP/CV	12	0.34	4	2	0.06	2
VT - no therapy	39	1.11	3	12	0.34	1
VT - total	88	2.51	8	16	0.46	5
VF - CV	11	0.31	2	1	0.03	1
VF - therapy aborted	0	0	0	0	0	0
VF - total	11	0.31	2	1	0.03	1
Total Non-ventricular events	99	2.82	9	17	0.48	5
<i>Unknown events</i>	28	0.83	2	0	0	0

[†] total number of events / total number of years of follow-up for all subjects combined

Abbreviations: VT - ATP, ventricular tachycardia with anti-tachycardia pacing; VT - ATP/CV, ventricular tachycardia with anti-tachycardia pacing followed by cardioversion; VF - CV, ventricular fibrillation with cardioversion

Table 3.11 Annualized arrhythmia rates in 33 subjects completing ≥ 6 months follow-up with equal baseline and follow-up periods

	Baseline					Intervention				
	Percentiles					Percentiles				
	0	25	50	75	100	0	25	50	75	100
<i>Confirmed from episode data</i>										
VT - ATP	0 ¹	0	0	0	45.34	0	0	0	0	3.73
VT - ATP/CV	0	0	0	0	1.58	0	0	0	0	3.21
VT - no therapy	0	0	0	0	0	0	0	0	0	0.98
VT - total	0	0	0	0	45.34	0	0	0	0	4.28
VF - CV	0	0	0	0	4.80	0	0	0	0	3.92
VF - therapy aborted	0	0	0	0	3.85	0	0	0	0	1.92
VF - total	0	0	0	0.78	8.65	0	0	0	0.77	3.92
Total arrhythmias	0	0	0.61	1.07	45.34	0	0	0	1.74	7.50
<i>Confirmed from case notes</i>										
VT - ATP	0	0	0	0	19.57	0	0	0	0	0
VT - ATP/CV	0	0	0	0	0.91	0	0	0	0	0
VT - no therapy	0	0	0	0	0	0	0	0	0	0
VT - total	0	0	0	0	19.57	0	0	0	0	0
VF - CV	0	0	0	0	1.44	0	0	0	0	1.92
VF - therapy aborted	0	0	0	0	2.13	0	0	0	0	0
VF - total	0	0	0	0	0.98	0	0	0	0	1.92
VTVF unknown therapy	0	0	0	0	15.19	0	0	0	0	0
Total arrhythmias	0	0	0	0.83	19.57	0	0	0	0	1.92
<i>Total ventricular events</i>										
VT - ATP	0	0	0	0	45.34	0	0	0	0	3.73
VT - ATP/CV	0	0	0	0	1.58	0	0	0	0	3.21
VT - no therapy	0	0	0	0	0	0	0	0	0	0.98
VT - total	0	0	0	0.79	45.34	0	0	0	0	4.28
VF - CV	0	0	0	0.61	4.81	0	0	0	0	3.92
VF - therapy aborted	0	0	0	0	3.85	0	0	0	0	1.92
VF - total	0	0	0	0.78	8.65	0	0	0	0.77	5.77
VTVF unknown therapy	0	0	0	0	15.19	0	0	0	0	0
Total	0	0	0.75	2.13	45.34	0	0	0	1.74	7.50
<i>Non-ventricular events</i>										
VT - ATP	0	0	0	0	20.56	0	0	0	0	1.07
VT - ATP/CV	0	0	0	0	3.66	0	0	0	0	0.80
VT - no therapy	0	0	0	0	25.30	0	0	0	0	7.37
VT - total	0	0	0	0.50	49.01	0	0	0	0	7.37
VF - CV	0	0	0	0	6.78	0	0	0	0	0.61
VF - therapy aborted	0	0	0	0	0	0	0	0	0	0
VF - total	0	0	0	0	6.78	0	0	0	0	0.61
Total Non-ventricular events	0	0	0	0.79	49.02	0	0	0	0	7.98
<i>Unknown events</i>										
	0	0	0	0	17.39	0	0	0	0	0

¹ individual annualized arrhythmia rate calculated for each subject

Abbreviations: VT - ATP, ventricular tachycardia with anti-tachycardia pacing; VT - ATP/CV, ventricular tachycardia with anti-tachycardia pacing followed by cardioversion; VF - CV, ventricular fibrillation with cardioversion

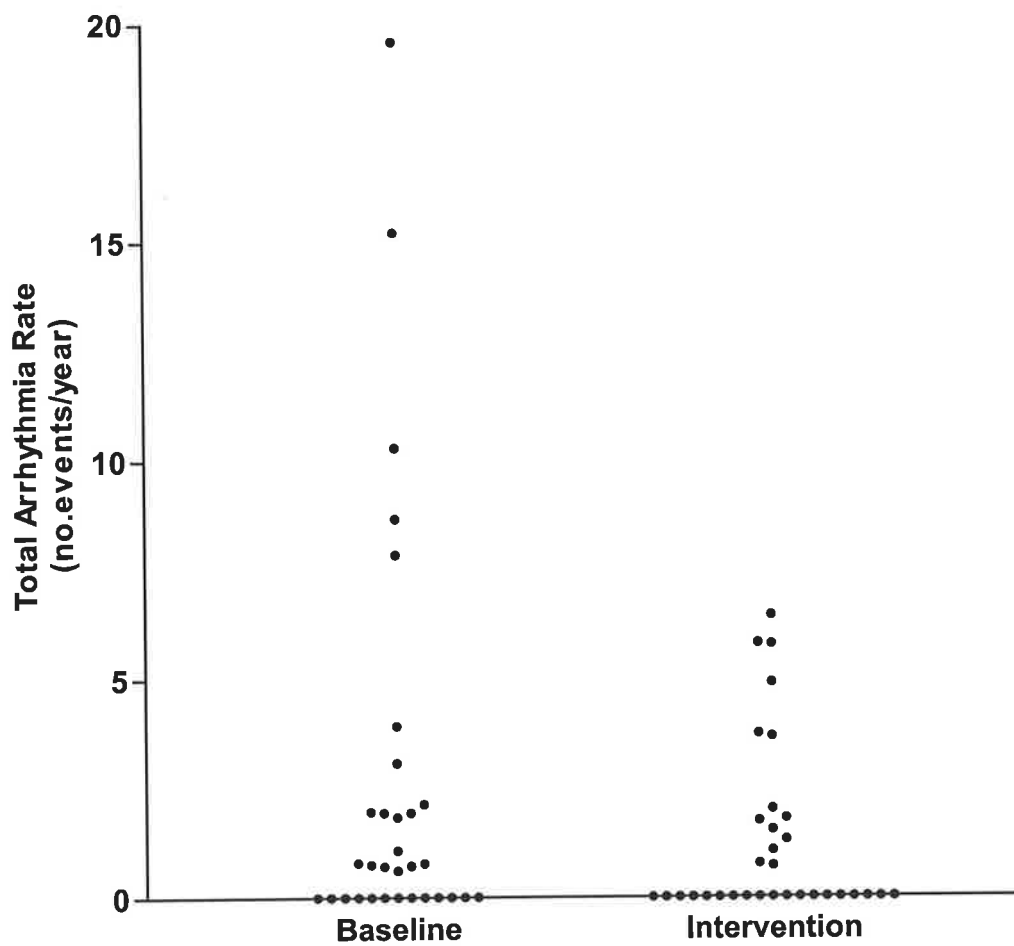


Figure 3.9 The total arrhythmia rate (no. of events/year) for baseline and intervention periods for the cohort with 33 subjects with ≥ 6 months follow-up and with equalized baseline and follow-up periods

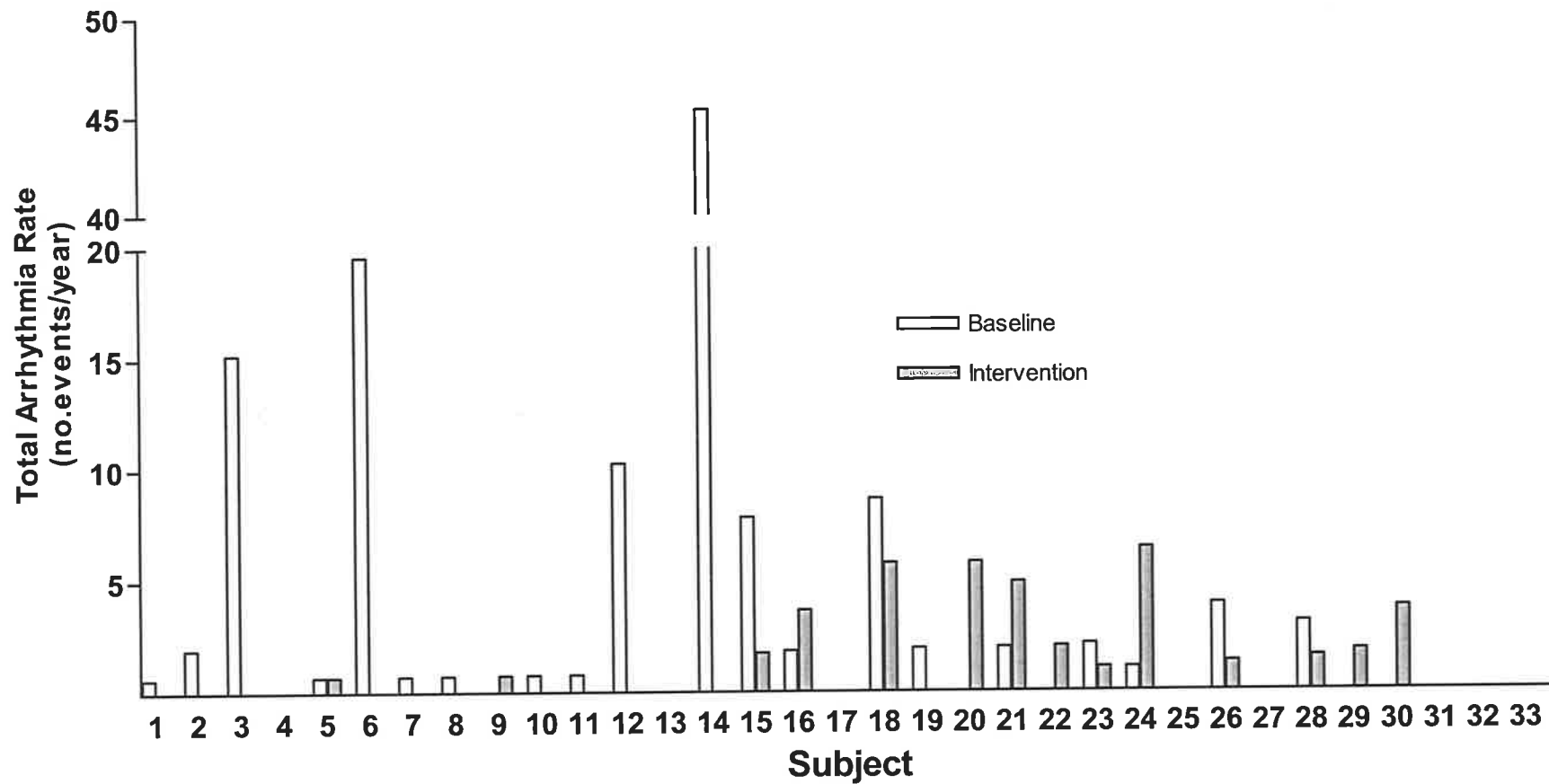


Figure 3.10 Comparison of the annualized arrhythmia rates for each of the 33 subjects who had completed at least 6 months of follow-up with equal baseline and follow-up periods.
The absence of a bar indicates that no arrhythmias were recorded

3.3.4.1.2 Non-ventricular events

There were 99 events distributed among 9 subjects recorded by the ICDs in the baseline period which were considered to be not ventricular arrhythmias, and 17 events in 5 subjects in the follow-up period. Of these, a total of 12 events resulted in cardioversion following unsuccessful ATP therapy, and 11 resulted in cardioversion as a first therapy, resulting in a total of 23 events throughout the baseline and intervention periods for which inappropriate ICD discharges were recorded (Table 3.10).

3.3.4.1.3 Unknown events

There were a total of 28 unknown arrhythmic events in this analysis, subject EG with 6 events and subject AJ with 22 events. After including 2 of the unknown events of subject EG as described in section 3.3.2.1.3, there was still no statistically significant difference between the baseline and follow-up periods in terms of annualized arrhythmia rates ($p=0.091$, Wilcoxon's Signed Rank Test), or number of events ($p=0.056$, Wilcoxon's Signed Rank Test).

3.3.5 Natural History of Ventricular Arrhythmias (i.e. no intervention)

A total of 24 subjects were included in the analysis for the first two 6 month periods, of which 18 had received no anti-arrhythmic drug therapy with the remaining 6 subjects receiving anti-arrhythmic drug therapy throughout the baseline period. In the total group analysis, there was a consistent proportion of subjects having events throughout the first 3 years from implant, ranging from 24% of subjects having at least 1 event in the period 18-24 months post implant to 54% of subjects having at least 1 event in the period 2 - 2.5 years post implant. There were no statistically significant differences found in any of the analyses undertaken (Wilcoxon's Sign Rank Test for paired comparisons between periods

1 and 2 and Friedmans's Test for Repeated Measures for the other group analyses).

Therefore, in the absence of intervention, the event rate does not appear to change significantly in any 6 month period from the time of implant.

Table 3.12 Natural history of confirmed ventricular arrhythmias from implant of ICD to commencement of n-3 PUFA supplemented diet

	Months from implant							
	0 - <6	6 - <12	12 - <18	18 - <24	24 - <30	30 - <36	36 - <42	42 - <48
Without anti-arrhythmics								
<i>n</i>	18 ²	18	17	13	11	8	6	1
<i>n</i> with events (% of total)	6 (33)	6 (33)	6 (35)	4 (31)	6 (55)	3 (37)	1 (17)	0
Total no. of events	52	10	18	6	24	16	2	0
No. events/person year	5.8	1.1	2.1	0.9	4.4	4.0	0.7	0
Percentiles (no. of events)								
0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
50	0	0	0	0	1	0	0	0
75	1	1	1	1	2	1	0	0
100	19	4	5	2	15	14	2	0
With anti-arrhythmics								
<i>n</i>	6	6	5	4	2	0	0	0
<i>n</i> with events (% of total)	0 (0)	1 (17)	3 (60)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)
Total no. of events	0	5	25	0	2	0	0	0
No. events/person year	0	1.7	10	0	0.5			
Percentiles (no. of events)								
0	0	0	0	0	0			
25	0	0	0	0	0			
50	0	0	1	0	1			
75	0	0	1	0	1.5			
100	0	5	23	0	2			
Total Group								
<i>n</i>	24	24	22	17	13	8	6	1
<i>n</i> with events (% of total)	6 (25)	7 (29)	9 (41)	4 (24)	7 (54)	3 (37)	1 (17)	0 (0)
Total no. of events	52	15	43	6	26	16	2	0
No. events/person year	4.3	1.3	3.9	0.7	4.0	4.0	0.7	0
Percentiles (no. of events)								
0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
50	0	0	0	0	1	0	0	0
75	1	1	1	0.5	2	0.5	0	0
100	19	5	23	2	15	14	2	0

¹ time periods are calculated in 6 month intervals from the date of implant

² number of subjects with baseline data extending into each 6 month period from implant

3.3.6 Premature Ventricular Complexes (PVCs)

There were 12 subjects with ICDs equipped with PVC counters (Medtronic models 7227 and 7271, and Guidant model Ventak Prizm DR 1851). Seven subjects recorded a decrease and 5 subjects recorded an increase in the mean daily rate of PVCs (Figure 3.11), while for runs of PVCs, 8 subjects recorded a decrease and 4 subjects recorded an increase (Figure

3.12). The distributions of the mean daily rates of PVCs in the intervention period, and of runs of PVCs in both baseline and intervention periods were not normal, and therefore these data (including PVCs in the baseline period) are presented as percentiles (Table 3.13). There were no statistical differences in the mean daily rates of PVCs or runs of PVCs, between the baseline and intervention periods ($p = 0.92$ and 0.17 respectively, Wilcoxon's Signed Rank Test).

The percentage change in the mean daily rate of PVCs ranged from an 82% decrease to a 174% increase from the baseline to follow-up periods, while runs of PVCs ranged from a 90% decrease to a 97% increase (Figure 3.13). The mean percentage change in the daily rate of PVCs was a 3.6% decrease, while for runs of PVCs, it was a 15.2% decrease.

There were large intra-subject variations in mean daily rates of both PVCs and runs of PVCs between interrogations of the ICD, and changes in individual rates are presented in figures 3.14 (PVCs) and 3.15 (runs of PVCs).

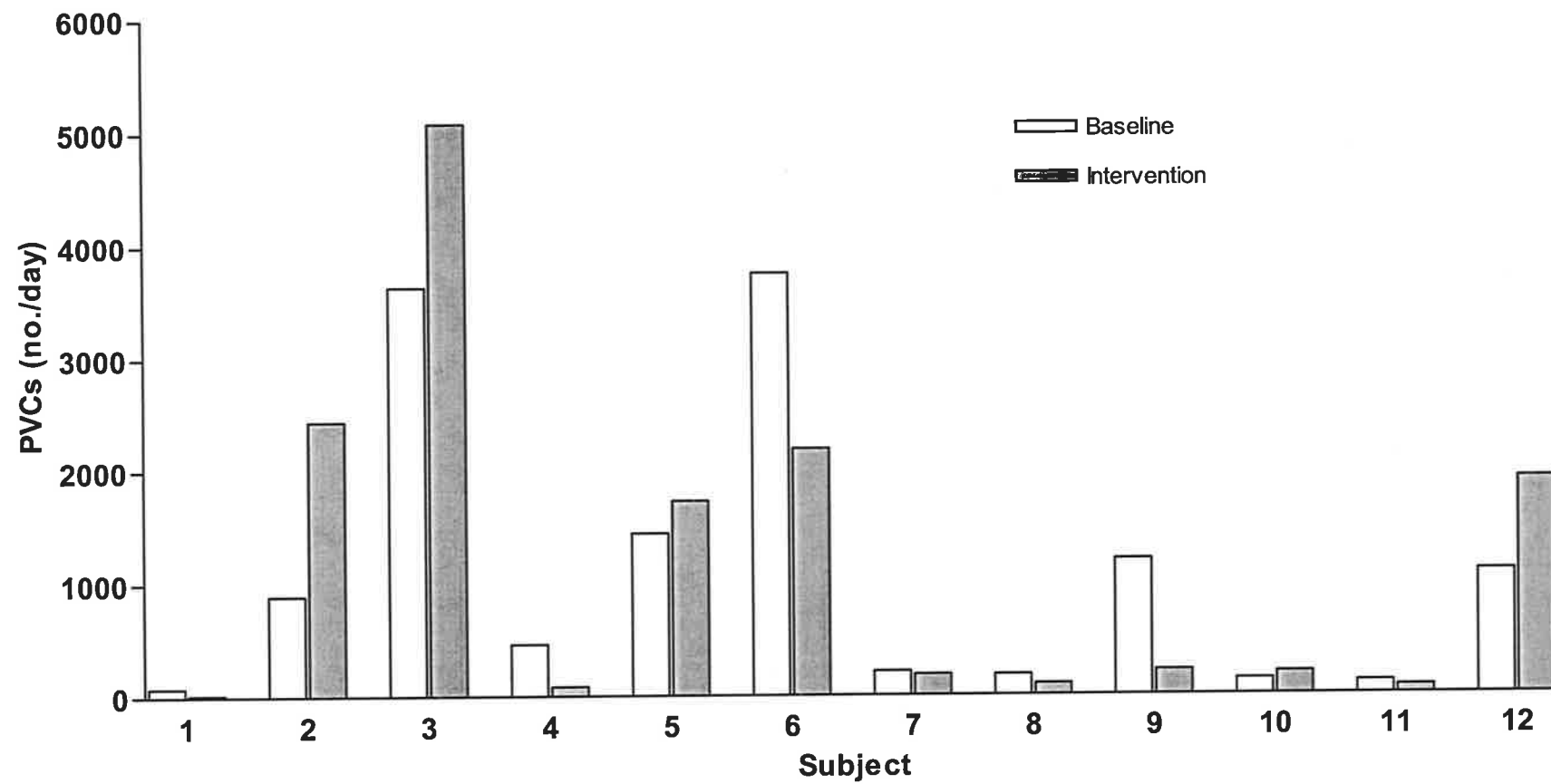


Figure 3.11 Comparison of the mean daily PVC rates in the baseline and follow-up periods for each of the 12 subjects with an ICD with a PVC counter

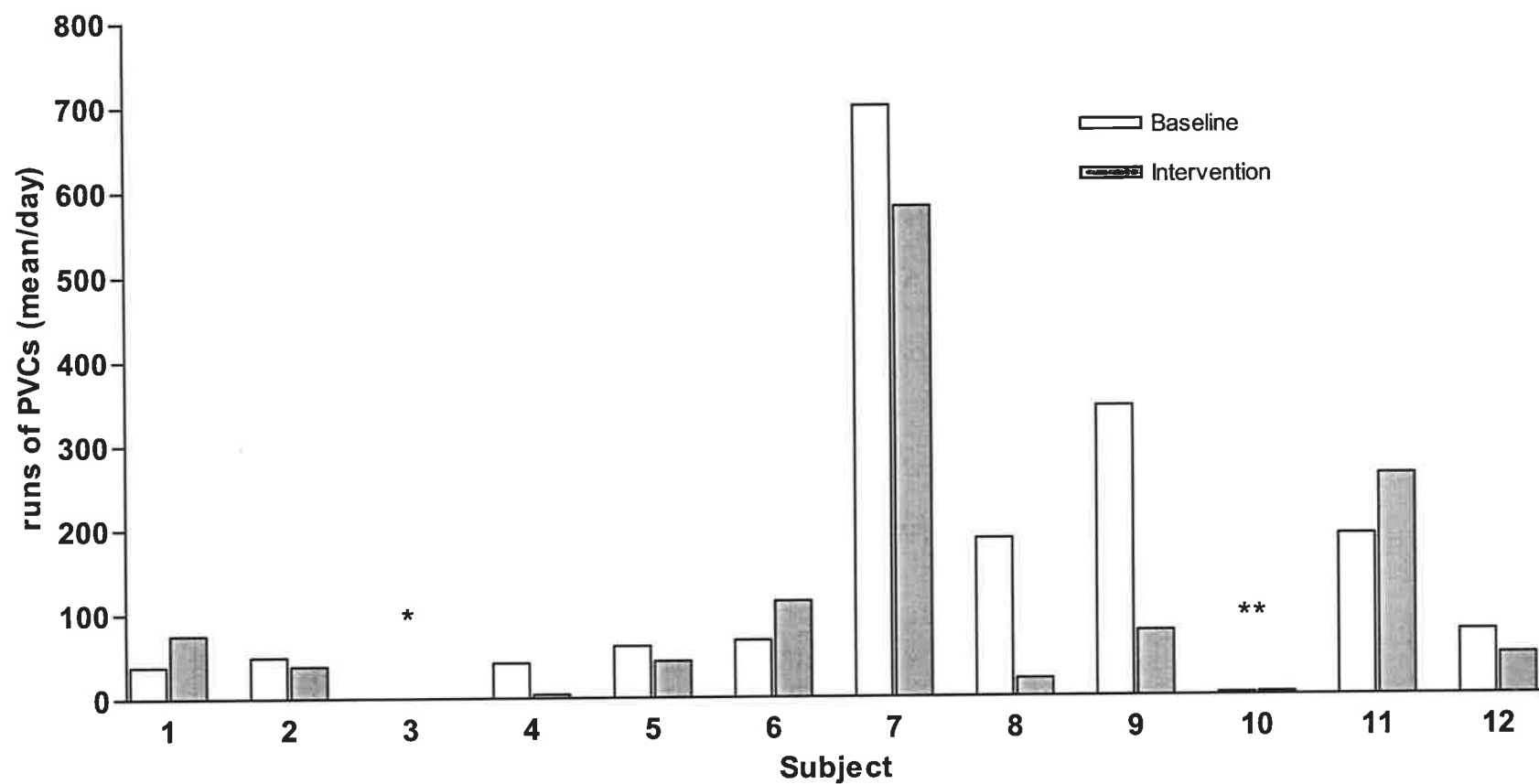


Figure 3.12 Comparison of the mean daily rates of runs of PVCs for each of the 12 subjects with an ICD with a PVC counter in the baseline and follow-up periods

* the rates for subject 3 were 0.18/day and 0.06/day for the baseline and follow-up period respectively

** the rates for subject 10 were 2.7/day and 3.8/day for the baseline and follow-up period respectively.

Table 3.13 Percentile distribution of mean daily rates of PVCs and runs of PVCs in the baseline and follow-up periods of the study

	percentile				
	Min	25	50	75	max
PVCs					
Baseline	78.2	172.5	675.9	1261.6	3755.4
Intervention	19.0	96.6	207.9	1777.0	5084.0
Runs of PVCs					
Baseline	0.18	40.9	64.3	188.3	700.6
Intervention	0.06	16.9	45.6	86.5	581.8

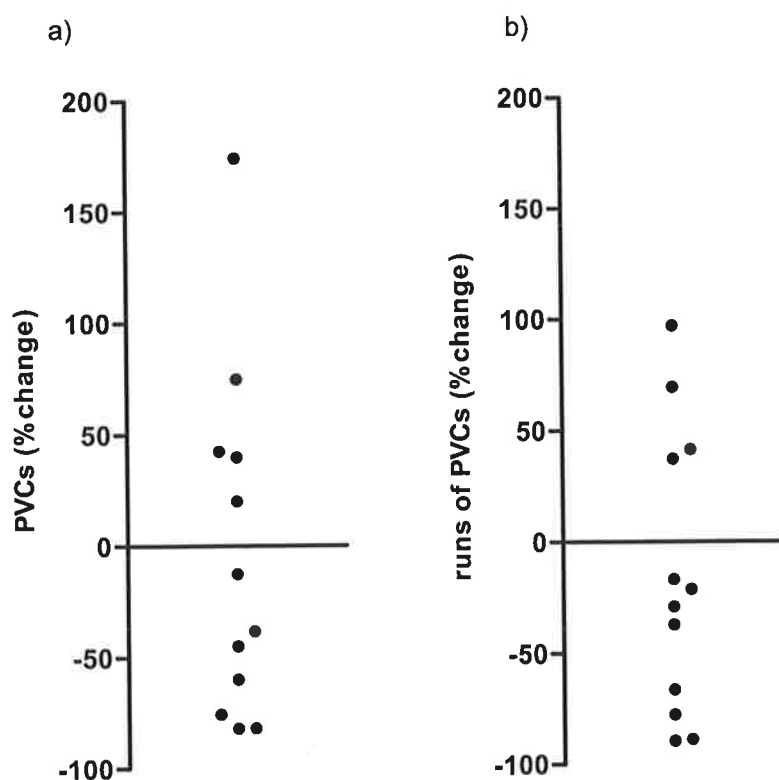


Figure 3.13 Percentage change from the baseline to follow-up periods in individual daily rates of; (a) PVCs and (b) runs of PVCs in 12 subjects with ICDs with PVC counters

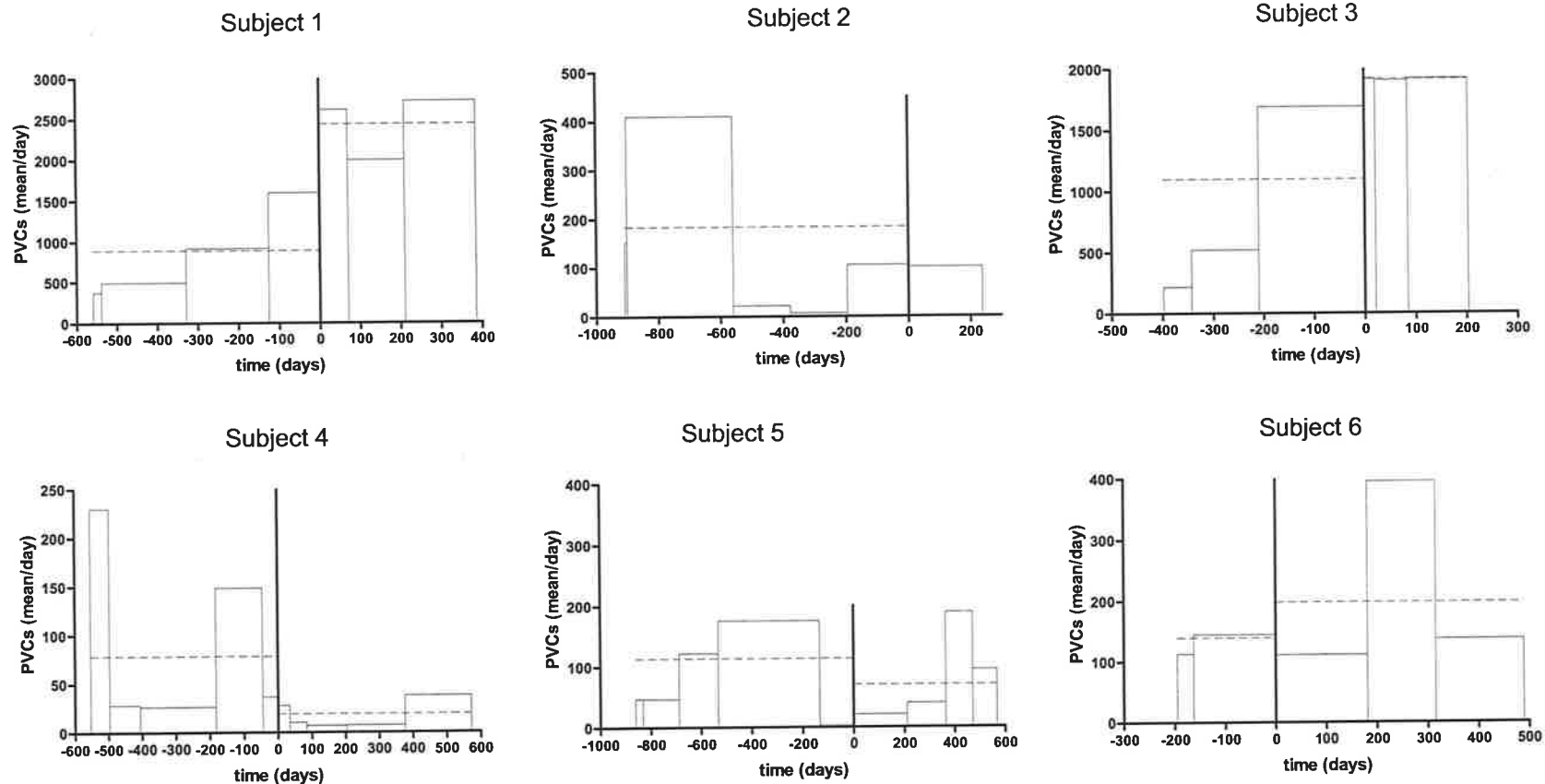
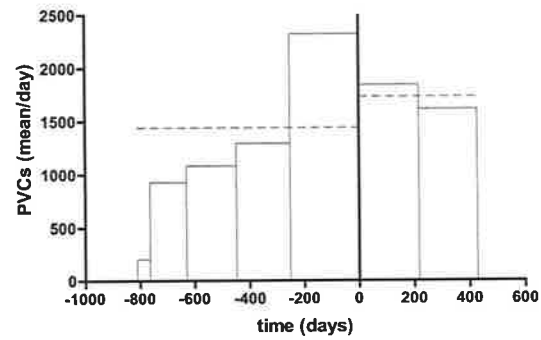
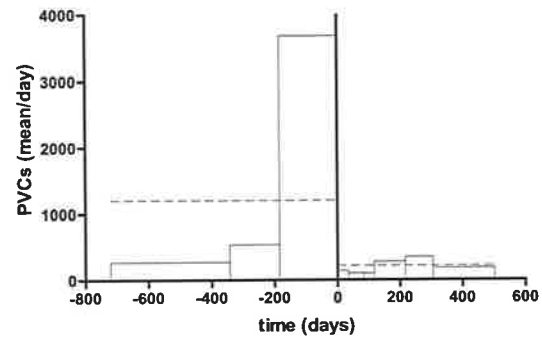


Figure 3.14 Changes in the mean number of PVCs/day for each of the 12 subjects with ICDs equipped with PVC counters, calculated from the number of PVCs recorded and the number of days between each interrogation. Time 0 is the day of enrolment into the study, with negative days counting back to the day of implant. The width of each bar represents the period between each interrogation of the ICD. The dashed lines represent the mean number of PVCs recorded throughout the baseline and intervention periods. Note the variation in the scale of the y-axis. (continued next page)

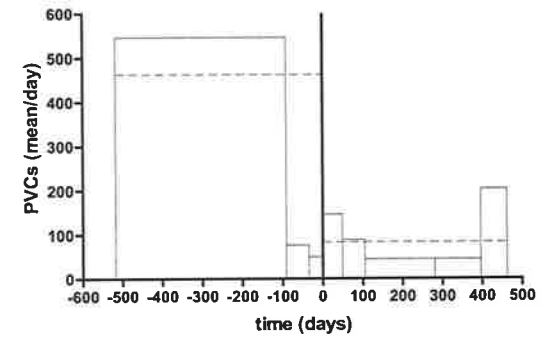
Subject 7



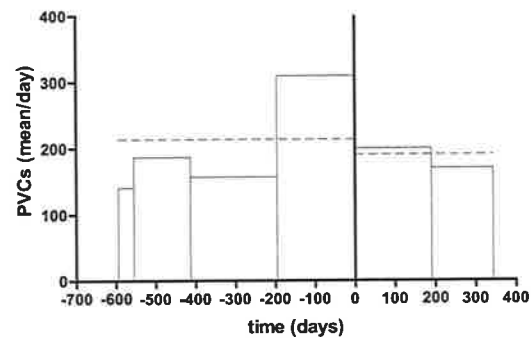
Subject 8



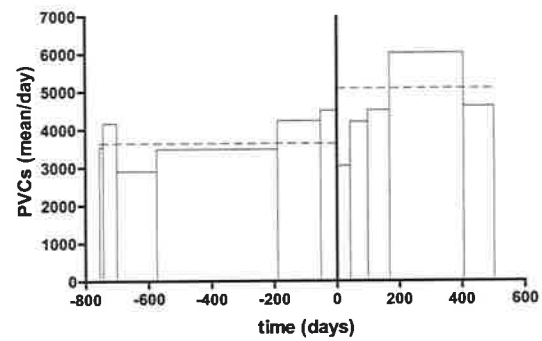
Subject 9



Subject 10



Subject 11



Subject 12

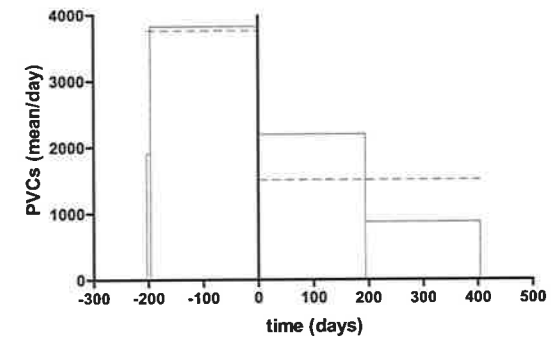


Figure 3.14 Continued

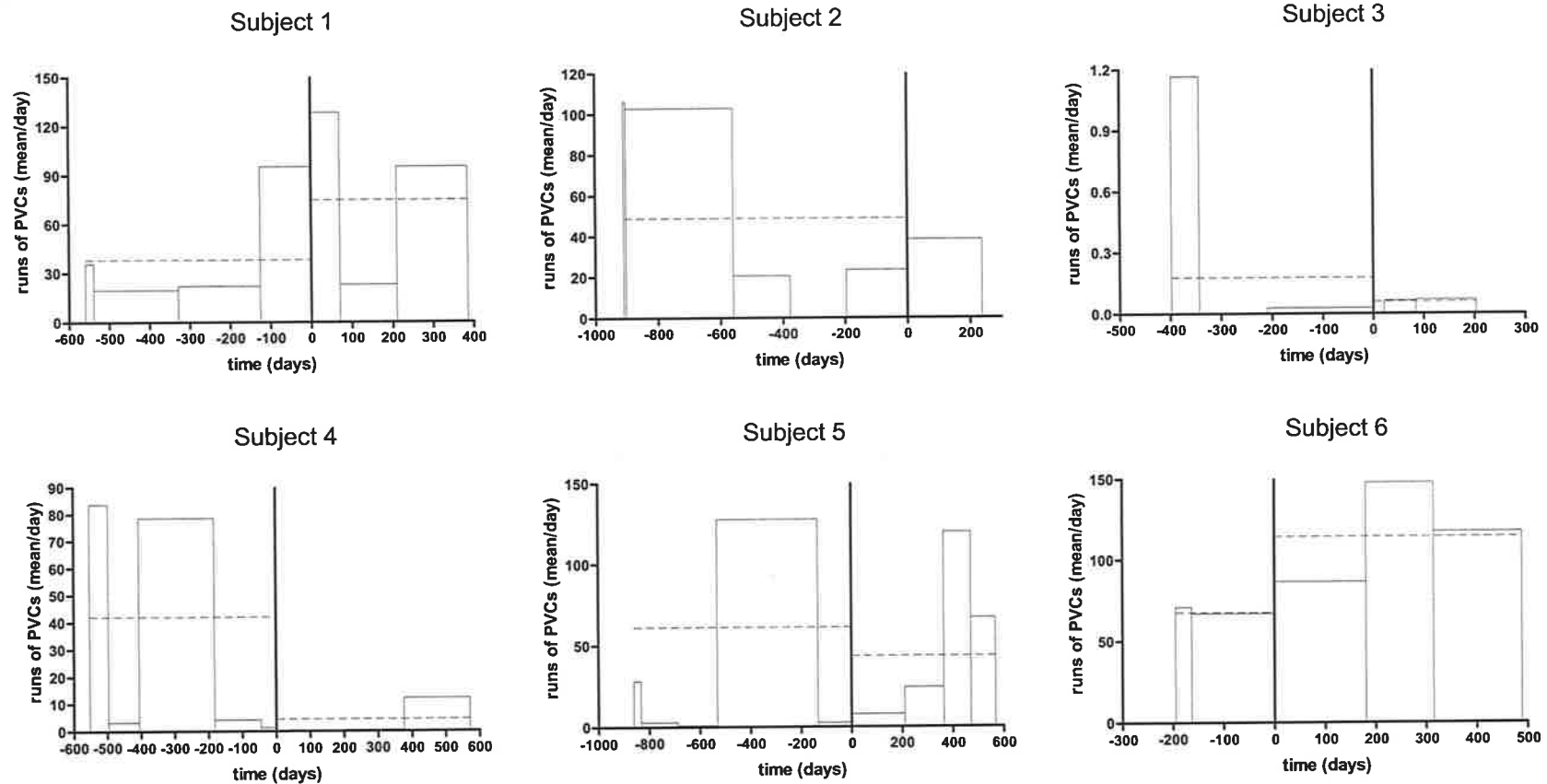
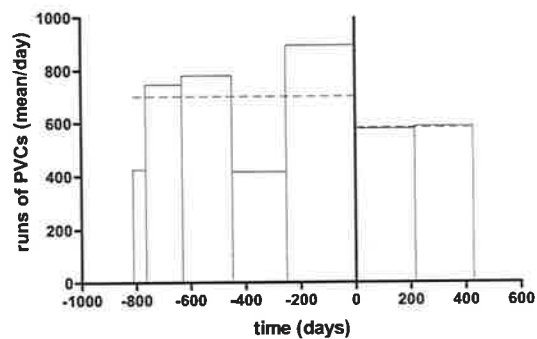
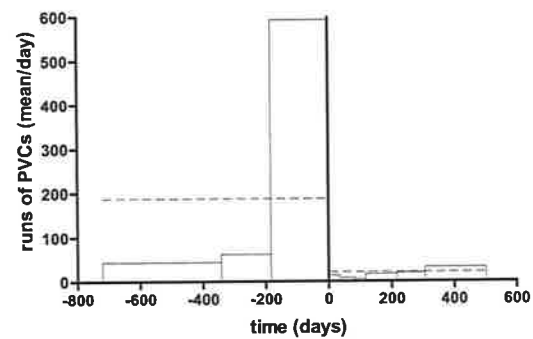


Figure 3.15 Changes in the mean number of runs of PVCs/day for each of the 12 subjects with ICDs equipped with PVC counters, calculated from the number of runs of PVCs recorded between each interrogation. Time 0 is the day of enrolment into the study, with negative days counting back to the day of implant. The width of each bar represents the period between each interrogation of the ICD. The dashed lines represent the mean number of runs of PVCs recorded throughout the baseline and intervention periods. Note the variation in the scale of the y-axis (continued next page)

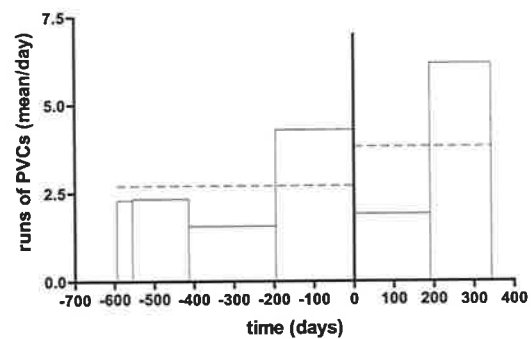
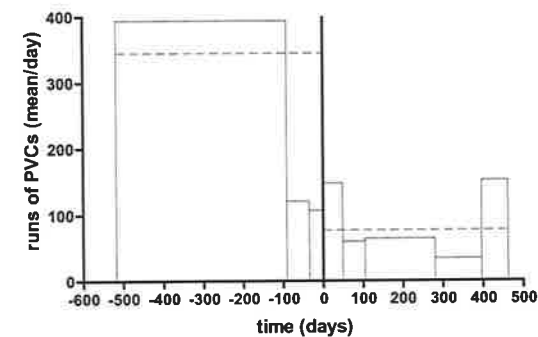
Subject 7



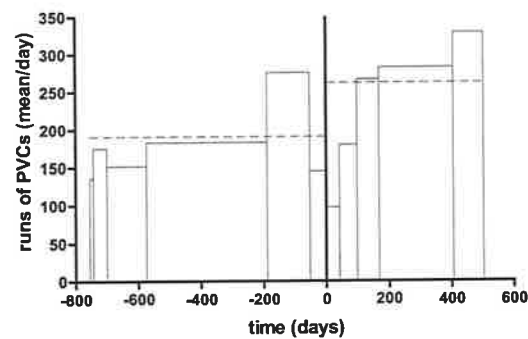
Subject 8



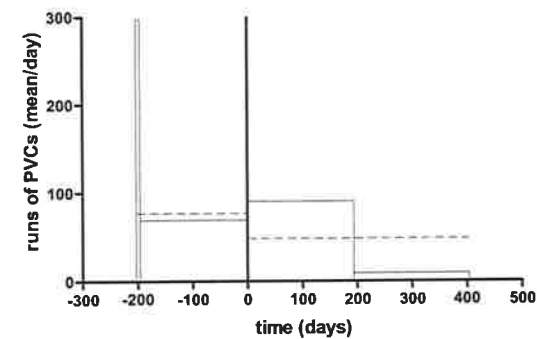
Subject 9



Subject 10



Subject 11



Subject 12

Figure 3.15 Continued

3.3.7 Ventricular Effective Refractory Period (VERP)

There was no effect of n-3 PUFA on VERP until after 12 months of supplementation, when there was a significant 7.5 ms extension (Table 3.14). Following approximately 3 months of n-3 PUFA supplementation, the VERP had increased in 50% of subjects compared to baseline, decreased in 30% and was unchanged in the remaining 20% of subjects (Figure 3.16). After about 6 months of follow-up, VERP had increased in 41% of subjects compared to baseline, decreased in 18% and was unchanged in the remaining 41%, whereas after 12-18 months of n-3 PUFA supplementation, VERP had increased from baseline in 50% of subjects, decreased in 8% of subjects, and was steady in the remaining 42% of subjects.

Table 3.14 VERP at approximately 3 months, 6 months and 12-18 months of follow-up.

Follow-up period	n	No. days	VERP (ms)	
			baseline	Follow-up
3 months	10	79±20	267.0±26.3	272.0±16.2
6 months	22	196±30	264.5±22.4	269.1±22.2
12-18 months	12	436±88	270.8±20.7	278.3±19.5*

*p<0.05 (Paired t-test)

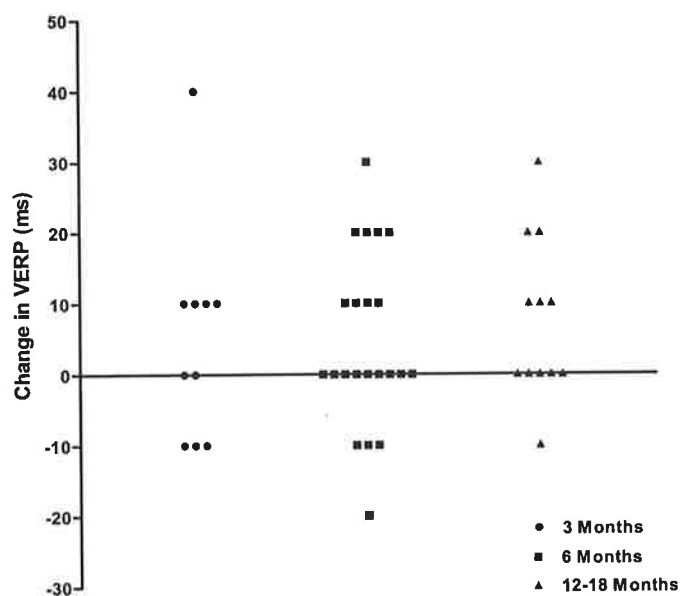


Figure 3.16 Changes in VERP from baseline for individual subjects at each time point.

3.4 DISCUSSION

This study is one of the first to examine the long-term anti-arrhythmic effects of LC n-3 PUFA in humans, using patients at high risk of suffering arrhythmias, and utilizing ICD technology for the recording of arrhythmic events. This chapter describes the results of 3 separate interim analyses of this study, and shows that the LC n-3 PUFA EPA and/or DHA are probably effective in reducing ventricular arrhythmias in this population of patients.

The study has a currently active cohort of 41 subjects. For the purpose of preparing this thesis, interim analysis was conducted on 2 sub-groups of the cohort;

- 20 subjects who had completed at least 12 months of intervention with n-3 PUFA
- a further 13 subjects (33 in total) who had completed at least 6 months of follow-up.

At the time of analysis, only 13 subjects had completed the full intervention period.

The 2 analyses conducted with the cohorts that had completed ≥ 12 months and ≥ 6 months of follow-up, and with the baseline period commencing at implant, both found significant decreases in the annualized arrhythmia rates when analyzed using Wilcoxon's Sign Rank Test, a non-parametric paired rank method. While this method of analysis compares the annual rate of arrhythmias in each period, for the majority of the subjects, the baseline period was considerably longer than the follow-up period, and given that arrhythmias do not occur at regular intervals, there was a greater period of time for arrhythmic events to occur in the baseline period.

To allow for this discrepancy, the 3rd analysis was undertaken, where the baseline period was adjusted back from the day of enrolment, to equal the number of days in the follow-up period, thereby having exactly the same amount of time in each period. This analysis did not show a statistically significant difference in total ventricular arrhythmia rate between

the two periods. However, this analysis included 13 subjects with baseline and follow-up periods of between 6 and 12 months with about 33% of subjects recording no events in the adjusted baseline period. However, there was a trend towards a reduction in annualized arrhythmia rate in the intervention period which may become significant when all 41 subjects currently enrolled have completed the study.

There are at least 3 studies currently underway which are also utilizing patients with ICDs to examine the long term effects of n-3 PUFA on arrhythmias in humans, in Oregon USA, in Boston USA, and in the Netherlands. None of these studies have yet reported any results. All of these studies are fundamentally different to the current study in that they are all randomized, placebo controlled studies, where the subjects are recruited at the time of implant. By contrast, this study employs a sequential design where the subjects are acting as their own controls. The study design for this thesis was finalized after considerable discussion. Although the concurrent studies in other countries are placebo controlled, it was considered that this would require advice to not eat fish or to minimize fish intake. Given the extensive evidence for benefits of fish and/or fish oil on cardiac mortality with relatively low intakes of fish, *this approach was considered unethical*. Therefore, a sequential design was chosen which would allow for paired analyses for individuals between baseline and intervention periods. This study design has a number of potential weaknesses, primarily of not allowing for time effects (i.e. the intervention always followed the control period), where there may be time related changes in treatments, lifestyle, and patient compliance with therapy that may lead to changes in arrhythmia rate. In addition, changes in arrhythmia rate may be a manifestation of the Hawthorne Effect, where subjects in a clinical trial do better in response to participation in the trial rather than as result of the intervention. The time effect was addressed in other ways. This included an examination of the natural variation in cardiac arrhythmias with time, and while there were

no significant differences found between the event rate in successive 6 month periods post implant, the potential exists for increasing arrhythmic activity associated with the natural progression of disease in this cohort of mainly elderly subjects, the majority of whom have significant underlying cardiac disease. It is likely that any bias introduced as a result of this time effect will bias against the intervention. Changes in anti-arrhythmic drug therapy were accounted for by including only periods of time where there was consistent anti-arrhythmic drug therapy between the baseline and intervention periods.

This study design is only possible due to the availability of retrospective data on arrhythmic events, with the regular storage of events onto disk being a standard practice prior to the initiation of this study. Given the practical problems of such a trial where such data is collected prior to the development of the study, or where subjects may be admitted to another hospital on an emergency basis, there were a high number of events identified only from casenotes (91 events in 9 subjects in the cohort of 20 subjects with ≥ 12 months intervention and 117 events in 13 subjects in the cohort of 33 subjects with ≥ 6 months intervention) which prevented independent verification of the events.

There were also a considerable number of ICD-recorded events in the baseline period for which no data could be found (209 events in 5 subjects in the cohort of 20 subjects with ≥ 12 months intervention and 212 events in 7 subjects in the cohort of 33 subjects with ≥ 6 months intervention). While these events were excluded from the main analyses, it is probable that some were ventricular arrhythmias, thereby biasing the results against the intervention as demonstrated in sections 3.3.2.1.3, 3.3.3.1.3 and 3.3.4.1.3, where inclusion of events identified as probable ventricular arrhythmias resulted in a lowering of the p value.

At the time of these interim analyses, 5 subjects had been lost to the study, 3 to gastrointestinal side effects shortly after commencing the intervention. One subject had anti-arrhythmic drug therapy commenced following a number of discharges from her ICD about 4.5 months after commencing the intervention. She went on to suffer incessant, intractable VT before eventually having a Left Ventricular Assist Device inserted, at which time her ICD was switched off, and finally receiving a heart transplant. The data from this subject was not included in the analysis as she did not have the minimum 6 months of intervention prior to the introduction of anti-arrhythmic drug therapy required to qualify for the analysis. If the data from this subject had been included, it would have favoured the apparent intervention effect as she had had a large number of asymptomatic, anti-tachycardia pacing terminated VTs prior to the commencement of the intervention.

The 1 death that has occurred in this cohort was in a 71 year old male with severe ischaemic heart disease, who died 82 days following commencement of the intervention, again not having the required 6 months of intervention to qualify for the analysis. Had the data from this subject been included, it would have favoured the null effect.

To date, the only published report of the anti-arrhythmic effects of n-3 PUFA in humans has been a small study demonstrating a significantly higher proportion of subjects given fish oil had a >70% suppression of PVCs compared to placebo in a very low risk population, where the exclusion criteria included patients with ventricular tachyarrhythmias of ≥ 3 PVCs (Sellmayer *et al*, 1995). There have been no published reports of studies examining the anti-arrhythmic effects of n-3 PUFA in higher risk patients. The development of the ICD has provided a mechanism to allow studies on the efficacy of anti-arrhythmic treatments in high risk patients, while offering protection from any arrhythmia that may occur.

It has previously been shown in humans that the prolongation of VERP by class III anti-arrhythmic drugs is associated with the prevention of VT and VF and increases in VERP of about 20% have been reported for amiodarone (Man *et al*, 1994; Naitoh *et al*, 1998).

Studies in isolated rat cardiomyocytes have demonstrated an effect of n-3 PUFA on prolonging VERP (Kang *et al*, 1995). Therefore, one of the original aims of this study was to determine whether n-3 PUFA has an effect on VERP in humans, thereby demonstrating some Class III anti-arrhythmic effects. This study failed to show any short-term effect of n-3 PUFA on VERP, and a significant effect was only seen after about 12-18 months where a mean 2.8% increase was observed. Even with larger numbers in the data set when the study is concluded, it is highly unlikely that an increase in VERP will be observed which is considered clinically significant and of the order seen with amiodarone.

In conclusion, this study has demonstrated that LC n-3 PUFAs are probably effective in suppressing ventricular arrhythmias in cardiac patients with ICDs. At the commencement of this study there was no information available in the literature to provide estimations of a treatment effect of n-3 PUFA in suppressing arrhythmias to allow sample size calculations. The study has a currently active cohort of 41 subjects, and while only 13 subjects had completed the full intervention period at the time of preparation of this manuscript, interim analysis suggests that the study will be sufficiently powered to detect a significant reduction in the incidence of ventricular arrhythmias as a result of increased intake of LC n-3 fatty acids.

CHAPTER 4

A PILOT STUDY TO INVESTIGATE THE EFFECTS OF N-3 FATTY ACIDS ON INDUCIBLE, SUSTAINED VENTRICULAR TACHYCARDIA IN PATIENTS UNDERGOING ELECTROPHYSIOLOGY TESTING

4.1 INTRODUCTION

A number of studies have shown an association between fish consumption, long chain n-3 polyunsaturated fatty acid (PUFA) intake or blood n-3 PUFA levels and sudden cardiac death (SCD) (Siscovick *et al*, 1995; Albert *et al*, 1998; GISSI Investigators, 1999; Albert *et al*, 2002). Studies in animal models of ischaemia/reperfusion and cell culture studies with rat cardiomyocytes have demonstrated a clear anti-arrhythmic effect of n-3 PUFAs (Leaf *et al*, 1999b), and it is believed that this is the mechanism of protection from SCD. To date, there have been no reported studies examining the anti-arrhythmic effects of n-3 PUFAs in humans. Therefore, this study was conducted as a pilot with the aim of establishing whether n-3 PUFAs have direct anti-arrhythmic effects in humans using an electrophysiological indicator of efficacy, i.e. the suppression of a previously inducible ventricular tachyarrhythmia at electrophysiology (EP) testing. If there is a direct effect found, these data could be used to design randomised trials for (1) a dose response relationship for fish oil on inducibility of ventricular tachyarrhythmia, and (b) a direct comparison with amiodarone.

4.2 METHODS

4.2.1 Ethical Approval

Ethical approval for the dietary intervention study was obtained from the Royal Adelaide Hospital Research Ethics Committee. All volunteers were fully informed of the nature of the study, and received written information regarding the study. Each subject was provided with the opportunity to discuss their participation in the study with a member of their family or a friend. Written consent was received from each participant prior to the commencement of the study. All personal information and results were kept confidential.

4.2.2 Subjects

Patients who underwent clinically indicated EP testing, and in whom a sustained ventricular arrhythmia was induced, were approached to participate in the study. The baseline EP testing was undertaken in the cardiac catheter laboratory using conventional, invasive diagnostic EP testing procedures as outlined in section 4.2.4. Some of these patients may have had more than 1 baseline EP study, if anti-arrhythmic drug testing had been undertaken. Subjects who consented to the study were provided with fish oil capsules (Natures Way, Roche Vitamins, Sydney Australia) containing 180 mg of EPA and 120 mg of DHA per capsule, and instructed to consume 3/day, a dose which provided 900 mg/day of LC n-3 PUFA.

4.2.3 Study design

As this was a pilot study involving patients with a potentially life-threatening disorder, and using a therapy with no demonstrated efficacy in humans, clinical determinations governed the course of treatment in all cases, and therefore, the clinical course varied

from patient to patient. The primary requirement was that the patient have an inducible ventricular arrhythmia at EP testing (either in the presence or absence of anti-arrhythmic drug therapy), and have at least 3 weeks of fish oil supplementation prior to a repeat EP study. In the case of patients having an ICD implanted on the basis of their baseline EP study, the post fish oil EP was conducted non-invasively via the PES function of the ICD. On this basis, only patients who had arrhythmias induced at the right ventricular apex (RVA) were included in the study.

4.2.4 Invasive electrophysiology testing

In the catheter laboratory, electrodes are inserted into the right ventricle via the right femoral vein, and positioned at the right ventricular outflow tract (RVOT) and the RVA. Electrical stimulation is applied directly to the inner myocardial wall at a current intensity of twice diastolic threshold, which causes a ventricular contraction. The EP stimulation protocol consisted of a paced 8 beat drive chain at 2 cycle lengths (600 ms and 400 ms), and sensed beats, with up to 3 extra stimuli, at both the RVOT and RVA until either a sustained ventricular arrhythmia was induced, or the end of the protocol was reached. Coupling intervals were reduced in 10 ms decrements until ventricular refractoriness occurred. When multiple extra stimuli were necessary, the initial extra stimulus was fixed at 20 ms beyond refractoriness. Sustained ventricular arrhythmia was defined as an arrhythmia lasting at least 30 secs, or requiring cardioversion/defibrillation.

4.2.5 Non-invasive electrophysiology testing via ICD

EP studies undertaken via the ICD used the same protocol as outlined above except that only 1 site (the RVA) is used and only paced drive chain was used, ie commencing with

an 8 beat drive chain at a cycle length of 600 ms with 1 extra stimulus and progressing through to a cycle length of 400 ms with 3 extra stimuli.

All EP studies conducted via the ICD were undertaken in the catheter laboratory, with the patients slightly sedated with fentanyl and/or benzodiazepines.

4.2.6 Analytical Methods

Plasma and erythrocyte fatty acids were processed as described in section 3.2.7.

4.2.7 Statistical Analysis

The EP stimulation protocol was ranked in order of the increasing aggressiveness required for induction of a ventricular arrhythmia, with non-inducible being ranked highest. Details are outlined in table 4.1.

Table 4.1 Ranking of stimulation protocol required for induction of ventricular arrhythmias at EP studies

Pacing protocol	rank
Inducible	
600/1 ¹	1
400/1	2
600/2	3
400/2	4
600/3	5
400/3	6
Non-inducible	7

¹pacing cycle length/no. extra stimuli

Paired analysis was then conducted using Wilcoxon's Sign Rank Test between the last EP study induced at the RVA prior to n-3 PUFA supplementation and the EP study following n-3 PUFA supplementation.

4.3 RESULTS

4.3.1 Subjects

A total of 11 patients completed this small pilot study by undergoing EP testing following fish oil supplementation. Nine of the subjects were male, and 7 subjects had left ventricular ejection fraction (LVEF) measured. Seven subjects presented with VT, 3 subjects with syncope and the remaining subject had unstable angina about 2 weeks following an MI. The clinical characteristics of the subjects are provided in Table 4.2.

Table 4.2 Baseline clinical characteristics of the subjects

n	11
Age	55.1±17.1 (26-75) ¹
Sex (% male)	88
LVEF (%) (n=7)	32.4±10.0
Presenting symptoms	
VT	8 ² (73)
syncope	3 (27)
Drug therapy	
amiodarone	7 (64)
sotalol	2 (18)
aspirin	6 (55)
β-blockers	7 (64)
ACE inhibitors	9 (82)
Angiotensin II receptor antagonist	1 (9)
Ca ⁺⁺ channel blockers	0
Statin	10 (91)
Warfarin	0
Anti-platelet agent	2 (27)
Digoxin	1 (9)

¹ $\bar{x} \pm SD$ (range in parentheses)

² n (% in parentheses)

4.3.2 Timing of EP studies

Of the eleven subjects in this study, seven subjects had 2 baseline EP studies, with the remaining four subjects having 1 baseline EP study. The details of the timing of EP studies is provided in Table 4.3. All subjects had inducible arrhythmias at their initial

EP study. A total of 6 subjects had baseline EP studies in the absence of anti-arrhythmic drugs (Table 4.3, subjects 1, 2, 6, 7, 4, 10).

In 4 of these subjects (subjects 1, 2, 6, 7), repeat invasive EP studies were performed following loading with anti-arrhythmic drugs (amiodarone in 3 cases and sotalolol in the other) and in all subjects the drug therapy failed to suppress the arrhythmia. The stimulation protocol required to induce the arrhythmia was identical in 1 subject, required 1 extra stimulus in 1 subject (600ms with 2 extras to 600ms with 3 extras), required the same number of extra stimuli but at a shorter drive cycle length (600ms with 2 extras to 400ms with 2 extras) in 1 subject. In the remaining subject (subject 6), the arrhythmia was induced with sensed triple extra stimuli at the RVOT, however, he was included in the study as his first EP study had been induced at the RVA. Following the continued inducibility of the arrhythmia, these 4 subjects were all recommended for ICD therapy, and commenced fish oil supplementation immediately, and subsequently had further EP studies performed non-invasively via the ICD, 3 (subjects 1, 6 and 7) at the time of implant, while the remaining subject (subject 2) lived in a country town (about 400 km from Adelaide) and returned after about 12 weeks following implant.

Of the remaining 2 subjects who had baseline EP studies free of anti-arrhythmic drugs, 1 subject commenced fish oil supplementation (subject 4) and had a repeat EP study invasively, while the other subject (subject 10) was recommended for immediate ICD implantation and had a repeat EP study non-invasively through the ICD lead system at the time of implant, commenced fish oil supplementation at the time of implant and had a repeat non-invasive EP study 6 weeks post implant.

Of the 11 subjects in this study, the remaining 5 subjects (subjects 3, 5, 8, 9 and 11) all had their initial EP study following administration of empiric anti-arrhythmic drug therapy (amiodarone in 4 cases and sotalol in the other). Following the continued inducibility the arrhythmia following drug therapy, all of these subjects had ICDs implanted. Three subjects (subjects 3, 5, and 8) commenced fish oil immediately following their EP study, and had their post fish oil repeat EP study performed non-invasively via the ICD. Two subjects (subjects 3 and 5) had the EP performed at the time of implant, and subject 8 had the EP performed 7 weeks post implant as his ICD was implanted only 1 week after his first EP study.

The remaining 2 subjects (subjects 9 and 11) had non-invasive repeat baseline EP studies at the time of ICD implant and commenced fish oil supplementation at the time of implant. They then returned for further non-invasive EP studies following 7 and 6 weeks of fish oil supplementation respectively.

Table 4.3 Ventricular arrhythmia induction during EP studies at baseline and following fish oil supplementation

Subject	Baseline EP studies						EP study following fish oil supplementation			
	EP1 ¹		EP2				Days on n-3	Anti-Arrhythmic Treatment	Induction	Method
	Anti-Arrhythmic Treatment	Induction	Days post EP1	Anti-Arrhythmic Treatment	Induction	Method				
1 ²	-	600/2 ³	14	Amiodarone	600/2	invasive	21	-	600/ 3	Non-invasive (at implant)
2	-	600/2	42	Sotalol	600/3	invasive	67	-	Non-inducible	Non-invasive (12 weeks post implant)
6	-	400/3	35	Amiodarone	NSR/3 ⁴	invasive	24	-	Non-inducible	Non-invasive (at implant)
7	-	600/2	7	Amiodarone	400/2	invasive	49	-	Non-inducible	Non-invasive (at implant)
10	-	400/2	28	-	400/3	Non-invasive (at implant)	42	-	600/3	Non-invasive
9	sotalol	600/2	28	Sotalol	400/2	Non-invasive (at implant)	49	-	400/2	Non-invasive
11	Amiodarone	600/2	42	Amiodarone	600/2	Non-invasive (at implant)	42	Amiodarone	400/3	Non-invasive
4	-	600/3	-	-	-	-	28	-	Non-inducible	Invasive
3	Amiodarone	400/2	-	-	-	-	21	-	Non-inducible	Non-invasive (at implant)
5	Amiodarone	400/1	-	-	-	-	42	-	400/2	Non-invasive (at implant)
8	Amiodarone	600/2	-	-	-	-	56	-	Non-inducible	Non-invasive (7 weeks post implant)

¹ all studies at EP1 were conducted invasively

² the subjects are sorted to group those subjects together with similar treatment protocols

³ drive chain cycle length and number of extra stimuli required to induce the arrhythmia

⁴ Normal Sinus Rhythm

4.3.3 Effects of anti-arrhythmic drugs on inducibility of arrhythmias

By definition, all subjects who received anti-arrhythmic drug therapy (sotalol or amiodarone) in this study were non-responders to that therapy in terms of its ability to suppress the arrhythmia.

4.3.4 Duration of n-3 supplementation

The mean duration of n-3 supplementation 40.1 ± 15.1 days with the minimum being 21 days and the maximum 67 days (Table 4.3).

4.3.5 Effects of n-3 PUFA on inducibility of arrhythmias

Following fish oil supplementation, the previously inducible arrhythmia was no longer inducible in 6 subjects. In 3 subjects, an extra stimulus was required to induce the arrhythmia, with subject 1 increasing from 600/2 to 600/3, subject 5 increasing from 400/1 to 400/2 and subject 11 increasing from 600/2 to 400/3). In 1 subject (subject 9), the stimulation protocol was identical following fish oil to that immediately prior to fish oil, while in the remaining subject (subject 10), the same number of extra stimuli were required, however, the arrhythmia was induced at a longer drive chain cycle length following fish oil (Table 4.3). The changes in stimulation protocol required to induce an arrhythmia are presented graphically in Figure 4.1.

There was a statistically significant shift towards non-inducibility, and where the arrhythmia was still inducible, towards an increase in the aggressiveness of the stimulation protocol required to induce the arrhythmia ($p < 0.01$, Wilcoxon's Sign Rank Test).

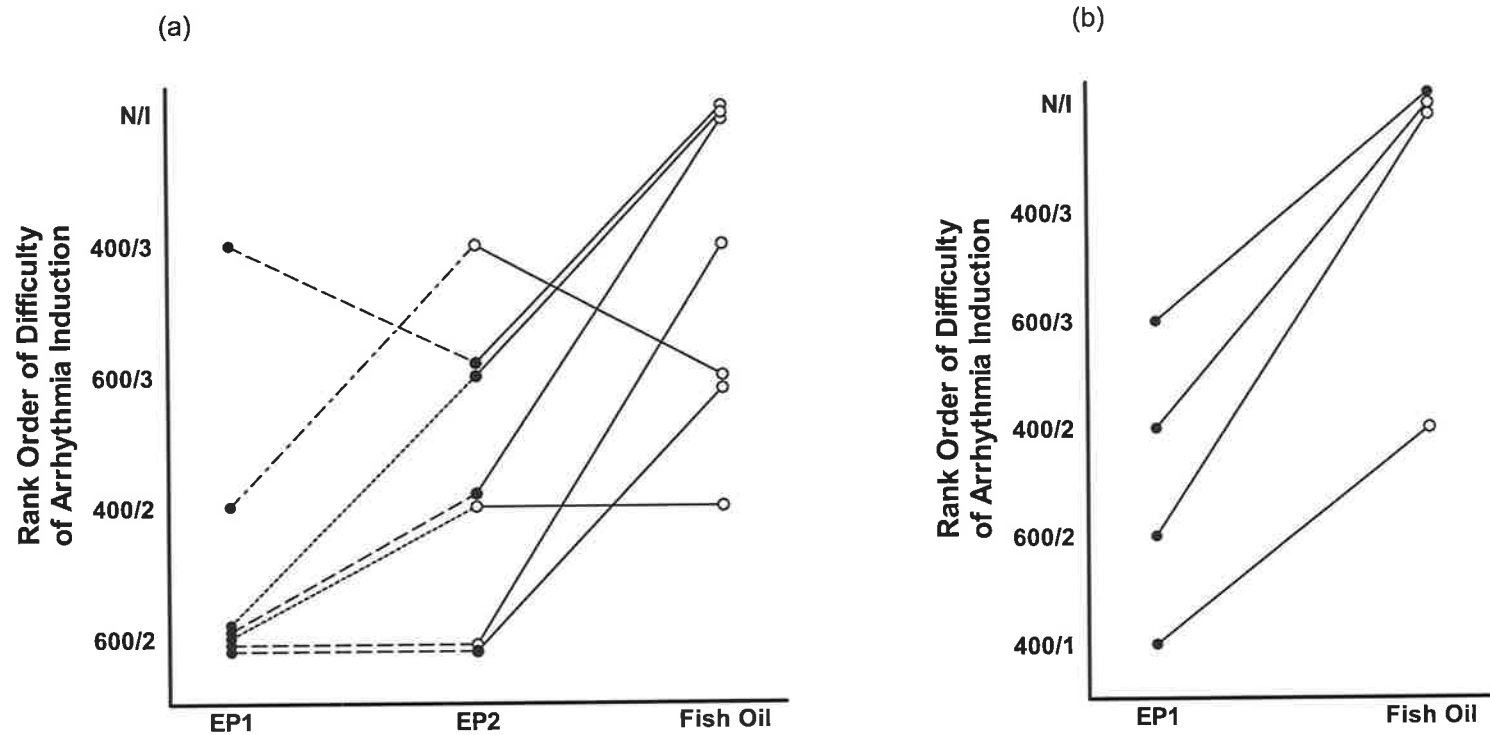


Figure 4.1 Ventricular arrhythmia induction during EP studies at baseline and following fish oil supplementation for subjects undergoing 2 baseline EP studies (a) and one baseline EP study (b). EP studies conducted invasively are represented by filled circles and those conducted non-invasively are represented by open circles. Dotted lines (.....) represents anti-arrhythmic treatment with sotalol, dashed lines (-----) represents anti-arrhythmic treatment with amiodarone, dot/dashed lines (-.-.-.-) represents no treatment, and a solid line represents treatment with fish oil. N/I = non-inducible.

4.3.6 Comparison of invasive and non-invasive EP studies

Three subjects had both invasive and non-invasive EP studies while drug therapy remained constant. In 1 subject, the stimulation protocol was identical between both studies. In 1 subject, the same number of extra stimuli were required to initiate the arrhythmia, but at a shorter cycle length was required in the non-invasive test, while in the last subject, an extra stimulus was required to initiate the arrhythmia. There was insufficient data to determine whether the inducibility of arrhythmias is the same by invasive and non-invasive methods.

4.3.7 Fatty acids

Following supplementation with 3 g/day fish oil, there were significant increases in plasma phospholipid and red blood cell EPA (134% and 115% respectively), DHA (51% and 30% respectively) and total n-3 PUFA (63% and 31% respectively) (Table 4.4).

There were significant decreases in plasma phospholipid and red blood cell total n-6 PUFA of 14% and 9% respectively (Table 4.4) while the changes in LA and AA were not significant. There were no changes in total saturates, OA or total monounsaturated fatty acids in either plasma phospholipids or red blood cells.

Table 4.4 Proportion of selected fatty acids in plasma phospholipids and red blood cells at baseline and following fish oil supplementation

Fatty Acid	baseline	Fish oil
<i>Plasma Phospholipids</i>		
Total Sats	43.1±1.0 ¹	43.6±0.3
18:1 n-9	10.6±1.4	10.8±1.8
Total Monos	14.0±1.1	14.3±1.9
n-6		
18:2	16.0±3.4	13.5±2.8
20:4	12.9±2.2	11.1±2.2
Total	34.2±2.5	29.3±3.3*
n-3		
20:5	1.34±0.41	3.14±1.56
22:5	1.35±0.31	1.89±0.47
22:6	3.89±0.40	5.89±1.12
Total	6.95±0.63	11.30±2.93
<i>Red Blood Cell Phospholipids</i>		
Total Sats	41.9±1.2	42.3±0.7
18:1 n-9	13.9±0.9	13.8±1.2
Total Monos	18.0±1.1	17.8±1.4
n-6		
18:2	8.1±1.4	7.7±1.2
20:4	15.0±1.3	14.0±2.2
Total	29.9±1.7	27.1±2.5*
n-3		
20:5	0.86±0.26	1.85±0.85*
22:5	2.88±0.86	3.25±0.87
22:6	4.66±0.68	6.08±1.24*
Total	8.96±0.94	11.70±2.46*

¹ $\bar{x} \pm SD$

*p<0.05 (paired t-test)

4.4 DISCUSSION

The primary aim of this pilot study was to examine whether LC n-3 PUFA at a dose of 900 mg/d of EPA + DHA in the form of encapsulated fish oil has any effect in suppressing previously inducible, sustained ventricular tachyarrhythmias at EP testing. Any observed effect would then allow sample size calculations for more definitive studies of the effects of fish oil in suppressing inducible arrhythmias in a dose response study.

Overall, we found 55% of subjects no longer had inducible arrhythmia following at least 3 weeks of n-3 PUFA supplementation. This result compares favourably with suppression rates reported for class III anti-arrhythmics, with 36% of patients having inducible arrhythmia suppressed by *d,l*-sotalol (Kühlkamp *et al*, 1999); 45% of patients suppressed by *d,l*-sotalol, amiodarone or E-4031/MS-551 (Naitoh *et al*, 1998), and 24% of patients taking sotalol and 41% taking amiodarone had an adequate response to therapy (in this case defined as the inability to induce VT or the ability to induce only relatively slow haemodynamically stable VT at repeat EP study) (Man *et al*, 1994). This result suggests that n-3 PUFAs probably have an anti-arrhythmic effect, and the observation in this study that of 9 patients who had inducible arrhythmia despite anti-arrhythmic drug therapy, 5 patients were no longer inducible following fish oil supplementation, raises the possibility of a synergistic association between n-3 PUFAs and class III anti-arrhythmics.

It is acknowledged that a potential weakness of this study is the range of EP protocols under which patients were examined, particularly where the repeat EP was conducted non-invasively following an invasive initial study. In 5 of the patients who were non-

inducible following fish oil supplementation, the initial EP was conducted invasively and the repeat EP study was conducted non-invasively. While there may be fundamental differences in the inducibility of ventricular tachyarrhythmias between invasive and non-invasive methodologies, the arrhythmia is initiated by the PVC that results from the extra stimulus, not the extra stimulus itself. Studies using early third generation ICDs, some of which utilized older first and second generation lead systems, reported that VF and VT were initiated non-invasively in 80% to 90% of patients in whom it had been previously induced invasively (Kleiman *et al*, 1994; Pinski *et al*, 1994; Costeas *et al*, 2000). On the other hand, the only patient to have an invasive EP study following fish oil was also non-inducible at repeat EP.

A potentially more important factor in the variability in the reproducibility of EP studies is the placement of the pacing electrode, with a new electrode being used for each EP study, the lead will not be placed in exactly the same position each time.

It is well recognized that the reproducibility of the induction of ventricular tachyarrhythmias at EP testing is less than 100%. An analysis of a number of studies which have examined the reproducibility of the induction of tachyarrhythmias at invasive EP studies indicates that overall, when the arrhythmia is induced with up to 4 extra stimuli at the initial EP study, about 61% of subjects required the same number of extra stimuli at the subsequent study, about 17% required fewer extra stimuli, about 20% required additional extra stimuli, and the remaining 2% were no longer inducible (Schoenfeld *et al*, 1984; McPherson *et al*, 1985; Lombardi *et al*, 1986; Beckman *et al*, 1988; Cooper *et al*, 1988; Cooper *et al*, 1989; Brembilla-Perrot *et al*, 2000). Therefore,

a rate of 55% of patients having their arrhythmia suppressed suggests that the n-3 PUFAs are probably having an anti-arrhythmic effect.

It has previously been reported that n-3 PUFA decreased the heart rate adjusted QT interval (QTc) in dogs (Billman *et al*, 1997). However, a recent report on the effects of n-3 PUFA on electrophysiological parameters in healthy men and women found no change in QTc, QRS duration, T-peak to T-end duration or T-loop morphology (Geelen *et al*, 2002). Amiodarone is also known to affect the sinus cycle length, QRS duration, QTc and VERP (Naitoh *et al*, 1998). As most of the subjects had previously been administered amiodarone, and with an elimination half life of 3-15 weeks (Kowey *et al*, 2000) there was insufficient washout prior to the EP study to examine the effects of n-3 PUFA on a range of electrophysiological parameters in this study.

The prognostic value of the suppression of inducible ventricular arrhythmias at EP testing in predicting a decreased risk of adverse outcomes has recently been questioned (Brodsky *et al*, 2002; Curtis, 2002). Data from the Electrophysiologic Study Versus Electrocardiographic Monitoring (ESVEM) study suggests that patients who continue to have inducible ventricular arrhythmias with programmed stimulation despite taking anti-arrhythmic drugs, are at increased risk of further arrhythmias and/or sudden death (Mason & The Electrophysiologic Study versus Electrocardiographic Monitoring Investigators, 1993).

In a subgroup analysis of the Multicentre Unsustained Tachycardia Trial (MUSTT) (Buxton *et al*, 2002) those patients with sustained VT at EP testing had a significantly higher (22%) risk of total mortality and a 63% increased risk of arrhythmic death or

cardiac arrest over 5 years than patients without inducible VT. None of these patients however, received any anti-arrhythmic drug therapy, and therefore provides no information on the question of whether rendering an inducible VT non-inducible has an effect on long-term outcome. On the other hand, data from the Anti-arrhythmics Versus Implantable Defibrillators (AVID) Trial found that inducibility of VT or VF did not predict death or recurrent VT or VF (Brodsky *et al*, 2002). However, the primary purpose of the present study was not to investigate the use of the EP study to predict long term efficacy of fish oil in preventing future VT or VF, but rather to determine whether fish oil suppressed inducible arrhythmias in the short term.

Overall, this pilot study provides suggestive evidence that a short course of fish oil has a cardiac stabilizing effect, and while there are acknowledged shortcoming in this study, it provides guidance for the development of future studies on the anti-arrhythmic and electrophysiological properties of n-3 PUFAs in humans.

CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

There is considerable evidence from epidemiological and randomized dietary intervention studies that the consumption of fish and/or fish oil, and to a lesser extent the vegetable n-3 fatty acid ALA, are beneficial in CHD. Evidence from animal and cell culture studies indicate that one of the mechanisms by which n-3 fatty acids confer these benefits is by the suppression of potentially fatal ventricular arrhythmias. However, the intake of n-3 fatty acids (the presumed active ingredient in fish and fish oil which confer these benefits) in the majority of people in western countries is quite low, and this probably is due to the limited range of foodstuffs which contain these fatty acids. The only sources of ALA are canola oil, flaxseed (linseed) oil, soybean oil and walnuts, while the long-chain n-3 fatty acids EPA and DHA occur in substantial quantities only in fish. This thesis contains 3 clinical studies, each concerned with increasing the health benefits of n-3 fatty acids.

5.1 STRATEGIES TO INCREASE CONSUMPTION OF N-3 PUFA

The dietary intervention study examined the potential of using a variety of foods to increase the consumption of n-3 fatty acids, both ALA and the long chain fatty acids EPA and DHA. This dietary approach utilized a number of everyday food items fortified with fish oil, as well as some items naturally high in n-3 fatty acids. In addition, a number of products high in monounsaturated fatty acids were provided to decrease the background intake of LA. Using this approach, the daily intake of ALA increased by almost 200%, and EPA and DHA intakes increased by 1600% and 1000% respectively. The dietary n-6:n-3 PUFA ratio decreased from 8.3:1 to 1.6:1.

Significant increases in the proportions of EPA + DHA in the phospholipid pools of plasma, platelets and mononuclear cells were achieved over 4 weeks of dietary intervention.

The Nutrition Committee of the American Heart Association (AHA) recently published recommendations for n-3 PUFA intakes in relation to people with and without documented heart disease (Kris-Etherton *et al*, 2002). For people without documented heart disease, at least 2 portions of fish (preferably oily) per week in addition to increased intakes of ALA are recommended. Two servings per week of an oily fish such as Atlantic salmon (200 g portions) would provide an intake of about 2.4 g/week of EPA + DHA (340 mg/d). In the study presented in this thesis, this n-3 intake was provided by consuming one cup of fish-oil fortified milk and one tablespoon of fish-oil fortified spread per day.

For patients with documented CHD, the AHA recommendation is a daily intake is about 1 g of EPA + DHA, preferably from oily fish. This would require a daily intake of about 170 g of Atlantic salmon, or 300 g of a less oily fish such as mullet. Such intakes clearly are unachievable for the majority of the population. Substitution of foods which comprise a normal part of the diet with fish-oil fortified alternatives enables increased consumption of n-3 PUFAs without radical alterations to dietary habits. For example, a daily intake of about 1 g of EPA + DHA could be achieved with 18 g (1 tablespoon) of spread (providing about 160 mg of EPA+DHA), 2 cups of milk (providing about 350 mg of EPA+DHA), 15 g of dip (providing about 120mg of EPA+DHA), 50 g luncheon meat (providing about 125 mg of EPA + DHA) and 4 sausages per week (providing an average daily intake of about 250mg EPA+DHA). Thus, the first study presented in this thesis demonstrates a practical alternative to fish consumption for achieving the intakes recommended by the AHA.

5.2 EFFECTS OF DIETARY N-3 PUFA ON THE INCIDENCE OF VENTRICULAR ARRHYTHMIAS

The remaining 2 studies examined the effects of increased consumption of EPA and DHA on ventricular arrhythmias in humans. The first study is a long term dietary intervention study in cardiac patients with implanted cardioverter-defibrillators (ICD), which aims to examine the effects of n-3 fatty acids on the incidence of arrhythmias over 18 months of intervention. This study employs a sequential design in which subjects act as their own controls using data recorded between implant and the commencement of the study (minimum of 12 months data) as baseline data.

A target intake of EPA + DHA of 900 mg/d was established, based on the level of supplementation provided in the GISSI study (GISSI Investigators, 1999). Because of the long-term design of this study, it was considered important to have dietary strategies which not only elevated n-3 fatty acid intake, but which were sustainable over the long term. Dietary n-3 fatty acids were supplied as fish-oil fortified foods developed in the diet study above (spread and long-life milk), along with canned fish. Subjects were also encouraged to increase their intake of fresh fish, and LC n-3 PUFA intake was estimated from a simplified diet diary. Fish oil capsules were provided to subjects unable to achieve the recommended intakes by dietary means alone and to those living in country areas.

This study is still ongoing, with 41 subjects currently enrolled. For the purpose of this thesis, 3 separate interim analyses have been conducted, (a) 20 subjects with at least 12 months intervention, (b) 33 subjects with at least 6 months intervention and (c) the 33 subjects from part (b) with equalised baseline and intervention periods. The first two analyses have shown significant reductions in the incidence of arrhythmias following

commencement of the fish oil intervention, with 70% of subjects in both analyses having a reduction in the incidence of arrhythmias and 25% having an increase in arrhythmia incidence. In both of these analyses, the total time of the baseline period was about double that of the intervention period, possibly allowing a greater opportunity for arrhythmias to occur in the baseline period.

The third analysis was conducted to equalise the baseline and intervention periods, and in this analysis, 48% of subjects had a decrease in arrhythmia incidence, 24% of subjects had an increase in arrhythmia incidence with the remaining 28% of subjects having no change in arrhythmia incidence. In this analysis, there was a high proportion of subjects who had arrhythmic events in the baseline period which were not included in the analysis due to the equalisation process. However, as the study continues, these events will be included as the baseline period increases to match the increasing intervention period. While the change in arrhythmia incidence was not statistically significant in this analysis ($p=0.10$), it is predicted that at the conclusion of this study we will be able to show a significant decrease in the incidence of arrhythmias following the intervention which indicate that the long chain n-3 fatty acids EPA and DHA have an effect in suppressing ventricular arrhythmias in patients with ICDs.

5.3 EFFECTS OF DIETARY N-3 PUFA ON INDUCIBLE ARRHYTHMIAS

The last study is a short-term pilot study examining the effects of fish-oil supplementation on inducible ventricular tachyarrhythmias in patients undergoing cardiac electrophysiology (EP) testing. Eleven patients with inducible, sustained ventricular tachyarrhythmias at EP testing (9 subjects previously inducible on anti-arrhythmic drug therapy) were

supplemented with fish-oil (3 g/d) for a minimum of 3 weeks. On retesting, 6 subjects were not able to have an arrhythmias induced, while 3 subjects required a more aggressive stimulation protocol to induce the arrhythmia.

While there were some weaknesses with this study, most notably the variation in study protocols used and in particular the variation between invasive and non-invasive EP studies, the principle purpose of this study was to determine whether fish oil supplementation has any effect on inducible ventricular arrhythmias. Any effect of fish-oil in suppressing inducible arrhythmias, would then allow sample size calculations for more definitive studies of the effects of fish oil in suppressing inducible arrhythmias.

These studies have demonstrated that n-3 fatty acids probably have some anti-arrhythmic properties in humans, with two studies, utilising two different methodologies and drawing similar conclusions. The studies also demonstrate that significant increases in the consumption of n-3 fatty acids can be achieved by incorporating n-3 fatty acid fortified foods into the everyday diet.

5.4 FUTURE DIRECTIONS

The two studies detailed in this thesis examining the effect LC n-3 PUFA on cardiac arrhythmias in humans indicate that EPA and DHA at a combined intake of about 1-1.5 g/d probably have anti-arrhythmic properties in humans.

The pilot study examining the effects of encapsulated fish oil on the inducibility of arrhythmias in patients undergoing electrophysiology testing indicated that fish oil is probably having an effect in suppressing inducible arrhythmias. Epidemiological data

suggests that LC n-3 PUFA intakes of as little as 100 - 150 mg/d provide protection from SCD with a 66% reduction in risk associated with this level of intake among relatively healthy men in the Physicians Health Study (Albert *et al*, 1998). Among survivors of acute MI, LC n-3 PUFA supplementation of 850 mg/d was associated with a 45% reduction in risk of SCD after 3.5 years of follow-up in the GISSI study (GISSI Investigators, 1999), while 1.8 g/d was associated with a non-significant (due to low number of events, with 2 events in the intervention group vs 8 events in the placebo group) 75% reduction in risk of SCD after 1 year in the Indian Infarct Survival Study (Singh *et al*, 1997). Dose response relationships have been reported between both fish intake and blood levels of LC n-3 PUFA, and the odds of primary cardiac arrest in a case-control study (Siscovick *et al*, 1995) and the risk of SCD in a cohort study (Albert *et al*, 1998; Albert *et al*, 2002). All of these studies suggest an anti-arrhythmic effect of n-3 PUFA. The reported benefits of LC n-3 PUFA have occurred at widely varying intakes, and further investigation into the dose response relationship between LC n-3 PUFA intake and anti-arrhythmic effects in a randomized controlled trial, using the treatment effect observed in the EP study is warranted.

A time-course analysis of the GISSI study found little divergence of the survival curves for SCD between n- PUFA and placebo during the first 60 days post enrolment, with survival becoming statistically significant at 120 days (Marchioli *et al*, 2002). A possible explanation for the delay in divergence of the survival curves is the time required to increase the proportion of n-3 PUFA in membrane phospholipids at a dose of 850 mg/d.

Rapid tissue incorporation of LC n-3 PUFAs following an oral loading dose of 10 g LC n-3 PUFA has recently been demonstrated, with a 130% increase in platelet phospholipid EPA and a 30% increase in platelet phospholipid DHA observed 14 hours after the loading

dose (Svaneborg *et al*, 2002). As patients admitted to coronary care units following MI are at high risk of arrhythmias, a high initial loading dose of fish oil may be beneficial in suppressing arrhythmias and reducing mortality during the acute post MI phase. Thus, a study of early intervention with n-3 fats in acute coronary syndrome is warranted.

Funding applications have been made for these suggested studies, in order to develop the information on n-3 fats and ventricular arrhythmias which has been provided by this thesis.

APPENDIX A

Dietary Information Provided to Subjects in the Diet Study (Chapter 2) to Reduce Consumption of n-6 PUFA.

FOODS ALLOWED	FOODS TO AVOID
FATS & OILS Only use the oil, spread, mayonnaise and salad dressing that you have been provided with.	FATS & OILS Avoid all other oils, cooking sprays, dressings, mayonnaises, lards, dairy blends, drippings, cooking margarines and spreads
NUTS & LEGUMES Hazelnuts, macadamias - only in limited amounts - one handful (~30g) 1-2 a week	NUTS & LEGUMES Peanuts (peanut butter), almonds (marzipan), cashews, brazil nuts, pecan nuts, pine nuts, pistachios, walnuts, sesame seeds (tahini paste and hommous), sunflower seeds, soybeans (tofu, soya milk and soya flour)
	MEAT Offal - heart, kidney, brain, lung, liver (pate), tongue, tripe etc.
FISH Mullet, Bream, Snook, Flathead, Trevally Canned pink or red salmon (in brine, water or canola oil) Canned sardines in tomato sauce Frozen fish: (cooked in canola oil) <i>Watties</i> - Crumbed Hoki, Batterd Hoki, Chargrilled Hoki <i>I&J</i> - Light & Crispy range	FISH Other packaged frozen fish
FRUITS & VEGETABLES Frozen Chips <i>McCain</i> - Healthy Choice: Straight Cut or Country Style <i>Birds Eye</i> - Steak House, Crinkle Cut, Home Style, French Fries, Flavour Crisp (Seasoned Crunchy Wedges only) <i>Logan Farm</i> - Straight Cut, Crinkle Cut	FRUITS & VEGETABLES All other varieties of frozen chips, Avoid those that are tinned in dressings (i.e. potato salad)
CEREALS Use flaxseed meal provided on breakfast cereals	CEREALS Toasted muesli, Kellogs' Balance, Kellogs' Komplete, Muesli bars
SNACK FOODS & CONFECTIONERY Dry biscuits - Salada, Sao Potato crisps - Kettle Chip Company	SNACK FOODS & CONFECTIONERY Other dry biscuits (i.e. BBQ shapes) Other potato crisps & cheese snacks (i.e. Cheezels) Health Food Bars - sesame bars, nut bars, muesli bars <u>Only</u> those chocolate bars which contain nuts or seeds as listed above (i.e. Snickers, Picnic)
FAST FOODS McDonalds, Hungry Jacks, Pizza Haven, Kentucky Fried Chicken, Fasta Pasta (except for sauces containing pesto)	FAST FOODS Pizza Hut

APPENDIX B

Example of a Portion of a Text File Conversion Obtained From Medtronic File Translation of Arrhythmia Episode Data

"Entry Number","Time of Episode","Episode Type","FVT Via","Average Cycle(ms)","Last Therapy","Last Therapy Efficacy","Duration","TDI (ms)","FDI (ms)","FTI (ms)","Onset Criterion","Onset","Stability Criterion","EGM Width Criterion","EGM Width","Interval Stability Low","Interval Stability High","Triggered Via Combined Count","Therapies Aborted Charge Time Out","Therapies Aborted Rate Limit","Therapies Aborted Sync Fail","Therapies Aborted User","Therapies Skipped Rate Limit","EGM/Episode Record Data","Episode In Progress"

24,"Jun 29, 2001 05:08:39","VF","NA", 290,"(No Rx Delivered)","NA"," 31 sec",370,300,300,"On","NA","On","Passive","NA","NA","NA","Yes","None","None","One or More","None","None","Yes","No"

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"Interval Data",1110

"Marker Data","VS"

"Interval Data",1050

"Marker Data","VS"

"Interval Data",1050

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"Interval Data",1120

"Marker Data","VS"

"Interval Data",1140

"Marker Data","VS"

"Interval Data",1060

"Marker Data","VS"

"Interval Data",1120

"Marker Data","VS"

"Interval Data",1130

"Marker Data","VS"

"Interval Data",1090

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"Interval Data",1130

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"Interval Data",1110

"Marker Data","VS"

"Interval Data",1140

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"Interval Data",1120

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"Interval Data",1050

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"Interval Data",1080

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1, 2, 1, 1, 3, 3, 3, 3, 4, 5, 6, 6, 6, 6, 6, 5, 4, 1, 0, 254, 252, 252, 252
"EGM
Data", 251, 252, 252, 253, 254, 254, 254, 254, 255, 255, 255, 255, 255, 255, 255, 0, 255, 0, 255, 0, 0, 0, 0, 0,
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"EGM
Data", 1, 1, 0, 1, 1, 0, 1, 0, 1, 0, 0, 0, 1, 0, 1, 1, 0, 1, 0, 1, 0, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 1,
1, 1, 1, 1, 1, 0, 251
"EGM Data", 245
"Interval Data", 1170
"Marker Data", "VS"
"End of Episode"

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APPENDIX C

n-3 PUFA Diet Diary for Estimation of n-3 PUFA Intake in the ICD Study

(Chapter 3)

INSTRUCTIONS

Please be as accurate and as comprehensive as possible.

Each page covers a 4 week period, and there are 20 pages to cover the whole period of the study.

MARGARINE & MILK

We would like you to restrict consumption of these foods to yourself only.

Place a tick alongside each day that you start a new tub of margarine or carton of milk.

Target intakes: Margarine – 20 gm/day (1 tub about every 2-3 weeks)

Milk – 2 cups (1 carton)/day

CANNED FISH

Place a tick against each day that you have a can of fish. If the canned fish is incorporated into a recipe, and you eat only a portion of the dish, write down the estimated amount that you have eaten (eg 1 can of salmon made into a mornay and you had about ¼ of the amount).

Target intake: at least 1.5 can/week

OTHER FISH/SEAFOOD

If you eat any other fish or seafood, could you write down the type, and an estimate of the amount (raw weight would be the best, however this is not always possible, so the number of fillets or pieces).

FISH OIL CAPSULES

In some instances we may supply you with fish oil capsules (eg going away on holidays, or to supplement intake). Please record the number of capsules taken each day.

Month: 1

	Date	Margarine	Milk	Canned fish		Other fish		Fish oil capsules (no.)
				Salmon	Sardines	Type	Qty	
Fri								
Sat								
Sun								
Mon								
Tues								
Wed								
Thurs								
Fri								
Sat								
Sun								
Mon								
Tues								
Wed								
Thurs								
Fri								
Sat								
Sun								
Mon								
Tues								
Wed								
Thurs								
Fri								
Sat								
Sun								
Mon								
Tues								
Wed								
Thurs								

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