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N-3 POLYUNSATURATED FATTY ACIDS IN HEART FAILURE: MECHANISMS AND RECENT CLINICAL EVIDENCE

R. MARCHIOLI✉, M.G. SILLETTA, G. LEVANTESI, R. PIOGGIARELLA AND G. TOGNONI

Consorzio Mario Negri Sud, Santa Maria Imbaro, Chieti, Italy
E-mail: marchioli@negrisud.it

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Abstract – Over the past 20 years, there has been significant progress in our knowledge of the pathophysiology of heart failure (HF) with consequent considerable development of both pharmacological and non pharmacological approaches. Despite improved therapeutic strategies, HF still remains burdensome in terms of mortality, quality of life, and hospitalization costs. A new and promising medical treatment to improve survival in HF patients stems from the recent results of the Italian study, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Heart Failure (GISSI-HF). GISSI-HF was a randomized, large scale, double-blind, placebo-controlled trial showing that n-3 PUFA (850–882 mg/d) reduced mortality and admission to the hospital for cardiovascular reasons in patients with chronic heart failure (HF) who were already receiving recommended therapies. The clinical benefit observed in GISSI-HF seemed to be mediated prominently by the antiarrhythmic effects of n-3 PUFA, though an effect on mechanisms related to HF progression cannot be excluded. This article presents the results of GISSI-HF study and reviews the previous clinical evidence on n-3 PUFA and risk of heart failure and discusses in depth the potential mechanisms through which n-3 PUFA treatment can improve clinical outcome in HF patients.

Key words: Heart failure; n-3 PUFA, arrhythmias, atherothrombosis, cardiac haemodinamics, inflammation.

INTRODUCTION

Heart failure (HF) is a complex, multifactorial clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with, or eject blood (101).

The early stage of HF is often clinically asymptomatic due to physiological compensatory mechanisms, including the activation of the adrenergic nervous system, the renin-angiotensin system, the cytokine system and the development of cardiomyocyte hypertrophy. With time, however, the sustained activation of these compensatory mechanisms can lead to secondary end-organ damage within the ventricle, with worsening left ventricle remodelling (increased LV chamber volume, wall thinning, and myocardial fibrosis) and subsequent cardiac decompensation (17). Overall, this syndrome represents a relatively common disorder being today the single most important cardiovascular public-health burden in developed countries: approximately 4.9 million person in Unites States are being treated for HF, and 550,000 new cases are diagnosed each year (9).

Over the past 20 years, there has been considerable progress in the knowledge of HF, that lead to supplant the view of chronic myocardial failure as an irreversible, end-stage process by the idea that it is possible to determine a true, biologically-based improvement in the intrinsic defects of function and structure that afflict the chronically failing heart. This concept has been developed because of the progress in the pharmacologic treatment of patients with chronic systolic heart failure and it is mainly related with the possibility to suppress the neurohormonal iper-activation of the renin–angiotensin–aldosterone system of HF patients, i.e., an intervention that can improve systolic function, may reverse cardiac remodeling (57), and improves clinical outcomes, including prolonged survival and reduced hospitalizations (18, 65, 100, 120).

Despite these successful therapies, morbility and mortality of patients with HF remain substantially high. Almost 50% of discharged patients are rehospitalized within 6 months, (3,

66) and the prognosis for even those optimally treated remains poor with annual mortality rate of 10% (34, 35, 95).

Approximately two-thirds of all HF patients have a history of ischemic heart disease (111) and the major clinical risk factor for HF include age, sex male, hypertension, diabetes, valvular heart disease and obesity (195). The mechanism of death from HF follows two broad courses partially related to the severity of left ventricular (LV) dysfunction: progressive congestive HF, more frequent in patients with severe reduction of LV function, or sudden cardiac death (SCD). This latter is thought to be due to ventricular tachyarrhythmias and it claims approximately 335,000 lives each year in the United States alone accounting for about 50% of all deaths associated with coronary heart disease (241). Compared with the general population, the incidence of SCD is 6 to 9 times higher in HF patients, especially those with a non-severe reduction of LV function (9). In daily clinical practice the prognosis is even worse as reported in 5,517 outpatients with congestive HF from different causes enrolled in the Italian Network on Congestive Heart Failure (IN-CHF) registry. One-year mortality rate for all-cause death was 11.9% with 46% of deaths being classified as sudden. The risk of death at 1 year was significantly increased according to functional NYHA class, all-cause mortality being significantly increased by 93% and sudden cardiac death by 52% in patients classified as NYHA III to IV versus NYHA I to II (11).

To change the natural history of HF one should not only target the remodelled left ventricle with neurohormonal modulators but also the pathophysiological processes leading to an increased risk of SCD.

In this respect, a new and promising medical treatment to improve survival in HF patients stems from the recent results of an Italian study, the GISSI-HF (215). The aim of this paper is to present the results of GISSI-HF study, to review the clinical context of the cardioprotective effects of n-3 PUFA, and present possible mechanisms through which n-3 PUFA treatment can improve clinical outcome in HF patients.

GISSI-HF RESULTS: A POTENTIAL BREAKTHROUGH IN THE TREATMENT OF HEART FAILURE

Between 2002 and 2005 the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) performed the GISSI-HF trial to test the efficacy of oral administration of n-3 polyunsaturated fatty acids (PUFA) on morbidity and mortality in patients with symptomatic HF of any cause and with any level of left ventricular ejection fraction (LEVF).

GISSI-HF was a multicenter, randomized, double-blind, placebo-controlled trial, carried out on 7046 patients in class II–IV chronic heart failure randomized to 1g daily of n-3 PUFA (850–882 mg eicosapentaenoic acid and docosahexaenoic acid as ethyl esters in the ratio of 1·2:1) or matching placebo (216). The prespecified two co-primary endpoints were 1) time to death and 2) time to death or admission to hospital for cardiovascular reasons. The results of GISSI-HF indicate that n-3 PUFA was able to improve long-term HF prognosis to a clinically important extent essentially through a decrease in arrhythmic events/sudden cardiac death and other events related to progression of heart failure. During a median follow-up of 3.9 years, treatment with n-3 PUFA, compared to placebo, was associated with statistically significant 9% reduction in the risk of death (955 *vs* 1014 patients; 95.5% confidence interval 0.833-0.998, P=0.041) and 8% reduction in all-cause mortality or hospitalization for a cardiovascular cause (1981 *vs* 2053 patients; 99% confidence interval 0.849-0.999, P=0.009) in patients already receiving evidence-based medical therapy for HF (215) (Figure 1). These effects were consistent in any prespecified subgroups, including patients with either an ischaemic or non-ischaemic cause of heart failure and those with reduced and preserved left ventricular systolic function.

With the exception of stroke, a lower rate of all secondary outcome events was observed in patients receiving n-3 PUFA treatment (Table 1). Patients treated with n-3 PUFA were less likely to die of SCD than those in the placebo group, though such effect was not statistically significant. In addition, a significant 28% reduction of hospitalizations for ventricular arrhythmias with n-3 PUFA treatment was observed (97 *vs* 132 patients; 95% CI 0.55-0.93, P=0.013) and this accounted for almost half the absolute risk reduction in first hospitalization for cardiovascular reasons. The number of presumed arrhythmic deaths and deaths from worsening of heart failure (both accounted for most deaths) were lower in the n-3 PUFA treatment compared to placebo group, 274 *vs* 304 and 319 *vs* 332 respectively. Treatment with n-3 PUFA was remarkably well tolerated, adverse drug reactions

being rare and similar in the active and placebo groups. By the end of the study, 1004 (29%) of patients in the n-3 PUFA group and 1029 (30%) in the placebo group permanently discontinued the study drug for various reasons (p=0·45), gastrointestinal disturbance being the most frequent cause in both groups.

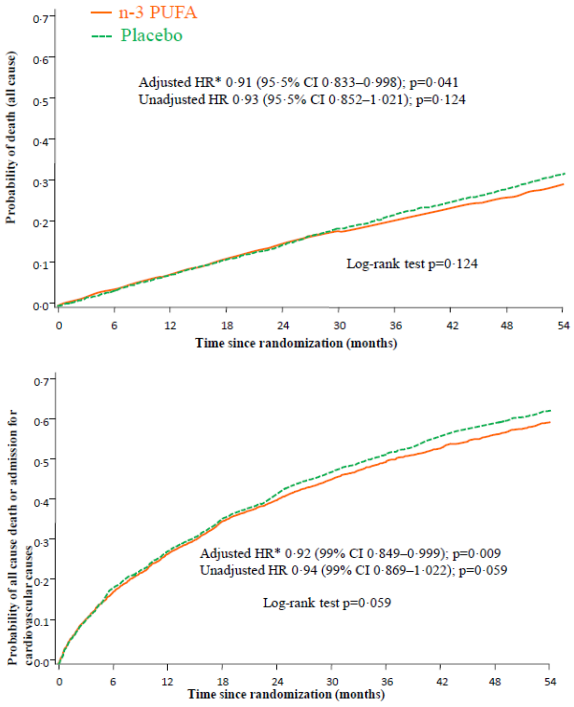

Figure 1. Kaplan-Meier curves for time to all-cause death and for time to all-cause death or admission to hospital for cardiovascular reasons.
PUFA=polyunsaturated fatty acids. *Estimates were calculated with a Cox proportional hazards model, with adjustment for admission to hospital for heart failure in the previous year, previous pacemaker, and aortic stenosis.

According to the aforementioned, the results of GISSI-HF indicate that in HF patients treatment with n-3 PUFA, given on top of evidence-based medical therapy, produce a clinical benefit by saving lives and reducing cardiovascular hospitalization and therefore is likely to improve quality of life. In fact, treatment of 1000 patients with n-3 PUFA 1 g/day for 3.9 years saved 18 lives and prevented 17 cardiovascular hospitalizations (Figure 2). Further, n-3 PUFA treatment was safe in a large population of patients with HF of any cause, who were recruited in a nation-wide network of clinical practice hospital and ambulatory facilities including over 300 sites. Overall, these aspects assure the transferability of the results to daily clinical practice for heart failure.

The practical implications of these findings would be the endorsement of n-3 PUFA as a therapy for heart failure, thus extending the benefit of n-3 PUFA beyond the setting of secondary prevention of coronary heart disease.

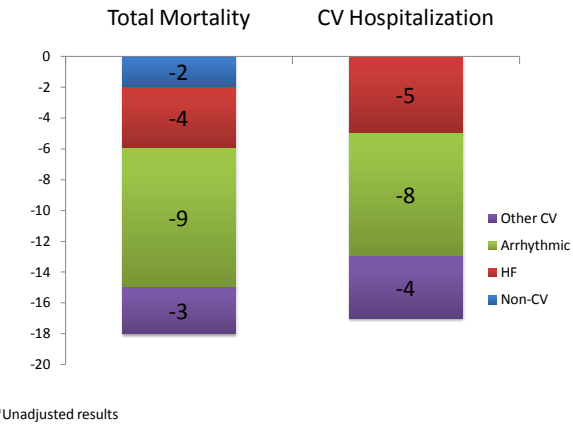

Figure 2. Deaths and cardiovascular hospitalization avoided every 1,000 gissi-hf patients treated for 4 year with n-3 PUFA 1g daily.

CLINICAL EPIDEMIOLOGY OF N-3 PUFA IN HEART FAILURE

The results of GISSI-HF appear in line with those coming from the few, previous studies evaluating the association between fish or n-3 PUFA intakes and risk of heart failure. The Cardiovascular Health Study, involving 4,738 men and women aged ≥65 years found an inverse association of baked/broiled fish intake with congestive heart failure; the HR (95% CI) for baked/broiled fish intake of ≥5 times a week versus <1 time a month was 0.68 (0.45 to 1.03), and the test for trend across the frequency categories was statistically significant (p = 0.009). A similar inverse association was observed for quintiles EPA and DHA intake (152). A GISSI-Prevenzione trial post-hoc analysis showed that the benefit from n-3 PUFA treatment 1g/d on total mortality were preserved irrespective of the worsening of left ventricular systolic function while those on sudden death seemed to increase patients with low ejection fraction (131). Akin findings were observed in a cohort of 57,972 Japanese free of cardiovascular disease at recruitment, followed up for 12.7 years. Reduced risk of death due to heart failure was associated with fish and n-3 PUFA dietary intake (236). Similar results have been reported in the ARIC (Atherosclerosis Risk in Communities) study, showing an

Table 1. Secondary outcomes and cause of death.
Data are number (%) unless otherwise stated. PUFA=polyunsaturated fatty acids. SCD=sudden cardiac death. MI=myocardial infarction. *95% CI was calculated with a Cox proportional hazards model with adjustment for admission to hospital for heart failure in the preceding year, previous pacemaker, and aortic stenosis.

	n-3 PUFA (N=3494)	**Placebo** (N=3481)	**Adjusted HR** (95%CI)*	**P value**
Patients who died of a cardiovascular cause	712 (20·4%)	765 (22·0%)	0·90 (0·81–0·99)	0·045
Patients who had an SCD	307 (8·8%)	325 (9·3%)	0·93 (0·79–1·08)	0·333
Patients admitted	1986 (56·8%)	2028 (58·3%)	0·94 (0·88–1·00)	0·049
for a cardiovascular reason	1635 (46·8%)	1687 (48·5%)	0·93 (0·87–0·99)	0·026
for heart failure	978 (28·0%)	995 (28·6%)	0·94 (0·86–1·02)	0·147
Patients who died of a cardiovascular cause or admitted for any reason	2157 (61·7%)	2202 (63·3%)	0·94 (0·89–0·99)	0·043
Patients with fatal and non-fatal MI	107 (3·1%)	129 (3·7%)	0·82 (0·63–1·06)	0·121
Patients with fatal and non-fatal stroke	122 (3·5%)	103 (3·0%)	1·16 (0·89–1·51)	0·271
Cause of death				
Acute MI	20 (0·6%)	25 (0·7%)	0·77 (0·43-1·39)	0·382
Worsening heart failure	319 (9·1%)	332 (9·5%)	0·92 (0·79-1·07)	0·275
Presumed arrhythmic	274 (7·8%)	304 (8·7%)	0·88 (0·75-1·04)	0·141

inverse association between plasma long-chain n-3 PUFA and incident HF among women (237).

N-3 PUFA BIOCHEMISTRY

n-3 (also known as omega-3) and n-6 (also known as omega-6) PUFA are two important classes of dietary polyunsaturated fatty acids which are required for normal growth, development and for the optimal function of many organs such as brain and heart. Both classes are substrates of the same enzymes and undergo the same metabolic oxidation pathways, and cannot be synthesized “de novo” by mammals or be interconverted but must be obtained from the diet and are thus essential nutrients.

Alpha-linolenic acid (C18:3 n-3; ALA) and linoleic acid (C18:2 n-6; LA) are the precursors of the omega-3 and omega-6 families, respectively. ALA is formed in the chloroplasts of green leaves, plankton, and algae by the desaturation of C18:2 n-6; vertebrates lack the desaturase to convert n-6 to n-3 PUFA.

Both ALA and LA can be elongated and desaturated in our bodies to yield different active metabolites. ALA can be converted to eicosapentaenoic acid (C20:5 n-3; EPA), the first of the important fish oil fatty acids. EPA can be further elongated and desaturated to form the other physiologically active fish oil fatty acids namely docosahexaenoic acid (C22:6 n-3; DHA), which is the major storage form of n-3 PUFA in cell membrane phospholipids of heart and neurons. LA is converted to arachidonic acid (C20:4 n-6; AA). However, the extent to which ALA is converted to EPA and DHA in humans is modest, ranging from 0.2% (170) to 15% (58) and varies considerably among different individuals for example, the conversion of α ALA acid to EPA and DHA in women is greater than that observed in men, possibly due to the regulatory effects of oestrogen (21). Furthermore, cell membranes storage of ALA is virtually nil, as it seems to be largely metabolized for energy. n-3 PUFA play an important role in modulating the physical state of biological membranes; in fact, the incorporation of n-3 PUFA increases the fluidity of the membrane phospholipid bilayer. This is considered as a possible means by which n-3 PUFA might affect the activity of membrane-associated proteins such as ion channels, carriers

and enzymes. A direct interaction between n-3 PUFA and membrane proteins and/or direct alteration of lipid rafts and specific membrane microdomains have also been suggested as alternative mechanisms by which n-3 PUFA modify membrane protein activities (125). Both EPA and AA are the eicosanoid precursors of the cyclooxygenase and lypoxygenase pathways, leading to the synthesis of prostaglandins, prostacyclins, thomboxans and leukotrienes. EPA competes with AA for the same eicosanoid synthetic pathways yielding generally less biological active metabolites or in some case antagonizing or attenuating the activity of AA metabolites. The series-2 prostaglandins and series-4 leukotrienes derived from AA promote platelet aggregation, enhance vasoconstriction and the synthesis of inflammatory mediators in response to physiological stressors. The series-3 prostaglandins and series-5 leukotrienes that are derived from EPA, work to attenuate excessive series-2 prostaglandins effects. Considering all the above, it is likely that an optimal dietary ratio between omega-6 and omega-3 might be important since an unbalanced omega-6/omega-3 ratio (20-30:1, as in the modern Western diet) has been suggested to be a contributing factor in the onset and progress of many inflammatory diseases including cardiovascular disease (203). Compared with saturated fatty acids and trans fatty acids, however, omega-6 fatty acids, particularly LA, have been attributed with a cardiovascular benefit, as highlighted in the latest AHA advisory. Aggregate clinical and experimental data indicate that the consumption of at least 5% to 10% of energy from omega-6 PUFA reduces the risk of CHD relative to lower intake (90).

POTENTIAL MECHANISMS OF ACTION OF N-3 PUFA

Many processes and mechanisms have been identified as underlying heart failure including atherothrombosis, arrhythmias, abnormalities of excitation-contraction coupling, abnormalities in energy metabolism, altered expression or function of contractile proteins, alterations in β-adrenergic receptor signal transduction, cytoskeletal abnormalities, hypertrophy and neurohormonal-cytokine changes. Taken together, these effects lead to a vicious circle that perpetuates and intensifies heart failure (17). The beneficial effect of n-3 PUFA in GISSI-HF on fatal events and on hospital admissions for cardiovascular cause suggests that this treatment might influence HF prognosis through antiarrhythmic mechanisms as well as those leading to the development/progression of HF. We will review the large body of evidence on the effects of n-3 PUFA with emphasis on the mechanism which could be more relevant in HF following the order that is summarized in Table 2. Though some mechanisms are correlated, evidence will be summarized in separated sections to facilitate the reading of single pathophysiological process.

Table 2. Pathophysiologic processes/mechanisms underlying HF development/progression

- Arrhythmias
- Atherothrombosis
  - Platelets
  - Clotting Factors and Fibrinolysis
  - Lipids
  - Oxidative Stress
- Neurohormonal System
- Cardiac Haemodinamics
- Cardiac Hypertophy
- Energy Metabolism
- Excitation-Contraction Coupling
- Inflammation
  - Proinflammatory Cytokines
  - Other Markers of Inflammation

Arrhythmias

The results of GISSI-HF appear to confirm the initial core hypothesis of a beneficial effect of n-3 PUFA in patients with HF by reducing arrhythmic events.

The first large-scale clinical trial, published in 1989 on 2033 patients who had recently suffered from MI, showed that those who had received as dietary advice to eat a modest intake of fatty fish (200-400g/week) had a 29% reduction of total mortality, primarily due to reduction in CHD death. This benefit appeared early (within 6 months after MI) and persisted up to 2 years of follow-up (23). The authors suggested the findings could be due to a

reduction in arrhythmic deaths, of more, 50–60% of deaths in the setting of CHD are sudden cardiac deaths (deaths within 1 hr of an acute MI) attributed to sustained ventricular arrhythmias (8). By far, the largest published trial was the GISSI-Prevenzione study that tested with an open design the efficacy of 850 mg/d omega 3 fatty acids (such as EPA and DHA) in 11,323 patients with recent myocardial infarction (MI<3 months) (79). This was on top of optimal pharmacological treatments and lifestyle advice. In GISSI-Prevenzione, n-3 PUFA treatment significantly reduced total and cardiovascular mortality by 20% and 30%, respectively with no significant changes in the rates of non-fatal cardiovascular events (myocardial infarction and stroke) across the treatment groups. The most affected cause of death among cardiac causes, was sudden cardiac death with a reduction of 45%; the benefit of n-3 PUFA was already significant after 4 months of treatment. These findings suggested an antiarrhythmic effect of n-3 PUFA as the prominent mechanism of action (133).

Few trials with n-3 PUFA have been carried out in patients with implanted cardioverter defibrillator and who therefore were at high risk of fatal ventricular arrhythmias. They have produced conflicting results, but their limited sample size and number of events might account for such discrepancies (19, 122, 182). In a subgroup analysis of the SOFA trial, however, a tendency towards a beneficial effect of fish oil on event free-survival (ICD intervention or all-cause death) arrhythmias was found in patients with prior MI (HR 0.76; 95% CI, 0.52-1.11) and ejection fraction <30% (HR 0.82; 95% CI, 0.50-1.33). This implies that the benefit of fish oil against life-threatening arrhythmias might specifically apply to patients with prior MI and HF patients (19).

Siscovick et al. demonstrated a relationship between risk of primary cardiac arrest and n-3 PUFA in a population-based cohort of adults aged between 25 and 74 years who were free of prior heart disease, major comorbidity, or history of fish-oil use. Among 334 patients who had experienced primary cardiac arrest and 493 age- and sex-matched control subjects, risk of primary cardiac arrest was decreased significantly by 50% for those who consumed an estimated 5.5 g of n-3 PUFA over the prior month compared with those who consumed no fish at all, after adjustment for potential confounders ($p = 0.05$). In a subset of 82 cases and 108 controls from this study, erythrocyte membrane n-3 PUFA were 4.3% ± 1.1 % for cardiac arrest cases and 4.9% ± 1.4% for controls ($p = 0.002$). Risk of primary cardiac arrest was significantly decreased by 70% for those in the third quartile of membrane n-3 PUFA concentration (mean, 5.0% of total fatty acids) compared with those in the lowest quartile (mean, 3.3% of total fatty acids) (205, 206).

In a double-blind placebo-controlled study in 65 patients with cardiac arrhythmias but without evidence of coronary heart disease or HF, the incidences of atrial and ventricular premature complexes, couplets, and triplets were reduced over a 6-month period among those randomized to treatment with 3 g/day of fish oil providing 1 g of n-3 PUFA compared with those randomized to 3 g/day of olive oil as a placebo (204).

In 39 patients who were free of complex ventricular arrhythmias and severe heart failure at baseline, a significant trend toward a reduction in ventricular premature complexes was also reported after 16-week treatment with 15 ml/day of fish oil providing 0.9 g EPA and 1.54 g DHA or with a placebo of sunflower seed oil; a 48% decrease in ventricular premature complexes in the fish oil group compared with a 25% decrease in the placebo group (197). In a study in 40 patients with dual-chamber pacemakers who had paroxysmal atrial tachyarrhythmia recorded at periodic monitoring, treatment with 1 g/day of n-3 PUFA for 4 months significantly reduced the number of atrial tachyarrhythmia episodes by 59% and the burden by 67% without change in device programming or pharmacologic therapy. During the 4-month follow-up after discontinuation of the n-3 PUFA therapy, both the number of episodes and burden of duration increased to levels comparable to pretreatment values (16). The incidence of atrial fibrillation has also been reported to be reduced by n-3 PUFA when used following coronary artery bypass surgery. In a prospective study, 160 patients were randomized to receive 2 g/day of n-3 PUFA or placebo control, starting 5 days before surgery and continuing until hospital discharge. The incidence of atrial fibrillation (33.3% in the control group and 15.2% in the n-3 PUFA group) and hospital stay (1 day shorter in the n-3 PUFA group) were significantly reduced. (24).

Intake of n-3 PUFA may also improve heart rate variability (HRV), suggesting potential effects on autonomic tone (increase of HRV

predict a lower risk mortality due to arrhythmic events in post-MI patients) (31, 32, 33). Further, fish or fish oil consumption has been reported to decrease heart rate, a major indipendent cardiovascular risk factor for CV death particularly sudden death (40, 78). Elevated resting heart rate has also been associated with increased risk of hospitalization in patients with heart failure (67).

Solid evidence that n-3 PUFA affect cardiac electrophysiology in humans comes from a meta-analysis pooling data of 30 clinical trials. n-3 PUFAs reduced HR particularly in those with higher baseline HR or longer treatment duration (153). Supplementation of n-3 PUFA at modest doses (810 mg) significantly reduced HR at rest and improved postexercise heart recovery in men with history of myocardial infarction, thus suggesting that low dose of n-3 PUFA may have a direct effect on cardiac physiology in part by improving autonomic sympathovagal balance (165). Additional data are availble for n-3 PUFA and ECG findings like corrected QT interval (71) and premature ventricular complexes (70). The anti-arrhythmic effect of n-3 PUFA suggested by the results of clinical studies is supported by many in vitro and in vivo experimental models, particularly in the ischemia-related ones. Diets rich in tuna fish oil reduced the incidence and severity of arrhythmias preventing ventricular fibrillation in rats (96, 137, 138, 141). In vivo and ex-vivo experiments in marmoset monkeys (140) and in dogs (15) showed that diets rich in tuna fish oil increased the ventricular fibrillation threshold. Various concurring mechanisms have been proposed to explain the antiarrhythmic effect of n-3 PUFA. Solid evidence suggests that n-3 PUFA stabilize the electrical activity of myocytes by elevating the action potential threshold (therefore a stronger electrical stimulus is required to elicit an action potential) and by prolonging the relative refractory time (106, 107). This occurs mainly by the inhibition of fast voltage-dependent Na^+ currents (232, 234, 235), and L-type calcium currents (124, 231).

n-3 PUFA also appear to have a protective effect against intracellular Ca^{2+} overload, probably by acting directly on Ca^{2+} homeostasis enzymes such as Ca^{2+}-Mg^{2+} ATPase (116). Recently, it has been shown that acute administration of n-3 PUFA inhibits triggered arrhythmias and reduces the number of afterdepolarization and calcium aftertransient (prolonged action potentials leading to spontaneous sarcoplasmatic reticulum calcium release) in myocytes from rabbits in response to noradrenalin and patients with HF (50). This effect occurs possibly through the inhibition of the electrogenic Na^+-Ca^{2+} exchanger (233). In vitro studies provided insights on how n-3 PUFA act on ion channels by showing that an essential role is played by the free carboxyl group of n-3 PUFA (non esterified form) in influencing the partitioning into sarcolemmal phospholipids (107, 108, 109, 110, 227). During ischemia, phospholipases and lipases have been reported to quickly release n-3 PUFA from phospholipids rather than other fatty acids thus increasing the pool of free n-3 fatty acids (NEFA) in the myocardium where they can exert their antiarrhythmic effect (156). There is also evidence that n-3 PUFA incorporation affects ion channels directly, by modifying the membrane microenviroment rather than via a generalized change in membrane fluidity (177). n-3 PUFA might exert their antiarrhythmic action also through the eicosanoid metabolism shift, namely a decreased production of TxA2 (27) following changes in cardiac phospholipids and NEFA (27, 138, 141). Of note, there is evidence TxA2 promotes arrhythmias (37, 128, 168) although the exact mechanisms are not known.

Atherothrombosis

The link between n-3 PUFA and cardiovascular diseases dates back to 1970 when Dyberg and Bang correlated the low rate of coronary heart disease (CHD) in Greenland Eskimos with their typical marine-based diet high in n-3 PUFA (12). Since then, the cardioprotective effects of fish consumption as well of EPA, DHA, and to a lesser extent ALA, were supported by large-scale epidemiological studies (118, 191). Most studies suggest that low n-3 PUFA intake (fish once or twice per week corresponding to 20-30 g/d) is protective against CHD (42, 56, 99, 119, 185) especially fatal CHD and sudden cardiac death (6, 7, 155, 206). These findings have been confirmed in a meta-analysis from 13 cohort studies including a total of 222,364 patients. Fish consumption was inversely and dose dependently related to fatal CHD, with each 20 g/day increase in fish intake being associated to a 7% lower risk of fatal CHD (92).

A more direct evidence of the cardioprotective role of n-3 PUFA comes from randomized controlled clinical trials in patients with a history of myocardial infarction (20, 86, 118, 192).

The cardioprotective effect of n-3 PUFA on ischemic events is somewhat supported by the results of secondary prevention trials like DART, GISSI-Prevenzione, and JELIS (240). Such benefit might be partially attributable to the plaque stabilizing effect of n-3 PUFA (219). Plaque rupture is the primary determinant of thrombosis-mediated acute cardiovascular events. Therefore, enhanced plaque stability could have contributed to the reduction ischemic events in these studies. JELIS is a trial on more than 18,000 Japanese patients (a population with a high mean fish intake) suggesting that the EPA group (1.8 g/d) experienced, after a mean of follow-up of 4.6 years, a reduction (19%) in non fatal coronary events (nonfatal MI, unstable angina, or coronary artery bypass grafting or percutaneous transluminal coronary angioplasty) vs control (240). The difference in sudden cardiac death between the GISSI- Prevenzione study and the JELIS study could be a function of the very low incidence (0.2%) of SCD in the Japanese population, i.e., approximately ten times less than in the GISSI-Prevenzione control group. The already high dietary intake of n-3 PUFA in the Japanese population (which also explains the very low incidence of SCD) probably masked any further benefit of n-3 PUFA. Similarly in a cohort study on 41,578 Japanese followed up for 11 years a high consumption of fish (8 times per week, median intake 180g/d) was inversely associated with the risk of coronary heart disease, especially non fatal coronary events (HR 0.43, 0.23 to 0.81) compared with a modest fish consumption (once a week, median intake 23 g/d) (103). To explain the contrasting results on nonfatal CHD, fatal CHD death and sudden cardiac death, it has been suggested that the slope of the dose-response curve for the antiarrhythmic effect of n-3 PUFA is steep at modest levels (<750 mg EPA plus DHA) but plateaus thereafter. This threshold effect explains findings among Japanese populations, in whom high background fish intake (eg, median 900 mg/d of EPA and DHA) is associated with very low CHD death rates (only 35 of the 18,645 participants in the JELIS experienced SCD during the 5 years of follow-up) and additional n-3 PUFA intake predicts little further reduction in CHD death; thus, most of the population is already above the threshold for prevention of fatal coronary events or sudden cardiac death (151). On the other side, the threshold effect for other mechanisms of n-3 PUFA (e.g., antinflammatory, see below) could be higher and close to the level of intake of n-3 PUFA in the Japanese population of the JELIS trial. Some studies however have not reported positive outcomes with n-3 PUFA. A randomized trial carried out on 3114 men with stabile angina either given advice to eat oily fish or fish oil capsules found no benefit on total mortality during 3-9 y follow-up. Surprisingly, patients who were sub-randomly assigned to fish oil capsules had higher rates of sudden cardiac death than control group (22).

On the basis of this evidence, the 2002 American Heart Association guidelines recommend that healthy subjects should be advised to consume fatty fish at least twice a week (i.e. approximately 500 mg/d of DHA and EPA) and patients with CHD should consume 1g/day of EPA/DHA. For patients with clinically relevant hypertrigliceridemia, the AHA recommends 2.0 to 4.0 g/d of DHA and EPA (118). Whether the benefit of fish oil supplementation extends to patients free of CHD, however, remains an open question.

1. Platelets

Platelet aggregability is reduced by n-3 PUFA. This could be explained by a shift in metabolism toward the production of vasodilatatory eicosanoids (PGH2) and weak platelet aggregants (TXA3) as indicated by in vitro experiments and clinical studies (76, 77, 127, 225, 226) on both atherosclerotic (117) and healthy individuals (222, 223). Furthermore DHA and EPA can act as antagonists of the TXA2/PGH2 receptor in human platelets (211). DHA, in addition, might also act by reducing the affinity of platelet TXA2/PGH2 receptor for its ligand (14). Many studies have shown that fish oil supplementation significantly prolongs bleeding time, whereas others showed no change or only non significant trends to prolongation (36, 158, 161, 190). These discrepancies are probably due to the different doses utilized: a dosage lower than 2 g/d of fish oils did not prolong bleeding time whereas more than 4 g/d can increase it (190). However in human studies, prolongation of bleeding time after fish oils intake has been usually found to be modest and there have been no reports of serious bleeding or any adverse effects even in patients undergoing angioplasty or coronary-artery-bypass graft surgery (46, 47). Accordingly FDA has ruled that intakes of up to 3 g/d of marine omega-3 fatty acids are GRAS (Generally Recognized As Safe) for inclusion in the diet (54). A recent

comprehensive report concluded that no increased risk for adverse bleeding episodes has been seen in patients with dose of n-3 PUFA up to 7g/die of EPA and DHA, even when taken in combination with other antiplatelet drugs (89).

2. Clotting factors and fibrinolysis

It is unclear whether n-3 PUFA act on clotting factors and the fibrinolytic processes. Some clinical evidence suggests a reduction in factor VII and fibrinogen with n-3 PUFA supplementation (158) and an epidemiologic study described an association between fish intake and decreased factor VIII, fibrinogen and von Willebrand factor (200). Ex vivo experiments also indicated that n-3 PUFA reduced TF activity in unstimulated or endotoxin-stimulated adherent monocytes from both healthy and hypertriglyceridemic individuals (220). These effects were not confirmed by others albeit using slightly different models (84, 85, 171, 193).

On the fibrinolytic front, n-3 PUFA supplementation increased vascular concentration of plasminogen activator (PAI) and reduced the concentration of vascular plasminogen activator inhibitor 1 (PAI-1), plasmin and alpha 2-antiplasmin (alpha 2-AP) in human volunteers (13). Still debated is the effect of n-3 PUFA on PAI-I activity. Some studies suggested that fish oil reduces PAI-1 ativity (145, 207) although others did not (83, 93, 161, 180, 214).

3. Lipids

n-3 PUFA affect a spectrum of systems and classes of molecules that are intimately related to the pathogenesis of atherosclerosis and thrombosis.

n-3 PUFA reduce plasma triglycerides through two main mechanisms of triglyceride homeostasis: synthesis and clearance of chylomicrons and VLDL lipoprotein (87, 88, 160, 176, 189). In rat hepatocytes n-3 PUFAs inhibited the activity of key enzymes in triacylglycerol formation, namely acyl CoA:1,2-diacylglycerol acyltransferase, phosphatidate phosphohydrolase and triacylglycerol and diacylglycerol hydrolases (134, 187). EPA has also been reported to inhibit *de novo* fatty acid synthesis (102) and to increase β-oxidation (186, 230, 238). Evidence from kinetic studies also indicated that fish oils increase the fractional catabolic rate (FCR) of VLDL (160, 189).

The hypolipidemic effects of EPA and DHA is mediated by binding and activation of peroxisome proliferator-activated receptor α activation (PPARα a nuclear hormone receptors that regulates genes involved in fatty acid β-oxidation) (104) and by decreasing the expression of sterol response element-binding protein-1c (SREBP-1c; a transcription factor required for acid and triglyceride synthesis) (135). Further, fish oils decrease triglyceride levels by reducing plasma apo C-III levels (apo C-III inhibits VLDL lipolysis and uptake by cellular receptors)(39).

The effect of n-3 PUFA on plasma cholesterol levels is rather controversial as both an increase or a reduction in low-density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) levels have been observed after fish oil intake (159, 181). Overall, n-3 PUFA (3-4g/d) seem not to significantly change total cholesterol levels, while HDL-C could be raised by 5-10%. An increase in LDL particle size, which are less susceptible than smaller LDL to oxidative damage, has been reported after fish oil supplementation (208, 210).

n-3 PUFA have been shown to decrease serum Lp (a), a genetically determined protein that has atherogenic and thrombogenic properties (94, 202).

4. Oxidative stress

The reduction of oxidative stress by n-3 PUFA might exert an antiatherosclerotic activity (64) and represent a potential mechanism limiting myocardium damage after ischemia and reperfusion in animal experimental models (209). Accordingly there is evidence that n-3 PUFA may act as scavengers to remove specific free radicals (1, 29, 149) and by decreasing platelet oxidative stress (28, 221). There is also evidence that LDL-cholesterol was less susceptible to oxidation in rabbits on a diet supplemented with EPA (188). The reduction of oxidative stress by n-3 PUFA seem to be realized stimulating antioxidant enzymes (49), by up-regulating their gene expression and concomitantly down-regulating genes associated with a production of reactive oxygen species (212). Supplementation of human endothelial cells with n-3 PUFA resulted in lower reactive oxygen/nitrogen (ROS/RNS) species production compared saturates, monounsaturates, or polyunsaturates of the omega 6 series fatty acids. Also n-3 PUFA were the most effective superoxide anion scavenger in fatty acids micelles. Although n-3 PUFA fatty acids have generally been regarded as susceptible to oxidation due to the presence of

a number of double bonds, it is more likely that in situ these PUFAs act as scavengers for free radicals. n-3 PUFA might indirectly act as anti- rather than pro-oxidant in vascular endothelial cells, hence diminishing inflammation and, in turn, the risk of atherosclerosis and cardiovascular disease (183).

Neurohormonal system

DHA has been shown to act at level of adrenal synthesis of aldosterone thereby producing antihypertensive effects on spontaneously hypertensive rats (SHR) probably mediated by blunting of the renin-angiotensin-aldosterone (RASS) system (60). DHA was also shown to inhibit the expression of the renin gene, in stroke-prone SHR, thus contributing to the hypotensive and renal protective effects of DHA (148). In addition, clinical studies have shown that EPA treatment suppressed vascular reactivity to both angiotensin II and norepinephrine (2, 30).

There is also evidence that fish oils increase endothelium-dependent vasodilatation in peripheral and coronary arteries through the stimulation of systemic nitric oxide synthesis in healthy volunteers (41, 91). In addition EPA inhibits mesangial cell endothelin-1 (ET-1) production, when stimulated by platelet-derived growth factor (PDGF-BB) or Epidermal Growth Factor (EGF) (162).

Cardiac haemodinamics

Several lines of clinical and experimental evidence indicate that n-3 PUFA might positively affect several haemodynamic parameters which are involved in HF progression. They include reduction of systemic vascular resistance (SVR) (48, 154) possibly through the induction of nitric oxide production (166), reduced vasoconstrictive response to norepinephrine and angiotensin II (30, 112, 150), improvement of arterial wall compliance (143), enhancement of vasodilatory responses (150) and reduction in systolic and diastolic blood pressures (72). In experimental models, 24 months of fish oil enriched diet improved LV diastolic filling, increasing both LV end-diastolic volume and stroke volume, thus improving myocardial efficiency (27, 139). A small size clinical trial in healthy adults, showing improvement in LV diastolic function after 7 week of n-3 PUFA supplementation (78). The association between n-3 PUFA and stroke volume appears at least partly mediated by the effect on reducing heart rate (HR), increasing filling time and possibly by enhancing early and late diastolic filling, as indicated by data in non-human primates (27, 139) and human beings (154).

Blood rheology can be involved among the pathophysiologic mechanisms of HF. Plasma and blood viscosity appears to be improved by n-3 PUFA supplementation. This could occur through a reduction in fibrinogen levels (36, 98, 179, 194), increased erythrocyte flexibility (a major determinant of whole blood viscosity) (61, 218) and decreased erythrocyte osmotic fragility (80).

These findings are therefore broadly consistent with the GISSI-HF results on death and hospitalization from worsening of HF.

Cardiac Hypertrophy

n-3 PUFA may attenuate cardiac hypertrophy through different mechanisms. In vivo experiments in mice indicate that dietary fish oil supplementation have inhibitory effects on cardiac hypertrophy by inhibiting Ras -Raf-1 - extracellular regulated kinase 1 and 2 -p90 ribosomal s6 kinase pathaway (201), and possibly via modification of the molecular composition of myocardial 1,2-diacylglicerol and the consequent inhibition of protein kinase C (PKC) redistribution (213) .

n-3 PUFA have been reported to modulate in vitro extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2) (51, 52) probably via inhibition of PKC (53). It was also reported that n-3 PUFA inhibited the in vitro cAMP-dependent PKC, protein kinase C, Ca(2+)/calmodulin-dependent PKC II, and the mitogen-activated protein kinase (MAPK) (147). n-3 PUFA may reduce platelet derived growth factor PDGF levels (68), possibly via a regulation of gene expression (105). Furthermore, EPA has been shown to prevent the binding of PDGF to its surface receptors and thus suppressing PKC activation (217). EPA also inhibits the growth of vascular smooth muscle cell (VSMC) from spontaneously hypertensive rat through the suppression of TGF-beta (157).

n-3 PUFA may exert pro-apoptotic and anti-apoptotic effects depending on the experimental models. In neuronal cells, DHA appeared to protect cells from apoptotic death induced by different stimuli (4, 115, 130). DHA, also, induce VSMC apoptosis, and this could explain the beneficial role in hypertension possibly in part by influencing vascular structure. Two distinct mechanisms are involved in DHA triggered

apoptosis: activation of PPAR-*a* via p38 mitogen-acitvated protein kinase-dependent pathway; 2) dissipation of mitochondrial membrane potential with following cytochrome c release from mitochondria, a key signal that initiates the irreversible death sequence (55).

Energy metabolism

Treatment with n-3 PUFA, a stimulator of fatty acid oxidation, has been reported to improve myocardial mechanical efficiency as by in vivo and ex vivo experiments. In experimental animal models, dietary fish oils have been reported to improve myocardial energy efficiency (139) and post-ischemic recovery of hemodynamic and metabolic functions (5, 97, 142, 169, 239).

A diet rich in n-3 PUFA can increase the left ventricular ejection fraction at rest in the marmoset monkey by increasing ventricular filling. Lower pressure–rate indices (indicator of oxygen consumption) and greater cardiac minute work (as indicator of energy input/output) suggest higher myocardial energy efficiency in fish oil fed animals (139). On isolated working hearts, dietary fish oil was found to increase the efficiency of oxygen use reducing myocardial oxygen consumption at any given work output, particularly with high workloads (174). A recent findings on athletes indicate that n-3 PUFA may act within the heart and skeletal muscle to reduce both whole-body and myocardial oxigen demand during exercise, without a decrement in performance (172). Therefore, the efficiency of oxigen use by the heart and skeletal muscle is increased after n-3 PUFA supplementation in rats (173, 174) but also in humans.

Excitation-contraction coupling

n-3 PUFA have been found to inhibit plasma membrane L-type CA currents ($I_{Ca\text{-}L}$) (82, 123, 175, 231) and reduce Ca^{2+} release from the sarcoplasmic reticulum (SR) (184) in rat myocytes, hence preventing Ca^{2+} overload. Preservation of voltage-sensitive calcium release mechanism, the other mechanism proposed for SR Ca^{2+} release (62) may explain the ability of n-3 PUFA to exert protective effects inhibiting Ca^{2+} influx through ($I_{Ca\text{-}L}$) while preserving cardiac contractile function (63).

Fish oils may influence myocardial contraction force by increasing the sensitivity of myofilaments to calcium (167) and by preventing cardiac glycoside ouabain toxicity and promoting its positive inotropic effects (73, 74, 81, 132). Finally, the substitution of arachidonic acid with EPA in cellular membranes blocks the formation of leukotriene B4 (LTB4), known to have a negative inotropic effect in isolated papillary muscle preparation (164).

Inflammation

1. Proinflammatory cytokines

EPA and DHA inhibit the production of proinflammatory cytokines, such as tumour necrosis factor–α (TNF-α), interleukin-1 (IL-1), interleukin-2 (IL-2)(25, 59, 121, 146, 178), and interleukin-6 (113), possibly through the inactivation of the NF-kB signal transduction pathway (129, 163). Furthermore n-3 PUFA can reduce TNF-α and IL-1ß production by inhibiting thromboxane A_2 (TXA_2) (25, 26). The anti-inflammatory actions of fish oils were also confirmed by in vivo experiments in dogs with heart failure: n-3 PUFA decreased IL-1 concentrations and improved cachexia, known to be due to an increase in the circulating levels of TNF-α (69). Additionally, very high dose of omega 3-fatty acid (8g/die) have been shown to decrease TNF-alpha production and to improve body composition in patients with advanced heart failure (144).

Reduction of C-reactive protein (CRP) during long-term fish oil supplementation has been reported in cancer patients (228, 229).

2. Other markers of inflammation

EPA can prevent the conversion of arachidonic acid into the active proinflammatory eicosanoids prostaglandin E_2 (PGE_2) and LTB4 by substrate competition thus leading to the production of less potent mediators (75, 127, 196, 226).

A number of studies also reported modulatory effects of n-3 PUFA on the expression of adhesion molecules. DHA and EPA reduced the expression of vascular cell adhesion molecule-1 (VCAM-1), of endothelial cells adhesion molecule-1 (ELAM-1/E-selectin) and of intracellular adhesion molecule-1 (ICAM-1), possibly through reduction of specific mRNA induction by IL-1 or TNF (38, 43, 44, 45, 114, 126, 224).

Monocyte binding to endothelium was decreased by oxidized n-3 PUFA, probably by preventing the activation of the transcription factor NF-kB and consequent induction of cell adhesion molecule expression. This modulation may represent a natural mechanism whereby inflammation-mediated oxidation of endothelial

PUFA may retard entry of phagocytes and thereby unrestrained phlogistic responses (199). Finally, in vitro experiments showed that EPA and DHA suppressed rolling and adherence of monocytes on TNF-α stimulated human endothelial cells by suppressing platelet activating factor synthesis (136). Clinical evidence indicated that n-3 PUFA displayed anti-inflammatory effects by incorporation in atherosclerotic plaques with subsequent changes of plaque morphology and degree of macrophage infiltration (indicating an improvement of plaque's stability) (219). Stability of plaques could explain reductions in non-fatal and fatal cardiovascular events associated with increased n-3 PUFA intake.

Finally, a new class of aspirin-triggered bioactive lipids called resolvins (Rv) were found in vivo during the resolution phase of inflammation in exudates from mice treated with aspirin and EPA. Endothelial cells expressing COX-2, treated with aspirin transform vascular EPA to RvE1 (the main bioactive component of the resolvins). RvE1 may be considered a novel component in endogenous antiinflammatory underling some of the beneficial effects of omega-3 PUFA (10, 198).

CONCLUSIONS

Current data suggest that patients with known coronary heart disease should consume at least 1.0 g/d of long-chain omega-3 fatty acids; people without disease, at least 250 to 500 mg/d. Both DHA and EPA should be consumed. The results of GISSI-HF suggest that HF patients should increase their intake of long-chain n-3 PUFA and consume at least 1.0 g/d like patients with known coronary heart disease. Because of the many potentially beneficial effects, the real mechanisms underlying the benefit of n-3 PUFA in HF are unknown. The three largest trials available to date suggest that daily doses up to 1g/d are likely to exert mainly antiarrhythmic effects (GISSI-P and GISSI-HF), while higher dosages of n-3 PUFA could be required to determine a significant reduction of atherothrombotic events (JELIS).

Other articles in this theme issue include references (242-253).

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