Dietary Fish Oil Confers Direct Antiarrhythmic Properties on the Myocardium of Rats[1]

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ABSTRACT This study tested the hypothesis that in vivo antiarrhythmic effects of dietary fish oil can be attributed directly to changes in myocardial properties. Sixty adult male rats were fed a fish oil diet (FO), an isoenergetic saturated fat diet (SAT) or a low fat reference diet (REF) for 16 wk. hearts isolated from these rats were perfused with washed porcine erythrocytes (0.4 hematocrit) in working heart mode. Dietary fish oil prevented reperfusion-induced ventricular fibrillation (VF) (% of rats with VF: REF 50%, SAT 80% $P = 0.35$, FO 0% $P < 0.05$ $n = 10$) and reduced arrhythmias in ischemia. In a separate set of hearts from rats fed the three diets, FO increased while SAT reduced the stimulation threshold for programmed electrical induction of VF during control perfusion compared with REF (mean ± SD: REF 7.1 ± 0.2 mA; SAT 5.8 ± 0.2 mA, $P < 0.001$; FO 15.1 ± 1.0 mA, $P < 0.001$, $n = 10$) and during subsequent ischemia (REF 5.9 ± 0.2 mA; SAT 3.8 ± 0.3 mA, $P < 0.001$; FO 8.9 ± 0.2 mA, $P < 0.001$, $n = 10$). The isolated working heart model used physiological workload and oxygenation but excluded extracardiac influences. Dietary fish oil prevented the initiation and reduced the severity of arrhythmias in the isolated hearts in response to a variety of stimuli. These results establish that irrespective of any effects on blood pressure or platelet function in vivo, dietary fish oil directly affects myocardial properties which may contribute to observed clinical reductions in cardiac mortality associated with fish consumption. J. Nutr. 126: 34–42, 1996.

INDEXING KEY WORDS:

- *dietary fats (n-3) fatty acids*
- *myocardial ischemia* • *arrhythmia* • *rats*

The epidemiological study of Kromhout et al. (1985) shows an inverse relationship between the consumption of fish and heart disease mortality. The biological activity is believed to reside with the (n-3) fatty acid content of the fish, and numerous potential mechanisms have been proposed (Kinsella et al. 1990, Leaf and Weber 1988). One factor that has been identified experimentally is the prevention of ventricular fibrillation (Hock et al. 1990, McLennan et al. 1988). Supporting these experimental observations are two recent clinical studies showing that consumption of fish (Burr et al. 1989) or the terrestrial 18 carbon (n-3) polyunsaturated fatty acid (PUFA)[4] α-linolenic acid (de Lorgeril et al. 1994) is associated with reduced mortality in post-infarction patients normally at high risk of sudden cardiac death. This then suggests clinical reductions in the incidence of fatal ventricular fibrillation (VF).

Experimental studies using rats (Hock et al. 1990, McLennan 1993, McLennan et al. 1988), dogs (Culp et al. 1980) and nonhuman primates (McLennan et al. 1993) demonstrate that dietary fish oils can confer protection against ventricular fibrillation induced by a variety of stimuli in vivo, including coronary artery occlusion and reperfusion or programmed ventricular stimulation. Altered myocardial vulnerability to arrhythmogenic stimuli is implicated. However, in addition to the incorporation of (n-3) fatty acids into myocardial membranes and the potential changes in

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[4] Abbreviations used: AA, arachidonic acid; BHT, butylated hydroxytoluene; CAST, Cardiac Arrhythmia Suppression Trial; CSIRO, Commonwealth Scientific and Industrial Research Organisation; DHA, docosahexaenoic acid; ECG, electrocardiogram; EPA, eicosapentaenoic acid; FO, fish oil-supplemented diet; LA, linoleic acid; P:S, ratio of polyunsaturated to saturated fatty acids; PUFA, polyunsaturated fatty acids; REF, low fat reference diet; SAT, saturated fat (sheep perirenal fat)-supplemented diet; VF, ventricular fibrillation; VPB, ventricular premature beats; VT, ventricular tachycardia.

0022-3166/96 $3.00 © 1996 American Institute of Nutrition.
Manuscript received 5 April 1995. Initial review completed 5 May 1995. Revision accepted 15 August 1995.

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myocardial cell function, it is well established that fish oils can modify platelet function, leukocyte function, blood pressure and heart rate (Kinsella et al. 1990, Leaf and Weber 1988), any of which may influence the response of the heart to arrhythmogenic stimuli or, indeed, generate arrhythmogenic stimuli.

This study was designed to test the hypothesis that the in vivo antiarrhythmic effects of dietary fish oil are attributable to direct alteration of myocardial properties. The antiarrhythmic properties of dietary fish oil were investigated using a system in which neural and humoral components or differences in cardiac loading conditions and coronary perfusion pressure could be excluded. In attempting to replicate in vivo conditions of metabolic supply and demand, a working heart preparation was used. Hearts were perfused with a high oxygen-carrying capacity solution containing washed porcine red blood cells.

MATERIALS AND METHODS

Animals and diets. Sixty male Hooded-Wistar rats [Commonwealth Scientific and Industrial Research Organisation (CSIRO) Adelaide, SA, Australia] were divided into three dietary groups (n = 20 per group) at 16 wk of age and fed one of two lipid-supplemented diets or a low fat reference diet (REF) for 16 wk. The REF diet, which was the nonpurified diet fed to all rats prior to experimental intervention, consisted of commercially available, unrefined rat pellets (Milling Industries, Adelaide, Australia) composed of the following (expressed as g/100 g): moisture, 11.6; protein, 20.5; fat, 7.6; crude fiber, 5.3. The REF diet was prepared by the manufacturer by blending all of the ingredients as a dry mix base and spraying with beef tallow at the time of extrusion pelleting. The fat in the dry mix base was present as a natural component of the wheat, oats, lucerne, soybean meal, meat meal, and fish meal contained in the diet. For the preparation of the lipid-supplemented diets the dry mix base (3.7 g fat/100 g) without added tallow was obtained. The saturated fat–supplemented diet (SAT) was prepared by adding to the dry mix 12 g/100 g of sheep perirenal fat, which is rich in saturated fatty acids (stearate, 18:0 and palmitate, 16:0). The fish oil–supplemented diet (FO) was prepared by adding (12 g/100 g) purified fish oil (Shaklee EPA, Shaklee, San Francisco, CA), which is rich in (n-3) PUFA: eicosapentaenoic acid [EPA, 20:5 (n-3)] and docosahexaenoic acid [DHA, 22:6 (n-3)]. The fish oil also included 1 g/100 g of the antioxidant all-*rac*-α-tocopherol, 21 μg/g retinol palmitate, and 1 μg/g cholecalciferol. All diets contained 0.05 g/100 g butylated hydroxytoluene (BHT) to prevent peroxidation of fats during storage. α-Tocopherol was added to the SAT diet to the same level as the FO diet. All diets were stored at 0–4°C following repelleting and drying, and new diet was prepared every 14 d. Thus, the fat-supplemented diets contained 15.3 g/100 g total fat (both isoenergetic), and the REF diet contained 7.6 g/100 g fat. **Table 1** shows the fatty acid profile of each diet analyzed by gas liquid chromatography (5710A Hewlett Peckard, Palo Alto, CA) according to a previously published method (Charnock et al. 1992). The rats were housed five per cage at 23°C with constant 55% humidity on a 12-h light:dark cycle and allowed free access to food and water. To avoid the possibility of fat peroxidation, leftover food was discarded daily prior to refilling food containers. Food was replenished daily in the late afternoon to minimize the period between exposure to room temperature, light and air and its consumption.

The study was approved by the Animal Care and Ethics Committee of the CSIRO Division of Human Nutrition according to the National Health and Medical Research Council and CSIRO "Australian code of practice for the care and use of animals for scientific purposes."

Isolated erythrocyte perfused working heart. Rats were randomly selected, and food was withheld overnight prior to each experimental day. The experiments were performed with the experimenter un-

TABLE 1

Fatty acids measured in reference and lipid-supplemented diets fed to rats for 16 wk prior to isolated working heart experiments[1]

Fatty acids	REF[2]	SAT	FO
		mol/100 mol	
14:0	1.09	2.66	6.06
16:0	19.38	22.82	10.50
16:1	1.89	1.60	9.83
18:0	7.61	28.49	4.57
18:1	20.71	27.10	11.37
18:2(n-6)	33.85	6.69	5.64
18:3(n-3)	3.34	1.52	1.20
20:0	0.21	0.48	3.77
20:1	0.47	0.46	1.48
20:4(n-6)	0.62	0.09	0.96
20:5(n-3)	2.23	0.35	24.28
22:5(n-3)	—	0.12	1.52
22:6(n-3)	6.17	0.76	11.84
24:0	0.76	—	0.37
% Total	98.36	93.65	93.59
Σ Saturated fatty acids	29.06	54.92	25.30
Σ PUFA	46.23	9.57	45.45
P:S Ratio	1.59	0.17	1.80
Σ(n-6) PUFA	34.48	6.79	6.60
Σ(n-3) PUFA	11.75	2.78	38.85
Total fat (*g/100 g*)	7.6	15.3	15.3

[1] Data represent the relative occurrence of fatty acids in the diets.
[2] Abbreviations used: REF, low fat reference diet; SAT, sheep perirenal fat-supplemented diet; FO, fish oil–supplemented diet; PUFA, polyunsaturated fatty acids; P:S, ratio of PUFA to saturated fatty acids.

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aware of the dietary treatment of each rat until the end of each experimental series. The animals were killed by cervical dislocation, and the hearts removed and prepared for working heart perfusion by the method of Neely et al. (1973) with the erythrocyte buffer (0.40 hematocrit) as described previously (Pepe and McLennan 1993). Briefly, following cannulation of the aorta, hearts were initially perfused for 10 min with Krebs-Henseleit solution in Langendorff mode, during which time pulmonary artery cannulation, pacing electrode placement, and residual blood washout took place. The electrocardiogram (ECG) was recorded continuously with two platinum alloy subdermal needle electrodes (Grass Instruments, Quincy, MA) placed at the surface of the ventricular apex and the aortic stump, and attached to a Grass preamplifier and ECG signal unit in a Grass polygraph. Hearts were switched to working heart mode and perfused with the porcine erythrocyte buffer (0.40 hematocrit) (Pepe and McLennan 1993).

Ischemia and reperfusion. Hearts from 10 rats of each dietary group were used for this protocol. All hearts were atria-paced in working heart mode at 300 beats/min, with square wave pulses of 2 ms duration at twice the threshold current with two Grass subdermal platinum alloy needle electrodes and a Narco Biosystems SI-10 stimulator (Narco Biosystems, Houston, TX) to remove the influence of variable heart rate on arrhythmia vulnerability. The hearts were perfused in working heart mode for 10 min to stabilize, with a left atrial filling pressure (preload) of 10 mmHg and an aortic pressure head (afterload) of 75 mmHg. The coronary perfusion pressure was equal to the afterload. At the end of this period, low flow global ischemia was induced by selectively lowering the coronary perfusion pressure to 35 mmHg while keeping the afterload and preload at 75 mmHg and 10 mmHg, respectively. After 15 min, reperfusion was initiated by raising the coronary perfusion reservoir back to 75 mmHg and was monitored for 10 min. Details of the apparatus and methodology for producing low flow global ischemia have been published previously (Pepe and McLennan 1993).

Programmed electrical stimulation. Hearts from a second group of 10 rats from each dietary group were perfused in working heart mode as described above but were allowed to beat spontaneously. A programmed electrical stimulation protocol was employed using square wave pulses (2 ms duration) at a stimulation intensity of twice the mid-diastolic excitation threshold. Pulses were delivered by a Grass S8800 digital stimulator via a Grass SIU5 stimulus isolation unit and a Grass CCU1 constant current unit. For each programmed stimulation event, ventricles were driven with a constant pacing train of 10 pulses (S_1) at 200-ms intervals, then a single extra stimulus (S_2) was introduced after a S_1/S_2 coupling interval which was decreased from 140 ms until a response was not elicited within the effective refractory period limits. The intensity of the extra stimulus was increased in the vulnerable period by raising the current in 2.5- to 5.0-mA steps until ventricular arrhythmias were induced or maximal current (43 mA) had been delivered. This program of stimulation was conducted first during control working heart perfusion, and then during low flow global ischemia (15 min after induction of ischemia).

At completion of either protocol, hearts were quickly removed from the apparatus, blotted dry and weighed. A small piece of tissue was cut from hearts and dried for dry weight calculations. The ventricles were frozen in liquid nitrogen and stored at −70°C until fatty acid analyses could be performed (Charnock et al. 1992b).

Ventricular arrhythmia assessment. The following definitions were used in the assessment and scoring of arrhythmias according to the Lambeth Convention (Walker et al. 1988).

a) Ventricular Premature Beats (VPB): discrete QRS complexes that were premature in relation to the P wave. Bigeminy or trigeminy were scored as variants of this.
b) Ventricular Tachycardia (VT): four or more consecutive ventricular premature beats of similar morphology were deemed to be tachycardia.
c) Ventricular Fibrillation (VF): This was defined as a signal from which QRS deflections are no longer distinguishable and no sinus rhythm can be accurately measured (distinct from polymorphic VT and from the flat signal of asystole in which no sinus rhythm is evident).

Statistical analysis. Results were expressed as means ± SD, except for the incidence of ventricular tachycardia or fibrillation which was expressed as the percentage of hearts in which episodes were observed. For percent incidence data the effect of dietary treatment was tested by Fisher's exact test; other data were evaluated by one- or two-way ANOVA as appropriate, with Tukey's test for comparisons between pairs of means (Siegel 1992). Statistical significance was accepted at the $P < 0.05$ level. Statistical analyses were conducted using the Statistix computer program (Analytical Software, St Paul, MN).

RESULTS

Ischemia and reperfusion. During the low flow ischemic period in atria-paced hearts, only infrequent VPB were observed, most of which occurred between 10 and 15 min of ischemia. Although infrequent in all dietary groups, significantly more ($P < 0.05$) VPB were recorded during ischemia in the SAT hearts (11 ± 1) than in the REF hearts (7 ± 1), whereas the lowest number was seen in FO hearts (3 ± 1, $P < 0.05$). Upon

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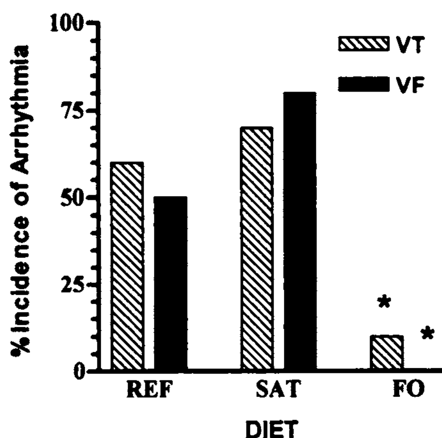

FIGURE 1 Percentage of rat, atria-paced isolated working hearts exhibiting spontaneous episodes of ventricular tachycardia (VT) and ventricular fibrillation (VF) during reperfusion after 15 min of low flow global ischemia following 16 wk feeding of fish oil (FO) or sheep perirenal fat (SAT) supplemented or a low fat reference (REF) diet, (n = 10/group). *, $P < 0.05$ vs. REF and SAT; Fisher's exact test.

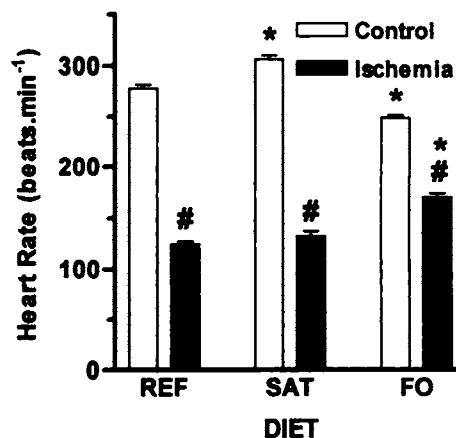

FIGURE 2 Spontaneous beat rate of rat, unpaced isolated working hearts during control perfusion and after 15 min of low flow global ischemia following 16 wk feeding of fish oil—(FO) or sheep perirenal fat—(SAT) supplemented or a low fat reference (REF) diet. Mean ± SD, n = 10/group. *, $P < 0.001$ vs. REF and the other dietary group; ANOVA, Tukey's test. #, $P < 0.0001$ vs. control; ANOVA.

reperfusion, arrhythmias were more conspicuous and included VPB, VT and VF. The incidence of VT was significantly lower in the FO dietary group compared with the REF group (**Fig. 1**). Ventricular fibrillation occurred in REF and SAT hearts in reperfusion but was not observed in any FO hearts (Fig. 1). In addition, 5 of 8 VF episodes in SAT hearts, and 3 of 6 in REF hearts were sustained (>30 s VF).

Programmed electrical stimulation. Under control working heart perfusion conditions, the mean spontaneous beat rate of SAT hearts was significantly higher ($P < 0.001$) than that of REF hearts, whereas FO hearts exhibited significantly lower beat rates ($P < 0.001$) (**Fig. 2**). After 15 min of low flow ischemia, the mean spontaneous heart rates had fallen significantly in all dietary groups (Fig. 2). The heart rate in the FO group fell to a lesser extent and thus was significantly higher than those of SAT and REF hearts during ischemia.

The programmed stimulation protocol induced VF in all hearts when sufficient current was applied. Under control conditions, significantly higher stimulation current was required to induce VF in FO hearts compared with REF or SAT hearts (**Fig. 3**). The threshold current for VF induction was reduced in all dietary groups in ischemia but remained significantly higher in the FO group and lower in the SAT group than in REF hearts (Fig. 3).

Ventricular fibrillation of a sustained duration (>30 s) occurred in 4 of 10 SAT hearts during the control period but in none of the REF or FO hearts ($P = 0.087$). Moreover, in 8 of 10 FO hearts, the stimulated VF episodes lasted for less than 5 s in contrast with only 2 of 10 REF ($P = 0.023$) and 2 of 10 SAT hearts having such brief VF episodes, resulting in a significantly lower mean VF duration in FO hearts (**Fig. 4**). The mean duration of VF episodes was significantly increased during ischemia in all dietary groups (Fig. 4). The longest mean duration of VF was observed in REF and SAT groups, whereas all FO hearts quickly defibrillated spontaneously, giving a significantly lower mean VF duration, even during ischemic insult (Fig. 4). In a few hearts from the REF and SAT groups, sinus rhythm did not resume following programmed stimulation at 15 min ischemia. Attempts were made to defibrillate the hearts by overdrive pacing after 45 s of VF but were unsuccessful. For statistical evaluation, these VF episodes were assigned a duration equal to

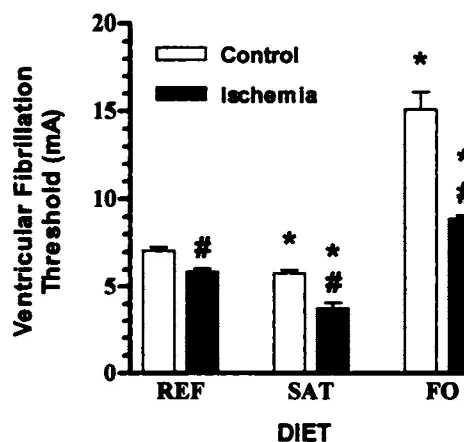

FIGURE 3 Threshold electrical current for induction of ventricular fibrillation in rat isolated working hearts during control perfusion and after 15 min of low flow global ischemia following 16 wk feeding of a fish oil—(FO) or a sheep perirenal fat—(SAT) supplemented or a low fat reference (REF) diet. Mean ± SD, n = 10/group. *, $P < 0.001$ vs. REF and the other dietary group; ANOVA, Tukey's test. #, $P < 0.0001$ vs. control; ANOVA.

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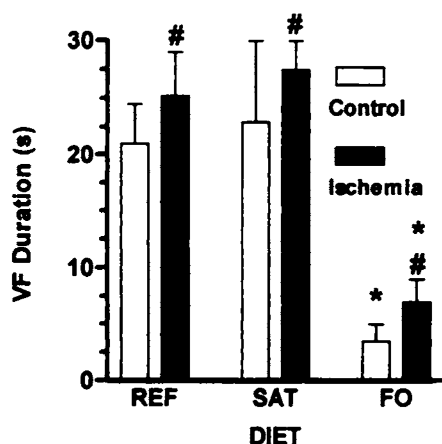

FIGURE 4 Mean duration of ventricular fibrillation (VF) episodes elicited by programmed electrical stimulation in rat isolated working hearts during control perfusion and after 15 min of low flow global ischemia following 16 wk feeding of a fish oil—(FO) or a sheep perirenal fat—(SAT) supplemented or a low fat reference (REF) diet. Mean ± SD, $n = 10$/group. *, $P < 0.001$ vs. REF and SAT; ANOVA, Tukey's test. #, $P < 0.0001$ vs. control; ANOVA.

the longest nonsustained VF (30 s). Notably, sustained VF was observed in 4 of 10 SAT hearts and 3 of 10 REF hearts, but no FO hearts had VF sustained for more than 30 s after 15 min of global ischemia ($P < 0.1$). Even in ischemia, VF episodes lasted for less than 5 s in 8 of 10 FO hearts compared with one REF heart ($P = 0.005$) and no SAT hearts ($P < 0.001$) having VF episodes of less than 5 s.

Diet and membrane fatty acids. Analyses of the three diets (Table 1) showed that both the REF and FO diets contained approximately 45% PUFA. In the REF diet they were predominantly (n-6) but included 12% (n-3), whereas in the FO diet the (n-3) PUFA (25% EPA and 12% DHA) predominated. The SAT diet contained only 10% PUFA (<0.5% EPA). The SAT diet also contained 55% saturated fatty acids while FO had 25%. The REF diet had only half the total fat content of the supplemented diets. The total myocardial phospholipid fraction fatty acid profiles in **Table 2** show that FO hearts incorporated a significantly larger proportion of the (n-3) PUFA EPA (20:5) and especially DHA (22:6) into the myocardial membranes, replacing both linoleic acid [LA, 18:2(n-6)] and arachidonic acid [AA, 20:4(n-6)]. While the SAT diet produced a significantly lower LA level than the REF diet, the level of AA was greater. The proportion of the total saturated fatty acids (33–35 mol/100 mol) and the proportion of the total PUFA (55–57 mol/100 mol) incorporated into myocardial membranes did not differ due to diet and thus neither did the PUFA to saturated fat (P:S) ratio. However, the ratio of [meanΣ(n-6):meanΣ(n-3) PUFA] was lower in FO hearts than in REF or SAT hearts due to the significantly greater incorporation of (n-3) PUFA and lower incorporation of (n-6) PUFA into membranes. In particular, the ratio of mean AA incorporation to mean EPA incorporation was modified by FO (4.52) relative to the REF (54.4) and SAT (71.4) dietary groups.

DISCUSSION

Our results show that dietary fish oil prefeeding was antiarrhythmic in isolated perfused hearts. This conclusion is based on several indices of arrhythmia vulnerability. Dietary fish oil prevented VT and VF in reperfusion after ischemia and elevated the electrical current required to elicit VF during a programmed ventricular stimulation protocol under control or ischemic conditions. Furthermore, when arrhythmias occurred in the hearts from fish oil-fed animals following any stimulus, the hearts always rapidly and spontaneously reverted to sinus rhythm. These observations indicate both a reduced likelihood of the heart to respond to an initiating arrhythmogenic trigger and

TABLE 2

Influence of dietary lipids on major fatty acids of myocardial phospholipids in rats fed lipid supplemented diets for 16 wk[1]

Fatty acids	REF[2]	SAT	FO	Pooled SD
		mol/100 mol		
14:0	0.10	0.11	0.15	0.04
16:0	11.23^{a}	9.90^{ab}	9.42^{b}	0.91
16:1	0.56	0.45	0.79	0.46
18:0	21.26^{b}	23.29^{a}	24.23^{a}	1.02
18:1	7.61	7.66	6.36	0.94
18:2(n-6)	21.63^{a}	13.86^{b}	10.20^{c}	1.67
18:3(n-3)	0.12	0.09	0.13	0.07
20:0	0.05	0.04	0.04	0.01
20:1	0.28	0.16	0.40	0.06
20:2	0.14	0.11	0.09	0.01
20:4(n-6)	17.67^{b}	20.33^{a}	14.13^{c}	1.29
20:5(n-3)	0.32^{b}	0.28^{b}	3.12^{a}	0.25
22:4(n-6)	0.36	0.27	<0.1	0.20
22:5(n-3)	1.41^{b}	1.84^{a}	1.83^{a}	0.18
22:6(n-3)	15.37^{c}	19.46^{b}	27.22^{a}	1.36
24:0	0.32	0.29	0.23	0.05
% Total	99.33	99.07	99.20	0.23
Σ Saturated fatty acids	33.88	34.76	34.88	1.57
Σ PUFA	57.04	55.97	56.97	2.12
P:S Ratio	1.68	1.65	1.63	0.07
Σ(n-6) PUFA	39.80^{a}	34.58^{b}	24.65^{c}	1.25
Σ(n-3) PUFA	17.24^{c}	21.39^{b}	32.32^{a}	1.05

[1] Data represent mean occurrence of fatty acids in myocardial total phospholipid ($n = 5$) and the pooled SD from ANOVA. Within a row, values not sharing a common superscript were significantly different ($P < 0.05$) by ANOVA, with Tukey's test for multiple pairwise comparisons.

[2] Abbreviations used: REF, low fat reference diet; SAT, sheep perirenal fat-supplemented diet; FO, fish oil-supplemented diet; PUFA, polyunsaturated fatty acids; P:S, ratio of PUFA to saturated fatty acids.

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a reduced probability of the myocardium sustaining arrhythmic activity in the event that it is initiated.

In vitro studies are often compromised by poor physiological relevance (Taegtmeyer et al. 1980). The hearts in this study were subjected to workloads in the physiological range (Neely et al. 1973, Pepe and McLennan 1993), and these conditions were maintained throughout ischemic and reperfusion periods. Importantly, these working hearts were perfused with a high oxygen-carrying perfusate containing red blood cells to adequately support their oxygen demands (Olders et al. 1990, Topping and Trimble 1985). While physiological levels of work output, oxygen demand and delivery were maintained, the hearts were free of potentially confounding neural and humoral influences, variable blood pressure (preload, afterload and coronary perfusion pressure), circulating fatty acids or other extracardiac effects that may have been influenced by dietary lipid intake. Thus, these in vitro findings support the whole-animal coronary artery occlusion studies in rats and dogs and programmed stimulation in marmoset monkeys (Culp et al. 1980, Hock et al. 1990, McLennan 1993, McLennan et al. 1988 and 1993) in which the extracardiac roles of fatty acids and potential compensatory humoral or neural mechanisms could not previously be excluded. They establish that the antiarrhythmic properties of fish oil reside within the heart itself even without extracardiac influences. Our study could not confirm the earlier report of Riemersma and Sargent (1989) (using Langendorff buffer-perfused hearts of low work output and low oxygen supply and demand), that fish oil does not prevent arrhythmias during reperfusion in vitro. Moreover, our findings are supported by the more recent study of Yang et al. (1993b), who also used Langendorff perfused hearts, but were able to prevent in vitro occurrence of reperfusion arrhythmias by fish oil prefeeding.

The antiarrhythmic effects of fish oil were established relative to either a diet supplemented with saturated animal fat or a low fat reference nonpurified diet. There was also a consistent trend for the SAT diet to increase arrhythmia incidence, severity or vulnerability to electrical stimulation in relation to the low fat diet; however, differences often were not significant. We previously observed significant pro-arrhythmic effects of the SAT diet (McLennan et al. 1988); however, the observations of the present study are in line with the clinical observation that regular inclusion of fish in the diet reduces mortality in post-infarction patients but a low fat diet is without significant effect (Burr et al. 1989).

The spontaneous ventricular arrhythmias that occurred in ischemia, although significantly reduced by the FO diet as seen previously in vivo (McLennan 1993, McLennan et al. 1988), were confined to a few extrasystoles. This model imposed only partial (low flow) ischemia with a well-oxygenated perfusion medium. It provided a twofold advantage, allowing both the programmed stimulation protocol to be followed without impediment from spontaneous VT or VF on the one hand and the assessment of spontaneous arrhythmic responses in reperfusion uninfluenced by the occurrence of antecedent ischemic arrhythmias. Moreover, the model ensured that the hearts continued to work throughout the ischemic period, unlike total global ischemia models in which prolonged asystole often occurs with the consequence of reduced metabolic demand (Yanagisawa et al. 1988, Yang 1993b).

We observed that the VF threshold (VFT) was reduced during ischemia in all dietary groups, supporting the previous findings that ischemic and hypoxic insult of the myocardium increases its vulnerability to arrhythmia induction and, hence, lowers the VFT (Lubbe et al. 1975). Nevertheless, the VFT remained higher in FO hearts during ischemia than was observed in REF or SAT hearts during either control or ischemic working heart perfusion, further emphasizing the low vulnerability to arrhythmia generation with FO feeding.

The inherent sinus rhythm was lower in hearts from fish oil–fed animals. Although this may be expected to contribute to altered arrhythmia vulnerability, several factors mitigate against this being the underlying basis of the antiarrhythmic effects of fish oil in the present study. The suppression of spontaneous ventricular arrhythmias in ischemia and reperfusion by dietary FO was observed in hearts with constant sinus rhythm, electrically driven at the same rate in hearts from all dietary groups. Premature electrical stimulation in spontaneously beating hearts was also immediately preceded by a constant interval pacing train to momentarily standardize ventricular beat rate in all hearts. When ischemia reduced the spontaneous beat rate across all dietary groups, the bradycardia was less evident in FO hearts, such that heart rate became lower in the REF and SAT hearts during ischemia. Despite the higher beat rate in FO hearts, the threshold current for fibrillation induction remained elevated and VF episodes remained very brief. Clearly, a low heart rate is not an essential determinant of the antiarrhythmic effect of fish oil although it may reflect the inherent excitability of the myocardial cells. This is in contrast, for example, to the antiarrhythmic properties of the β-adrenoceptor blocker metoprolol which are lost when its bradycardic action is overdriven in isolated hearts (Tosaki et al. 1987).

The mechanism of the antiarrhythmic effect of fish oil (n-3) fatty acids is unknown, but it most likely resides in the altered fatty acid composition of myocardial membranes or intracellular free fatty acid pools (Charnock et al. 1992a, Katz and Messineo 1981). The FO diet used in the present study increased incorporation of EPA and DHA into cardiac cell membranes principally in exchange for the (n-6) PUFA and eicosanoid precursor arachidonic acid [20:4(n-6)] as reported

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previously for similar dietary interventions (Charnock et al. 1992b, McLennan 1993, McLennan et al. 1993, Yang et al. 1993b). More specific and extensive changes in (*n*-6) and (*n*-3) fatty acid incorporation occur in individual cardiac phospholipids with fish oil or other dietary interventions (Abeywardena et al. 1987, Swanson and Kinsella 1986). Hence, the total phospholipid fatty acid composition may underestimate some changes in the specific local phospholipid environment of individual membrane proteins.

There are numerous putative chemical mediators of ischemia or reperfusion-induced arrhythmias (Curtis et al. 1993) which could be influenced by changes in the lipid environment of membrane ion channels, enzymes and receptors or by altered substrate or inhibitor fatty acid availability for eicosanoid production (Katz and Messineo 1981). Increased extracellular accumulation of K^+ occurs with the onset of ischemia, and abnormally high K^+ levels are associated with reduced conduction, altered refractoriness and arrhythmogenesis (Kleber 1984). Whether caused by increased K^+ efflux or reduced K^+ influx, this not only reduces membrane potential and alters the action potential but also leads to increased intracellular Ca^{++}, either in direct exchange for K^+ or in exchange for intracellular Na^+, which accumulates as a result of raised extracellular K^+ (Wilde and Aksnes 1995). We have found that dietary fish oil largely prevents the rise in venous K^+ in ischemia (Pepe and McLennan 1992). Excessive intracellular accumulation of Ca^{++} is also putatively arrhythmogenic, possibly by spontaneous release from overloaded sarcoplasmic reticulum, causing oscillatory local membrane depolarizations which in turn modulate the threshold for arrhythmia induction or trigger spontaneous action potentials (Lakatta and Guarnieri 1993). This excessive accumulation is blocked in isolated cardiomyocytes by acute application of the (*n*-3) fatty acid DHA (Pepe et al. 1994) or in isolated papillary muscles by dietary fish oil (McLennan et al. 1987). Ischemic venous acidosis represents extracellular proton accumulation in exchange for Na^+ which may be arrhythmogenic through modulation of Na^+/Ca^{++} exchange with a rise in intracellular Ca^{++} (Orchard and Cingolani 1994). Venous acidosis is also limited in isolated working hearts with FO prefeeding (Pepe and McLennan 1992). Another recent study showed that acute infusion of a lipid emulsion of fish oil fatty acids can prevent ischemia-induced VF in infarcted dogs in vivo (Billman et al. 1994). This could mimic the release and metabolism of endogenous fatty acids that occur in vitro following fish oil prefeeding. Bogdanov et al. (1995) reported that DHA decreases the amplitude of the transient outward K^+ current (I_{to}) and accelerates the inactivation of I_{to} in isolated adult rat cardiomyocytes. Accordingly, DHA may inhibit arrhythmogenesis by minimizing the contribution of I_{to} to the cardiac electrical heterogeneity that is intensified during ischemia. Thus, there is accumulating evidence for a variety of possibly interrelated mechanisms whereby fish oil may limit arrhythmias, at least in ischemia.

Fatal cardiac arrhythmias are often the result of the complex interplay among: *1)* pathological structural changes such as infarction or hypertrophy; *2)* functional changes or trigger events such as ischemia, electrolyte imbalance or proarrhythmic drugs and; *3)* the myocardial substrate or basic underlying membrane properties. Myerburg (1986) proposed that increased myocardial substrate vulnerability is a critical clinical factor that predisposes a patient to sudden cardiac death in conjunction with a trigger event or factors which modulate the severity of the trigger or the sensitivity of the myocardium to the effect of the trigger event. Curtis et al. (1993) further speculated that if the many putative chemical mediators of arrhythmias are capable of acting independently and in parallel, then inhibition of any individual mediator might be ineffective in inhibiting arrhythmias in ischemia or reperfusion. Arrhythmia vulnerability was altered by dietary lipid supplementation across a range of stimuli and stimulus intensities. It was effective even under control perfusion conditions in the absence of pathological structural changes or functional changes. We therefore propose that dietary fatty acids can directly modulate myocardial substrate vulnerability as well as diminish potential trigger levels in ischemia and reperfusion. The (*n*-3) fatty acids may provide a basis for the development of new antiarrhythmic agents effective in reducing the occurrence of sudden cardiac death as well as receiving greater emphasis for regular inclusion in the diet.

The potential for dietary lipids containing (*n*-3) fatty acids such as EPA and DHA in fish oils or α-linolenic acid from terrestrial sources (McLennan and Dallimore 1995) to prevent fatal cardiac arrhythmias is of particular interest in light of the Cardiac Arrhythmia Suppression Trial (CAST) outcomes for classical antiarrhythmics (Echt et al. 1991). In the CAST, suppression of day to day benign arrhythmias in post-infarction patients by flecainide or encainide did not translate to a reduced incidence of sudden cardiac death. Mortality was actually increased. In contrast, other recent studies have found that dietary (*n*-3) fatty acids reduce mortality in similar groups of post-myocardial infarction patients at high risk of sudden cardiac death (Burr et al. 1989, de Lorgeril et al. 1994). Risk factors such as body weight, body mass index, blood pressure, serum triglycerides, total, LDL and HDL cholesterol and apoprotein levels were unchanged in these studies, consistent with a direct myocardial effect.

Considerable evidence indicates that fish oil can reduce ischemic injury (Hock et al. 1990, Pepe and McLennan 1992, Yanagisawa et al. 1988, Yang et al. 1993a and 1993b) which in itself could contribute to the inhibition of arrhythmias in ischemia and reper-

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fusion. However, the antiarrhythmic effects were observed in response to such diverse triggers as ischemia, reperfusion, premature electrical stimulation and a combination of premature electrical stimulation with myocardial ischemia. We have previously demonstrated that the cardiac effects of fish oil in rats are not due to dietary-induced differences in coronary artery pathology (Turner et al. 1990). Therefore, direct modulation of myocardial substrate vulnerability to arrhythmogenic stimuli and the ability of the myocardium to sustain life-threatening arrhythmic activity are implicated. Fish oil also potentially modulates blood pressure, plasma triglycerides, platelet aggregation and other factors (Leaf and Weber 1988) which may also be expected to reduce cardiovascular risk and act synergistically with any direct antiarrhythmic action. In extending previous in vivo experimental findings (Hock et al. 1990, McLennan 1993, McLennan and Dallimore 1995, McLennan et al. 1988 and 1993), the present results strongly support an antiarrhythmic theory at least as a component of (*n*-3) fatty acid modulation of cardiac mortality.

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