Dietary (n-3) Long-Chain Polyunsaturated Fatty Acids Inhibit Ischemia and Reperfusion Arrhythmias and Infarction in Rat Heart Not Enhanced by Ischemic Preconditioning

Grace G. Abdukeyum, Grace G. Abdukeyum, Alice J. Owen, Peter L. McLennan

Abstract
Ischemic preconditioning (IPC) and (n-3) PUFA are both cardioprotective. This study compared effects of dietary fish oil, IPC, and their interactions on heart function and injury during myocardial ischemia and reperfusion. Male Wistar rats were fed diets containing 10% wt:wt fat comprising either 7% high-docosahexaenoic acid (DHA) [22:6(n-3)] tuna fish oil + 3% olive oil [(n-3) PUFA]; 5% sunflower seed oil + 5% olive oil [(n-6) PUFA]; or 7% beef tallow + 3% olive oil [saturated fat (SF)] for 6 wk. In control experiments, isolated perfused hearts were subjected to 30-min regional ischemia and reperfused for 120 min. The IPC hearts were subjected to 3 cycles of 5-min global ischemia before the ischemia and reperfusion. Control (n-3) PUFA hearts had significantly lower heart rate, coronary flow, end diastolic pressure, maximum relaxation rate, and ischemic and reperfusion arrhythmias. In reperfusion, they had greater developed pressure and maximum relaxation rate and smaller infarct (10.9 ± 0.6% ischemic zone, n = 6) than (n-6) PUFA (47.4 ± 0.3%, n = 6) or SF (50.3 ± 0.3%, n = 6). Compared with control, IPC significantly improved heart function and reduced infarct in (n-6) PUFA (11.8 ± 0.4%, n = 6) and SF hearts (13.1 ± 0.1%, n = 6). Heart function and infarct [(n-3) PUFA 9.6 ± 0.1%, n = 6] did not differ among dietary IPC groups. Arrhythmias, significantly reduced by IPC in (n-6) PUFA and SF hearts, were significantly lower in (n-3) PUFA IPC hearts. Dietary fish oil induces a form of preconditioning, nutritional preconditioning, limiting ischemic cardiac injury, and myocardial infarction and endows cardioprotection as powerful as IPC, which provides no additional protection in (n-3) PUFA hearts.

Introduction
Dietary (n-3) PUFA provide cardiovascular protection, with regular intake of (n-3) long-chain PUFA through fish or fish oil associated with reduced mortality from heart disease in both epidemiological studies and clinical trials (1–4). Experimental evidence suggests that regular consumption of (n-3) PUFA is particularly effective in protecting against the damaging effects of myocardial ischemia (heart attack). Clinical intervention studies and animal studies have shown (n-3) PUFA is associated with prevention of fatal cardiac arrhythmias that can occur following an ischemic episode (sudden heart attack death), even though the incidence of ischemic events may not be affected (5,6). Animal studies have shown antiarrhythmic effects as well as enhanced early posts ischemic recovery of heart function and provide indirect evidence of protection against ischemia-reperfusion injury (7,8).

An alternative approach to deliver cardioprotection may be through the phenomenon of ischemic preconditioning (IPC), wherein brief periods of acute myocardial ischemia provide some protection for the heart against the damaging effects of subsequent prolonged episodes of ischemia. IPC, which was first demonstrated in dogs (9), has subsequently been confirmed in rats, rabbits, and other animal models. Its demonstration in humans has invigorated a search to establish a viable means to utilize preconditioning therapeutically (10). The protective influences of IPC include lower heart muscle oxygen demand, improved recovery of post ischemic heart function, reduction in the incidence of ischemia-reperfusion–induced arrhythmia, and reduction of infarct size (11–14).

Ischemia generates numerous metabolites, autacoids, and cell-signaling molecules that can act as triggers of preconditioning (15) and many are under investigation for the development of potential therapeutic approaches to preconditioning, so called pharmacological preconditioning (16,17). Despite advances in

1 High-DHA tuna fish oil was the gift of Nu-Mega Ingredients, Altona, Australia.
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5 Abbreviations used: AA, arachidonic acid; AS, arrhythmia score; DHA, docosahexaenoic acid; EDP, end diastolic pressure; %en, percent energy; EPA, eicosapentaenoic acid; IPC, ischemic preconditioning; LA, linoleic acid; LVDP, left ventricular developed pressure; SF, saturated fat diet; VF, ventricular fibrillation; VT, ventricular tachycardia.
identifying pharmacological approaches to mimic IPC, their lack of cardiac specificity and inherent potential for side effects, together with poor prospects of predicting ischemic episodes, brings into question the feasibility of such an approach and largely limits their potential use to specific situations, such as during coronary artery by-pass graft and other planned cardiac surgery.

The clinical and epidemiological evidence for the cardioprotective effects of fish oil, together with specific experimental evidence of preconditioning, such as effects on heart function, suggests that (n-3) PUFA provide protection that might be the nutritional equivalent of IPC or pharmacological preconditioning. This study aimed to evaluate the efficacy of dietary fish oil in providing cardioprotection under the same conditions as IPC and to evaluate their potential synergy. It specifically tested the hypothesis that (n-3) PUFA would provide sustained recovery of myocardial function and reduce infarct size following ischemia-reperfusion. This is preparatory to investigating the cell-signaling mechanisms underpinning (n-3) PUFA-mediated cardioprotection. A dietary approach with a safe and effective nutritional component would abrogate the need to predict the onset of ischemic episodes, which currently constrains the potential of pharmacological therapies.

### Methods

Fifty-four male Wistar rats were randomly assigned to 3 dietary groups. They received for 6 wk 1 of 3 iso-energetic diets containing either predominantly saturated fat, (n-6) PUFA, or (n-3) PUFA containing all essential vitamins and minerals but with gelatin replacing casein as part of the protein source. Rats were fed fabricated diets based on the AIN-93 M diet (18) containing (g/100 g dry weight): cornstarch, 57; sucrose, 10; casein, 9; gelatin, 5; cellulose, 5; fat, 10; mineral mix, 3.5; and vitamin mix, 1 (19). The diet provided 64.6% of energy (%en) as carbohydrate, 13.6% as protein, and 22% as fat. The fatty acid profile of each diet is shown in Table 1. All diets were consumed iso-energetically by the rats, delivering a similar total fat intake to all rats. The diet was prepared with (g/100 g dry weight) fat, 10, comprising fish oil, 7 [NuMega high-docosahexaenoic acid (DHA) tuna fish oil] plus olive oil, 3 (Meadow Lea Foods) [SF (n-3) PUFA diet] or sunflower seed oil, 5 (Meadow Lea Foods) plus olive oil, 5 [SF (n-3) PUFA diet] or beef tallow, 7 (Meadow Lea Foods) plus olive oil, 3 (SF diet). The oil blends in the (n-3) PUFA diet and the (n-6) PUFA diet were designed to deliver similar total fatty acids. The oil blends in the (n-3) PUFA diet and the SF diet were designed to deliver similar total (n-6) PUFA. Gelatin replaced some casein to permit the diets to be mixed wet, then set in trays at 4°C until use.

Animal care and experiments were conducted with the approval of the local animal ethics committee according to the guidelines of the National Health and Medical Research Council, Australia, Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (20). Following 6 wk of feeding, rats were anesthetized with pentobarbital (a.i.), intraperitoneal) and their hearts were rapidly re-perfused in Langendorff mode under constant pressure of 75 mm Hg. A 6–0 silk suture was passed through the myocardium closely drawn tight for coronary artery occlusion. A thin-walled, fluid-filled plastic balloon catheter connected to a pressure transducer (Cobe) was introduced into the left ventricle via the left atrium. Balloon volume was adjusted to produce an end diastolic pressure (EDP) of 6–8 mm Hg. Left ventricular hemodynamics were constantly monitored using a computer acquisition and processing program LabView for Windows (National Instruments)

Hearts were allowed to beat spontaneously and equilibrated for 30 min and then we took baseline measurements of cardiac function prior to initiation of ischemia. Coronary flow was measured by timed collection of the coronary effluent. Heart rate, maximum rate of pressure development, maximum rate of relaxation, and left ventricular developed pressure (LVDP) were determined by analyzing pressure tracings using LabView for Windows.

We recorded the electrocardiogram. Ventricular tachycardia (VT) was assessed as ≥4 consecutive beats of similar morphology with no preceding P-wave and with a basic cycle length at least 20% less than that of preceding complexes. Ventricular fibrillation (VF) was assessed as chaotic morphology of the repetitive complexes for at least 4 cycles accompanied by a precipitous drop in developed pressure. Arrhythmias were also assessed by counting the number of ventricular premature beats and the incidence and total duration of all episodes of VT and VF. Global severity of arrhythmias was assessed during ischemia and reperfusion using hierarchical scores (6). The arrhythmia score (AS) awarded points on a hierarchical scale of 0–9. A score of 0–5 represents increasing degrees of reverting arrhythmias. A score of 6–9 represents the occurrence of nonreverting VF of progressively earlier onset.

Control experiments were defined as those in which hearts were subjected to regional ischemia and reperfusion, and infarct size determination, but in which no IPC was imposed. They commenced with 30-min equilibration, after which we induced regional ischemia by coronary artery occlusion, then 120-min reperfusion. Separate groups of hearts were subjected to an IPC protocol prior to the 30-min ischemia. This consisted of 3 cycles of 5-min global ischemia (aortic inflow stopped), each followed by 5-min reperfusion before the onset of 30-min regional ischemia by coronary occlusion, then 120-min

### Table 1: Fat and fatty acid concentrations of the rat diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>SF (g/kg of diet)</th>
<th>(n-6) PUFA (g/kg of diet)</th>
<th>(n-3) PUFA (g/kg of diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish oil</td>
<td>–</td>
<td>–</td>
<td>70</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>–</td>
<td>50</td>
<td>–</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>70</td>
<td>–</td>
<td>70</td>
</tr>
<tr>
<td>Olive oil 2</td>
<td>30</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>12:0</td>
<td>0.6</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>14:0</td>
<td>2.6</td>
<td>n.d.</td>
<td>2.1</td>
</tr>
<tr>
<td>16:0</td>
<td>20.6</td>
<td>8.1</td>
<td>17.1</td>
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<tr>
<td>16:1</td>
<td>3.2</td>
<td>0.4</td>
<td>3.4</td>
</tr>
<tr>
<td>18:0</td>
<td>14.1</td>
<td>3.6</td>
<td>4.5</td>
</tr>
<tr>
<td>18:1 (oleic acid)</td>
<td>47.9</td>
<td>50.0</td>
<td>33.5</td>
</tr>
<tr>
<td>18:2(n-6) (LA)</td>
<td>4.7</td>
<td>38.0</td>
<td>3.5</td>
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<tr>
<td>18:3(n-3) (α-linolenic acid)</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
</tr>
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<td>20:4(n-6) (AA)</td>
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<td>n.d.1</td>
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<tr>
<td>20:5n-3 (EPA)</td>
<td>n.d</td>
<td>n.d</td>
<td>4.9</td>
</tr>
<tr>
<td>22:5n-3 (docosapentaenoic acid)</td>
<td>n.d.</td>
<td>n.d</td>
<td>0.8</td>
</tr>
<tr>
<td>22:6n-3 (DHA)</td>
<td>n.d</td>
<td>n.d</td>
<td>20.2</td>
</tr>
<tr>
<td>Total SFA</td>
<td>38.1</td>
<td>12.6</td>
<td>25.5</td>
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<tr>
<td>Total monounsaturated fatty acids</td>
<td>51.4</td>
<td>50.6</td>
<td>39.2</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>5.4</td>
<td>38.6</td>
<td>31.4</td>
</tr>
<tr>
<td>Total(n-6) PUFA</td>
<td>4.8</td>
<td>38.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Total(n-3) PUFA</td>
<td>0.6</td>
<td>0.5</td>
<td>26.4</td>
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<tr>
<td>P:S ratio 2</td>
<td>0.25</td>
<td>3.18</td>
<td>1.14</td>
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<tr>
<td>(n-6):(n-3) PUFA</td>
<td>8.3</td>
<td>74</td>
<td>0.19</td>
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</tbody>
</table>

1 Not detected.
2 PUFA:SFA ratio.

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reperfusion. On completion of the reperfusion period, the coronary artery was reoccluded and the heart was infused with Evans Blue dye to reveal the ischemic zone (unstained region of the heart). Hearts were sliced and incubated in a buffer containing triphenyl-tetrazolium chloride and sodium phosphate (pH 7.4), then stored in 10% formalin until photographed and analyzed for infarct size using the Imager program. Infarct size was reported as a percentage of the zone at risk.

Data handling and statistical analysis. Results were expressed as means ± SEM. For each measured hemodynamic parameter, 2-way repeated-measures ANOVA was conducted with diet and IPC main effects. Missing values due to arrhythmias produced unequal numbers at some time points and precluded post hoc analysis of repeated-measures ANOVA for interactions between diet and IPC. Therefore, for hemodynamic measures, 2-way ANOVA was conducted at each time point with diet and IPC main effects and diet × IPC interaction. Individual comparisons between diet × IPC were conducted using Tukey’s post hoc test. The percentage of isolated hearts acutely exhibiting VT or VF during ischemia and reperfusion were tested using Fisher’s exact test. Values were considered to be significantly different at P < 0.05.

Results

Effects of diet on body and organ weights. The body weights of the groups at wk 0 or at the time of experimentation following 6 wk of dietary manipulation did not differ. Body weight gain, calculated as weight at 6 wk minus initial weight, and body size as indicated by tibia length did not differ between diets. Heart size, as determined by heart mass, heart weight:body weight ratio, or heart weight:tibia length ratio, did not differ between diet groups (Table 2).

Effects of diet on baseline hemodynamics. Baseline hemodynamics were measured after perfused hearts had equilibrated for 30 min. Baseline spontaneous heart rate was lower (P < 0.01) in (n-3) PUFA hearts (203 ± 8 bpm; P < 0.01) than SF (203 ± 8 bpm) or (n-6) PUFA hearts (201 ± 8 bpm). Compared with SF (Fig. 1A,D) and (n-6) PUFA hearts (Fig. 1B,E), coronary flow was lower in (n-3) PUFA hearts (P < 0.001) (Fig. 1C), and developed pressure was greater (Fig. 1F) (P < 0.01). The EDP, initially set at 6–8 mm Hg in all hearts, was lower in the (n-3) PUFA hearts after equilibration (Fig. 2C) (P < 0.01) than in SF (Fig. 2A) and (n-6) PUFA hearts (Fig. 2B). The maximum rate of ventricular relaxation was greater in (n-3) PUFA hearts (Fig. 3F) (P < 0.01) than in the SF (Fig. 3D) or (n-6) PUFA hearts (Fig. 3E). The maximum rate of ventricular pressure development (Fig. 3A–C) and the rate-pressure product, which is the product of heart rate and systolic pressure (data not shown), did not differ between dietary groups at equilibrium.

Effects of IPC on baseline heart function. After 3 5-min periods of global IPC, ventricular developed pressure increased in the SF (Fig. 1A) and (n-6) PUFA hearts (Fig. 1B) (P < 0.05), as did maximum rate of relaxation (Fig. 3D,E) (P < 0.05). End diastolic pressure was lower after IPC (P < 0.05) compared with control SF and (n-6) PUFA hearts (Fig. 2 A, B). Heart function measures did not differ between the (n-3) PUFA control and IPC hearts (P > 0.05) (Figs. 1–3).

Effects of regional ischemia and reperfusion. Occlusion of the left anterior descending coronary artery reduced the coronary flow and produced regional ischemia in all hearts (Fig. 1A–C). Measures of heart function including heart rate (data not shown), LVDP (Fig. 1D–F), rate pressure product (data not shown), and maximum rates of pressure development and relaxation (Fig. 3) were reduced in all hearts during ischemia (P < 0.001). The EDP rose in all hearts during ischemia (Fig. 3) (P < 0.001).

When the occlusion was released to allow reperfusion, coronary flow rapidly increased to a mean of 131.5 ± 3.4% of the preocclusion level across all diets, then returned gradually toward the preischemic value over time (Fig. 1A–C). Measures of heart function including heart rate (data not shown), LVDP (Fig. 1D–F), rate pressure product (data not shown), and maximum rates of pressure development and relaxation (Fig. 3), all returned toward equilibrium values after reperfusion. The EDP, which was elevated during ischemia, remained elevated for the entire 120 min of reperfusion in all groups (Fig. 2) (P < 0.001).

During ischemia, 59 and 41% of all control hearts exhibited VT or VF, respectively. All episodes of VT and VF spontaneously reverted to sinus rhythm. On reperfusion, VT and VF occurred in 63 and 37% of hearts, respectively (Table 3).

Effects of diet on responses to ischemia and reperfusion. Coronary artery occlusion reduced coronary flow in all dietary groups by a similar percentage, leaving residual flows of 78.2 ± 2.1% (SF); 70 ± 3.1% (n-6 PUFA); and 71 ± 2.2% (n-3 PUFA) through the nonischemic region in each of the dietary control groups. During ischemia and reperfusion, coronary flow (Fig. 1C) and spontaneous heart rate (data not shown) were lower and developed pressure (Fig. 1D) was higher in the (n-3) PUFA control hearts compared with the SF and (n-6) PUFA control hearts (P < 0.01), which did not differ. The maximum rate of ventricular relaxation was greater in (n-3) PUFA (Fig. 3F) than in the SF (Fig. 3D) and (n-6) PUFA (Fig. 3E) groups (P < 0.01). The EDP, which became elevated during ischemia, remained lower in the (n-3) PUFA hearts during ischemia and reperfusion (Fig. 2C) than in the SF (Fig. 2A) or (n-6) PUFA hearts (Fig. 2B) (P < 0.01). The rate-pressure product was not significantly different between dietary groups during ischemia and reperfusion (data not shown). The incidence of the arrhythmias VT and VF during either ischemia or reperfusion was lower in (n-3) PUFA hearts than in the SF hearts (P < 0.05) (Table 3). The duration of arrhythmia episodes in (n-3) PUFA hearts also appeared to be lower, but their very low occurrence prevented statistical comparison. The cumulative AS was lower in (n-3) PUFA control hearts compared with either SF and (n-6) PUFA control hearts in ischemia (P < 0.05) and reperfusion (P < 0.05) and the (n-6) PUFA AS was significantly lower than SF in ischemia (Table 3).

TABLE 2 Body weight and heart weight characteristics of rats fed SF, (n-6) PUFA, or (n-3) PUFA diets for 6 wk.1

<table>
<thead>
<tr>
<th>Diet</th>
<th>SF</th>
<th>(n-6) PUFA</th>
<th>(n-3) PUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight at 0 wk, g</td>
<td>340 ± 11</td>
<td>346 ± 14</td>
<td>354 ± 12</td>
</tr>
<tr>
<td>Body weight at 6 wk, g</td>
<td>473 ± 8</td>
<td>471 ± 6</td>
<td>470 ± 9</td>
</tr>
<tr>
<td>Body weight gain, g/6 wk</td>
<td>133 ± 9</td>
<td>125 ± 5</td>
<td>116 ± 6</td>
</tr>
<tr>
<td>Heart weight, g/6 wk</td>
<td>435 ± 3.4</td>
<td>433 ± 0.2</td>
<td>449 ± 0.5</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>3.1 ± 0.2</td>
<td>3.0 ± 0.3</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>Heart weight/tibia length, mg/mm</td>
<td>33.3 ± 1.2</td>
<td>33.0 ± 1.3</td>
<td>31.8 ± 0.6</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. n = 18.
2 Ventricular weight measured after ischemia-reperfusion protocol with atria and major vessels removed.

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**Interaction between diet and IPC in responses to ischemia and reperfusion.** IPC influenced cardiac function during reperfusion with lower coronary flow \((P < 0.05)\) (Fig. 1), heart rate \((P < 0.05)\) (data not shown), and EDP \((P < 0.05)\) (Fig. 2). Developed pressure \((P < 0.05)\) (Fig. 1) and maximum rate of ventricular relaxation were also higher in reperfusion after IPC \((P < 0.05)\) (Fig. 3). There were interactions between diet and IPC \((P < 0.01)\) and pairwise comparisons showed that the changes in heart function occurred in the SF and \((n-6)\) PUFA hearts only and not in the \((n-3)\) PUFA hearts. For example, the developed pressure was higher during ischemia in SF IPC and \((n-6)\) PUFA IPC hearts compared with their control hearts and recovered to a level not significantly different to the baseline levels during reperfusion. Developed pressure during ischemia or reperfusion did not differ between \((n-3)\) PUFA control and \((n-3)\) PUFA IPC hearts. Left ventricular developed pressure, EDP, and maximum rate of ventricular relaxation did not differ between diets in IPC hearts. Arrhythmias in both ischemia and reperfusion were reduced in IPC compared with control hearts such that episodes of VT or VF were of shorter duration \((P < 0.05)\) and cumulative AS were lower \((P < 0.05)\) (Table 3). There were interactions between diet and IPC \((P < 0.01)\), and pairwise comparisons showed that these changes in arrhythmia occurred in the SF and \((n-6)\) PUFA IPC hearts only. The AS for both ischemia and reperfusion remained significantly lower in \((n-3)\) PUFA IPC hearts than in SF IPC or \((n-6)\) PUFA IPC hearts.

**Effects of diet and IPC on infarct size.** The infarct size as percent ischemic zone at risk was smaller in \((n-3)\) PUFA control hearts than in the SF or \((n-6)\) PUFA control hearts \((P < 0.05)\) (Fig. 4). The infarct size in \((n-6)\) PUFA and SF control hearts did not differ. The ischemic zone at risk did not differ between groups (data not shown), indicating an equivalent ischemic insult.

**FIGURE 1.** Effect of ischemia (30 min) and reperfusion (120 min) on coronary flow \((A–C)\) and LVDP \((D–F)\) in control and IPC-isolated perfused hearts from rats that consumed the SF, \((n-6)\) PUFA, or \((n-3)\) PUFA diets for 6 wk. Filled bar on time-axis shows 30-min ischemia duration. Values are means \(\pm\) SEM, \(n = 6–9\). *IPC and control groups differ at that time, \(P < 0.05\). Differences between diet groups at each time are not shown. Within control or IPC groups, curves for a variable without a common letter differ between diets, \(P < 0.01\).

**FIGURE 2.** Effect of ischemia (30 min) and reperfusion (120 min) on left ventricular EDP in control and IPC isolated perfused hearts from rats that consumed the SF, \((n-6)\) PUFA, or \((n-3)\) PUFA diets for 6 wk. Filled bar on time-axis shows 30-min ischemia duration. Values are means \(\pm\) SEM, \(n = 6–9\). *IPC and control groups differ at that time, \(P < 0.05\). Differences between diet groups at each time are not shown. Within control or IPC groups, curves for a variable without a common letter differ between diets, \(P < 0.01\).
Infarct size was smaller in SF IPC ($P < 0.05$) and (n-6) PUFA IPC hearts ($P < 0.05$) compared with their controls, representing $\sim 35\%$ reduction in infarcted ventricle. Infarct size did not differ between (n-3) PUFA IPC and (n-3) PUFA control hearts or between diets within the IPC hearts (Fig. 4).

**Discussion**

The present study demonstrates that dietary fish oil, providing a source of (n-3) PUFA, protects the rat heart against myocardial infarction and arrhythmias and improves postischemic recovery of heart function when hearts are subjected to occlusion of a major coronary artery in the simulation of an acute heart attack. The mechanisms for this comprehensive cardioprotection likely reside within the myocardium, dependent upon the (n-3) PUFA incorporation into cellular membranes (6–8,21,22), because neither blood platelets (23) nor fatty acids (24) were circulating in the isolated heart perfusate. Our 6-wk feeding period ensured that the time necessary for the concentration of the major myocardial (n-3) PUFA, DHA [22:6(n-3)], to reach equilibrium within cellular membranes was exceeded (19) and it represents a habitual dietary intake. This study verified by direct measurement that (n-3) PUFA limit lethal myocellular injury and infarct size, previously suggested indirectly through the reduced release of cellular markers of ischemic damage (8,22,25). Reduced infarct size, regarded as the ultimate indicator of cardioprotection by IPC (26), was also observed in this study after IPC, but in SF and (n-6) PUFA hearts only. The reduced infarct size was comparable to that after fish oil feeding with or without IPC; the additional imposition of IPC in (n-3) PUFA hearts did not further reduce

**FIGURE 3** Effect of ischemia (30 min) and reperfusion (120 min) on maximum rate of pressure development (+dP/dt$_{max}$) and maximum rate of relaxation (-dP/dt$_{max}$) in control and IPC-isolated perfused hearts from rats that consumed the SF, (n-6) PUFA, or (n-3) PUFA diets for 6 wk. Filled bar on time-axis shows 30-min ischemia duration. Values are means $\pm$ SEM, $n = 6–9$. *IPC and control groups differ at that time, $P < 0.05$. Differences between diet groups at each time are not shown. Within control or IPC groups, curves for a variable without a common letter differ between diets, $P < 0.01$.

**TABLE 3** Effect of IPC on arrhythmia during ischemia and reperfusion in isolated hearts from rats fed SF, (n-6) PUFA, or (n-3) PUFA diets for 6 wk$^1$

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control hearts (no IPC)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>IPC hearts</th>
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<tr>
<td></td>
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</tr>
<tr>
<td>SF</td>
<td>9</td>
<td>89$^a$</td>
<td>39.6 $\pm$ 5.5</td>
<td>78$^a$</td>
<td>38.7 $\pm$ 7.1</td>
<td>4.8 $\pm$ 0.4$^a$</td>
<td>9</td>
<td>67$^a$</td>
<td>24.0 $\pm$ 2.1$^a$</td>
<td>44</td>
<td>12.7 $\pm$ 0.8</td>
</tr>
<tr>
<td>(n-6) PUFA</td>
<td>9</td>
<td>67$^{ab}$</td>
<td>18.5 $\pm$ 3.4</td>
<td>44$^{ab}$</td>
<td>12.3 $\pm$ 3.4</td>
<td>3.2 $\pm$ 0.3$^b$</td>
<td>9</td>
<td>44$^{ab}$</td>
<td>7.8 $\pm$ 0.8$^a$</td>
<td>33</td>
<td>1.3 $\pm$ 0.3</td>
</tr>
<tr>
<td>(n-3) PUFA</td>
<td>9</td>
<td>22$^b$</td>
<td>9.0 $\pm$ 7.0</td>
<td>0$^b$</td>
<td>n.d.$^a$</td>
<td>2.0 $\pm$ 0.0$^a$</td>
<td>9</td>
<td>11$^b$</td>
<td>2.0 $\pm$ 0.0$^a$</td>
<td>0</td>
<td>n.d.$^a$</td>
</tr>
<tr>
<td>All</td>
<td>27</td>
<td>59</td>
<td>27.8 $\pm$ 4.3</td>
<td>41</td>
<td>28.0 $\pm$ 6.0</td>
<td>3.8 $\pm$ 0.3</td>
<td>27</td>
<td>41</td>
<td>16.0 $\pm$ 3.0$^a$</td>
<td>30</td>
<td>7.0 $\pm$ 2.2$^a$</td>
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<tr>
<td>Reperfusion</td>
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<td></td>
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<td></td>
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<tr>
<td>SF</td>
<td>9</td>
<td>89$^a$</td>
<td>20.1 $\pm$ 4.0$^a$</td>
<td>67$^a$</td>
<td>29.8 $\pm$ 6.0</td>
<td>4.8 $\pm$ 0.5$^a$</td>
<td>9</td>
<td>56</td>
<td>14.0 $\pm$ 1.8$^a$</td>
<td>44</td>
<td>9.8 $\pm$ 2.4$^a$</td>
</tr>
<tr>
<td>(n-6) PUFA</td>
<td>9</td>
<td>78$^{ab}$</td>
<td>18.8 $\pm$ 0.7$^{ab}$</td>
<td>33$^{ab}$</td>
<td>16.0 $\pm$ 4.5</td>
<td>3.7 $\pm$ 0.4$^b$</td>
<td>9</td>
<td>56</td>
<td>6.4 $\pm$ 1.2$^a$</td>
<td>22</td>
<td>7.5 $\pm$ 2.5$^a$</td>
</tr>
<tr>
<td>(n-3) PUFA</td>
<td>9</td>
<td>22$^b$</td>
<td>2.5 $\pm$ 0.5$^b$</td>
<td>11$^b$</td>
<td>5.0$^b$</td>
<td>2.0 $\pm$ 0.0$^b$</td>
<td>9</td>
<td>11</td>
<td>4.0 $\pm$ 0.0$^a$</td>
<td>11</td>
<td>4.0 $\pm$ 0.0$^a$</td>
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<tr>
<td>All</td>
<td>27</td>
<td>63</td>
<td>17.5 $\pm$ 2.3</td>
<td>37</td>
<td>23.2 $\pm$ 4.6</td>
<td>3.9 $\pm$ 0.3</td>
<td>27</td>
<td>41</td>
<td>9.6 $\pm$ 1.5$^a$</td>
<td>26</td>
<td>8.2 $\pm$ 1.6$^a$</td>
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</table>

$^1$ Values are means $\pm$ SEM, $n = 9$ or 27 (all) or %. *Different from control in that diet group, $P < 0.05$. Within ischemia or reperfusion, diet group values with superscripts without a common letter differ, $P < 0.05$.

$^2$ All represents the collective incidence and duration of arrhythmias for all rats under control and IPC conditions.

$^3$ n.d., Not detected. a, Incidence of VT or VF too low to conduct statistical analysis on duration.

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The sustained increase in left ventricular EDP, a characteristic of myocardial ischemia also reflected in the impaired rate of relaxation, is often indicative of impaired intracellular calcium handling and was inhibited in (n-3) PUFA control hearts compared with SF and (n-6) PUFA hearts and after IPC. Improved calcium handling is also implicated in the antiarrhythmic effects and protection against mitochondrial damage by either IPC (28) or fish oil (8,29). The low coronary flow in (n-3) PUFA control hearts, with a similar trend after IPC, occurred paradoxically without detriment to cardiac function and therefore corroborates previously reported low oxygen demand and high coronary flow reserve of those hearts (8,14,22,30). When considered together with the enhanced recovery of contractile function in reperfusion, this suggests improved energy efficiency in both (n-3) PUFA control and in IPC hearts, perhaps due to underlying changes in mitochondrial metabolism. Reduced rates of mitochondrial respiration in hearts from fish oil-fed rats (29), together with membrane fatty acid modulation, can modify a number of intracellular and membrane events (31). These findings suggest that both IPC and (n-3) PUFA might decrease or delay calcium overload by affecting calcium influx, efflux, or intracellular redistribution. The low resting heart rate observed in (n-3) PUFA hearts in this study and also found in the human heart in association with regular fish intake (32) further supports the involvement of calcium handling in the cardioprotective effects of (n-3) PUFA. Low heart rate is associated with reduced cardiovascular risk, including reduced risk of arrhythmic sudden death (33). Although heart rate may be influenced by many physiological mechanisms, there is evidence that (n-3) PUFA modulate pacemaker cell calcium channels (34). Thus, dietary (n-3) PUFA modulation of calcium handling through altered cardiac membrane composition may affect oxygen use and arrhythmia vulnerability (8), heart rate and recovery in reperfusion, and contribute to longer postinfarction survival (35).

In our study, the antiarrhythmic effect of (n-3) PUFA was most clearly demonstrable compared with the SF diet, with nonsignificant trends between (n-3) PUFA and (n-6) PUFA (control: ischemic VF P = 0.08, reperfusion VT P = 0.06), except in overall AS. An often-observed trend toward reduced arrhythmia with (n-3) PUFA compared with (n-6) PUFA was established as a significant difference through meta-analysis of a large number of animal studies (36) and in a dietary study that carefully balanced total PUFA intake (21). Although there are some cardioprotective effects attributable to PUFA in general (34), the effects of fish oil in this study are clearly attributable to the (n-3) PUFA, because the (n-6) PUFA hearts did not perform in any way significantly differently to the SF hearts. The in vitro nature of the present study isolates these effects to intrinsic properties of the heart following membrane composition change rather than to autonomic nervous function or peripheral vascular effects that could contribute in vivo to reduced heart rate and arrhythmia, a conclusion reinforced by lower heart rate with fish supplement in human heart transplant patients (37).

The fish oil consumed by rats in this study was extracted from tuna fish without enrichment and although its high content of DHA relative to eicosapentaenoic acid [EPA, 20:5(n-3)] (38) contrasts with the EPA-rich oils often used experimentally or for human supplementation (39), it does in fact reflect the balance of (n-3) PUFA found in many common table fish, including most salmon species, canned or fresh (40,41), in which DHA usually exceeds EPA content. Furthermore, DHA is the predominant (n-3) PUFA obtained from fish in the usual human diet (42,43). Therefore, the high-DHA tuna fish oil may better reflect the common food sources contributing to preventative cardioprotection in humans described in epidemiological studies. Irrespective of their ratio of EPA:DHA, the main effect of dietary fish oils on myocardial membrane phospholipids is to elevate incorporation of DHA (7,19,21,44–46). By comparison, little EPA is incorporated into rat heart, even when fish oil with an EPA:DHA ratio of 2:1 is consumed (7). In human (47), nonhuman primate (21), and rat myocardium (19,35), DHA is the principle (n-3) PUFA. In both human (48) and animal studies (46) in which the purified (n-3) PUFA, EPA, and DHA, have been administered, the direct cardiac effects are attributed to DHA. Therefore, although antiarrhythmic effects and ischemic protection are observed with either high-DHA (6,21) or high-EPA (7,8) fish oils, DHA rather than EPA appears to be responsible for the direct cardioprotective effects (35,46).

As a consequence of using fish oil with olive oil, we produced an (n-3) PUFA diet with 0.8% energy as linoleic acid (LA), which is less than recommended for growing rats (18,49). The SF diet therefore provided a low LA (1.0%en) control for this study. Animal growth data and heart weights did not differ between either of these diets and the LA-replete (LA 7.9% en) (n-6) PUFA diet. Heart function, which differed little between (n-6) PUFA and SF hearts, illustrates that n-6 PUFA deficiency was not a contributing factor to either the poor functional recovery or infarct size in reperfused SF and (n-6) PUFA hearts or the beneficial effects of the (n-3) PUFA diet. Cunnane and Anderson (1997) demonstrated that only a small proportion of dietary LA is incorporated into tissue during rat growth and suggested that the essential requirements are substantially <2% energy, as long as adequate α-linolenic acid or oleic acid are provided (49,50). At 18 wk and 340 g, our rats were mature at commencement yet continued to gain >3 g/d during the study, comparable to nondeficient rats of that age (50). The fatty acid composition of hearts (analyzed from a parallel group of animals) illustrated that neither LA nor arachidonic acid (AA) concentrations were reduced after feeding the low-LA, SF diet for 6 wk (percent total phospholipid fatty acids: LA, 17.5 ± 0.2%; AA, 23.3 ± 0.3%) compared with the (n-6) PUFA diet (LA, 18.7 ± 0.2%; AA, 23.5 ± 0.2%; IPC and control differ within diet group, *P < 0.05. Within control or IPC groups, panels for a variable without a common letter at the 150-min point differ, P < 0.01.

**FIGURE 4** Influence of IPC on infarct size after 30-min ischemia and 120-min reperfusion in isolated perfused hearts from rats that consumed the SF, (n-6) PUFA, or (n-3) PUFA diets for 6 wk. Values are means ± SEM, n = 6. *IPC and control differ within diet group, P < 0.05. Within control or IPC groups, panels for a variable without a common letter at the 150-min point differ, P < 0.01.
PUFA may underpin the low cardiovascular morbidity and ischemic events in the human population when (n-3) PUFA support an IPC-like effect for the reduced consequences of reductions in new cardiac events (1,3). The present observations in blood pressure, blood lipids, and, most significantly, without regular intake of fish or fish oil reduces mortality without changes with human intervention trials in postinfarction patients, fish consumption have been described in many settings (41,54, none are available in clinical practice, largely because of the need contrast, whereas many pharmacological agents mimic IPC (53), infarct size that would require no prediction of ischemic events. In the time window for rapid coronary revascularization to limit solution for preventative ischemic cardioprotection or increase secondary cardioprotection. It could thus represent a low-risk conditioning'' of the heart, just as pharmaceuticals, heat, and ischemia prevent ventricular fibrillation following coronary artery occlusion and after any ischemic episode. We can define the cardioprotection attributable to dietary (n-3) PUFA as “nutritional pre-conditioning” of the heart, just as pharmacologicals, heat, and ischemia produce pharmacological, thermal, and IPC. Fish oils from numerous sources are classified by the U.S. FDA as “generally regarded as safe” for addition into foods (52) and fish has a history of safe consumption associated with both primary and secondary cardioprotection. It could thus represent a low-risk solution for preventative ischemic cardioprotection or increase the time window for rapid coronary revascularization to limit infarct size that would require no prediction of ischemic events. In contrast, whereas many pharmacological agents mimic IPC (53), none are available in clinical practice, largely because of the need to administer the treatment prior to an ischemic event (17). Over the past decade or more, the protective cardiovascular effects of fish consumption have been described in many settings (41,54, 55). With human intervention trials in postinfarction patients, regular intake of fish or fish oil reduces mortality without changes in blood pressure, blood lipids, and, most significantly, without reductions in new cardiac events (1,3). The present observations support an IPC-like effect for the reduced consequences of ischemic events in the human population when (n-3) PUFA are a regular part of the diet (2-4) without invoking reduced coronary atherosclerosis and prevention of new ischemic episodes. Nutritional pre-conditioning by membrane incorporation of (n-3) PUFA may underpin the low cardiovascular morbidity and mortality associated with regular fish and fish oil consumption.

**Literature Cited**


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