Dietary (n-3) long chain polyunsaturated fatty acids inhibit ischemia and reperfusion arrhythmias and infarction in rat heart not enhanced by ischemic preconditioning

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Abstract
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Keywords
Ischemic preconditioning; arrhythmia; heart rate; hemodynamics; ischemia; reperfusion; infarct size; fish oil; dietary lipids

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Key words: ischemic preconditioning; arrhythmia; heart rate; hemodynamics; ischemia; reperfusion; infarct size; fish oil; dietary lipids
Introduction:

Dietary omega-3 (n-3) polyunsaturated fatty acids (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 and animal studies have shown (n-3) PUFA to be 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 enhanced early post-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, wherein brief periods of acute myocardial ischemia provide some protection for the heart against the damaging effects of subsequent prolonged episodes of ischemia. Ischemic-preconditioning (IP), 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 of utilising preconditioning therapeutically (10). The protective influences of ischemic preconditioning include lower heart muscle oxygen demand, improved recovery of post-

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1 Abbreviations used: AA, arachidonic acid; ALA, alpha linolenic acid; AS, arrhythmia score; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ECG, electrocardiogram; EFA, essential fatty acid; EPA, eicosapentaenoic acid; FO, fish oil; GRAS, generally regarded as safe; IP, ischemic preconditioning; LA, linoleic acid; MI, myocardial infarction; n.d., not detected; PUFA, polyunsaturated fatty acid; sat, saturated fat; SF saturated fat diet VF, ventricular fibrillation; VPB, ventricular premature beats; VT, ventricular tachycardia
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 signalling molecules that can act as triggers of preconditioning (15), and many are under investigation for the potential development of therapeutic approaches to preconditioning, so called pharmacological preconditioning (16,17). Despite advances in identifying pharmacological approaches to mimic ischemic preconditioning, 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 bypass graft and other planned cardiac surgery.

The clinical and epidemiological evidence for cardioprotective effects of fish oil, together with specific experimental evidence of preconditioning–like effects on heart function suggests that (n-3) PUFA provide a form of protection that might be seen as nutritional equivalent of ischemic preconditioning. This study aimed to evaluate the efficacy of dietary fish oil in providing cardioprotection under the same conditions as ischemic preconditioning 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 signalling mechanisms underpinning (n-3) PUFA-mediated cardioprotection. A dietary approach with a safe and effective nutritional component could overcome the need to predict the onset of ischemic episodes, which currently constrains the potential effectiveness of pharmacological therapies.
Methods:

Fifty four male Wistar rats were randomly assigned to three dietary groups. They received, for 6 weeks, one of three iso-energetic diets containing either predominantly saturated fat, (n-6) PUFA or (n-3) PUFA based on the American Institute of Nutrition AIN97 rat diet, containing all essential vitamins and minerals but with gelatine replacing casein as part of the protein source. Animals were fed fabricated diets based on the AIN-97 M diet (18) containing (% dry weight) 57% cornstarch, 10% sucrose, 9% casein, 5% gelatine, 5% cellulose, 10% oil, 3.5% mineral mix and 1% vitamin mix (19). The diet provided 64.6% of energy (%en) as carbohydrate, 13.6%en as protein and 22%en 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 animals. The diet was prepared with 10% (dry wt) fat consisting of 7% fish oil (NuMega high DHA tuna fish oil) plus 3% olive oil ((n-3) PUFA diet) or 5% sunflower seed oil plus 5% olive oil ((n-6) PUFA diet) or 7% beef tallow plus 3% olive oil (SF diet). All diets contained sufficient PUFA to prevent EFA deficiency. The oil blends in the (n-3) PUFA diet and the (n-6) PUFA diet were designed to deliver similar total PUFA. Gelatine was included in place of some casein to permit the diets to be mixed wet then set in trays at 4°C, sliced into cubes and kept frozen at –20°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 six weeks of feeding, rats were anesthetised with pentobarbital sodium (60mg/kg⁻¹ i.p.) and their hearts were rapidly
removed through a thoracic incision by transecting all major vessels, and then
submerged in cold perfusate to arrest beating.
The heart was subsequently attached to a perfusion apparatus via the aorta; perfusion
was initiated immediately with Krebs-Henseleit bicarbonate buffer (NaCl 118mM, KCl
4.7mM, MgSO₄·7H₂O 1.6mM, KH₂PO₄ 1.2 mM, NaHCO₃ 24.9 mM, CaCl₂ 2.5mM, and
glucose 11.1mM). The solution was gassed with 5%CO₂ in O₂ at 37°C. The myocardial
temperature was maintained near 37°C throughout the experiment. The heart was
perfused in the Langendorff mode under constant pressure of 75 mmHg set by adjusting
the height of the reservoir. 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 of 6-8mmHg. Left
ventricular haemodynamics were constantly monitored using data acquisition and
processing program Lab View for Windows (National Instruments).
Hearts were allowed to beat spontaneously and equilibrated for 30min and then baseline
measurements of cardiac function were taken 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 were determined by analysing pressure tracings using Lab View for Windows.
The electrocardiogram (ECG) was recorded and ventricular tachycardia (VT) was
assessed as four or more consecutive beats of similar morphology with no preceding P
wave and with a basic cycle length at least 20% less than that of prevailing complexes.
Ventricular fibrillation (VF) was assessed as chaotic morphology of the repetitive
complexes for at least four cycles accompanied by a precipitous drop in developed
pressure. Arrhythmias were additionally assessed by counting the number of ventricular
premature beats (VPB) and the incidence and total duration of all episodes of VT
(Ventricular Tachycardia) and VF (Ventricular fibrillation). Global severity of arrhythmias was assessed using hierarchical scores for ischemia and reperfusion (6). The score 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 non-reverting VF of progressively earlier onset.

Control experiments commenced with 30min equilibration, after which regional ischemia was induced by occluding the left anterior descending coronary artery for 30min, followed by release of the occluding ligature and 120min reperfusion. Separate groups of hearts were subjected to an ischemic preconditioning protocol prior to 30min ischemia. This consisted of three cycles of 5min global ischemia, each followed by 5min reperfusion before the onset of 30min regional ischemia, then 120min reperfusion. On completion of the reperfusion period, the coronary artery was re-occluded 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 analysed 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 mean ± SEM. For each parameter, the effects of ischemic preconditioning and dietary treatment were tested by 2-way repeated-measures analysis of variance (ANOVA) with individual between-diet comparisons by Tukey’s post hoc test. The percent of isolated hearts acutely exhibiting spontaneous, sustained ventricular tachycardia (VT) or ventricular fibrillation (VF) was scored during ischemia or
reperfusion and group contrasts were tested by Fisher’s exact test. Values were considered to be significantly different at $P<0.05$.

**Results**

**Effects of diet at equilibrium**

Under control isovolumic conditions at equilibrium, compared with the saturated fat (Fig 1a) and (n-6) PUFA hearts (Fig 1b), the (n-3) PUFA hearts exhibited significantly lower coronary flow ($P<0.001$) (Fig 1c), spontaneous heart rate (Fig 2c) that was $\geq 20$ beats $\text{min}^{-1}$ lower ($P<0.01$) than SF (Fig 2a) or (n-6) PUFA (Fig 2b) and $\geq 30$ mmHg greater developed pressure (Fig 1f) ($P<0.01$) compared to SF (Fig 1d) or (n-6) PUFA (Fig 1e). These heart functions were not significantly different between SF and (n-6) PUFA hearts. The maximum rate of ventricular relaxation was significantly greater in (n-3) PUFA (Fig 4c) ($p<0.01$) than the SF (Fig 4a) or (n-6) PUFA hearts (Fig 4b). The end diastolic pressure, which was initially set at 6-8 mmHg in all hearts, was significantly lower in the (n-3) PUFA hearts (Fig 3c) ($P<0.01$) after equilibration than in SF (Fig 3a) and (n-6) PUFA hearts (Fig 3b). The maximum rate of ventricular pressure development (Fig 4a-c) and the rate-pressure product, which is the product of heart rate and systolic pressure (data not shown), were not significantly different between dietary groups at equilibrium.

**Effects of Ischemic Preconditioning on Heart Function at Equilibrium**

After three 5 min periods of global ischemic preconditioning, ventricular developed pressure increased significantly in the SF (Fig 1a) and (n-6) PUFA hearts (Fig 1b) ($P<0.05$), as did maximum rate of relaxation (Fig 4a,b) ($P<0.05$). End diastolic pressure
was significantly lower (Fig 3a,b) (P<0.05) compared to control SF and (n-6) PUFA hearts at equilibrium. Coronary flow and spontaneous heart rate tended to be lower but the differences did not reach significance. There were no significant differences between ischemia-preconditioned and control hearts in the (n-3) PUFA group (P>0.05) for any measure of heart function.

**Effects of Regional Ischemia and Reperfusion**

Occlusion of the left anterior descending coronary artery reduced the coronary flow producing regional ischemia in all hearts (Fig 1a-c). Measures of heart function, left ventricular developed pressure (Fig 1d-f), heart rate (Fig 2), rate pressure product (data not shown) and maximum rates of pressure development and relaxation (Fig 4) were significantly reduced in all hearts during ischemia (P<0.001). End diastolic pressure rose significantly 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 pre-occlusion level across all diets, then returned gradually towards the equilibrium value over time (Fig 1a-c). Measures of heart function including: heart rate (Fig 2), left ventricular developed pressure (Fig 1d-f), rate pressure product (data not shown) and maximum rates of pressure development and relaxation (Fig 4), all returned towards equilibrium values after reperfusion. The end diastolic pressure, which was elevated during ischemia, remained significantly elevated for the entire 120-min of reperfusion in all groups (Fig 3) (P<0.001).

Cardiac arrhythmias were common during ischemia, with 59% and 41% of all control hearts exhibiting ventricular tachycardia or ventricular fibrillation respectively. All
episodes of VT and VF spontaneously reverted to sinus rhythm. On reperfusion, ventricular tachycardia and ventricular fibrillation occurred in 63% and 37% of hearts respectively (Table 2).

**Effects of diet on responses to Ischemia and Reperfusion**

Coronary artery occlusion reduced coronary flow by a similar percentage in all dietary groups, leaving residual flows of 78.2 ± 2.1% (SF); 70 ± 3.1%,((n-6) PUFA); and 71 ± 2.2% ((n-3) PUFA) in each of the dietary control groups. During ischemia and reperfusion, the (n-3) PUFA control hearts exhibited significantly lower coronary flow (Fig 1c), significantly lower spontaneous heart rate (Fig 2c), significantly higher developed pressure (Fig 1d) compared with the SF and (n-6) PUFA control hearts (P<0.01), which were not significantly different to each other. The maximum rate of ventricular relaxation was also greater in (n-3) PUFA than the SF and (n-6) PUFA groups (Fig 4) (P<0.01). The end diastolic pressure, which did become elevated during ischemia, remained significantly lower in the (n-3) PUFA hearts during ischemia and reperfusion (Fig 3c) than in SF (Fig 3a) or (n-6) PUFA hearts (Fig 3b) (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 both ischemia and reperfusion was significantly lower in (n-3) PUFA hearts than in the SF hearts (P<0.05) (Table 2). The duration of arrhythmia episodes in (n-3) PUFA hearts also tended to be lower but the very low occurrence prevented statistical comparison. The cumulative arrhythmia score was significantly 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 n-6 PUFA arrhythmia score was significantly lower than SF in ischemia (Table 2)
Effects of Preconditioning on responses to Ischemia and Reperfusion

Ischemic-preconditioning significantly influenced cardiac function during reperfusion with lower coronary flow (P<0.05) (Fig 1), heart rate (P<0.05) (Fig 2) and a reduced rise in end diastolic pressure (P<0.05) (Fig 3). Developed pressure (P<0.05) (Fig 1) and maximum rate of ventricular relaxation were also higher in reperfusion after preconditioning (P<0.05) (Fig 4). These changes were observed in the SF and (n-6) PUFA hearts but not in the (n-3) PUFA hearts. For example, the developed pressure remained higher during ischemia in preconditioned SF and (n-6) PUFA hearts compared to control hearts and recovered to a level not significantly different to the equilibrium levels during reperfusion. There were no significant differences in developed pressure during ischemia or reperfusion between preconditioned and control (n-3) PUFA hearts (P>0.05). The incidence of arrhythmias VT and VF in ischaemia or reperfusion tended to be lower after preconditioning but did not reach significance (P=0.27-0.32). The episodes of VT or VF were of shorter duration (P<0.05) following preconditioning, and cumulative arrhythmia scores were significantly lower in both ischemia (P<0.05) and reperfusion (P<0.05) (Table 2).

Effects of Diet and Preconditioning on Infarct size

The infarct size as percent ischemic zone at risk was significantly smaller in (n-3) PUFA hearts than in SF or (n-6) PUFA hearts (P<0.05) (Fig 5). There were no significant differences in infarct size between (n-6) PUFA and SF hearts. There was no difference in the zone at risk between groups (data not shown), indicating that the degree of ischemia was equivalent.

Ischemic-preconditioning significantly reduced infarct size in SF (P<0.05) and (n-6) PUFA hearts (P<0.05) representing approximately 35% reduction compared with
control hearts. There were no significant differences in infarct size between preconditioned and control (n-3) PUFA hearts (P>0.05)(Fig 5).
Discussion

The present study demonstrates that dietary (n-3) PUFA fish oil protects the rat heart against myocardial infarction when hearts are subjected to occlusion of a major coronary artery, in a manner simulating an acute heart attack. Mechanistically this cardioprotection must occur within the myocardium following fatty acid incorporation into cellular membranes, since neither blood platelets nor fatty acids were circulating in the isolated heart perfusate (21). The 6-week feeding period undertaken in the present study exceeds the two to four weeks feeding required for the level of the major myocardial (n-3) PUFA, DHA to reach equilibrium within cellular membranes (19). Previous indirect evidence for (n-3) PUFA limitation of lethal myocellular injury, through the reduced release of cellular markers of damage in ischemia such as creatine kinase (8,22,23) was directly established in this study. Reduced infarct size is regarded as the ultimate indicator of cardioprotection by ischemic preconditioning (24) and in this study ischemic preconditioning also significantly reduced infarct size after ischemia-reperfusion in SF and (n-6) PUFA hearts. However, while infarction was equally inhibited by ischemic preconditioning or fish oil feeding, the infarct size was not further reduced by the concurrent imposition of ischemic preconditioning in (n-3) PUFA hearts. Fish oil feeding therefore appears to produce “dietary” or “nutritional” preconditioning equivalent to ischemic preconditioning in terms of infarct protection.

In the early stages of regional myocardial ischemia, before myocellular damage becomes fatal (infracted), heart function (especially relaxation and filling of diastolic heart function) becomes depressed. Ventricular end diastolic pressure gradually rises as hearts become stiff and less amenable to relaxation, and a high proportion of hearts become arrhythmic. Reduced rates of pressure fall after each beat and elevated end
diastolic pressure between heart beats are indicative of poor ability of the heart to relax and fill. However, in (n-3) PUFA hearts, the characteristic ischemia-induced rise in end diastolic pressure was less pronounced, arrhythmias were largely absent, and functional recovery during subsequent reperfusion was enhanced relative to SF and (n-6) PUFA hearts. Similarly, in ischemic preconditioned hearts, the end diastolic pressure remained low during ischemia and reperfusion and functional recovery was enhanced, but this was only evident in the SF and (n-6) PUFA hearts. These findings show that while both (n-3) PUFA and ischemic preconditioning protected the heart against the adverse effects of prolonged ischemia, the effects were not additive or synergistic. Indeed, in terms of arrhythmia generation, diastolic relaxation and end diastolic pressure, fish oil feeding produced greater cardioprotection than acute ischemic preconditioning alone. The isolated perfused hearts were free of circulating fatty acids throughout the in vitro evaluation of cardiac function, indicating that the fish oil effects were dependent on the prior incorporation of the (n-3) fatty acids into cellular membranes (6-8,22).

Ischemic preconditioning reduces the severity of reperfusion arrhythmias in the rat heart (13) and enhances the recovery of contractile function of the myocardial region-at-risk in rabbits (11). Importantly, we can demonstrate that neither the effects of nutritional- nor ischemic-preconditioning were attributable to differences in degree of ischemia, or variations in collateral flow, since all hearts exhibited equivalent reductions in coronary blood flow during the regional ischemia. This implied a similar ischemic region size in all hearts, which was later confirmed in stained slices. Paradoxically, both the (n-3) PUFA diet and ischemic-preconditioning reduced coronary flow at rest and during reperfusion, indicative of reduced myocardial oxygen delivery. When considered together with the maintained or enhanced contractile function, this suggests improved energy efficiency.
Both dietary fish oil (8,22) and ischemic preconditioning (14,25) have previously been shown to reduce myocardial oxygen consumption of the heart.

The mechanism of the antiarrhythmic effects (observed with either ischemic preconditioning (12,13) or dietary fish oil in isolated hearts, in whole animals and in human studies(6,8,26-28)) is still not clear. It has been suggested that ischemic preconditioning may attenuate electrophysiological effects associated with calcium-overload during ischemia and reperfusion and thereby protect against arrhythmias (29) and mitochondrial damage (30). Reduced intracellular calcium overload is also implicated in the cardiovascular protective effects of fish oil, affecting both arrhythmias and mitochondrial function (8,31). Modulation of the slowed maximum rate of relaxation and elevated end diastolic pressure by either ischemic preconditioning or dietary fish oil in the present study also suggests altered intracellular calcium handling during ischemia (32). These findings support the suggestion that both ischemic and nutritional preconditioning might decrease or delay calcium overload by affecting calcium influx, efflux or intracellular redistribution.

Further support for the involvement of calcium handling in the cardioprotective effects of ischemic preconditioning and (n-3) PUFA is provided by the low resting heart rate observed in (n-3) PUFA hearts in this study and that seen in the human heart in association with regular fish intake (33). Low heart rate is associated with reduced cardiovascular risk, including reduced risk of sudden death (34). Although heart rate may be influenced by many physiological mechanisms, evidence is accumulating that (n-3) PUFA modulate calcium channels(28). The in vitro nature of the present study isolates these effects from changes in autonomic nervous function or peripheral vascular effects that could contribute in vivo to reduce heart rate.(35).
The low coronary flow observed in (n-3) PUFA hearts occurring without detriment to cardiac function concurs with the low oxygen demand and high coronary flow reserve of those hearts (8,22). Underlying changes in mitochondrial metabolism may contribute to more efficient oxygen utilization in (n-3) PUFA hearts. Increased energy utilisation efficiency is similarly evident after ischemic preconditioning (14,25). Reduced rates of mitochondrial respiration are seen in hearts from fish oil fed rats (31) and membrane fatty acid modulation may modify a number of intracellular and membrane events. Thus, dietary fat modulation of calcium handling through altered cardiac membrane composition may affect oxygen use and arrhythmia vulnerability (8).

Since discovery of the powerful protective effects of ischemic preconditioning in animals and humans, research has focussed on identification of intracellular mediators and development of pharmacological mimetic agents to provide a therapeutic approach that might reliably protect the heart from ischemic insult (36,37). Examples of ischemic preconditioning mimetic agents include adenosine, adenosine agonists, protein kinase C agonists, K$_{ATP}$ channel openers, and NO donors (38). These agents are effective when given just prior to an ischemic episode to produce a genuine preconditioning effect, however, the need for administration of such agents immediately ahead of the onset of ischemia is a problem for clinical application, since the occurrence of an acute out of hospital ischemia event is rarely predictable. Such agents are also largely non-specific for myocardium and may produce unwanted side-effects unless administered by direct cardiac infusion. Dietary fish oil on the other hand, has long been classified by the US-FDA as “generally regarded as safe” (GRAS) and has a history of safe consumption associated with both primary and secondary cardioprotection. Fish oils are rich sources of the long chain (n-3) (or omega-3) PUFA; eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Because eating fish oil leads
to sustained elevated incorporation of DHA into myocardial membrane phospholipids (39), the evidence of cardioprotection persisting for periods of feeding ranging from 6 weeks in the present study and for longer periods ranging from four to 30 months (8,6,40) with regular fish or fish oil consumption, the (n-3) PUFA can be present at all times prior to any unexpected ischemic episode.

Dietary fish oil induces changes in membrane fatty acid composition that are sustained with regular consumption and produces cardioprotection that appears similar to ischemic preconditioning that by virtue of the continuous presence of (n-3) PUFA in myocardial membranes is not reliant on prediction of imminent ischemic episodes. Therefore, in light of its cardioprotective effects to reduce the consequences of ischemic events in the human population when a regular part of the diet (7,8,41), the (n-3) PUFA provided by dietary fish or fish oils may be effective “nutritional preconditioning” agents. Unlike pharmacological mimicking of ischemic preconditioning (3,42), nutritional preconditioning would require no prediction or prior knowledge of ischemic events and could thus represent a low-risk solution to ischemic cardioprotection.

Over the past decade or more, the protective cardiovascular effects of fish consumption have often been described and are now firmly established (43). Human intervention trials in post-infarction patients demonstrate reduced mortality with regular intake of fish or fish oil, without changes in blood pressure, blood lipids or, most significantly, without reductions in new cardiac events (44). These observations support a preconditioning-like effect, rather than the more obvious reduction in coronary atherosclerosis and prevention of new ischemic episodes. Nutritional preconditioning by membrane incorporation of (n-3) fatty acids may
underpin the low cardiovascular morbidity and mortality associated with regular fish and fish oil consumption.
**Literature cited**


20. Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th ed. National Health and Medical Research Council, Canberra, 2004 pp84


Fig 1
Influence of dietary supplements and Ischemic Preconditioning (IP) on coronary flow (a, b, c) and left ventricular developed pressure (d, e, f) during 30min ischemia and 120min reperfusion in rat isolated, spontaneously beating hearts. Filled symbols: control groups, open symbols: preconditioned groups. (a,d) saturated fat diet: ●○; (b,e) (n-6) PUFA diet: ▲∆; (c,f) (n-3) PUFA diet ■□. Filled bar on time-axis shows 30min ischemia duration. Results are expressed as mean ± SEM. n=6-9. Ischemic preconditioning vs control within dietary group (* P < 0.05). (n-3) PUFA vs saturated fat and (n-6) PUFA dietary groups (# P < 0.01).

Fig 2
Influence of dietary supplements and Ischemic Preconditioning on heart rate during 30min ischemia and 120min reperfusion in rat isolated, spontaneously beating hearts. Filled symbols: control groups, open symbols: preconditioned groups. (a) saturated fat diet: ●○; (b) (n-6) diet: ▲∆; (c) (n-3) diet ■□. Filled bar on time-axis shows 30min ischemia duration. Results are expressed as mean ± SEM. n=6-9. Ischemic preconditioning vs control within dietary group (* P < 0.05). (n-3) PUFA vs saturated fat and (n-6) PUFA dietary groups (# P < 0.01).

Fig 3
Influence of dietary supplements and Ischemic Preconditioning on left ventricular end diastolic pressure (EDP) during 30min ischemia and 120min reperfusion in rat isolated spontaneously beating hearts. Filled symbols: control groups, open symbols: preconditioned groups. (a) saturated fat diet: ●○; (b) (n-6) diet: ▲∆; (c) (n-3) diet ■□. Filled bar on time-axis shows 30min ischemia duration. Results are expressed as mean ±
SEM. n=6-9. Ischemic preconditioning vs control within dietary group (* P < 0.05). (n-3) PUFA vs saturated fat and (n-6) PUFA dietary groups (# P < 0.01).

Fig 4
Influence of dietary supplements and Ischemic Preconditioning on maximum rate of contraction +dP/dt\textsubscript{max} and maximum rate of relaxation –dP/dt\textsubscript{max} during 30min ischemia and 120min reperfusion in rat isolated spontaneously beating hearts. Filled symbols: control groups, open symbols: preconditioned groups. (a,d) saturated fat diet: ● ○ ; (b,e) (n-6) diet: ▲ △; (c,f) (n-3) diet ■ □. Filled bar on time-axis shows 30min ischemia duration. Results are expressed as mean ± SEM. n=6-9. Ischemic preconditioning vs control within dietary group (* P < 0.05). (n-3) PUFA vs saturated fat and (n-6) PUFA dietary groups (# P < 0.01).

Fig 5
Influence of dietary supplements and Ischemic Preconditioning (IP) on infarct size after 30min ischemia and 120min reperfusion in rat isolated hearts. Speckled bars: control groups, square hatched bars: preconditioned groups. Results are expressed as mean ± SEM. n=6. Ischemic preconditioning vs control within dietary group (* P < 0.05). (n-3) PUFA vs saturated fat and (n-6) PUFA dietary groups (# P < 0.05).

*: Significant difference to same dietary group preconditioned (p<0.05).
Table 1.

Fatty acid composition of rat diets

<table>
<thead>
<tr>
<th></th>
<th>(n-3) PUFA</th>
<th>(n-6) PUFA</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>% FO</td>
<td>7.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% SSO</td>
<td>5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% beef tallow</td>
<td></td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>% OO</td>
<td>3.0%</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td>g/kg of diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>2.1</td>
<td>0</td>
<td>2.6</td>
</tr>
<tr>
<td>16:0</td>
<td>17.1</td>
<td>8.1</td>
<td>20.6</td>
</tr>
<tr>
<td>16:1</td>
<td>3.4</td>
<td>0.4</td>
<td>3.2</td>
</tr>
<tr>
<td>18:0</td>
<td>4.5</td>
<td>3.6</td>
<td>14.1</td>
</tr>
<tr>
<td>18:1 (OA)</td>
<td>33.5</td>
<td>50.0</td>
<td>47.9</td>
</tr>
<tr>
<td>18:2 n-6 (LA)</td>
<td>3.5</td>
<td>36.0</td>
<td>4.7</td>
</tr>
<tr>
<td>18:3 n-3 (LNA)</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>20:4 n-6 (AA)</td>
<td>1.3</td>
<td>n.d</td>
<td>0.1</td>
</tr>
<tr>
<td>20:5 n-3 (EPA)</td>
<td>4.9</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>22:5 n-3 (DPA)</td>
<td>0.8</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>22:6 n-3 (DHA)</td>
<td>20.2</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>Total sat</td>
<td>25.5</td>
<td>12.6</td>
<td>38.1</td>
</tr>
<tr>
<td>Total Mono</td>
<td>39.2</td>
<td>50.6</td>
<td>51.4</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>31.4</td>
<td>36.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Total (n-6) PUFA</td>
<td>5.0</td>
<td>36.2</td>
<td>4.8</td>
</tr>
<tr>
<td>Total (n-3) PUFA</td>
<td>26.4</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>P:S ratio</td>
<td>1.14</td>
<td>3.18</td>
<td>0.25</td>
</tr>
<tr>
<td>(n-6):(n-3) PUFA ratio</td>
<td>0.19</td>
<td>74</td>
<td>8.3</td>
</tr>
</tbody>
</table>

P:S ratio = polyunsaturated to saturated fatty acid ratio
Table 2. Influence of diet and ischaemic preconditioning on cardiac arrhythmias in ischemia and reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Diet</th>
<th>n</th>
<th>%VT</th>
<th>Duration of VT (s)</th>
<th>%VF</th>
<th>Duration of VF (s)</th>
<th>AS</th>
<th>n</th>
<th>%VT</th>
<th>Duration of VT (s)</th>
<th>%VF</th>
<th>Duration of VF (s)</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SF</td>
<td>9</td>
<td>89</td>
<td>39.6 ±5.5</td>
<td>78</td>
<td>38.7 ±7.1</td>
<td>4.8 ±0.4</td>
<td>9</td>
<td>67</td>
<td>*24.0 ± 2.1</td>
<td>44</td>
<td>12.7 ±0.8</td>
<td>*3.5 ±0.2</td>
</tr>
<tr>
<td>(n-6) PUFA</td>
<td>9</td>
<td>67</td>
<td>18.5 ±3.4</td>
<td>44</td>
<td>12.3 ±3.4</td>
<td>#3.2 ±0.3</td>
<td>9</td>
<td>44</td>
<td>*7.8 ± 0.8</td>
<td>33</td>
<td>1.3 ±0.3</td>
<td>*2.1 ±0.3</td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>(n-3) PUFA</td>
<td>9</td>
<td>#23</td>
<td>#9.0 ±7</td>
<td>#0</td>
<td># n.d.</td>
<td>†2.0 ±0.0</td>
<td>9</td>
<td>#11</td>
<td>*2.0 ± 0.0</td>
<td>0</td>
<td>* n.d.</td>
<td>†1.0 ±0.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>27</td>
<td>59</td>
<td>27.8 ±4.3</td>
<td>41</td>
<td>28.0 ±6</td>
<td>3.8 ±0.3</td>
<td>27</td>
<td>41</td>
<td>*16.0 ± 3.0</td>
<td>30</td>
<td>*7.0 ±2.2</td>
<td>*3.0 ±0.2</td>
</tr>
<tr>
<td></td>
<td>SF</td>
<td>9</td>
<td>88</td>
<td>20.1 ±4</td>
<td>67</td>
<td>29.8 ±6</td>
<td>4.8 ±0.5</td>
<td>9</td>
<td>56</td>
<td>*14.0 ± 1.8</td>
<td>44</td>
<td>*9.8 ±2.4</td>
<td>*3.0 ±0.2</td>
</tr>
<tr>
<td>(n-6) PUFA</td>
<td>9</td>
<td>77</td>
<td>18.8 ±0.7</td>
<td>33</td>
<td>16.0 ±4.5</td>
<td>3.7 ±0.4</td>
<td>9</td>
<td>56</td>
<td>*6.4 ± 1.2</td>
<td>22</td>
<td>*7.5 ±2.5</td>
<td>*2.0 ±0.2</td>
<td></td>
</tr>
<tr>
<td>Reperfusion</td>
<td>(n-3) PUFA</td>
<td>9</td>
<td>#22</td>
<td>#2.5 ±0.5</td>
<td>#11</td>
<td># 5.0</td>
<td>†2.0 ±0.0</td>
<td>9</td>
<td>11</td>
<td>#4.0 ± 0.0</td>
<td>11</td>
<td>#4.0 ±0.0</td>
<td>†1.0 ±0.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>27</td>
<td>63</td>
<td>17.5 ±2.3</td>
<td>37</td>
<td>23.2 ±4.6</td>
<td>3.9 ±0.3</td>
<td>27</td>
<td>41</td>
<td>*9.6 ± 1.5</td>
<td>26</td>
<td>*8.2 ±1.6</td>
<td>*2.3 ±0.2</td>
</tr>
</tbody>
</table>

Incidence and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF), and on arrhythmia score (AS) during 30min ischemia or 120min reperfusion in rat isolated spontaneously beating hearts.

SF: saturated fat diet; (n-6) PUFA: (n-6) polyunsaturated fatty acid diet; (n-3): (n-3) polyunsaturated fatty acid diet; %: percent of animals. Duration of VT or VF represents the total duration in seconds of all episodes that occurred in each heart. Hearts with no VT or VF episodes could not be included for duration analysis. Results are expressed as mean ± SEM or % incidence. n=9 per dietary group except durations, where n= 0-8.n.d. not detected. * Significantly different Ischemic Preconditioning vs control within dietary group (P <
0.05). # Significantly different to SF (P < 0.05). † Significantly different to SF and (n-6) PUFA (P < 0.05). ø: incidence of VT or VF too low to conduct statistical analysis on duration;
Figure 1.

Figure 2.

Figure 3
Figure 4.

Figure 5