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The cardiovascular protective role of docosahexaenoic acid

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Abstract

Dietary fish oils rich in *n*–3 polyunsaturated fatty acids can modulate a diverse range of factors contributing to cardiovascular disease. This study examined the relative roles of eicosapentaenoic acid (20:5 *n*–3; EPA) and docosahexaenoic acid (22:6 *n*–3; DHA) which are the principal *n*–3 polyunsaturated fatty acids regarded as candidates for cardioprotective actions. At low dietary intakes (0.4–1.1% of energy (%en)), docosahexaenoic acid but not eicosapentaenoic acid inhibited ischaemia-induced cardiac arrhythmias. At intakes of 3.9–10.0%en, docosahexaenoic acid was more effective than eicosapentaenoic acid at retarding hypertension development in spontaneously hypertensive rats (SHR) and inhibiting thromboxane-like vasoconstrictor responses in aortas from SHR. In stroke-prone SHR with established hypertension, docosahexaenoic acid (3.9–10.0%en) retarded the development of salt-loading induced proteinuria but eicosapentaenoic acid alone was ineffective. The results demonstrate that purified *n*–3 polyunsaturated fatty acids mimic the cardiovascular actions of fish oils and imply that docosahexaenoic acid may be the principal active component conferring cardiovascular protection.

Keywords: Dietary fat; *n*–3 fatty acid; Arrhythmia; Blood pressure; Proteinuria; Thromboxane

1. Introduction

Evidence from human epidemiological studies and experimental animal studies suggests that the regular consumption of fish or fish oil can reduce morbidity and mortality from cardiovascular disease. Numerous potential contributary influences on the cardiovascular system have been proposed (Leaf and Weber, 1988; Kinsella et al., 1990; Israel and Gorlin, 1992). The protective effects reported for fish oils include the ability to prevent or suppress experimentally induced cardiac arrhythmias (McLennan et al., 1988, 1993; Hock et al., 1990; Pepe and McLennan, 1996), retard the development of excessive blood pressure in the spontaneously hypertensive rat (Schoene and Fiore, 1981; Mtabaji et al., 1988; Karanja et al., 1989; Singer et al., 1990; Howe et al., 1991a; Bexis et al., 1994), lower blood pressure in humans (Norris et al., 1986; Knapp and FitzGerald, 1989; Bonaa et al., 1990; Howe, 1995), markedly decrease the potential for the production of thromboxane in the circulation (Knapp and FitzGerald, 1989; Abeywardena et al., 1991) enhance endothelial cell mediated vascular relaxation and reverse impaired relaxation (Yin et al., 1991; Bexis et al., 1994) and provide some degree of protection against a wide range of nephropathies (Donadio, 1991; DeCaterina et al., 1994; Fujikawa et al., 1994).

It is widely accepted that the *n*–3 polyunsaturated fatty acids are the active constituents of fish oils. Eicosapentaenoic acid (EPA, 20:5 *n*–3) and docosahexaenoic acid (DHA, 22:6 *n*–3) which are both present in varying proportions in all fish oils, are the major *n*–3 fatty acids. Most commercial oils contain more eicosapentaenoic acid than docosahexaenoic acid. This predominance of eicosapentaenoic acid and its prominent role in eicosanoid metabolism as a substrate for cyclooxygenase producing biologically active prostacyclin but inactive thromboxane has put it to the fore as the putative active component of fish and fish oils (Leaf and Weber, 1988). To date, little evidence can be provided, however, to confirm either or both fatty acids as the principal active component.

In the present study we used purified fatty acid esters of eicosapentaenoic acid and docosahexaenoic acid in com-

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parative dietary experiments to determine whether the proposed cardiovascular protective roles could be demonstrated with either fatty acid alone and to compare their relative effectiveness. Test systems included the ability to suppress ischaemia-induced arrhythmias, to retard the development of hypertension in the spontaneously hypertensive rat, to offset thromboxane constrictor responses in isolated blood vessels and to reduce urinary protein excretion in an animal model of hypertension induced renal failure.

2. Materials and methods

2.1. General procedures

Male rats of the Hooded Wistar, spontaneously hypertensive (SHR), or stroke-prone spontaneously hypertensive (SHR-SP) strains were used in the study. All rats were obtained from the CSIRO breeding colony and, as indicated below, randomly assigned to fabricated diets containing test fatty acids.

High purity ethyl esters of eicosapentaenoic acid (95%) and docosahexaenoic acid (95%) and an *n*–3 mix containing 26% eicosapentaenoic acid + 37% docosahexaenoic acid ethyl esters, all with vitamin E contents of 3 $mg \cdot g^{-1}$ of oil were provided for this study by Hoffmann-La Roche, Basel, Switzerland. The purified fatty acids were incorporated into diets at the levels indicated below for the specific experiments. All diets contained 5% total fat which represented 11.65% of available energy (%en) as fat. The diets additionally contained 17.5% protein and 68% carbohydrate. The detailed composition, including complete vitamin and mineral components, was as previously published (Rayner and Howe, 1995). The total vitamin E content of the diets for the arrhythmia studies was 65 $mg \cdot kg^{-1}$ diet. The vitamin E content of diets for all of the remaining studies was 135 $mg \cdot kg^{-1}$. In selected experiments involving the influence of fatty acids on blood pressure, a comparison was also made with diets using an *n*–3 ethyl ester concentrate containing 38% eicosapentaenoic acid (20;5) and 29% docosahexaenoic acid (22;6) w/w (NIH/NOAA Biomedical Test Material Program, NMFS Southeast Fisheries Science Center, Charleston, USA). Olive oil was used throughout as a dietary control. In all cases, diets were consumed ad libitum for the periods indicated below for individual experiments. Body weights were measured at the start and completion of the dietary experiments. The procedures adopted for the use of animals in the experiments described were approved by the CSIRO Division of the Human Nutrition Animal Experimentation Ethics Committee.

2.2. Ischaemia-induced arrhythmias

Hooded Wistar rats were fed synthetic diets for 5 weeks. Groups of ten rats were fed the olive oil control diet (containing 5% olive oil as the sole source of fat) or a diet containing either 0.5% docosahexaenoic acid, 0.5% eicosapentaenoic acid, or 0.5% *n*–3 eicosapentaenoic acid + docosahexaenoic acid mix, with the remainder of fat (4.5%) provided by olive oil. The choice of 0.5% test fatty acid was based on preliminary evidence which suggested this level could reproducibly prevent arrhythmias. The individual fatty acid diets contained 1.11%en as docosahexaenoic acid or eicosapentaenoic acid while the mix diet contained 0.43%en as docosahexaenoic acid + 0.30%en as eicosapentaenoic acid. The diets were prepared as summarised in Table 1. The animals were fed ad libitum up to a maximum of 450 mg/kg body weight/day with the test fatty acids. This value is based on a normal consumption of 30 g/rat/day by animals weighing 300–350 g.

A common surgical procedure was used to induce myocardial ischaemia and has been described by us previously (McLennan et al., 1988). The rats were fasted overnight, anaesthetised with pentobarbitone sodium (60 mg/kg i.p.) and artificially ventilated with room air by an endotracheal tube. Blood pressure was monitored via a catheter in the left femoral artery. A left thoracotomy was performed, the heart exteriorised, and a silk ligature placed through the muscle surrounding the left anterior descending coronary artery. The heart was returned to the thoracic cavity. After a 10 min equilibration period the suture was tightened to produce regional myocardial ischaemia. Regional myocardial ischaemia produced a characteristic pattern of cardiac arrhythmias which commonly commenced after 5 min, peaked at 10 min, and usually subsided by 15 min. In that time, arrhythmias included isolated extrasystoles, patterns of extrasystoles, runs of tachycardia, and bursts of ventricular fibrillation (which may be sustained [fatal]). Arrhythmias were identified from lead 1 and lead 2 of an ECG and blood pressure traces. They were quantified according to a Gaussian-distributed arrhythmia score based on incidence and severity of arrhythmias in each animal as we have described previously (McLennan et al., 1988). The score awards points on a hierarchical scale of 0–9. A score of 0–5 represents increasing degrees of non-fatal arrhythmias. A score of 6–9 represents the occurrence of fatal ventricular fibrillation of progressively earlier onset.

2.3. Blood pressure – established hypertension

SHR rats aged 4 months were fed the synthetic diet for a period of 6 weeks. The diet groups consisted of: olive oil control (5% olive oil), 4.5% eicosapentaenoic acid + 0.5% safflower oil, and 4.5% docosahexaenoic acid + 0.5% safflower oil. The safflower oil was used to avoid essential fatty acid deficiency. The level of fatty acids was based on the effective doses determined in our previous studies (Bexis et al., 1994). The docosahexaenoic acid and eicosapentaenoic acid diets contained 9.96%en as the individual *n*–3 fatty acids. At the end of the diet feeding period indirect blood pressure was measured by a photoelectric

tail-cuff procedure (IITC, Life Sciences, Woodlands Hills, CA, USA) as described previously (Bexis et al., 1994).

2.4. Blood pressure – developmental phase of hypertension

SHR rats at 4 weeks of age (before the development of hypertension) were fed the synthetic diets described above for the established hypertensive group for a period of 12 weeks. An additional group was fed a diet containing 4.5% *n*–3 ethyl ester concentrate (29% docosahexaenoic acid and 38% eicosapentaenoic acid; NIH/NOAA Biomedical Test Materials Program, NMFS South East Fisheries Science Center, Charleston, USA) plus 0.5% safflower oil. The NIH oil diet contained 3.04%en docosahexaenoic acid and 3.98%en eicosapentaenoic acid. At the end of the 12-week period indirect blood pressure was measured by a Doppler tail-cuff procedure (Model 812, Parkes Medical Electronics, Aloha, OR, USA).

2.5. Non-endothelial cell vascular thromboxane production

The SHR animals used for the blood pressure experiments described above were also used in this study. After the blood pressure measurement, the animals were killed and the thromboxane constrictor responses generated in thoracic aortic ring preparations after inhibition of nitric oxide production were determined as we have described previously (Dyer et al., 1994). Aortic rings 3 mm in length were suspended in an organ bath chamber in 15 ml Krebs-Henseleit solution maintained at 37°C, and aerated with a mixture of 95% O_2 and 5% CO_2. The Krebs-Henseleit solution comprised 113 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_2O$, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 0.57 mM ascorbic acid and 11.2 mM glucose in de-ionised water. The tissues were equilibrated for 60 min under a resting tension of 4 g. Isometric contractions were measured using an FTO3 transducer (Grass Medical Instruments, Quincy, MA, USA) and recorded on a WR3701 Linearcorder (Graphtec). The contraction generated after the addition of 10^{-4} M N^{ω}-nitro-L-arginine (NOLA) was measured as we have described previously (Dyer et al., 1994).

2.6. Urinary protein excretion

The urinary protein excretion in 16-week-old SHR-SP rats with established hypertension was measured at 6, 9, and 12 weeks after they had commenced an experimental diet containing 2% sodium and one of the test fatty acids. Eicosapentaenoic acid, docosahexaenoic acid or the eicosapentaenoic acid + docosahexaenoic acid mix (26% eicosapentaenoic acid and 37% docosahexaenoic acid, ethyl esters) were included in the diet at a concentration of 4.5% by weight together with 0.5% safflower oil as described for the hypertension studies above. The mix diet contained 2.73%en as eicosapentaenoic acid and 3.88%en as docosahexaenoic acid. The energy levels of the other diets were as for the blood pressure studies. The olive oil control diet contained 5% olive oil. In addition, a test group comprising 5% safflower oil was used. Animals were placed in metabolic cages and overnight (16 h) urine samples collected (onto an ice-chilled container) from each animal the week before starting the diets and thereafter as indicated. Total urinary protein content was determined using a Lowry protein assay as modified for use on a COBAS autoanalyser against a standard curve constructed with bovine serum albumin.

2.7. Statistical analysis

Data are presented as the mean ± S.E.M. Statistical analyses were performed using Student's *t*-test, analysis of variance (ANOVA) or repeat measures ANOVA, with Bonferroni modification for comparison of multiple means. Significance was accepted at the $P < 0.05$ level.

2.8. Drugs and reagents

The following drugs and reagents were used: (−)-Arterenol [(−)-norepinephrine bitartrate], N^{ω}-nitro-L-arginine (NOLA) and bovine serum albumin were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Norepinephrine and KCl were dissolved in saline containing 0.01% ascorbic acid. Phenobarbitone sodium (Nembutal) was purchased from Boehringer Ingelheim (Artarmon, New South Wales, Australia). Analytical grade chemicals were used for all laboratory work.

3. Results

3.1. Ischaemia-induced arrhythmias

Regional myocardial ischaemia induced by occlusion of the left anterior descending coronary artery stimulated arrhythmias in olive oil fed control Hooded Wistar rats (Fig. 1). Of the dietary polyunsaturated fatty acids tested, docosahexaenoic acid and the mix of eicosapentaenoic acid and docosahexaenoic acid significantly reduced the incidence and severity of ventricular arrhythmias as indicated by the arrhythmia score (Fig. 1) but eicosapentaenoic acid was without effect. Ventricular fibrillation which is a major component of that score and occurred in 80% of olive oil fed control animals was significantly inhibited in docosahexaenoic acid (20%) ($P < 0.03$ χ^2) and eicosapentaenoic acid + docosahexaenoic acid mix fed animals (10%) ($P < 0.01$ χ^2) but not in those animals fed the eicosapentaenoic acid diet (70%).

The myocardial membrane phospholipid fatty acids of olive oil control hearts included 9.7 ± 0.8% docosahexaenoic acid. Incorporation was significantly higher with

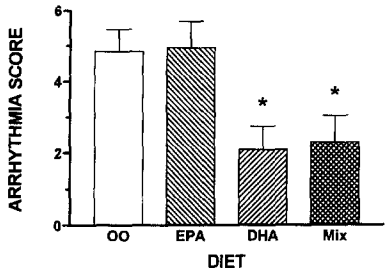

Fig. 1. Effect of dietary fatty acids on ischaemia-induced cardiac arrhythmias in Hooded Wistar rats. Myocardial ischaemia induced by in vivo coronary artery occlusion after 5 weeks feeding of fully fabricated diets with 5% w/w fat as olive oil or with partial replacement of olive oil by purified fatty acid ethyl esters (0.5% w/w) to a level of 150 mg·rat^{-1}·day^{-1}. OO: olive oil. EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid. Mix: blend of EPA + DHA. Mean ± S.E.M. Mean values were compared against the control group fed the olive oil diet. * $P < 0.02$, $n = 10$, ANOVA with Bonferroni correction for multiple comparisons of means.

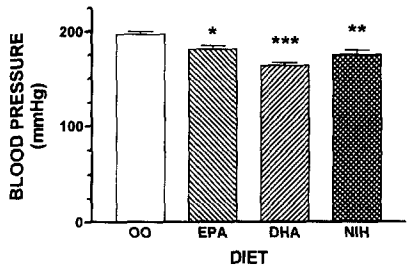

Fig. 3. The influence of purified eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) enriched diets (4.5% w/w) on blood pressure in young SHR during the development phase of hypertension. Animals were fed synthetic diets enriched in different fatty acids for 12 weeks before determination of blood pressure by a Doppler procedure as described in the text. Mean ± S.E.M. Mean values were compared against the control group fed the olive oil (OO) diet. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; $n = 8$, ANOVA, Bonferroni test for multiple comparison of means. NIH-NIH fish oil supplemented diet.

the docosahexaenoic acid diet (18.0 ± 0.8% $P < 0.001$ ANOVA), the eicosapentaenoic acid + docosahexaenoic acid mix diet (18.1 ± 0.9 $P < 0.001$ ANOVA) and with the eicosapentaenoic acid diet (14.7 ± 0.4% $P < 0.05$ ANOVA). Small changes in eicosapentaenoic acid incorporation (< 0.1% in OO control) were also recorded (docosahexaenoic acid diet 0.5 ± 0.1%, eicosapentaenoic acid + docosahexaenoic acid mix diet 0.7 ± 0.1%, eicosapentaenoic acid diet 1.9 ± 0.1%).

3.2. Established and developing hypertension

There were no significant differences in blood pressure between any of the diets as measured by indirect photoelectric (IITC) tail-cuff procedures in SHR with established hypertension (Fig. 2).

In contrast, the development of high blood pressure, as determined by indirect Doppler tail-cuff procedures in young SHR, was significantly retarded by feeding dietary eicosapentaenoic acid, NIH fish oil or docosahexaenoic acid (compared with olive oil controls). The docosahexaenoic acid diet was more effective than NIH oil, which in turn was more effective than eicosapentaenoic acid (Fig. 3). The predominant influence of docosahexaenoic acid in retarding the development of blood pressure in the SHR when compared to the other diets was also confirmed using the indirect photoelectric (IITC) tail-cuff procedure (results not shown).

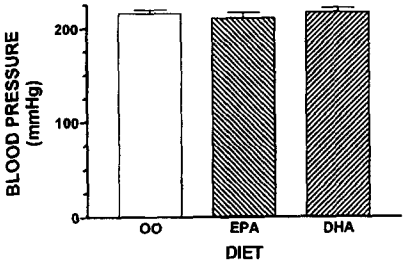

Fig. 2. The influence of purified eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) enriched diets (4.5% w/w) on blood pressure in adult spontaneously hypertensive rats with established hypertension compared to a control group fed an olive oil (OO) supplemented diet. Mean ± S.E.M. values are shown ($n = 8$; $P > 0.05$, ANOVA).

3.3. Non-endothelial cell vascular thromboxane constrictor response

Using adult SHR with established hypertension (Fig. 2), dietary administration of docosahexaenoic acid was found to significantly reduce the vascular, NOLA-induced, thromboxane-like contraction in isolated aortic ring preparations (Fig. 4). Dietary eicosapentaenoic acid also tended to inhibit this response but the effect was not statistically significant.

3.4. Urinary protein excretion

In SHR-SP with established hypertension, sodium loading (2% in diet) was associated with a dramatic and

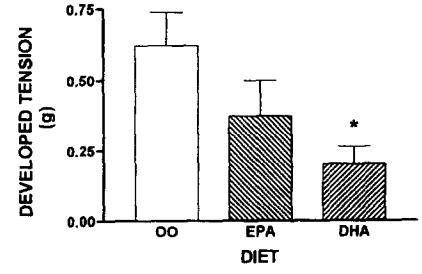

Fig. 4. The influence of purified eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) enriched diets (4.5% w/w) on tension developed in endothelium-intact aortic ring preparations from adult spontaneously hypertensive rats with established hypertension compared to the control group fed the olive oil (OO) diet. Mean ± S.E.M. values after incubation with NOLA (N^{ω}-nitro-L-arginine) are given. Mean values were compared against the OO control group. * $P < 0.05$; $n = 5$, ANOVA, Bonferroni test for multiple comparison of means.

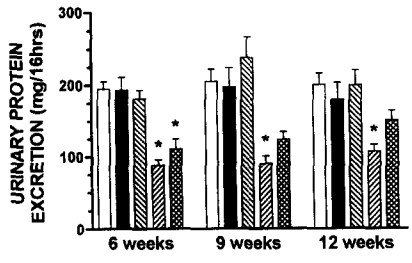

Fig. 5. Effect of dietary fatty acids on urinary protein excretion from salt-loaded, stroke-prone spontaneously hypertensive rats (SHRSP). SHRSP were salt-loaded at 4 months of age by the addition of 2% sodium to fully fabricated diets. Diets contained one of the following purified fatty acid ethyl esters (4.5% final concentration): eicosapentaenoic acid (3rd columns, $n = 8$), docosahexanoic acid (4th columns, $n = 7$), or a blend of eicosapentaenoic acid and docosahexaenoic acid (5th columns, $n = 8$) with control diets comprising 5% olive oil (1st columns, $n = 8$) or safflower oil (2nd columns, $n = 8$). Urine was collected overnight (16 h) onto ice and total protein content measured. Mean ± S.E.M. * $P < 0.05$, repeat measures ANOVA).

sustained increase in urinary protein excretion after 6 weeks in olive oil controls and safflower oil fed animals (Fig. 5). Dietary administration of docosahexaenoic acid or the docosahexaenoic acid + eicosapentaenoic acid mix retarded the increase in urinary protein excretion and the effect of docosahexaenoic acid alone was significantly sustained for a further 6 weeks. The eicosapentaenoic acid diet was associated with similar proteinuria to olive oil and safflower oil.

4. Discussion

This study has used highly concentrated sources of eicosapentaenoic acid and docosahexaenoic acid to confirm an active role of $n-3$ polyunsaturated fatty acids and to compare the relative activities of these fatty acids. Using a range of parameters associated with cardiovascular morbidity and mortality, the results of this study clearly show cardiovascular protective actions of the $n-3$ polyunsaturated fatty acids and strongly suggest that docosahexaenoic acid may be the principal active component of fish oils.

Previous studies have shown that docosahexaenoic acid is the major $n-3$ fatty acid incorporated into myocardial membranes following feeding of fish oils (Charnock et al., 1986; Pepe and McLennan, 1996), even those in which eicosapentaenoic acid predominates, suggesting an important role for docosahexaenoic acid in heart function. The finding that docosahexaenoic acid is effective as an antiarrhythmic is consistent with the results of our previous studies in which we have observed that the administration of fish oil (from several sources) is associated with a low incidence and severity of cardiac arrhythmias as well as a major incorporation of docosahexaenoic acid into cardiac tissue (McLennan et al., 1988, 1993; McLennan, 1993; Pepe and McLennan, 1996; Charnock et al., 1986). Most of the previous studies using fish oils have provided docosahexaenoic acid at levels between 3.8% and 5.4% of the available energy (%en) with two studies as low as 1.5%en (Charnock et al., 1991; McLennan et al., 1993). In this study we found that docosahexaenoic acid levels of 1.1%en (docosahexaenoic acid diet) and 0.4%en (eicosapentaenoic acid + docosahexaenoic acid mix diet, also including 0.3%en eicosapentaenoic acid) were both antiarrhythmic. Moreover an equivalent incorporation of docosahexaenoic acid into myocardial membranes was achieved with both docosahexaenoic acid-containing diets. Although docosahexaenoic acid incorporation was increased with the eicosapentaenoic acid diet containing 1.1%en as eicosapentaenoic acid, it was less than with the diets containing docosahexaenoic acid and the eicosapentaenoic acid diet did not prevent arrhythmias. Dietary fish oil has not been directly shown to reduce arrhythmias in man. However, Burr and coworkers (Burr et al., 1989) found that a modest intake of fish or fish oil is associated with reduced mortality in post-myocardial infarction patients. Such a population is recognised as a high risk group for ventricular fibrillation and sudden death. There were no differences in blood lipids, blood pressure or new cardiac events, consistent with an antiarrhythmic effect. Direct investigation in man is awaited with interest.

The demonstration of an effective role of docosahexaenoic acid in preventing arrhythmias, together with its ready disposition in cardiac tissue after dietary administration of fish oils, may be due to its ability to modulate intracellular Ca^{2+} overload in isolated cardiac myocytes (Hallaq et al., 1990, 1992; Pepe et al., 1994). Although eicosapentaenoic acid has been found to share the antiarrhythmic properties of docosahexaenoic acid in isolated neonatal myocytes (Hallaq et al., 1990) its failure to exhibit antiarrhythmic effects in the present study may reflect a threshold dose effect. The low dose of eicosapentaenoic acid fed to rats in this study produced only a small increase in membrane levels and although membrane incorporation of docosahexaenoic acid was increased to a greater extent it did not reach the level seen with docosahexaenoic acid or docosahexaenoic acid + eicosapentaenoic acid mix diets. It is evident, however, that a major proportion of eicosapentaenoic acid was metabolised either in the liver or in the heart to docosahexaenoic acid which is preferentially incorporated into myocardial membranes. A higher intake of purified eicosapentaenoic acid may produce a greater docosahexaenoic acid incorporation and a significant antiarrhythmic effect. A wide spectrum of potential chemical mediators has been implicated in ischaemic arrhythmias (Curtis et al., 1993). Amongst these factors, fish oils or fish oil fatty acids have been found to inhibit Ca^{2+} overload (Hallaq et al., 1990, 1992; Pepe et al., 1994), thromboxane production (Abeywardena et al., 1991), ischaemic acidosis and ischaemic K^+ loss (Pepe and McLennan, 1992).

In addition to its influence on cardiac rhythm disorders,

docosahexaenoic acid retarded the development of high blood pressure when fed to young pre-hypertensive SHR albeit at a higher dose (10.0%en as docosahexaenoic acid). This was consistent with the effects of dietary fish oils on hypertension development in SHR and SHR-SP models (Schoene and Fiore, 1981; Karanja et al., 1989; Singer et al., 1990; Howe et al., 1991a). Unlike the antiarrhythmic effects, however, the development of hypertension was also retarded by eicosapentaenoic acid. Nevertheless, there was a clear order of blood pressure retardation between the diets (docosahexaenoic acid diet > eicosapentaenoic acid + docosahexaenoic acid diet > eicosapentaenoic acid diet). Thus the presence of docosahexaenoic acid seemed to be most important, with eicosapentaenoic acid providing some effect if present at high enough concentrations. None of the *n*–3 rich diets modified the blood pressure in the adult SHR with already established hypertension. In man, fish oil produces small but significant blood pressure reductions (Knapp and FitzGerald, 1989) which are enhanced by sodium restriction (Howe, 1995). Similarly, the reduction of blood pressure in adult SHR is largely dependent on concomitant sodium restriction (Howe et al., 1991b) and sodium intake was not restricted in the present study. The mechanisms by which blood pressure may be reduced are not established. Although animal and human studies have shown fish oil inhibition of bioactive thromboxane (vasoconstrictor) and preservation of prostacyclin (vasodilator) production consistent with an eicosanoid mechanism, a direct relationship to blood pressure remains uncertain (Knapp and FitzGerald, 1989; Bexis et al., 1994).

We have previously identified a vascular constricting process in blood vessels of the SHR that is revealed only after the impairment of nitric oxide production (Dyer et al., 1994). This process is inhibited by agents that interfere with the production of thromboxane and by antagonists of the thromboxane receptor and can be regarded as thromboxane-like. While it could be speculated from known interactions with eicosanoid synthetic mechanisms that fish oils may interfere with this vascular contractile process, it has not previously been tested. The results of the present study showed that dietary pre-feeding with docosahexaenoic acid but not eicosapentaenoic acid reduced this thromboxane-like constrictor activity. This raises the possibility that docosahexaenoic acid may serve a role as a regulating lipid to prevent unwanted contraction generated through thromboxane and restore the constrictor/dilator balance following impairment of the normal functioning of the nitric oxide (relaxation) process. It remains to be determined whether docosahexaenoic acid is acting by inhibition of thromboxane synthetase (Abeywardena et al., 1991) or by inhibition of thromboxane A_2/prostaglandin H_2 receptor function (Swann et al., 1990). These dietary *n*–3 induced changes in aortic thromboxane-like activity were found in adult SHR in which blood pressure was not influenced by diet, once again highlighting an apparent lack of association between thromboxane production and hypertension. Nevertheless, such a mechanism may play a role in local vascular control at sites of endothelial damage.

It has been reported previously that fish oils may afford renal protection in models of renal disease other than hypertension (Donadio, 1991; DeCaterina et al., 1994; Fujikawa et al., 1994). In the present study we demonstrated that docosahexaenoic acid retarded the development and progression of proteinuria in the salt loaded SHR-SP with established hypertension but eicosapentaenoic acid was without effect. This effect occurred early and was evident throughout the 12-week period of the study. In contrast we recently found that both docosahexaenoic acid and eicosapentaenoic acid were able to suppress the development of proteinuria in young SHR-SP salt-loaded during the development of hypertension and this was associated with reduced renal thromboxane production (Rayner and Howe, 1995). Suppression of microalbuminuria in diabetic rats has been achieved by dietary supplementation with eicosapentaenoic acid alone (Fujikawa et al., 1994). This was attributed to inhibition of renal thromboxane A_2 production and is consistent with the effects of thromboxane synthetase inhibition in diabetes (Craven et al., 1992). The identification of docosahexaenoic acid as a potentially protective fatty acid in the kidney offers scope to pursue the mechanism of action of this lipid with respect to its renal protection.

The experiments summarised above highlight several cardiovascular actions of docosahexaenoic acid when administered in the diets of rats. In all four diverse areas studied, docosahexaenoic acid was more effective than eicosapentaenoic acid which was often without significant effect at doses used. Although eicosapentaenoic acid does exhibit cardioprotective properties, these may only be observed at higher intakes or after its conversion to docosahexaenoic acid in the body. Some of the findings for docosahexaenoic acid await evaluation at lower dietary intakes and the mechanisms of action can only be speculated. A common underlying factor may be the inhibition of thromboxane production. Collectively the findings suggest that docosahexaenoic acid is the principal effective cardiovascular protective fatty acid in fish oil. A significant physiological role for eicosapentaenoic acid cannot be eliminated and it may provide an important precursor pool for the production of docosahexaenoic acid. Irrespective of the mechanisms, the effects seen in this study offer the intriguing possibility that docosahexaenoic acid, consumed directly or formed from dietary precursors, functions over a wide spectrum in a general cardiovascular protective role.

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