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Blood pressure and vascular reactivity changes in spontaneously hypertensive rats fed fish oil

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1 To examine possible mechanisms of antihypertensive effects of feeding fish oil rich in n-3 fatty acids, we have studied vascular reactivity of aortic rings and perfused mesenteric resistance vessels of spontaneously hypertensive rats (SHR) given such a diet.

2 In two experiments, rats were fed a semi-synthetic diet containing either 'fish oil' (10 and 20% by weight) or hydrogenated coconut oil (control) (10 and 20%) for 4 weeks.

3 Blood pressure rose significantly less in the fish oil group than in controls in both experiments.

4 Aortic rings from control rats showed endothelium-dependent relaxations to low concentrations of acetylcholine (ACh) but relaxed less at higher concentrations. In contrast, rings from the fish oil group had relaxations which increased through the range of concentrations used. Indomethacin (10 μM) also increased the relaxation responses seen in rings from control rats, suggesting that fish oil inhibits a contractile cyclo-oxygenase product. This contractile substance may be thromboxane A_2 (TxA_2) or its endoperoxide precursor, prostaglandin H_2 (PGH_2) as aortic incubates and serum levels of TxB_2 (the stable product of TxA_2) were greatly reduced in fish oil-fed rats, and the decrease of relaxant responses to high concentrations of ACh were also blocked by a TxA_2/PGH_2 receptor blocker (SQ 29548).

5 In contrast to aortic rings, perfused preconstricted mesenteric resistance vessels of control rats relaxed to ACh in a similar fashion to tissues from fish oil-fed rats. However, in this preparation, fish oil feeding enhanced relaxations to sodium nitroprusside (SNP) and contractile responses to noradrenaline were less than controls. After removal of endothelium with 0.05% saponin, contractile responses to noradrenaline increased in both groups but responses from fish oil-treated rats were still attenuated. This suggests that fish oil feeding alters reactivity of mesenteric resistance vessels at the level of the smooth muscle.

6 The results indicate that fish oil feeding may reduce blood pressure by decreasing vascular smooth muscle reactivity to noradrenaline in resistance vessels. The effect may be enhanced by inhibition of an endothelium-derived cyclo-oxygenase product, such as TxA_2 or PGH_2 in conduit vessels.

Keywords: Fish oil; vascular reactivity; spontaneously hypertensive rats; blood pressure

Introduction

Dietary fish oils rich in eicosapentaenoic acid (EPA) have diverse effects. In man there is evidence that fish oil supplementation can lower blood pressure in subjects with essential hypertension (Norris *et al.*, 1986; Knapp & Fitzgerald, 1989; Bonaa *et al.*, 1990). The effects of dietary fish oils in rat models of hypertension are variable. Some reports have claimed that fish oil feeding lowers blood pressure (Schoene & Fiore, 1981) while others have shown no effect on blood pressure in spontaneously hypertensive rats (SHR) on a normal sodium intake but exacerbation of hypertension after salt loading (Codde *et al.*, 1987). Fish oil feeding has been reported to prevent dexamethasone induced hypertension (Codde & Beilin, 1985) but had no effect on Goldblatt 1-kidney, 1-clip hypertensive rats (Codde *et al.*, 1985).

Changes in blood pressure after fish oil feeding may be related to alterations in vascular reactivity. Fish oil supplementation has been reported to facilitate endothelium-dependent relaxations to bradykinin, adenosine diphosphate (ADP) and 5-hydroxytryptamine (5-HT) in porcine coronary arteries (Shimokawa *et al.*, 1987). Aortae of SHR treated with pure EPA also have augmented endothelium-dependent relaxation to acetylcholine (ACh) (Yin *et al.*, 1988). In this study we have further examined the effects of fish oil feeding on vascular reactivity of aortic rings and investigated the role of cyclo-oxygenase products in modulating relaxations to ACh in these vessels. In addition we have examined the effect of fish oil feeding on endothelium-dependent and independent reactivity of isolated perfused mesenteric resistance vessels of SHR.

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Methods

Male SHR (220–265 g) were studied in two separate experiments in each of which, rats were randomly divided into 2 equal groups matched for blood pressure and weight. In the first experiment, rats were fed a semi-synthetic diet containing 20% (by weight) of either 'Max EPA' fish oil (fish oil) or hydrogenated coconut oil containing 3% safflower oil (control).

In the second experiment, rats were fed 10% (by weight) of these two oil diets. The higher levels of oils were used initially to maximize effects on vascular reactivity but as rats on the diets with 20% fish oil gained less weight than controls (Table 1), 10% oils were used in the second experiment. The major fatty acid composition of these oils was analysed by gas chromatography. 'Max EPA' fish oil contained C16:0 (16%), C18:0 (3%), C18:1 (12%), C20:1 (4%), C20:5 (18%), C22:6 (12%). Safflower oil contained 16:0 (8%), 18:0 (2%), 18:1 (13%), 18:2 (77%). The hydrogenated coconut oil was free of unsaturated fatty acids and safflower oil was added so that the mixture contained 3% safflower oil to prevent essential fatty acid deficiency. The dry component of the feed consisted of (by weight): casein (22.2%), macrominerals (2.2%), vitamins and trace minerals (0.83%), cellulose (7.8%), choline chloride (0.28%), cornflour (50%) and sucrose (16.7%). The feed was mixed in our laboratories before being baked into small biscuits at 120°C for 20 min. Rat feed was kept at 4°C and made up weekly. The dietary period lasted 4 weeks. Systolic blood pressures were taken by tail-cuff sphygmomanometry on rats warmed to a temperature of 39°C for 15 min. Blood pressures were taken as an average of 3 readings before the start of the dietary period and at the end of 4 weeks. At the

Table 1 Summary of weight and blood pressure changes in both 10% and 20% oil fed spontaneously hypertensive rats (SHR)

	Control (20%) ($n = 20$)		*Fish oil* (20%) ($n = 20$)	
Expt. 1.	*start*	*end*	*start*	*end*
Weight (g)	247 ± 4	315 ± 3	250 ± 4	295 ± 4**
Blood pressure (mmHg)	195 ± 5	220 ± 5	190 ± 4	205 ± 3**
	Control (10%) ($n = 25$)		*Fish oil* (10%) ($n = 25$)	
Expt. 2	*start*	*end*	*start*	*end*
Weight (g)	237 ± 3	326 ± 4	237 ± 2	321 ± 3
Blood pressure (mmHg)	178 ± 2	231 ± 2	179 ± 2	222 ± 2**

Values are mean ± s.e.mean of rats at the start and the end of the 4 week dietary period.
Significantly less than control values ** $P < 0.01$.

end of the dietary period, the rats were anaesthetized with 0.1 ml 100 g^{-1} Nembutal (60 mg ml^{-1}) administered intraperitoneally. Two ml of blood was taken from the inferior vena cava for haematocrit, plasma electrolytes, serum fatty acid and serum thromboxane B_2 (TxB_2) analysis. In experiment 1 (20% oils), the reactivity of aortic rings alone was examined while in experiment 2, responses of both the aortic rings and perfused mesenteric resistance vessels were investigated.

Vascular reactivity in aortic rings

For experiment 1, a segment of thoracic aorta approximately 1.5 cm long was removed and cut into two rings of 5 mm length with a double bladed scalpel. One ring had its endothelium removed by gentle rubbing with a cotton probe. In histological studies carried out before this study it was seen that rubbing had clearly removed the endothelium from its adjacent smooth muscle ($n = 5$). Aortic rings were connected to force transducers (Grass FTO3) with platinum wires and mounted in 25 ml organ baths. The tissues were allowed to equilibrate for 1 h at 2 g tension in Krebs solution. Composition of the Krebs solution was (mM) NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.1 and calcium EDTA 0.026. The tissues were maintained at 37°C and bubbled continuously with 95% O_2:5% CO_2. The rings were then challenged twice with priming concentrations of 30 nM noradrenaline to evaluate the viability of the tissues. A recovery period of 15 min was allowed between challenges.

Cumulative concentration-effect curves to ACh and sodium nitroprusside (SNP) were constructed on rings precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (1–7 μM) that produced a tension of approximately 1.3 g which was 50–70% of maximum contraction of the aortic rings to $PGF_{2\alpha}$.

For experiment 2, the procedure for investigation of vascular reactivity in aortic rings was the same as in experiment 1, but in addition, the role of cyclo-oxygenase products on the endothelium-dependent responses to ACh were evaluated by constructing concentration-effect curves to ACh before and after addition of indomethacin (10 μM). The inhibitor was added 15 min before and during construction of a 2nd ACh concentration-effect curve. After maximum relaxation to ACh had been achieved, methylene blue (10 μM) an inhibitor of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) (Rapoport & Murad, 1983; Martin *et al.*, 1985) was added to assess the role of endothelium-derived relaxing factor (EDRF) in these vessels.

In a separate experiment, to investigate the possible role of thromboxane in ACh-stimulated responses in aortic rings of SHR, SQ 29548, a (TxA_2/PGH_2) receptor blocker (Ogletree *et al.*, 1985) was used. In this experiment, ACh concentration-effect curves were constructed with and without addition of SQ 29548 (1 μM) to the organ bath. This concentration of TxA_2/PGH_2 receptor blocker was used because it has been shown to inhibit platelet aggregation and contractile responses of rat aortic strips to the thromboxane-mimetic, 11,9-epoxymethano PGH_2 (Ogletree *et al.*, 1985). The precontractile agent used in these experiments was phenylephrine (30–100 nM) to give a contraction of 1.3 g. $PGF_{2\alpha}$ was not used because SQ 29548 inhibited contractile responses to this prostaglandin.

Vascular reactivity in the perfused mesenteric bed preparation

The superior mesenteric artery was isolated and cannulated with a polyethylene cannula of dimensions 0.5 mm (internal diameter) and 1.5 mm (outer diameter), (SP 40). Warmed Krebs solution (37°C) was immediately perfused at a flow rate of 2.8 ml min^{-1}. The Krebs solution used was of the same composition as that described above except that EDTA was not added. The thoracic aorta was then quickly removed for organ bath studies (procedure is described above). The small intestine was carefully dissected away from the mesenteric vessels and the preparation mounted in a water jacketed organ bath maintained at 37°C. Krebs solution was pumped by a 3-channel peristaltic pump (Pharmacia P-3) through polyethylene tubing (SP 70) connected to a debubbler and to the cannula (Figure 1). The debubbler was of 2 ml volume where at least 1 ml was always filled with perfusion fluid. Perfusion pressure was measured by a pressure transducer (Gould-Statham P 23ID) via a side arm and connected to a Grass polygraph (model 7B). All tubing was immersed in the water bath at 37°C.

All three channels of the peristaltic pump were working simultaneously to deliver solutions at a constant flow rate. When drug solutions were to be perfused, tubing running through channel B was simply removed from the Krebs solution and placed in a tube containing the drug. Similarly, if two drugs were to be perfused simultaneously, then channel B and C were used. This enabled us to perfuse two drug solutions simultaneously without altering the flow rate.

Basal pressure of mesenteric preparations of fish oil-fed rats (15 ± 0.6 mmHg, $n = 15$) were similar to that of control preparations (16 ± 0.8 mmHg, $n = 15$). After 30 min equilibration, the preparation was perfused with 5 μM phenylephrine to ensure the viability of the preparation. This perfusion was repeated before concentration-effect curves to ACh, SNP and noradrenaline were constructed in all tissues. The order was as mentioned and was strictly maintained. When examining the relaxation responses to ACh and SNP, the tissues were preconstricted with phenylephrine (10–40 μM) to obtain a

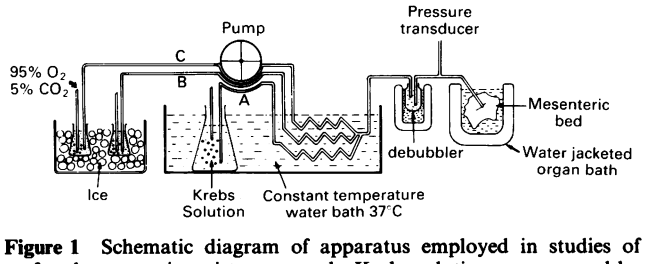

Figure 1 Schematic diagram of apparatus employed in studies of perfused mesenteric resistance vessels. Krebs solution was pumped by a 3-channel peristaltic pump connected to a debubbler and to the cannula. Perfusion pressure was measured by a pressure transducer via a side arm. To maintain a constant temperature all tubing was immersed in the water bath at 37°C. Krebs solution was pumped through all three channels simultaneously to deliver solutions at a constant flow rate of 2.8 ml min^{-1}. When drugs were to be perfused, the end of the tubing running through either channel B or channel C was simply placed in a flask containing the specific drug dissolved in Krebs solution. This method allowed the perfusion of several drugs simultaneously without alteration in flow rate.

background perfusion pressure of 70 mmHg above baseline. This was between 50–70% of maximum contraction response to phenylephrine. After construction of the relaxation and constrictor curves mentioned above, 0.05% saponin was perfused through the preparation for 90 s to remove the endothelium. Saponin has previously been reported to remove endothelium without damaging the underlying smooth muscle (Chiba & Tsukada, 1984; DeMey & Gray, 1985). In preliminary experiments, it was seen that the preparations did not relax to ACh after saponin perfusion but relaxations to SNP were unimpaired ($n = 4$). In histological sections stained with Factor VIII, saponin had significantly damaged the endothelium ($n = 3$). Basal pressure after saponin perfusion was slightly raised but was not significantly different between groups (fish oil: 21 ± 0.8 mmHg, $n = 15$; control: 21 ± 1 mmHg, $n = 15$). Lastly, a second concentration-effect curve to noradrenaline was constructed.

Serum thromboxane B_2 and fatty acid analysis

Blood samples were allowed to clot by incubation for 30 min at 37°C and were then centrifuged at 2500 r.p.m. for 15 min. Aliquots of 100 μl for serum TxB_2 and 200 μl were taken for fatty acid analysis and stored at −20°C. Serum TxB_2 was measured by direct radioimmunoassay (RIA). The relative fatty acid composition of serum was determined by gas chromatography of the corresponding methyl esters. Fatty acid methyl esters were prepared by treatment of serum extracts with 4% H_2SO_4 in methanol at 100°C for 20 min. Methyl esters were analysed by gas chromatography (Hewlett-Packard 5890A) on a 10 m × 0.53 m Superox 11 Column (Alltech), temperature programmed from 190°C to 245°C at 5° per min with nitrogen carrier gas and a split ratio of 30:1. Peak areas were calculated automatically with a Hewlett-Packard 3393A computing integrator.

Aortic thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ analysis

Segments of abdominal aorta (1 cm long) were also removed from the rats for analysis of eicosanoid production. The segments of aorta were cleared of fat and connective tissue before being incubated in Krebs solution (without EDTA) for 1 h at 37°C. The segments were then removed, blotted dry and weighed. Tissue incubates were stored at −20°C until analysis by direct RIA (Codde *et al.*, 1984).

Haematocrit and plasma electrolytes

Part of the plasma collected was placed in microhaematocrit tubes and centrifuged in a microfuge. The rest of the plasma was placed in heparin-lined tubes and sent for electrolyte analysis at the Biochemistry Department of Royal Perth Hospital.

Drugs and solutions

Acetylcholine chloride, noradrenaline (arterenol) bitartrate, sodium nitroprusside, $PGF_{2\alpha}$ and indomethacin were all purchased from Sigma chemicals. Saponin was from BDH chemicals and methylene blue was from AJAX chemicals. A stock solution of $PGF_{2\alpha}$ (10 mg ml^{-1}) was made up in ethanol and stored at −20°C. On the days of the experiment, $PFG_{2\alpha}$ was made up fresh by drying down the required amount with nitrogen and reconstituting in Krebs solution. Indomethacin was made up fresh everyday by dissolving in equimolar sodium carbonate. The TxA_2/PGH_2 receptor blocker, SQ 29548 ([1S-[1α,2β(5Z),3β,4α]]-7-[3-[[2-(phenylamino) carbonyl] hydrazino]methyl]-7- oxabicyclo[2.2.1] hept-2-yl]-5-heptenoic acid) was a donation from Squibb. A solution of 10 mg ml^{-1} was made up in 95% ethanol and then diluted down to the required concentration with 2 mM sodium carbonate. Concentration of ethanol in the organ bath was 0.0095%. All other drugs were made up in Krebs solution. Concentrations mentioned in the text are final bath concentrations.

Statistics

Statistical differences between concentration-effect curves of responses from different dietary groups were ascertained by calculating mathematically the area under the curve by the methods outlined by Matthews *et al.* (1990). The areas were then tested by either unpaired or paired t tests. Relaxations are expressed as the percentage of the precontractile agent. EC_{50} is taken as the mean of 50% of maximum responses of individual concentration-effect curves. Means were then compared by either paired or unpaired t tests. All results are expressed as mean ± s.e.mean with n = number of rats. P values of less than 0.05 were considered significant.

Results

Blood pressure

In the first experiment, systolic blood pressure of both groups increased over the 4 week dietary period but rats fed 20% fish oil had 15 mmHg lower systolic pressure (Table 1) compared to control rats ($P < 0.01$). Body weights also increased less with fish oil feeding (Table 1). In the second experiment when the rats were given 10% oils, there was a smaller (9 mmHg) but significant difference ($P < 0.01$) in the blood pressure rise between the two groups while body weights of fish oil-fed rats increased to a similar extent compared to controls.

Vascular reactivity

Aortic rings: Rings with intact endothelium from control rats fed 20% saturated fats relaxed to 0.1 μM ACh (Figure 2) but relaxed less to higher concentrations. Responses were significantly different between 0.1–3 μM ACh ($P < 0.01$) with the highest concentration causing a relaxation of $41 \pm 6\%$ ($n = 14$) in rings from control SHR while rings from fish

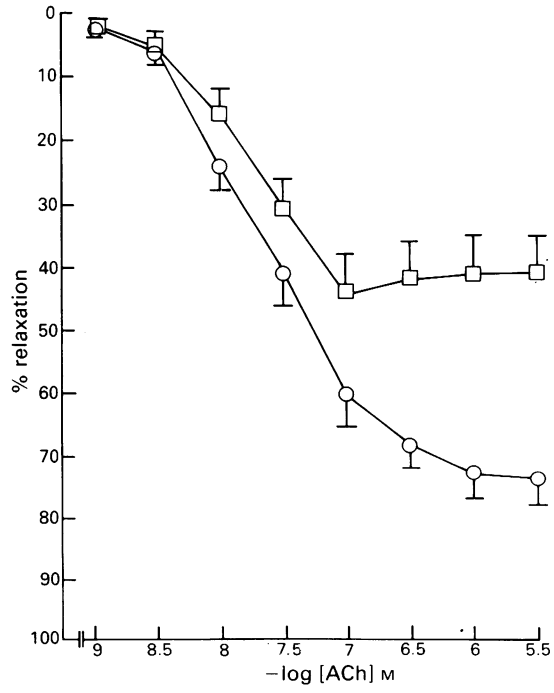

Figure 2 Effects of fish oil (20%) feeding on endothelium-dependent relaxation of aortae to acetylcholine (ACh). Cumulative concentration-effect curves were constructed on rings from fish oil-fed rats (○; $n = 19$) and control (□; $n = 14$) rats. Aortic rings were precontracted with prostaglandin $F_{2\alpha}$ (1–7 μM) to a tension of 1.3 g.

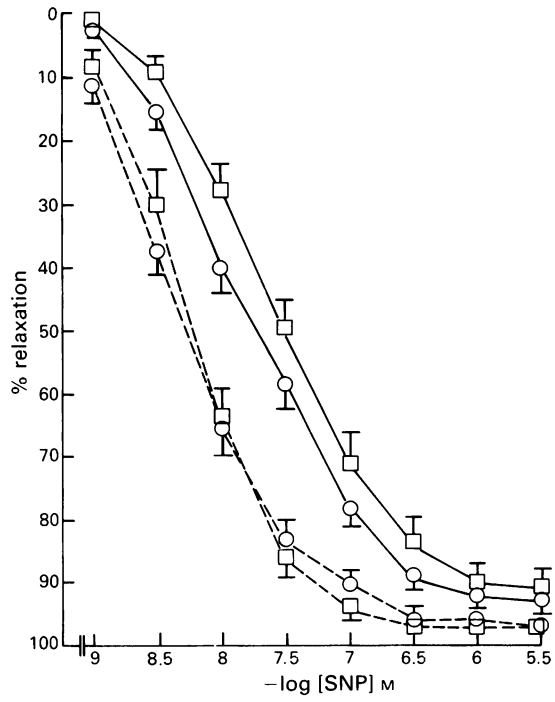

Figure 3 Cumulative concentration-effect curves to sodium nitroprusside (SNP) of aortae from 20% fish oil [(○), continuous lines = with endothelium ($n = 15$); broken lines = without endothelium ($n = 17$)] and control rats, [(□), solid lines = with endothelium ($n = 15$), broken lines = without endothelium ($n = 15$)]. Aortic rings were precontracted with prostaglandin $F_{2\alpha}$ (1–7 μM) to a tension of 1.3 g before cumulative concentrations of sodium nitroprusside were added.

oil-fed rats relaxed to 74 ± 4% of $PGF_{2\alpha}$ precontraction ($n = 19$). Rings denuded of endothelium did not relax or contract in response to ACh. Endothelium-dependent relaxations to SNP were similar in both groups of rats (Figure 3). The rings without endothelium were more sensitive to SNP than aortae with endothelium. The EC_{50}s were as follows: control (with endothelium), 70.4 ± 20.1 nM ($n = 15$), fish oil (with endothelium), 20.7 ± 5 nM ($n = 15$); control (without endothelium), 6.9 ± 1.1 nM ($n = 17$; $P < 0.01$ as compared to rings with endothelium), fish oil (without endothelium), 8.0 ± 2.0 nM ($n = 15$; $P < 0.01$ as compared to rings with endothelium).

In the second experiment, intact aortic rings from control rats fed 10% saturated fat, showed responses similar to those seen in rats fed 20% saturated fats (Figure 4). Rings from fish oil-fed rats relaxed more than controls between 0.3 μM–30 μM ACh ($P < 0.01$). At 0.3 μM, rings from fish oil-fed rats (53 ± 3%) relaxed to a greater extent than controls (42 ± 4%). At higher concentrations of ACh, control rings relaxed less but rings from fish oil-fed rats continued to relax. Preincubation with indomethacin (10 μM) increased the relaxations to high concentrations of ACh in control tissues. Indomethacin enhanced relaxations to ACh in rings from fish oil-fed rats (68 ± 3% at 30 μM) compared to control rings preincubated with indomethacin (58 ± 4% at 30 μM; $P < 0.05$). Methylene blue (10 μM) completely reversed the relaxations in rings from both groups of rats (data not shown). Rings without endothelium did not relax to ACh.

In the presence of the TxA_2/PGH_2 receptor blocker SQ 29548, relaxations to 0.3–30 μM ACh continued to increase in aortic rings taken from SHR ($n = 7$; $P < 0.01$) (Figure 5).

Perfused mesenteric resistance vessels Tissues from fish oil-fed rats were more sensitive (2 fold shift in EC_{50} vs controls) and relaxed more to concentrations of SNP between 1 nM and 10 μM (Figure 6a) as compared to control tissues. In contrast to the aortic rings, control preparations showed no enhancement of relaxation to ACh by fish oil (Figure 6b).

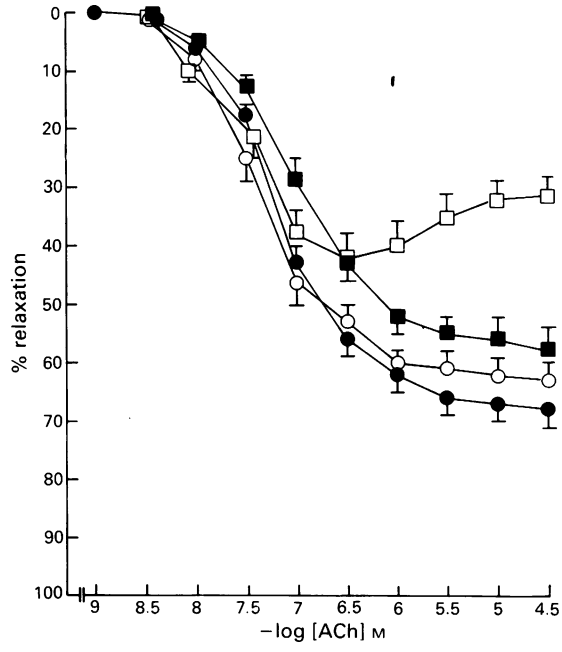

Figure 4 Endothelium-dependent relaxations to acetylcholine (ACh) of aortic rings from 10% fish oil (○)-fed ($n = 14$) and control (□) rats ($n = 16$). Cumulative concentration-effect curves were constructed on rings precontracted with prostaglandin $F_{2\alpha}$ (1–7 μM) to a tension of 1.3 g. Indomethacin (10 μM) was incubated with rings from fish oil-fed (●) rats ($n = 15$) and control (■) rats ($n = 15$), 15 min before and throughout construction of a 2nd concentration-effect curve to ACh. Areas under the curve were calculated and statistical significance tested by unpaired t tests when assessing differences between aortic rings. Paired t tests were only used when assessing the reactivity to ACh within the same tissue before and after incubation with indomethacin.

Tissues with intact endothelium from fish oil-fed rats contracted less to noradrenaline between 3–30 μM ($P < 0.05$) than tissues from control rats. Contractile responses to 30 μM noradrenaline in fish oil-fed rats (163 ± 18 mmHg) were almost

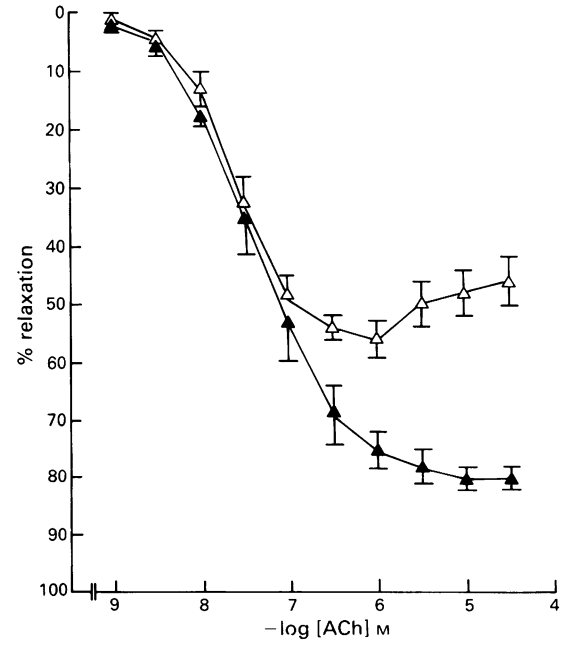

Figure 5 Concentration-effect curves to acetylcholine (ACh) of aortic rings in spontaneously hypertensive rats (SHR) with (▲) and without (△) the thromboxane A_2/prostaglandin H_2 receptor blocker, SQ 29548. Cumulative concentration-effect curves were constructed in rings precontracted by phenylephrine (30–100 nM) to give a contraction of 1.3 g. Values are mean of $n = 7$ rats in each group; s.e.mean shown by vertical bars.

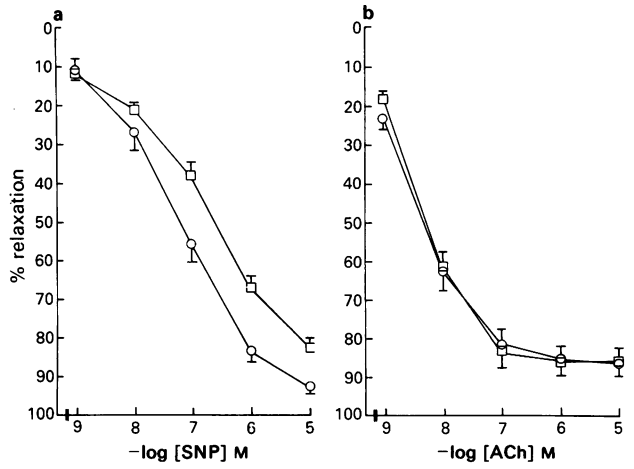

Figure 6 (a) Concentration-effect curves to sodium nitroprusside (SNP) of perfused mesenteric resistance vessels from 10% fish oil-fed spontaneously hypertensive rats (SHR, ○) and controls (□), $n = 15$ in both groups. EC_{50} of tissues from fish oil-fed rats ($0.09 \pm 0.02\,\mu M$) was significantly less than control tissues ($0.2 \pm 0.03\,\mu M$; $P < 0.01$). Mesenteric resistance vessel preparations were perfused with phenylephrine (10–40 μM) to achieve a perfusion pressure of 70 mmHg above baseline before construction of a cumulative concentration effect curve to SNP. (b) Concentration-effect curves to acetylcholine (ACh) of perfused mesenteric resistance vessels from 10% fish oil-fed SHR (○) and controls (□). Points are mean of 15 rats in both groups; s.e.mean shown by vertical bars.

half that of controls (92 ± 15 mmHg; Figure 7) but the EC_{50} was not different between groups. This lack of difference in EC_{50} could have been due to the large difference in contractile responses to the maximum concentration of 30 μM noradrenaline used. Both sensitivity and contractions to 30 μM noradrenaline were increased in endothelium-denuded preparations as compared to endothelium-intact preparations of the two dietary groups (Figure 7). Nevertheless, fish oil-fed tissues still contracted less in response to noradrenaline (between 1–10 μM; $P < 0.01$) than control preparations. Sensitivity of preparations from fish oil-fed rats to noradrenaline was significantly less than (2 fold rightward shift in EC_{50} vs controls) controls in tissues denuded of endothelium.

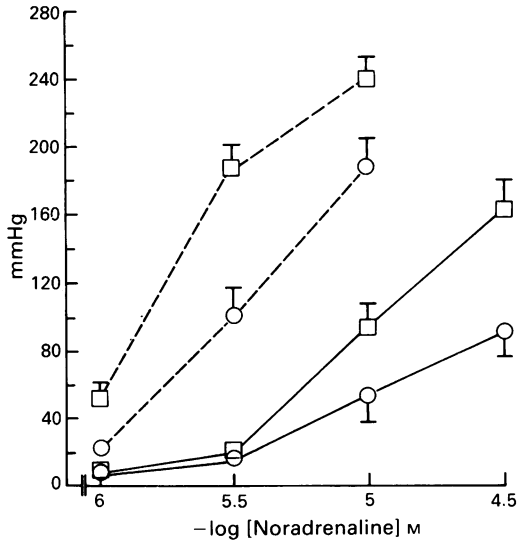

Figure 7 Concentration-effect curves to noradrenaline of perfused mesenteric resistance vessels from 10% fish oil-fed spontaneously hypertensive rats (SHR). [(○), solid lines = with endothelium, broken lines = without endothelium)] and controls [(□), solid lines = with endothelium, broken lines = without endothelium)], $n = 15$ for all groups. To investigate the role of endothelium in modulating the responses of these preparations to noradrenaline, the endothelium was removed by perfusion of 0.05% saponin for 90 s before construction of a second concentration-effect curve to noradrenaline. Areas under the curve were analysed statistically by unpaired t tests for preparations from different dietary groups. Paired t tests were only used to assess significant differences within the same preparation before and after saponin perfusion. EC_{50} of tissues with intact endothelium from fish oil-fed rats ($9.2 \pm 0.9\,\mu M$) was similar to control tissues ($9.2 \pm 0.6\,\mu M$). After saponin perfusion, sensitivity to noradrenaline was increased in tissues from both groups (fish oil, EC_{50}: $2.0 \pm 0.1\,\mu M$; control, EC_{50}: $4.7 \pm 0.6\,\mu M$) as compared to intact preparations but EC_{50} of control tissues was more than that from fish oil-fed rats ($P < 0.01$ for both comparisons).

Table 2 Serum fatty acids of 10% oil-fed spontaneously hypertensive rats (SHR)

	Control ($n = 8$)	*Fish oil* ($n = 10$)
16:0	29.0 ± 0.8	33.0 ± 1.5*
18:0	22.5 ± 0.8	24.0 ± 1.6
18:1	18.0 ± 0.8	12.9 ± 0.7**
18:2	9.6 ± 0.8	6.6 ± 0.7**
20:4	15.3 ± 1.2	8.2 ± 0.6**
20:5	trace	6.3 ± 1.1**
22:6	1.0 ± 0.2	5.6 ± 0.7**

Results expressed as % serum fatty acid composition.
Significantly different from control values. * $P < 0.05$; ** $P < 0.01$.

The following biochemical analyses were carried out in animals fed 10% fish oil or hydrogenated coconut oil.

Serum fatty acids

Arachidonic acid (20:4) levels were 54% lower in serum from 10% fish oil-fed rats than in controls (Table 2). Eicosapentaenoic acid (20:5) levels were much greater in the serum of fish oil-fed rats than in control rats.

Aortic and serum eicosanoid production

Aortae of 10% fish oil-fed rats had a reduced capacity to generate TxB_2 and 6-keto $PGF_{1\alpha}$ than controls (Table 3) by 25% and 43% respectively. TxB_2 levels of serum from fish oil-fed rats were approximately 3 times lower than controls.

Plasma electrolytes and haematocrit

There were no differences in plasma Na, urea, creatinine or haematocrit between dietary groups.

Discussion

These results demonstrate two possible mechanisms by which fish oil may have an antihypertensive effect and attenuate the blood pressure rise in SHR. Firstly, there was a reduction in vascular reactivity to noradrenaline of resistance vessels and secondly enhanced endothelium-dependent relaxation to ACh in conduit vessels.

In perfused mesenteric resistance vessels the major findings were that fish oil feeding (i) reduced the contractile responses to noradrenaline by a mechanism independent of endothelium, (ii) facilitated the relaxations to SNP and (iii) did not affect the relaxations to ACh. Contractile responses to 30 μM noradrenaline were decreased in fish oil-fed animals. After the

Table 3 Eicosanoid production in tissues of 10% oil-fed spontaneously hypertensive rats (SHR)

	Control	*Fish oil*
Aortic TxB_2 ($ng\,mg^{-1}$)	0.48 ± 0.08 ($n = 16$)	0.12 ± 0.02** ($n = 16$)
Serum TxB_2 ($ng\,ml^{-1}$)	769 ± 60.9 ($n = 18$)	213 ± 13.5** ($n = 18$)
Aortic 6-keto $PGF_{1\alpha}$ ($\mu g\,mg^{-1}$)	17.5 ± 2.5 ($n = 16$)	7.6 ± 1.0** ($n = 16$)

Significantly less than control values; $P < 0.01$.

endothelium was removed, the contractile responses to noradrenaline increased in both groups, while both the sensitivity and contractions to 30 μM noradrenaline were still reduced in tissues from fish oil-fed rats. The increased reactivity to noradrenaline after endothelium removal is similar to that reported in deoxycorticosterone acetate (DOCA) hypertensive rats (King & Webb, 1988) and may be attributable to stimulation by noradrenaline of endothelium-derived relaxing factor (EDRF) release (Cocks & Angus, 1983) or the basal release of EDRF may inhibit the contractile response to noradrenaline (Bullock *et al.*, 1986).

The mechanism by which the fish oil diet decreased mesenteric vascular reactivity to noradrenaline is unclear at present. It is interesting to speculate that its effect might be due to changes at the receptor level, alterations to the excitation/contraction coupling or changes to the contractile process. When the α_1-adrenoceptor is occupied, inositol-1,4,5 trisphosphate is released into the cytoplasm and stimulates the release of calcium from the sarcoplasmic reticulum (Abdel-Latiff *et al.*, 1986). The inositol-1,4,5 trisphosphate-stimulated calcium release is large enough to cause myosin light chain kinase activation (Van Breemen *et al.*, 1986) and hence smooth muscle contraction. Locher and co-workers (1988) reported that vascular smooth muscle cells treated for 4 weeks with either 'Max EPA' fish oil or pure eicosapentaenoic acid significantly reduced low-density lipoprotein stimulated synthesis of inositol-1,4,5 trisphosphate. Thus it could be possible that fish oil decreased the reactivity of resistance vessels to noradrenaline by reducing phosphoinositide activity and hence inositol-1,4,5 trisphosphate-stimulated calcium release from the sarcoplasmic reticulum. It is also possible that fish oil feeding could affect the structure and alter the phosphorylation/dephosphorylation regulation of the α_1-adrenoceptors. Investigation into whether the decreased responses were specific to noradrenaline may help to confirm this hypothesis.

The increased relaxations after fish oil feeding of resistance vessels to SNP which acts directly on the smooth muscle, are difficult to explain but may be linked to the mechanism involved in the decreased reactivity of these vessels to noradrenaline. SNP is believed to cause relaxation through the generation of nitric oxide via a cysteine-dependent enzyme system at the smooth muscle level (Moncada *et al.*, 1988). It is possible that fish oil feeding enhanced the production of nitric oxide through this cysteine-dependent enzyme system.

In contrast to aortic rings, perfused mesenteric resistance vessels of controls did not exhibit a decrease in relaxation at high concentrations of ACh. This indicates that there is less (if any) production of the contractile cyclo-oxygenase metabolite in the resistance vessels than in the aortic rings. As ACh stimulates the production/release of EDRF from the mesenteric resistance vessels, it appears that fish oil did not affect the stimulated production/release of EDRF from these vessels.

It was recently reported that isolated segments (3rd order) of mesenteric resistance vessels of SHR with intact endothelium contract in response to high concentrations of ACh and that this effect is abolished by indomethacin (Watt & Thurston, 1989) suggesting that the contractions are caused by a cyclo-oxygenase product. In another study in which resistance vessels were taken just proximal to the gut wall of the stroke-prone SHR, the results were similar to ours in that no decrease in relaxation to high concentrations of ACh was observed (Tesfamariam & Halpern, 1988). A possible explanation for these discrepancies could be the size of the vessels used, with larger vessels producing more of the contractile factor.

Aortic rings (with intact endothelium) of control SHR in both experiments (20% and 10% oils) relaxed to 0.1 and 0.3 μM ACh respectively but relaxed less to higher concentrations. These rings preincubated with indomethacin relaxed to 0.1 μM ACh and continued relaxing at higher concentrations of ACh. A similar effect of indomethacin has been reported in precontracted aortae of adult SHR (Luscher & Vanhoutte, 1986) and old WKY rats (Koga *et al.*, 1989). Indomethacin reduced ACh-induced contractions in intact quiescent rings of canine basilar arteries (Katusic *et al.*, 1988), rabbit pulmonary arteries (Altiere *et al.*, 1986) and aortae of SHR (Luscher & Vanhoutte, 1986). Our results confirm the hypothesis that ACh stimulates the production of a contractile cyclo-oxygenase substance that decreases relaxation of aortic rings in response to ACh.

Rings from fish oil-fed rats relaxed to ACh in an essentially similar fashion to control rings preincubated with indomethacin. This would suggest that fish oil enhanced endothelium-dependent relaxation to ACh by inhibiting the production of a contractile cyclo-oxygenase product. That the contractile cyclo-oxygenase product may be TxA_2 or an endoperoxide precursor is supported by the demonstration of a reduced capacity of serum and aortae of fish oil-fed rats to produce TxB_2 as has been demonstrated in other rat tissues (Croft *et al.*, 1985). A previous report has shown that both basal and ACh-stimulated production of TxB_2 is greater in aortae of SHR than normotensive WKY controls (Luscher *et al.*, 1986). Whereas the use of imidazole as a thromboxane-synthetase inhibitor failed to block the ACh-induced contractions (Luscher & Vanhoutte, 1986), the thromboxane synthetase inhibitor, CV-4151 significantly reduced the ACh-induced contractions in aortae of old WKY rats (Koga *et al.*, 1989). In our hands, the TxA_2/PGH_2 receptor blocker, SQ 29548 increased the endothelium-dependent relaxations to ACh and stopped the decrease in relaxant responses of aortic rings to high concentrations of ACh. Recently, it has been suggested that the endothelium-derived contracting factor released by ACh in aortae of SHR may be PGH_2 (Kato *et al.*, 1990) because the TxA_2/PGH_2 receptor blocker SQ 29548 inhibited the contractile responses to ACh but the thromboxane synthetase inhibitor OKY 046 did not. This provided indirect evidence that the contractile cyclo-oxygenase product is PGH_2 but direct evidence is required to prove this hypothesis. Thus our results lend support to the hypothesis that the aortic endothelium of SHR produces a contractile cyclo-oxygenase product in response to high concentrations of ACh. We postulate that this contractile product may be TxA_2 or PGH_2 and that their synthesis is inhibited by fish oil feeding.

The active component of dietary fish oils that is involved in mediating these possible changes in cyclo-oxygenase is probably EPA. Dietary EPA has been reported to depress the production of TxA_2 by reducing platelet phospholipid arachidonic acid stores and by competitively inhibiting cyclo-oxygenase (Needleman *et al.*, 1979). Another fatty acid found in fish oils is docosahexaenoic acid (DHA) but this fatty acid is probably not as active as EPA. Croft and co-workers reported that a diet rich in DHA did not alter arachidonic acid content of either liver or kidney phospholipids (Croft *et al.*, 1987). Whole blood thromboxane and vascular prostacyclin was also unchanged in rats fed a DHA-enriched diet.

It is unlikely that the enhanced relaxations to ACh could be attributed to the increased production of vasodilator prostaglandins, as indomethacin would have blocked the production of such prostanoids and one would then expect to have even less relaxation occurring when adding indomethacin to aortic rings of fish oil-fed rats. Another alternative is that fish oil feeding increased the production/release of EDRF. This was postulated as the mechanism for augmented endothelium-dependent relaxations in coronary arteries (Shimokawa *et al.*, 1987) and microvessels (Shimokawa *et al.*, 1988) of pigs fed fish oil. This explanation may be plausible in our experiment since methylene blue which inhibits guanylate cyclase and hence the action of EDRF (Martin *et al.*, 1985) completely reversed the endothelium-dependent relaxations to ACh of all tissues. However, this finding does not provide any information on whether the enhanced endothelium-dependent relaxations seen in aortae of fish oil-fed rats was due to increased production/release of EDRF, it simply indicates that ACh caused endothelium-dependent relaxations which were mediated by EDRF. One way to resolve the issue of the role of

EDRF in aortae of fish oil-fed rats is to assay the release of the substance.

Relaxations to ACh are probably due primarily to the production/release of EDRF (Peach *et al.*, 1985) and to a much lesser extent prostacyclin which has been shown to be the main vasodilator prostanoid released from ACh stimulation (Luscher *et al.*, 1986; Luscher & Vanhoutte, 1986). In our experiment, fish oil-treated rings relaxed more between 0.1 and 0.3 μM ACh than control rings with indomethacin. This could be caused by fish oil increasing EDRF production/release or by indomethacin inhibiting both the cyclo-oxygenase derived contractile factor as well as prostacyclin.

In summary, our results suggest that fish oil feeding could attenuate the blood pressure rise in SHR by a decrease in reactivity of resistance vessels to noradrenaline. The blood pressure lowering effect could be enhanced by an effect on compliance of conduit vessels caused by the inhibition of an endothelium generated contractile cyclo-oxygenase product such as TxA_2 or PGH_2.

These results may help explain the antihypertensive effect of fish oils in man and in some forms of experimental hypertension.

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