The influence of different amounts of *n*-3 polyunsaturated fatty acids on bleeding time and *in vivo* vascular reactivity

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Mesenteric bleeding time, mesenteric vascular reactivity, platelet and erythrocyte lipid fatty acid composition were measured at 2–3 weeks, 5–6 weeks and 11–22 weeks in normotensive Wistar rats, fed on high (6·5% energy) or moderate (1·6% energy) intakes of eicosapentaenoic acid (20:5*n*-3; EPA) as fish oil, compared with controls fed on a diet devoid of EPA. All diets contained the same level of linoleic acid (4% energy): the moderate- and high-EPA diets also contained 1·1 and 4·4% of the energy as docosahexaenoic acid (22:6*n*-3) respectively. Moderate, but not high, intakes of EPA increased mesenteric bleeding time. Similar reductions in erythrocyte and platelet arachidonic acid (20:4*n*-6) occurred in animals fed on either high or low amounts of EPA, but the proportion of EPA increased dose-dependently. At high intakes of EPA the proportion of oleic acid in platelets and erythrocytes was decreased. Blood pressure platelet counts, mesenteric vessel diameter and mesenteric vascular reactivity to vasopressin were unaffected by treatment. High intakes of fish oil led to a slight fall in packed cell volume. In a second experiment bleeding time and mesenteric vascular reactivity to noradrenaline were increased 2–4 weeks after receiving a moderate intake of EPA and these effects persisted 5–21 d after switching to a control diet. A similar increase in vascular reactivity to noradrenaline was observed in animals given indomethacin (6 mg/kg) but not in those given aspirin (20 mg/kg).

Haemostasis: Eicosapentaenoic acid: Docosahexaenoic acid: Noradrenaline

A prolongation of template bleeding time has been noted among Greenland Eskimos whose traditional diet consists of marine mammals, fish and caribou (Bang & Dyerberg, 1980). Human volunteer studies have also observed that bleeding time is prolonged following the consumption of oily fish (Thorngren & Gustafson, 1981) or fish oil supplements (Goodnight *et al.* 1981; Sanders *et al.* 1981). These effects have been attributed to eicosapentaenoic acid (20:5*n*-3; EPA) and docosahexaenoic acid (22:6*n*-3; DHA) which are present in the marine food chain. Measurements of platelet aggregation have yielded inconsistent results, although there is a trend for partial inhibition of platelet aggregation induced by low doses of collagen (Haines *et al.* 1986; Knapp *et al.* 1986). However, the degree of inhibition of platelet aggregation by fish oil is mild compared with the effects induced by aspirin. Human studies suggest that bleeding time is prolonged to a greater extent by a combination of aspirin and fish oil than by either individually (Thorngren & Gustafson, 1981; Harris *et al.* 1991). Several animal studies (Hornstra, 1982) have investigated the effects of fish oils on haemostatic function. However, the majority have been poorly controlled and have not fed chemically-defined diets. In some cases animals have been fed on fish oil as the only source of fat in the diet and the diets have contained inadequate intakes of linoleic acid.

Several human studies have reported a reduction in blood pressure with fish oil (Knapp & Fitzgerald, 1989; Bonaa *et al.* 1990). This effect seems to be more marked when

hypertension is present. Lorenz *et al.* (1983) claimed that this was accompanied by a decreased pressor response to noradrenaline. Animal studies have given conflicting results of the effects of fish oil on blood pressure. Scherhag *et al.* (1982) claimed that fish increased blood pressure in normotensive rats but this was not confirmed by Croft *et al.* (1984). Several studies (Schoene & Fiore, 1981; Ziemlanski *et al.* 1985; Yin *et al.* 1991) have shown that fish oil decreases blood pressure in spontaneously hypertensive rats or in salt-loaded rats. However, fish oil did not influence blood pressure in the Goldblatt 'one clip, one kidney' rat model (Mahoney *et al.* 1983; McGowan *et al.* 1985), where an inhibition of prostaglandin production would be expected to increase blood pressure. Yin *et al.* (1991) have tried to explain the blood pressure-lowering effect of fish oil in hypertensive rats by decreased vascular reactivity. They found that aortic rings from animals given fish oil showed enhanced relaxation to sodium nitroprusside and that they were less sensitive to noradrenaline.

In previous work (Roshanai & Sanders, 1985) we have shown that there is interaction between the intake of linoleic acid and the proportion of EPA and DHA in tissues. Moreover, most animal studies have involved feeding very large amounts of fish oil providing intakes of EPA and DHA considerably greater than those found even in Eskimos. We report the effects of diets containing varying amounts of fish oil on bleeding time and vascular reactivity in rats when care was taken to control feed intake and the intake of other nutrients, especially linoleic acid.

METHODS

Diets

Three semi-synthetic diets were used. All contained (g/kg): casein 200, starch 400, sucrose 100, fat 227, mineral mixture 40, vitamin mixture 20, cellulose powder 11, D,L-methionine 2. The diets provided 43·3% energy from fat and 41·0% energy as fatty acids. A fish oil concentrate (MaxEPA; Seven Seas Ltd, Hull) was used to supply the *n*-3 fatty acids and it contained 1 g L-α-tocopheryl acetate and 100 mg dodecyl gallate/kg. Refined olive oil (BP) was used as the source of fat in the control diet. The two experimental dietary fats were mixtures of MaxEPA, sunflower and olive oils (g/kg): 50 g MaxEPA/kg diet (50 MaxEPA) 220, 22, 758; 200 g MaxEPA/kg diet (200 MaxEPA) 881, 79, 40. The fatty acid composition of the three diets was determined by GLC (Table 1). All three diets provided an identical proportion of linoleic acid (4% of the dietary energy). The control, 50 MaxEPA and 200 MaxEPA diets contained (% total dietary energy): EPA 0, 1·64 and 6·56 respectively; DHA 0, 1·1 and 4·4 respectively. The control, 50 and 200 MaxEPA diets provided approximately 87, 136 and 284 mg vitamin E/kg diet respectively. Animals received 20 g portions of diet daily in order to reduce spillage and minimize oxidation of the diet. Diets were prepared regularly in batches stored in sealed bags under N_2 at $-20°$ until required. The oxidative status of the diet was checked regularly using the Kreis test (Egan *et al.* 1981).

Animals

Expt 1. Eighty-four male Wistar rats (3–4 weeks old) were individually housed in stainless-steel cages with raised floors and fed on powdered stock diet for 10 d. Feed intakes and weight gains were measured. Twelve rats showing low feed intake or poor weight gain were excluded from use in the trial. The remaining seventy-two rats were randomly allocated to the three experimental diets (twenty-four per group). The initial body weights (g) in the three groups were as follows: control 138 (SE 2·4), 50 MaxEPA 131 (SE 1·7), 200 MaxEPA 139 (SE 2·6). The animals were given 20 g diet/d. Growth rates and feed

Table 1. *Fatty acid composition of the experimental diets**

Diet...	Fatty acids (g/100 g total fatty acids)		
	Control	50 MaxEPA	200 MaxEPA
Saturated	17·0	19·2	25·9
Monounsaturated	73·3	62·7	31·1
18:2*n*-6	9·6	9·5	9·6
20:5*n*-3	0	4·0	16·1
22:5*n*-3	0	0·7	2·7
22:6*n*-3	0	2·6	10·6
Other polyunsaturated fatty acids	0	1·3	4·0

50 MaxEPA, 200 MaxEPA, experimental diets containing fat as a mixture of MaxEPA (Seven Seas Ltd, Hull), sunflower oil and olive oil in the following amounts (g/kg fat): 220, 22, 758 and 881, 79, 40 respectively.

* For details of diets and procedures, see pp. 44–46.

intakes were monitored throughout the study. Starting 15 d after introduction to the experimental diet measurements of vascular reactivity to vasopressin, bleeding time and blood sampling were carried out on one animal from each group, until eight animals from each group had been studied. After 5 weeks on the experimental diet conscious systolic blood pressure was measured in a further eight animals from each group. After 32 d on the diet a further set of measurements of vascular reactivity, bleeding and blood sampling were carried out. Blood pressure was measured on the remaining eight animals per group on weeks 8 and 10 of the experimental diet. Measurements of vascular reactivity, bleeding time and blood sampling were carried out starting from day 73 through to day 98.

Expt 2. Forty-five male Wistar rats were fed *ad lib* on powdered, pelleted diets for 10 d and the feed intake and weight recorded. The weight gain over this period was 34 (SD 20) g. All those animals with a weight increase below 14 g were excluded. Of the remaining rats, thirty-two were randomized into two groups to receive control or 50 MaxEPA diet. Blood pressure was measured on three occasions during this baseline period. Mean body weight (g) and blood pressure (mmHg) respectively of the animals allocated to control and experimental groups were 359 (SE 4·1), 114 (SE 1·6) and 356 (SE 4·0) and 116 (SE 1·8). As in the first study, feed intake was restricted to 20 g/d. Measurements of blood pressure, bleeding time and vascular reactivity to noradrenaline were made on one rat on the control diet and one on the experimental diet on each day at varying intervals, starting on day 15. After 30 d the remaining animals on the 50 MaxEPA diet were transferred to the control diet.

Expt 3. The influence of aspirin (20 mg/kg) injected intraperitoneally and indomethacin (6 mg/kg) on bleeding time and vascular reactivity was studied in adult rats fed on the stock diet.

Procedures

Blood pressure was measured in conscious animals using a tail cuff sphygmomanometer (Narco Biosystems electrosphygmomanometer; E & M Instrument Co. Inc., Houston, Texas, USA). All other procedures were carried out on anaesthetized animals. Animals were injected intraperitoneally with 60 mg sodium pentobarbital/kg body weight and were injected with an additional 30 mg/kg body weight hourly. The rat was positioned on a thermostatically-controlled pad set to 37·5° in a suction-drained tray. The abdomen was opened with a mid-line incision and the small intestine was displaced on a circular sloping

stage (300 mm in diameter). The preparation of exposed mesentery was superfused with physiological saline (NaCl 131·9 mM, KCl 4·7 mM, $NaHCO_3$ 18 mM, $CaCl_2$ 2 mM, pH 7·35) at 30° at 3·5 ml/min.

Bleeding time was estimated by puncturing with a scalpel blade (no. 15) the arteries at the junction of the mesentery and the intestine. The cessation of bleeding was observed through a binocular microscope (magnification × 10). This procedure was carried out on five different arteries in different sections of the posterior mesentery in each animal: the mean of these five estimates was recorded; the SD of the mean was typically 0·30 within the same animal.

Vascular reactivity was measured on branches of the main mesenteric artery. Vascular reactivity was defined as the percentage constriction brought about by a defined concentration of agonist. An artery was cleared of fat and connective tissue and separated from the adjacent vein with filter paper. After allowing 10 min for recovery, the external diameter of the artery was measured using an eyepiece graticule (magnification × 40). The agonist was administered by a slow-infusion pump into the superfusion fluid so as not to alter temperature or flow properties. The vascular response which resulted in constriction of the artery occurred within 15 s of drug superfusion and stabilized within 1 min. Internal vascular diameter measurements were taken at 2 min. This was then expressed as a percentage of the external diameter of the artery. In Expt 1 the vascular response to vasopressin in concentrations ranging from 0·46 to 4·6 units/ml was measured. In Expts 2 and 3 the vascular response to nine concentrations of noradrenaline ranging from 0·018 to 5·66 μM on two separate arteries was measured. A dose–response curve was constructed from a plot of percentage constriction of the artery *v.* dose of agonist and the dose of agonist giving the half-maximal response (ED_{50}) was estimated.

On completion of these measurements of vascular reactivity, blood was drawn from the abdominal aorta and anticoagulated with EDTA (1 mg/ml). The packed cell volume was measured, platelet counts were made using a Coulter counter and erythrocyte and platelet phospholipids were prepared and their fatty acids analysed by GLC.

Statistical treatment of results

Data from Expt 1 were analysed using the analysis of variance package on SPSS/PC (North Western University) with treatment and time as factors. The data in Expts 2 and 3 were analysed by one-way analysis of variance and Duncan's multiple-range test to compare between-group differences. The dose–response curves to noradrenaline in Expt 2 were compared using repeated-measures analysis of variance: duplicate measures of vascular reactivity were made for each animal, and treatment and dose were entered as factors.

RESULTS

Expt 1

Growth rates (Table 2) and food intakes (values not shown) were similar in all three groups. Platelet count, packed cell volume and blood pressure were not affected by the 50 MaxEPA diet (Table 2) but the packed cell volume was significantly lowered by the 200 MaxEPA diet. The animals receiving the 50 MaxEPA diet showed a prolongation of bleeding time ($P \sim 0{\cdot}002$) that became statistically significant after 6 weeks, but those receiving the 200 MaxEPA diet showed no change compared with the controls (Table 3). Vascular reactivity to vasopressin was unaffected by treatment but the ED_{50} increased with age ($P < 0{\cdot}01$) in both control and EPA-fed animals. This change was accompanied by an increase in blood vessel diameter ($P < 0{\cdot}01$). Changes in platelet and erythrocyte lipids occurred rapidly (Table 4). The proportion of arachidonic acid was decreased and that of EPA was

Table 2. *Expt 1. Platelet count, packed cell volume, systolic blood pressure and body weight in rats fed on control, 50 MaxEPA or 200 MaxEPA diets**

(Mean values with their standard errors)

Diet... Week of experiment	Control			50 MaxEPA			200 MaxEPA		
	n	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
					Platelets (10^8/l)				
2–3	8	282	27·3	8	220	28·9	8	320	26·7
6–7	8	281	33·8	7	330	74	8	277	55·3
11–12	8	253	30·7	8	247	13·1	8	304	25·1
					Packed cell volume				
2–3	8	43	2·0	8	40	1·5	8	38	1·1
6–7	8	48[ab]	1·3	7	46[bc]	2·8	8	44[c]	2·4
11–12	8	45[ab]	1·2	8	44[bc]	0·9	8	41[c]	1·1
					Systolic blood pressure (mmHg)				
5	8	127	4·5	8	132	2·4	8	127	3·9
8	8	118	2·8	8	121	3·3	8	119	2·4
10	7	118	2·9	8	118	2·3	8	117	3·1
					Body wt (g)				
2–3	8	255	9	8	250	7	8	254	8
6–7	8	349	14	8	346	11	8	360	12
11–12	8	455	12	8	454	11	8	460	16

[abc] Values with unlike superscript letters in the same row were significantly different ($P < 0·05$).

50 MaxEPA, 200 MaxEPA, experimental diets containing fat as a mixture of MaxEPA (Seven Seas Ltd, Hull), sunflower oil and olive oil in the following amounts (g/kg fat): 220, 22, 758 and 881, 79, 40 respectively.

* For details of diets and procedures, see pp. 44–46.

Table 3. *Expt 2. Bleeding time, vessel diameter and vascular reactivity to vasopressin in rats fed on control, 50 MaxEPA or 200 MaxEPA diets**

(Mean values with their standard errors)

Diet... Week of experiment	Control			50 MaxEPA			200 MaxEPA		
	n	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
					Bleeding time (s)				
2–3	8	137	4·7	8	149		8	132	5·5
6–7	8	105[a]	7·1	8	147[b]		8	117[a]	10·3
11–12	8	132[a]	8·6	8	161[b]		8	142[a]	9·2
					Vessel diameter (μm)				
2–3	8	108	3·9	8	101	6·0	8	104	4·4
6–7	8	125	4·9	8	122	5·6	8	116	3·9
11–12	8	136	5·0	8	138	4·2	8	133	4·5
					Vascular reactivity to vasopressin (ED_{50} units/ml)				
2–3	8	2·28	0·04	8	2·5	0·26	8	2·5	0·30
6–7	8	2·96	0·27	8	3·28	0·43	8	2·74	0·32
11–12	8	5·21	0·58	8	6·61	0·90	8	5·6	0·47

[ab] Values with unlike superscript letters in the same row were significantly different ($P < 0·05$).

50 MaxEPA, 200 MaxEPA, experimental diets containing fat as a mixture of MaxEPA (Seven Seas Ltd, Hull), sunflower oil and olive oil in the following amounts (g/kg fat): 220, 22, 758 and 881, 79, 40 respectively.

* For details of diets and procedures, see pp. 44–46.

Table 4. *Expt 1. Eicosapentaenoic acid (EPA) and arachidonic acid (AA) in platelet and erythrocyte lipids in rats fed on control, 50 MaxEPA or 200 MaxEPA diets**

(Mean values with their standard errors for four rats per group)

Week of experiment	Control		50 MaxEPA		200 MaxEPA	
	Mean	SE	Mean	SE	Mean	SE
			Platelet AA (g/100 g)			
2–3	22·1	1·91	10·9[a]	1·45	10·3[a]	0·67
6–7	27·2	1·37	11·7[a]	1·45	12·1[a]	1·10
11–12	26·8	1·28	14·5[a]	2·55	13·0[a]	1·16
			Platelet EPA (g/100 g)			
2–3	Trace		8·9[ab]	0·97	13·6[ab]	1·59
6–7	Trace		7·0[ab]	1·05	12·6[ab]	1·85
11–12	Trace		7·2[ab]	1·74	12·7[ab]	1·42
			Erythrocyte AA (g/100 g)			
2–3	22·0	3·07	15·2[a]	1·76	12·1[a]	0·28
6–7	20·0	1·87	13·3[a]	1·50	11·2[a]	1·48
11–12	23·7	3·19	13·1[a]	0·50	13·2[a]	1·18
			Erythrocyte EPA (g/100 g)			
2–3	0·4	0·20	3·8[a]	1·97	8·3[b]	0·50
6–7	0·3	0·06	5·3[a]	0·82	10·0[b]	1·50
11–12	0·2	0·08	5·5[a]	0·66	10·2[b]	0·77

[ab] Values in columns with unlike superscript letters were significantly different ($P < 0·05$).

50 MaxEPA, 200 MaxEPA, experimental diets containing fat as a mixture of MaxEPA (Seven Seas Ltd, Hull), sunflower oil and olive oil in the following amounts (g/kg fat): 220, 22, 758 and 881, 79, 40 respectively.

* For details of diets and procedures, see pp. 44–46.

Table 5. *Expt 2. Influence on bleeding time and* in vivo *vascular reactivity to noradrenaline of moderate intakes of fish oil providing 1·6% energy as eicosapentaenoic acid (50 MaxEPA) in adult rats*

(Mean values with their standard errors)

Dietary treatment†	Bleeding time (s)			ED_{50} to noradrenaline (μM)		
	n	Mean	SE	*n*	Mean	SE
50 MaxEPA: 14–30 d	9	144**	7·1	9	0·26**	0·021
30 d then control diet 5–17 d	7	137*	7·1	6	0·26*	0·045
Control diet only	16	109	3·7	16	0·35	0·018

50 MaxEPA, experimental diet containing fat as a mixture of MaxEPA (Seven Seas Ltd, Hull), sunflower oil and olive oil in the following amounts (g/kg fat): 220, 22, 758 respectively.

Mean values were significantly different from those of control group: * $P < 0·05$, ** $P < 0·01$.

† For details of diets and procedures, see pp. 44–46.

significantly increased ($P < 0·01$) in the MaxEPA-treated groups in both erythrocytes and platelet lipids. The proportion of arachidonic acid was similarly depressed by both the 50 and 200 MaxEPA diets. However, the proportion of EPA was clearly related to the amount provided by the diet. At the highest dose of EPA the increase in EPA in platelet and erythrocyte lipids was mainly at the expense of oleic acid (18:1*n*-9), and to a lesser extent

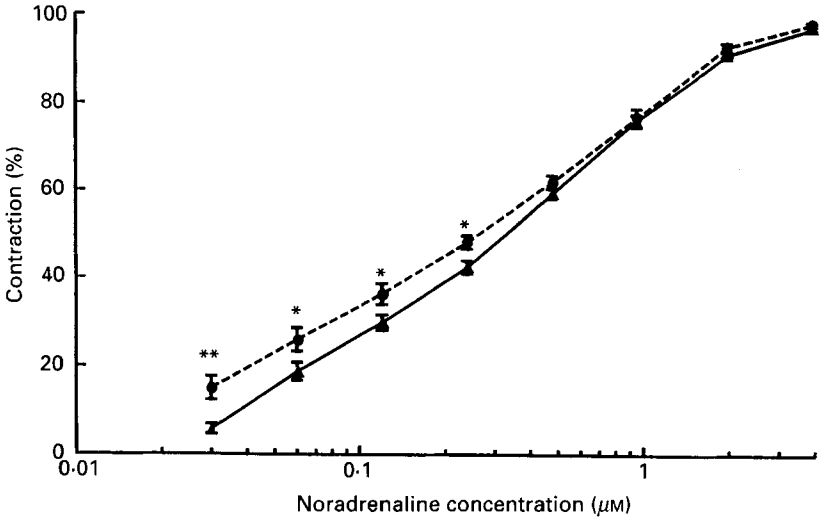

Fig. 1. Expt 2. Dose–response curve of reactivity of mesenteric vessels to noradrenaline in nine rats fed on diets containing 1·6% energy as eicosapentaenoic acid (●), or sixteen rats on control diet (▲). For details of diets and procedures, see pp. 44–46. Mean values were significantly different from those for the control diet: * $P < 0.05$, ** $P < 0.01$.

Table 6. *Expt 3. Influence of aspirin (20 mg/kg) and indomethacin (6 mg/kg) on bleeding time and vascular reactivity to noradrenaline in rats*†

(Mean values with their standard errors)

	Bleeding time (s)			ED_{50} (μM)		
	n	Mean	SE	*n*	Mean	SE
Aspirin	4	189*	4	4	0·32	0·04
Indomethacin	6	114	15·4	6	0·20*	0·02
Control	10	127	7·5	10	0·320	0·020

Mean values were significantly different from other values in the same column: * $P < 0.05$.
† For details of procedures, see pp. 45–46.

linoleic acid. For example, the proportions of oleic acid in platelet lipids for control, 50 MaxEPA and 200 MaxEPA diets respectively were: 15·5 (SE 0·09), 15·3 (SE 0·11) and 11·5 (SE 0·34) ($P < 0.001$) and corresponding values for erythrocyte lipids were 15·5 (SE 0·26), 15·2 (SE 0·18) and 11·5 (SE 0·21) ($P < 0.001$).

Expt 2

Growth rates, packed cell volumes, blood pressures and feed intakes were similar in both groups (values not shown). The animals fed on the 50 MaxEPA diet showed an increase in bleeding time (Table 5). This effect persisted after transferring the animals from the experimental to the control diet. There was a difference in vascular reactivity dose–response curves to noradrenaline and there was a significant dose × treatment ($P = 0.01$) interaction (Fig. 1). The maximum contraction induced by noradrenaline ranged between 78 and 82% of vessel diameter and did not differ between the experimental and the control groups. However, the constriction induced by threshold doses of noradrenaline was increased

by the 50 MaxEPA diet. Overall this increased sensitivity to noradrenaline at low concentrations was reflected in a slightly lower ED_{50} compared with the controls. The effect persisted after the animals were transferred from experimental to control diet.

Expt 3

Aspirin led to a prolongation of bleeding time ($P < 0.01$) but did not alter the vascular reactivity to noradrenaline (Table 6). Indomethacin on the other hand did not prolong bleeding time but decreased the ED_{50} to noradrenaline ($P < 0.01$).

DISCUSSION

We were careful to control the intake of linoleic acid in the present study as we have previously shown an interaction between the proportion of linoleic acid in the diet and the intake of EPA on platelet lipids and thromboxane B_2 production (Sanders *et al.* 1983; Roshanai & Sanders, 1985). The proportion of EPA in platelet and erythrocyte lipids increased rapidly with a clear dose–response effect being evident. The proportion of arachidonic acid in platelet and erythrocyte lipids was, however, decreased to a similar extent by both dietary levels of EPA. At higher intakes of EPA the proportion of oleic acid and, to a lesser extent, linoleic acid in platelets and erythrocyte lipids was decreased. This presumably arose because of competition between the different fatty acids for the acyl transferases. Erythrocyte lipids are believed to be good markers of tissue essential fatty acid status and it would be expected that similar changes would be seen in the arterial wall lipids.

A reduction in packed cell volume was noted with the highest intake of EPA. This may have been a consequence of increased cell turnover caused by lipid peroxides. Care was taken to ensure that the fish oil contained adequate amounts of antioxidant and vitamin E, in addition to the vitamin E provided by the experimental diet. However, this may not completely guard against *in vivo* lipid peroxidation. Lipid peroxides are believed to inhibit the production of prostacyclin, which possibly might explain why the high dose of EPA failed to prolong bleeding time.

The measurement of bleeding time is subject to a number of errors, especially observer bias. Bleeding time was measured in the present study on the mesenteric bed. This method has several advantages over other methods such as the tail bleeding time: the cessation of bleeding can be accurately gauged; replicate measurements can be made. In the first study we experienced some difficulties in carrying out the measurement in young animals owing to the small size of the vessel beds and were unable to find a significant increase in bleeding time after 2–3 weeks on the experimental diet, but were able to at 5–6 and 11–12 weeks. In the second study where the animals were 350 g on allocation to the experimental diet we found a significant increase in bleeding time after 2–4 weeks on the experimental diet. The prolongation of bleeding time persisted 5–12 d after withdrawal of the experimental diet. Human studies (Sanders *et al.* 1981) found that bleeding time reverted to normal 5–6 weeks after withdrawal of the EPA or fish oil from the diet. It is possible that a longer period of time is needed to allow for the normalization of bleeding time in this animal model. Several factors control bleeding time and these include platelet count and function, packed cell volume, blood pressure and vascular reactivity. The increase in bleeding time was not accompanied by any significant change in platelet count, packed cell volume or blood pressure. Platelet adhesiveness to the vascular endothelium may be a major determinant of bleeding time. EPA is a potent inhibitor of human platelet adhesion (Li & Steiner, 1991) but a weak inhibitor of platelet aggregation *ex vivo*. High doses of EPA, however, were less potent in inhibiting platelet adhesion. This might partly explain why the higher intake of EPA (approximately 6·5% of the dietary energy) failed to prolong bleeding time. Aspirin

is a drug that inhibits cyclo-oxygenase, and potently inhibits platelet prostaglandin production and aggregation and prolongs bleeding time.

We also investigated the influence of two cyclo-oxygenase inhibitors (aspirin and indomethacin) on bleeding time. Aspirin prolongs bleeding time at low doses but not at high doses (Amezcua *et al.* 1979). At low doses it is a potent inhibitor of platelet thromboxane production and a weaker inhibitor of endothelial prostacyclin production. Indomethacin, which is a more potent cyclo-oxygenase inhibitor, would be expected to inhibit both thromboxane and prostacyclin production at the doses used. Aspirin at a dose of 20 mg/kg prolonged bleeding time but did not influence vascular reactivity to noradrenaline.

Indomethacin, however, in our hands, had no effect on mesenteric bleeding time, but like the 1·6% energy EPA diet increased sensitivity to noradrenaline. Therefore, the results of the present study suggest that a mechanism other than inhibition of platelet prostaglandin production is responsible for the protracted bleeding time following diets containing fish oil. This view is supported by studies in man that find an augmentation of bleeding time by aspirin in subjects given dietary EPA (Thorngren & Gustafson, 1981; Harris *et al.* 1991). The activation of endothelial cells, besides stimulating prostacyclin production, leads to the production of endothelial-derived relaxing factor (EDRF), which has been identified as nitrous oxide. Both these mediators inhibit platelet aggregation and cause vascular dilatation. Our previous studies (Sanders *et al.* 1983) showed that this level of intake of EPA did not inhibit the generation of prostacyclin PGI_2 measured as its stable metabolite $PGF_{1\cdot\alpha}$ in aortic rings, but that anti-platelet activity was greater in the animals receiving EPA. Dietary supplementation of pigs with cod-liver oil has been found to augment endothelium-dependent relaxations to aggregating platelets in their coronary arteries (Van Houte *et al.* 1991). These relaxations were not mediated by prostacyclin since they were insensitive to indomethacin. Thus, it appears that EPA augments the release of EDRF activity and this might be another possible explanation for the observed prolongation of bleeding time.

Juan & Sametz (1989) found that guanethidine, an adrenergic neurone blocking agent, prolonged bleeding time strongly in control animals, even when treated with indomethacin, but not in fish oil-treated animals. This perhaps suggests that the dietary EPA (or possibly DHA) decreases the production or release of noradrenaline. Indeed Lefkowith & Schreiner (1987) have reported decreased release of noradrenaline in rats fed on EPA. Previous studies have examined the vascular sensitivity to noradrenaline in denervated tissues (Lockette *et al.* 1982). This may not reflect the *in vivo* situation, especially as it is known that supersensitivity to noradrenaline occurs if blood vessels are denervated. Yin *et al.* (1991) used spontaneously hypertensive rats and so their results are not comparable with the present study. Our results suggest that the intact mesenteric arterioles in normotensive rats receiving moderate amounts of EPA show supersensitivity to noradrenaline. These findings are consistent with a report from Gudbjarnason *et al.* (1978) who found increased sensitivity to exogenous noradrenaline in animals fed on cod-liver oil. Our paradoxical observation of supersensitivity to exogenous noradrenaline could reflect homeostatic upregulations of α-receptors.

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