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Journal of Nutritional Biochemistry 12 (2001) 258–265

THE JOURNAL OF Nutritional Biochemistry

Dietary n-3 fatty acids alter the contractile response to thromboxane A_2 agonists of porcine coronary arteries

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Received 12 June 2000; received in revised form 22 November 2000; accepted 7 December 2000

Abstract

Dietary supplementation with marine fish oils rich in n-3 fatty acids reduces circulating thromboxane A_2 (TxA_2). However, the effects on thomboxane A_2 receptor mediated vascular reactivity are uncertain. The aim of this study was to test the hypothesis that dietary modification of TxA_2 levels alters vascular responsiveness to TxA_2 analogues. Juvenile female white pigs were fed a diet enriched in either 5% (w/w) fish oil or beef tallow for 6 weeks. Serum and myocardial tissue levels of eicosapentaenoic and docosahexaenoic acid reached a plateau during this period. Vascular responses were measured in isolated coronary arterial rings with intact endothelium by isometric tension measurement. Arteries from pigs fed fish oil produced a greater maximum vasoconstrictor tension to the TxA_2 analogue U46619 than did rings from pigs fed beef tallow (120 ± 6% compared to 92 ± 8%, values represented as a percentage relative to the maximum vasoconstrictor effect obtained to KCl, regression analysis, analysis of variance, $P \leq 0.05$). The vasoconstrictor potency of U46619 was similar in both treatment groups. The vasoconstrictor EC_{50} was 10.3 (6.8–15.7) nmol/L (mean, 95% confidence interval) for fish oil and 9.5 (5.7–15.8) nmol/L for beef tallow treated animals. Changes in vascular responses to U46619 were associated with a fourfold difference in plasma thromboxane B_2 levels between treatment groups (12.1 ± 2.6 pg/mL fish oil, 48.3 ± 3.1 pg/mL beef tallow, Students' unpaired t-test $P \leq 0.05$). Vasoconstrictor responses obtained to endothelin-1, KCl and 5-hydroxtryptamine and the vasodilator response to sodium nitroprusside were not different between treatment groups. Dietary manipulation of thromboxane A_2 levels by n-3 fatty acids alters vascular reactivity to U46619, possibly as a result of agonist-induced desensitization of thromboxane A_2 receptors.

Keywords: n-3 polyunsaturated fatty acids; Thromboxane A_2; Pig; Coronary artery

1. Introduction

Dietary supplementation with n-3 polyunsaturated fatty acids (PUFA), such as is found in some marine fish oils, markedly reduces serum thromboxane A_2 (TxA_2) levels [1,2]. This occurs primarily by replacement of arachidonic acid derived TxA_2 with its conformational isomer but biologically inert TxA_3. However, n-3 PUFA ingestion may also depress the overall production of TxA_2, TxA_3 and other prostaglandins [3] as it is a less efficient substrate for cyclooxygenase than arachidonic acid. Dietary n-3 PUFA also inhibits arachidonic acid synthesis, thus reducing the amount available for eicosanoid formation [4,5]. TxA_2 is amongst the most potent vasoconstrictors known and a reduction in its circulating levels might be expected to have a beneficial effect on blood pressure. Similarly, chronic changes in circulating bioactive TxA_2 levels may be expected to induce changes in vascular smooth muscle reactivity to TxA_2.

Dietary n-3 PUFA supplementation has previously been reported to promote endothelium dependent relaxations to bradykinin, adenosine diphosphate (ADP) and 5-hydroxy-

Abbreviations: 5-HT, 5 hydroxytryptamine; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ET, endothelin; PUFA, polyunsaturated fatty acid(s); SNP, sodium nitroprusside; TxA_2, thromboxane A_2; BHT, butylated hydroxytoluene.

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0955-2863/01/$ – see front matter
PII: S0955-2863(00)00159-5

Table 1A
Serum fatty acid composition (% of total fatty acids) in pigs following treatment at 6 weeks on beef tallow and fish oil modified fat diets (*P ≤ 0.05, Student unpaired t-test, n = 5)

Fatty acid	Beef tallow	Fish oil
Myristic (C14:0)	0.56 ± 0.07*	0.80 ± 0.05
Palmitic (C16:0)	19.15 ± 0.33	19.66 ± 0.51
Stearic (C18:0)	14.08 ± 0.62	13.08 ± 0.49
Total saturated fatty acids	33.79 ± 1.02	33.54 ± 1.05
Palmitoleic (C16:1n-7)	1.51 ± 0.17*	2.16 ± 0.06
Oleic (C18:1n-9)	25.54 ± 2.44*	14.27 ± 0.38
C18:1n-7)	1.91 ± 0.14	2.07 ± 0.02
Total monounsaturated fatty acids	28.96 ± 2.75*	18.5 ± 0.46
Linoleic acid (C18:2n-6)	23.87 ± 0.89*	19.21 ± 0.93
Arachidonic acid (C20:4n-6)	9.51 ± 0.92*	3.89 ± 0.14
Total n-6 pUFA	33.38 ± 1.81*	23.1 ± 1.07
C18:3(n-3)	0.64 ± 0.05*	0.93 ± 0.03
Eicosapentaenoic (C20:5n-3)	0.62 ± 0.05*	16.69 ± 0.44
Docosapentaenoic (C22:5n-3)	1.08 ± 0.06*	2.98 ± 0.20
Docosahexaenoic (C22:6n-3)	1.52 ± 0.56*	4.26 ± 0.66
Total n-3 PUFA	3.86 ± 0.72*	24.86 ± 1.33

All values are the mean ± SEM.

tryptamine (5-HT) in pig coronary arteries [6] and to acetylcholine in the aorta of spontaneously hypertensive rats [7]. Despite the interest in the effects of n-3 fatty acids on vascular reactivity, particularly in regard to endothelial cell dependent responses, the changes in vascular responses to TxA_2 receptor mediated effects have been relatively poorly studied. This is surprising given that n-3 fatty acids can have very marked effects on the prostaglandin biosynthetic pathway. Accordingly, this investigation was designed to test the hypothesis that dietary n-3 PUFA supplementation, leading to a reduction in circulating bioactive thromboxane, would modulate TxA_2 receptor mediated vascular reactivity.

2. Methods

2.1. Animals and diet

Juvenile female white pigs (initial weight, 6.1 ± 1.2 kg) were fed a uniform amount of diet per day for 6 weeks. Experimental diets were prepared from a commercial pig feed (Grower diet for pigs YS Feeds, Young, NSW, Australia). This diet contained 2.5% fat (w/w) supplemented by an additional 5% fat in the form of either beef tallow (Allowrie Prime Beef Dripping, Bonlac Foods Ltd., Melbourne, Australia) or fish oil (MaxEPA, R.P. Scherer, Melbourne, Australia). All diets were nutritionally adequate and iso-energetic (the fatty acid composition of the diets is shown in Table 1C). Diets were prepared fresh weekly and stored at −20°C to prevent oxidation of n-3 fatty acids. Daily individual serves were packaged for each animal and serve sizes were adjusted to ensure adequate nutrition and uniform growth. Weight gain was similar in the beef tallow (17.3 ± 2.2 kg) and fish oil fed animals (16.9 ± 1.8 kg). Animals were individually fed once a day.

2.2. Tissue and plasma collection

Myocardial tissue and blood were collected from pigs under general anesthesia (2.5% Halothane, ICI Sydney, Australia). Coronary arteries were quickly removed from the heart, together with surrounding muscle, and placed immediately into ice-cold Krebs' buffer. Composition, in mmol/L, NaCl 97.0; $NaHCO_3$ 24.0; KCl 3.0; KH_2PO_4 1.2; $CaCl_2$ 1.89; $MgSO_4$ 1.0; D-glucose 5.5) at a pH of 7.35 maintained at 4°C, prior to being prepared into vascular rings for experimental purposes.

TxA_2 is highly unstable in plasma and can only be measured reliably as its stable metabolite TxB_2. Plasma for enzyme-immunoassay of TxB_2 was prepared from blood samples taken from a cannula inserted into the femoral artery and collected into 10 mL lithium heparin tubes containing indomethacin (3 μg/mL). Blood samples were centrifuged at 3000 g for 15 min at 4°C and plasma stored at −20°C until required for assay. TxB_2 radioimmunoassay was performed using commercially available enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI., USA). TxB_2 was extracted from plasma into 2 volumes of ethyl acetate. Ethyl acetate plasma extracts were pooled, lyophilized and reconstituted in assay buffer according to manufacturer recommendations prior to assay.

2.3. Fatty acid analysis

Lipids were fractionated into chloroform using the following procedure. Tissue samples were washed in ice-cold phosphate buffered saline of pH 7.4 and any blood, connective tissue or adipose tissue was removed. Coronary arterial tissue and serum samples were homogenised in chloroform: methanol 2:1 v/v [8], containing 0.005% butylated hydroxy toluene (BHT) as an antioxidant and vortexed for 1 minute. Five mL of saline was added and the extract vortexed again. The chloroform phase was collected and evaporated under N_2. Lipid extracts were stored in 1 mL of hexane at −20°C until further analysis. Fatty acid methyl esters were prepared using acetyl chloride [9] and analysed using gas chromatography (Hewlett Packard 5890 Palo Alto, CA, USA). A 30 m × 0.25 mm (ID) × 0.25 mM cyanopropyl column (J&W Scientific DB-225 Folsom, CA., USA) was used with H_2 as the carrier gas. The flow rate was 1.4 mL/min. Inlet and detector temperatures were 25°C. Column temperature was programmed from 190–220°C at a rate of 1°C/min up to 190°C and then at a rate of 3°C/min up to 220°C. Fatty acid peaks were identified by comparison of the retention times with those of authentic fatty acid standards.

2.4. Arterial ring bioassay

Segments (15–30 mm) of left descending and circumflex coronary artery were dissected from pig hearts (1.5–2.0 mm outside diameter) and cut into 5 mm cylindrical rings. The rings were mounted in organ baths on two horizontally arranged platinum wire pins (100 μm diameter) passed through the lumen of the vascular cylinder. One pin was fixed to the organ bath and the other was connected vertically to a strain gauge (Grass FTO3c, Grass Instrument Co., Quincy, Mass., USA) for isometric tension recording. Each arterial ring was set up in a 15 mL water-jacketed organ bath filled with Krebs' buffer (as described above) maintained at 37°C and gassed with 95% O_2; 5% CO_2.

Tissues were allowed to equilibrate for 50–60 min with a 15 mL washout every 10 min, during which they were placed under a resting tension of 50 mN. The isometric contraction of the isolated coronary arteries was recorded using a Grass model 79E polygraph (Grass Instrument Co., Quincy, Mass., USA). KCl concentration-response curves (2.5–75 mmol/L) were obtained at the beginning of each experiment to determine the maximal constriction of each vessel to a depolarizing concentration of KCl. Preparations producing a maximal vasoconstrictor response equivalent to, or less than, 20 mN tension to the maximum effective concentration of KCl were discarded. Following a wash-out period of 20–40 min, cumulative concentration-response curves to increasing semi-log concentrations of the thromboxane A_2 mimetic U46619 (0.1–450 nmol/L), endothelin-1 (ET-1, 0.1–100 nmol/L) and 5-HT, (0.1–45 μmol/L) were obtained. Contractile responses obtained to these agonists were expressed as a relative to the maximum response obtained to KCl. In a separate series of experiments, following KCl treatment and washout, the pig coronary rings were sub-maximally constricted (to approximately 80% of the maximal KCl induced vasoconstriction) with U46619 (5–15 nmol/L) and a concentration response curve was obtained to the vasodilator sodium nitroprusside (SNP, 0.03–300 mmol/L). Endothelium dependent relaxations were also examined in arterial rings to 5-HT (1.4–400 μmol/L) and bradykinin (0.03–300 nmol/L) using methods as otherwise described for SNP.

2.5. Statistical analysis

Linear regression analysis was performed on all concentration response curves. Differences in the linear portions of the concentration response curves to the various agonists examined were compared and tested for significant displacement and deviation from parallelism as described by Bowman and Rand, [10]. Unless stated otherwise, a probability value of $P \leq 0.05$ was considered significant. Non-parallel curves, and multiple comparisons of means were tested for differences using analysis of variance (ANOVA, Minitab, Pasadena, Ca., USA). Pairwise comparisons were made using Students' paired or unpaired t-test as appropriate, where indicated. All values are expressed as the mean (± standard error of the mean, S.E.M.) unless stated otherwise.

Table 1B
Myocardial tissue fatty acid composition (% of total fatty acids) in pigs following treatment of 6 weeks on beef tallow and fish oil modified fat diets (*$P \leq 0.05$, Students unpaired t-test, n = 5).

Fatty acid	Beef tallow	Fish oil
Myristic (C14:0)	0.77 ± 0.11	0.98 ± 0.09
Palmitic (C16:0)	18.96 ± 0.84	19.60 ± 0.35
Stearic (C18:0)	16.21 ± 0.53	16.23 ± 0.48
C20:0	0.67 ± 0.08	0.78 ± 0.10
C22:0	0.47 ± 0.15	0.51 ± 0.14
C24:0	0.52 ± 0.16	0.07 ± 0.07
Total saturated fatty acids	37.60 ± 1.87	38.17 ± 1.23
Palmitoleic (C16:1n-7)	1.86 ± 0.25	2.33 ± 0.24
Oleic (C18:1n-9)	25.56 ± 1.94	20.98 ± 1.07
C18:1n-7	3.56 ± 0.05*	4.03 ± 0.04
C20:1n-9	0.50 ± 0.06	0.23 ± 0.14
C22:1n-9	0.53 ± 0.33	0.00 ± 0.00
C24:1	0.52 ± 0.16	0.23 ± 0.15
Total monounsaturated fatty acids	32.53 ± 2.79	27.8 ± 1.64
Linoleic acid (C18:2n-6)	17.5 ± 0.86*	13.42 ± 0.31
C20:2n-6	0.50 ± 0.05*	0.08 ± 0.08
C20:3n-6	0.47 ± 0.14	0.48 ± 0.12
Arachidonic acid (C20:4n-6)	8.95 ± 1.35*	4.52 ± 0.43
Total n-6 PUFA	27.42 ± 2.14*	18.50 ± 0.94
Linolenic (C18:3n-3)	0.56 ± 0.04	0.60 ± 0.15
Eicosapentaenoic (C20:5n-3)	0.33 ± 0.10*	7.54 ± 0.60
Docosapentanoic acid (C22:5n-3)	0.92 ± 0.11*	2.99 ± 0.15
Docosahexaenoic (C22:6n-3)	0.82 ± 0.16*	4.40 ± 0.20
Total n-3 PUFA	2.63 ± 0.41*	15.53 ± 1.10

All values are the mean ± SEM.

3. Results

3.1. Plasma thromboxane serum and tissue fatty acid profiles

Serum and myocardial fatty acid profiles following 6 weeks dietary fat supplementation are described in Table 1A and B, respectively. Plasma levels of TxB_2 were significantly less in fish oil fed animals (12.1 ± 2.6 pg/mL) compared to those receiving beef tallow (48.2 ± 3.1 pg/mL) (Fig. 1). ($P \leq 0.05$, Students' unpaired t-test).

3.2. Vascular reactivity

The EC_{50} for the vasoconstrictor effect of U46619 in porcine coronary artery rings from animal fed either fish oil (10.3, 6.8–15.7 nmol/L), range and 95% confidence interval) or beef tallow (9.5, 5.7–15.8 nmol/L) enriched diets was not significantly different (Students' unpaired test). However, the maximum increase in tension obtained to U46619 in rings obtained from fish oil fed pigs (120 ± 6%)

Table 1C
Fatty acid composition of beef tallow and fish oil diets used in this study (% of total fatty acids).

Fatty acid	Beef tallow	Fish oil
Myristic (C14:0)	2.2	5.2
Palmitic (C16:0)	23.5	21.2
Stearic (C18:0)	14.9	5.7
Total saturated fatty acids	40.6	32.1
Palmitoleic (C16:1n-7)	0.9	5.7
Oleic (C18:1n-9)	38.1	20.8
C18:1n-7)	1.5	2.6
Total monounsaaturated fatty acids	41.5	29.1
Linoleic (C 18:2n-6)	15.1	19.9
Arachidonic (C20:4n-6)	0.1	0.1
Total n-6 PUFA	15.2	20.0
Linolenic (C18:3n-3)	1.3	1.6
Eicosapentanoic (C20:5n-3)	0.1	7.3
Docaspentanoic (C22:5n-3)		0.9
Decosahexanoic (C22:6n-3)		4.9
Total n-3 PUFA	1.4	14.7

All values are mean ± SEM.

was significantly greater than the maximum response obtained to U46619 in beef tallow fed animals (92 ± 8%) (results expressed relative to maximum KCl induced increase in tension, $P \leq 0.05$, Students' unpaired t-test). Concentration response curves obtained to U46619 in fish oil and beef tallow fed pigs were significantly different from parallelism but were not significantly displaced (Fig. 2), ($P \leq 0.05$, regression analysis, ANOVA, n = 6–11).

The vasoconstrictor response obtained due to depolarization of arterial vascular smooth muscle cell membranes by KCl was not significantly different in rings obtained from

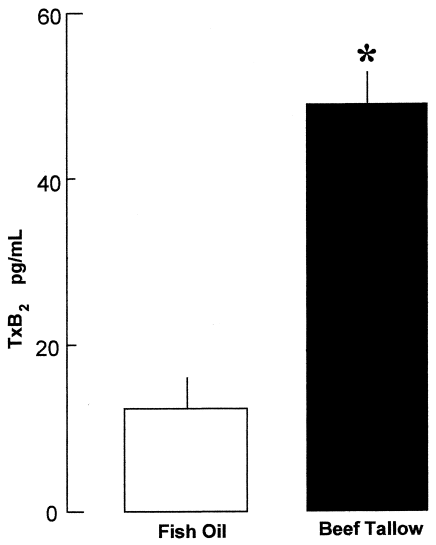

Fig. 1. Plasma thromboxane B_2 (TxB_2) levels in pigs fed modified fat diets (*$P < 0.05$ Students' unpaired t-test, n = 6). All values are the mean ± standard error of the mean (SEM).

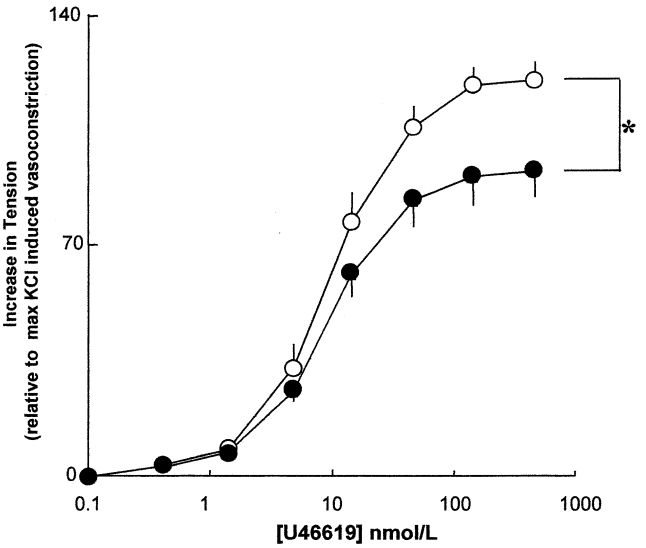

Fig. 2. Concentration dependent vasoconstrictor response curves obtained in pig coronary arteries to the thromboxane A_2 mimetic U46619, following treatment with modified fat diets. ○ Fish oil, ● beef tallow. Curves are not significantly displaced but differ significantly from parallelism, the maximum vasoconstrictor effect of U46619 is significantly different between fish oil and beef tallow treated animals (*$P \leq 0.05$, ANOVA, linear regression analysis, n = 6–11). All values are mean ±SEM.

pigs fed a diet enriched in either fish oil or beef tallow for a period of 6 weeks (Fig. 3). There were no significant differences in the concentration response curves or the maximal tension obtained to KCl in the fish oil (35 ± 8 mN) or the tallow (30 ± 5 mN) treated groups. The maximum KCl induced vasoconstriction was less than that produced by

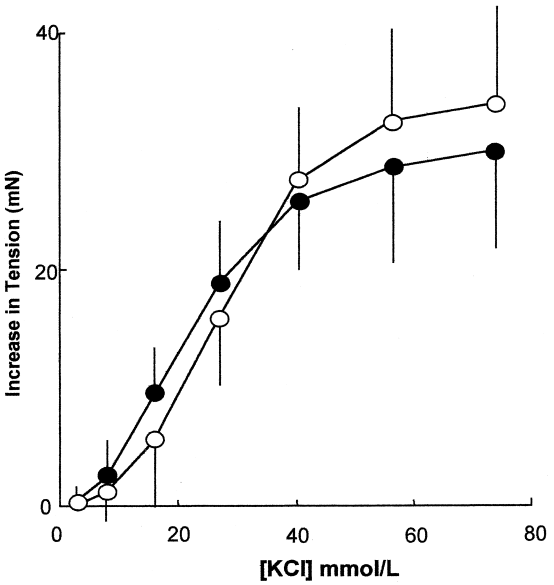

Fig. 3. Concentration dependent vasoconstrictor response curves obtained in pig coronary arteries to KCl, following treatment with modified fat diets. ○ Fish oil, ● beef tallow. Curves are not significantly displaced and are not significantly different from parallelism (ANOVA, linear regression analysis, n = 5–6). All values are the mean ±SEM.

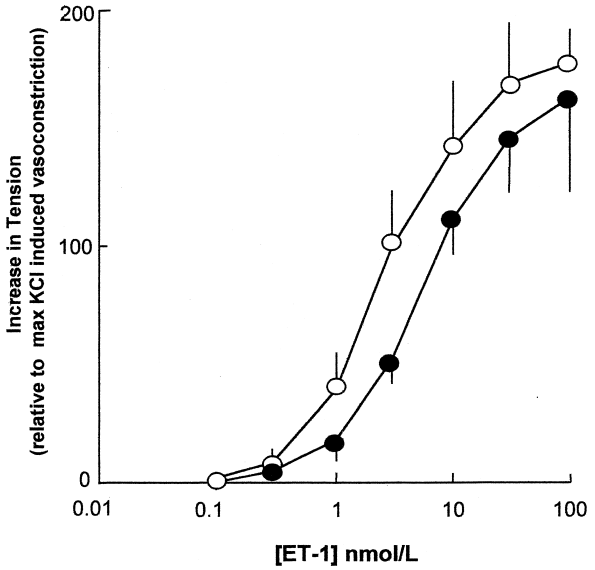

Fig. 4. Concentration dependent vasoconstrictor response curve obtained in pig coronary arteries to endothelin-1, following treatment with modified fat diets for 6 weeks. ○ Fish oil, ● beef tallow. Curves are not significantly displaced and are not significantly different from parallelism (ANOVA, linear regression analysis, n = 5–6). All values are the mean ±SEM.

either ET-1 or U46619 in both treatment groups (Students' paired t-test).

ET-1 caused a similar concentration dependent constriction of porcine coronary arterial rings in both beef tallow and fish oil groups (Fig. 4). The constriction observed to ET-1 in coronary arteries of both fish oils and beef tallow fed pigs was potent and of comparable magnitude to that caused by U46619 in arteries of fish oil fed pigs.

5-HT caused a weak concentration dependent constriction of porcine coronary arterial rings obtained from both beef tallow and fish oil treated groups. No significant differences were found in the vasoconstrictor concentration response curves to 5-HT between fish oil and beef tallow fed animals (Fig. 5A). There was also no significant difference in the maximum response obtained to 5-HT, fish oil (34 ± 14 mN, beef tallow, 37 ± 0.9 mN), following submaximal vasoconstriction by U46619. 5-HT caused vasorelaxation at concentrations ranging from (1–1000 μmol/L) (Fig. 5B) with a similar response in fish oil and beef tallow treated animals. Bradykinin (0.3–300 nmol/L) caused dose dependent vasorelaxation when studied under similar conditions as described for 5-HT (as described above). In fish oils treated pigs bradykinin caused a maximum vasorelaxation of 88.8 ± 13.8% of U46619 induced tone compared to 72.7 ± 10.5% in beef tallow fed animals (Fig. 6).

Concentration response curves for SNP obtained in arterial rings, constricted with U46619 were not significantly different between treatment groups (Fig. 7). SNP completely reversed the U46619 induced constriction in fish oil treated animals (maximal dilatation, 99 ± 6%). This was not significantly different compared to that obtained in beef tallow treated animals (112 ± 14%) (regression analysis, ANOVA).

4. Discussion

The results of this study indicate that short term dietary manipulation of n-3 PUFA can specifically affect porcine coronary artery vascular reactivity. In this study manipula-

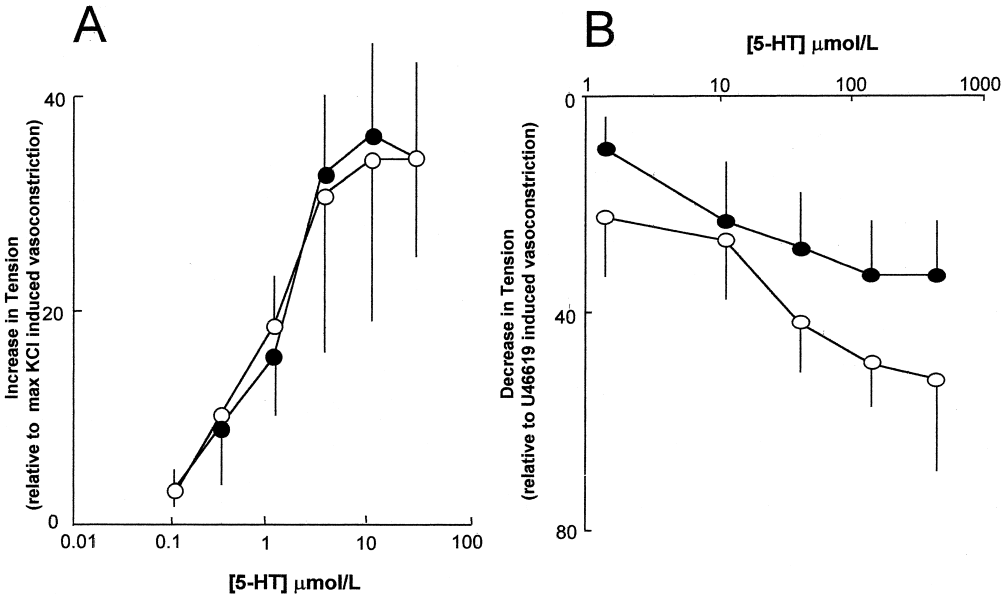

Fig. 5. (A) Concentration dependent vasoconstrictor response curves obtained in pig coronary arteries to 5-hydroxytryptamine, following treatment with modified fat diets ○ Fish oil, ● beef tallow (n = 5–6). (B) Concentration dependent vasodilator response curves obtained in pig coronary arteries constricted with U46619 to 5-hydroxytryptamine, following treatment with modified fat diets ○ Fish oil, ● beef tallow (n = 5–6). Curves in (A) and (B) are not significantly displaced and are not significantly different from parallelism (ANOVA, linear regression analysis). All values are the mean ±SEM.

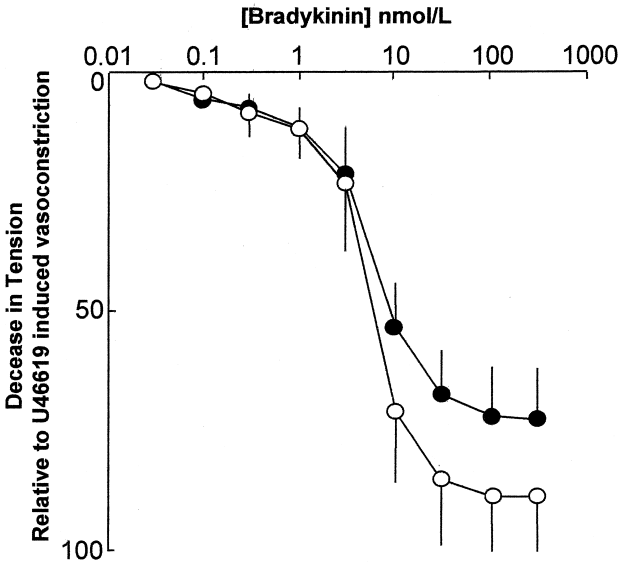

Fig. 6. Concentration dependent vasodilator response curves obtained in pig coronary arteries constricted with U46619 tobradykinin, following treatment with modified fat diets. ○ Fish oil, ● beef tallow (n = 3). All values are the mean ±SEM.

tion of fatty acid intake only affected TxA_2 receptor mediated vasoconstriction. Vasoconstrictor effects to KCl, endothelin and 5-HT were unaffected, as were vasodilator responses, in constricted vessels, to glyceryl trinitrate, 5-HT and bradykinin. Vasoconstrictor responses to the TxA_2 mimetic U46619 were significantly reduced in coronary arterial rings of pigs fed beef tallow in comparison to those fed fish oil. We observed a reduction in the maximum effect but

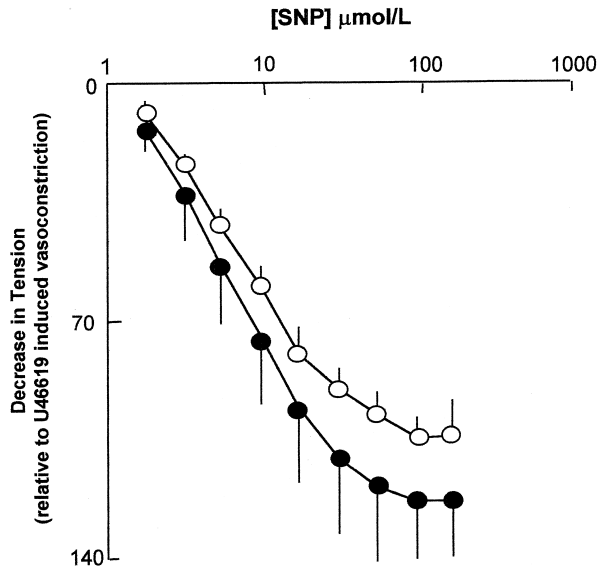

Fig. 7. Concentration dependent vasodilator response curve obtained in pig coronary arteries constricted with U46619 to sodium nitroprusside following treatment with modified fat diets. ○ Fish oil, ● beef tallow (n = 5–6). Curves are not significantly displaced and are not significantly different from parallelism (ANOVA, linear regression analysis). All values are the mean ±SEM.

there was no reduction in the sensitivity of pig coronary arteries to U46619. Therefore the elevated levels of plasma TxB_2 in beef tallow fed animals may be implicated in the reduced vasoconstrictor response of the coronary vasculature to the effects of TxA_2 agonists.

U46619 mediated coronary artery vasoconstriction in beef tallow treated animals may be reduced relative to those fed fish oil for a number of reasons. Our results are consistent with agonist-induced desensitization of TxA_2 receptors in the vascular smooth muscle of the porcine coronary artery. Agonist-induced desensitization of TxA_2 receptors has been demonstrated in a variety of preparations including platelets and various isolated cells *in vitro*, but whether a similar process occurs in intact blood vessels remains to be established [11,12]. It is also possible that post-receptor mediated events are involved.

A similar study to ours, although studying rat platelets, has shown that dietary n-3 PUFA results in an increase in U46619-induced stimulated platelet aggregation [13]. These investigators concluded that this response was part of a compensatory mechanism resulting from limited substrate availability for TxA_2 production. They further concluded that this effect was due to altered transduction of the TxA_2 signal as there were no significant differences in the receptor binding of U46619 or the TxA_2 antagonist SQ 29548 in platelets from animals fed n-3 or n-6 PUFA enriched diets.

It is clear that n-3 PUFA cause complex changes in the mechanism and structure of the vascular system, resulting in profound alterations in eicosanoid metabolism, plasma and tissue lipid levels, lipoprotein and triglyceride profiles and membrane fluidity [14]. These changes have the potential to affect TxA_2 receptor mediated responses by a variety of other mechanisms. n-3 PUFA can produce substantial changes in the membrane structure of vascular smooth muscle cells which could alter TxA_2 receptor configuration and function. n-3 PUFA and their derivatives may also have direct vasodilator effects on vascular smooth muscle [15] which could antagonize the effects of vasoconstrictors such as U46619. Additionally n-3 PUFA may interact with TxA_2 receptors directly leading to reductions in TxA_2 receptor mediated effects [16]. However, it would be unlikely that these effects would be specific for TxA_2 analogues as demonstrated in these experiments.

Karanian *et al*. [17,18] have demonstrated that 11-OH and 14-OH docosohexaenoic acid (DHA), but not DHA itself, can selectively and acutely antagonize the contractile effects of U46619 on the rat aorta and other tissues *in vitro*. Hydroxylated derivatives of both n-3 and n-6 PUFA can both antagonize the contractile actions of U46619 on vascular tissues [18]. It is of interest that the n-3 hydroxylated derivative of DHA, 14-OH-DHA (C22:6n3), is nearly twice as potent as the n-6 (C22:5n6) counterpart in this respect. More recent studies by Karanian *et al*. [18] have indicated that hydroxylated DHA derivatives produce a marked decrease in TxA_2 receptor affinity, but not receptor density in cerebral blood vessels of the rat. Increased dietary intake of

fish oil leads to chronic exposure of the vasculature to elevated levels of hydroxylated n-3 PUFA. If hydroxylated derivatives of eicosapentaenoic acid (EPA) and DHA from fish oil rich diets cause chronic receptor TxA_2 antagonism, one would expect TxA_2 mediated effect to be reduced whereas, the opposite effect was observed in our experiments.

It has been suggested that n-3 PUFA can raise the membrane potential of cells and thus, in the case of myocytes, require a greater electrical stimulus to produce an action potential and consequently cell contraction. This effect is believed to be largely mediated through fast, voltage dependent Na^+ channels although a similar effect of n-3 PUFA on outward K^+ and L-type Ca^{2+} channels has been suggested [20–22]. Despite these reports indicating marked changes in cellular excitability we observed no changes in KCl induced depolarization-contraction of porcine coronary arterial rings. We observed no differences in the maximum constriction of the arterial rings to KCl or the rate at which it was obtained.

Few studies have investigated interactions between ET-1 and n-3 PUFA in any organ or tissue and the few studies done have been complicated by pre-existing pathology. Uncertainty exists regarding the effects n-3 PUFA on circulating endothelin levels. Plasma ET-1 immunoreactivity has been reported to be increased following long-term dietary supplementation with n-3 fatty acids in microalbuminuric insulin dependent diabetes mellitus [23]. In contrast dietary fish oil supplementation in cyclosporin treated renal transplant patients has been associated with lowered endothelin levels [24]. In our study there appeared to be a tendency for a reduction in the ET-1 response of arterial rings in pigs fed beef tallow relative to those on fish oil, however this effect was not significant. The different responses obtained to the potent vasoconstrictors ET-1 and U46619, respectively between treatment groups would therefore tend to suggest that the effects we have obtained with U46619 are specific to U46619.

As with Shimokawa and Vanhoutte [25] we did not observe any differences for SNP-induced vasodilatation in the coronary arteries of either fish oil or tallow fed pigs. These results suggest that the vascular smooth muscle response to NO is not altered following dietary fat manipulation. Earlier studies by Shimokawa and colleauges [6,25, 26] also using porcine coronary arteries, indicated that dietary fish oils enhance the vasodilator response to bradykinin, a nitric oxide (NO) dependent dilator and 5-HT. The vasodilator response we obtained to 5-HT and bradykinin, in endothelium intact rings, from fish oil treated animals displayed a tendency to be augmented, consistent with these previous studies, but in our studies failed to reach statistical significance.

In conclusion, dietary n-3 PUFA supplementation results in a reduction in circulating bioactive thromboxane, which may subsequently modulate TxA_2 receptor mediated vascular responses. This effect is specific for the TxA_2 mimetic U46619, as examined in this study, similar effects did not occur with other vasoconstrictors such as ET-1, 5-HT and KCl.

Acknowledgments

We wish to thank Robert Blake for expert scientific and technical assistance. The research was supported by the National Heart Foundation of Australia and The University of Newcastle.

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