VASCULAR REACTIVITY AND HIGH DIETARY EICOSAPENTAENOIC ACID

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Abstract

Epidemiologic studies suggest that high dietary intake of
eicosapentaenoic acid (EPA), a precursor of the trienoic
prostaglandins, is associated with a low incidence and reduced
extent of myocardial infarction. Vascular reactivity of isolated
aortic strips from rats maintained for 3 weeks on a control diet or
on a diet supplemented with menhaden fish oil (17% EPA) was examined
with norepinephrine, sodium arachidonate, KCl, $PGF_2\alpha$ and
nitroprusside. Aortic strips from rats fed the fish oil diet were
significantly less responsive to the contractile effects of
norepinephrine and arachidonate compared to those from control diet
rats. Treatment of aortic strips with indomethacin decreased
responsiveness to norepinephrine. The magnitude of the decrease was
greater in control rats resulting in a similar vascular response
between the 2 groups after blockade. Contractions to arachidonate
were abolished by indomethacin. There were no differences in
vascular responses to KCl, $PGF_2\alpha$ and nitroprusside in aortic
strips from control diet rats and those from the fish oil diet
rats. Aortic strips from the fish oil diet rats contained more EPA
than those from the control diet rats. Thus, the contractile effect
of norepinephrine in isolated rat aortic strips is normally
augmented by intrinsic prostaglandins, and this augmentation is
diminished by dietary intake of EPA.

Introduction

Eicosapentaenoic acid (EPA) is found in high concentration in
fish of the Atlantic Ocean, and it has been suggested that a high
dietary intake of this fatty acid may be involved with the low
incidence of myocardial infarction in Greenland Eskimos (1).
Dietary supplementation with menhaden fish oil, an oil that is
relatively high in EPA (17%), reduces infarct size in experimental
models of cerebral artery ligation and coronary artery thrombosis
(2, 3). These reductions in infarct size were correlated with
changes in platelet aggregation. The goal of this study was to
determine the effects of fish oil supplementation on vascular
responsiveness in rats.

PROSTAGLANDINS

Methods

    Male Sprague-Dawley rats (200 g) were placed on the following
diet ad libitum (g/100 g diet): casein, 27.36; sucrose, 30.38;
cornstarch, 20.49; cellulose fiber, 6.87; soybean oil, 6.87; AIN-76
mineral mix, 6.05; AIN-76 vitamin mix, 1.40; choline chloride, 0.23;
and dl-methionine, 0.35 (AIN Ad Hoc Committee on standards for
nutritional studies, 1977). The fish oil-treated rats received 16.4
g/100 g of basal diet as menhaden fish oil (supplied by Dr. A.
Bimbo, Zapata Haynie Co., Reedville, Virginia). After three weeks
of either the control basal diet or the basal diet supplemented with
fish oil, the animals were terminated and their aortae were
excised. Helically cut strips of these aortae were mounted in organ
chambers and isometric contractions were recorded, as described
previously (4). The fatty acid content of aortic strips was
determined by gas liquid chromatography as described by Culp et al.
(3).
    Vascular responses were recorded for varying concentrations of
norepinephrine (Breon Laboratories, Inc.), sodium arachidonate
(Sigma Chemical Co.), $PGF_{2\alpha}$(Sigma Chemical Co.), PGE_2 (Sigma
Chemical Co.) and nitroprusside (Roche Laboratories) added
cumulatively to the muscle bath. Increasing potassium
concentrations in the muscle bath were achieved by equimolar
substitution of NaCl with KCl. Norepinephrine, PGF_2 and
nitroprusside were dissolved in water; sodium arachidonate was
dissolved in 100 mM Na_2CO_3 buffer in a concentration of 1.0
mg/ml and subsequently diluted in water. Indomethacin (Sigma
Chemical Co.) was dissolved in ethanol in a concentration of 5.0
mg/ml. In the experiments requiring inhibition of prostaglandin
cyclooxygenase, indomethacin was added to the muscle bath to achieve
a final concentration of 5.0 μg/ml ten minutes prior to the addition
of either norepinephrine or sodium arachidonate. Ethanol, in the
concentration used in these experiments (0.1%), had no effect on the
contractile responses of the aortic strips.

Results

    Cumulative addition of norepinephrine to the muscle bath
produced contractile responses in aortic strips from untreated rats
and fish oil-treated rats (Figure 1). Aortic strips from fish
oil-treated rats were less sensitive to the catecholamine than were
those from untreated rats. The concentration of norepinephrine
required to produce a half-maximal response (ED_{50}) was 20.4 x
10^{-10} M in the aortic strips from the fish oil-treated rats
compared to an ED_{50} of 7.4 x 10^{-10} M for aortic strips from
untreated rats ($p < 0.05$). The values for ED_{50} were determined by
logit-transformation and compared statistically by Student's "t"
test. Treatment of aortic strips with indomethacin (5 μg/ml), a
cyclooxygenase inhibitor, caused a significant shift to the right in
the dose-response relationship to norepinephrine in aortic strips
from both groups of rats. However, the magnitude of the shift was

greater in aortic strips from untreated rats; this resulted in similar vascular responses between the two groups of rats after cyclooxygenase blockade (fish oil-treated rats ED_{50} = 78.5 x 10^{-10} M versus untreated rats ED_{50} = 95.5 x 10^{-10}, p 0.05). There were no differences in the magnitude of maximal contractile responses to norepinephrine in aortic strips from either group of rats in the presence (fish oil-treated rats = 613 + 65 mg; untreated rats = 657 + 80 mg) or absence of indomethacin (fish oil-treated rats = 598 + 58 mg; untreated rats = 634 + mg).

Aortic strips from fish oil-treated rats were less responsive to the cumulative addition of sodium arachidonate to the muscle bath than were aortic strips from untreated rats (Figure 2). Indomethacin (5.0 μg/ml) completely inhibited contractile responses to sodium arachidonate in aortic strips from both groups of rats.

There were no differences in contractile responses to $PGF_{2\alpha}$ (Figure 3) or depolarizing concentrations of KCl (Figure 4) in aortic strips from the untreated and fish oil-treated rats. The magnitude of maximal contractile responses were: 1) $PGF_{2\alpha}$; fish oil-treated rats = 650 + 94 mg.; untreated rats = 650 + 110 mg; 2)
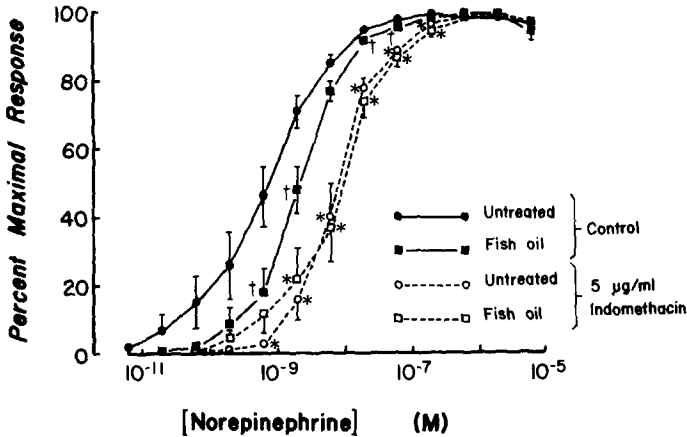

Figure 1. Dose-response to norepinephrine. Aortic strips from untreated and fish oil-treated rats were made to contract in response to the cumulative addition of norepinephrine to the muscle bath in the absence (control) and presence of 5.0 μg/ml indomethacin. Asterisks indicate a statistically significant difference (p < 0.05, Student's "t" test) between the control responses and those in the presence of indomethacin. The daggers indicate a statistically significant difference between responses in aortae from untreated rats and those from fish oil-treated rats. Values are the mean + SEM for 6 rats in each group.
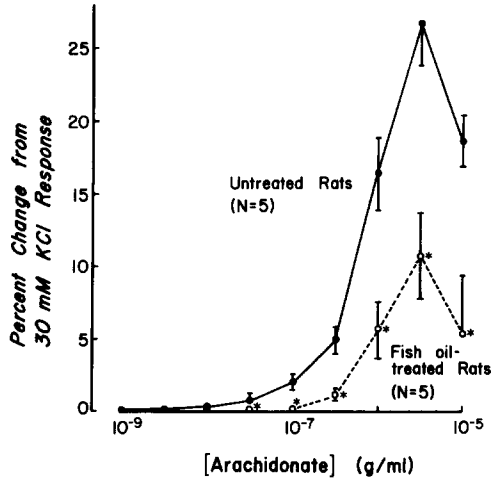
Figure 2. Dose-response to sodium arachidonate. Aortic strips from untreated and fish oil-treated rats were contracted with 30 mM KCl. After the contractile response had reached a plateau, sodium arachidonate was added cumulatively to the muscle bath. Asterisks indicate a statistically significant difference between aortae from untreated rats and those from fish oil-treated rats (p < 0.05, Student's "t" test). Values are the mean ± SEM for 5 rats in each group.
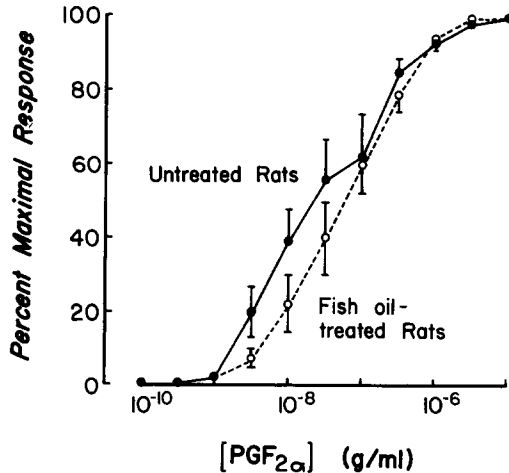

Figure 3. Dose-response to $PGF_2\alpha$. Aortic strips from untreated and fish oil-treated rats were made to contract in response to the cumulative addition of $PGF_2\alpha$ to the muscle bath. Values are the mean ± SEM for 5 rats in each group.

KCl; fish oil-treated rats = 559 $\pm$ 49 mg; untreated rats = 505 $\pm$ 42 mg. Treatment of the aortic strips with PGE_2 resulted in contractions and there were no differences between untreated and fish oil-treated rats (data not shown). There was no difference in the relaxing effect of nitroprusside on aortic strips from untreated and fish oil-treated rats that had been contracted with 30 mM KCl (Figure 5). Indomethacin (5.0 μg/ml) had no effect on the contractions induced by KCl nor the relaxing effect of nitroprusside, suggesting that the inhibitory effect on the norepinephrine and sodium arachidonate responses was not due to a non-specific action of the drug on the smooth muscle.

The fatty acid composition of aortae from the two groups of rats is depicted in Table 1. Aortae from the fish oil-treated rats had a higher percentage of 20:5 (n-3) (EPA), 22:5 (n-3), and 22:6 (n-3) fatty acids; these same aortae had decreased amounts of 18:2 (n-6) (linoleic acid), 18:1 (n-9) and 20:4 (n-6) (arachidonic acid) fatty acids compared to the aortae from untreated rats.

Discussion

These data show that a dietary supplement of menhaden fish oil decreases vascular responsiveness of isolated rat aortae to norepinephrine and sodium arachidonate, and this dietary manipulation results in a change of the fatty acid composition of the lipid fraction of blood vessel membranes.
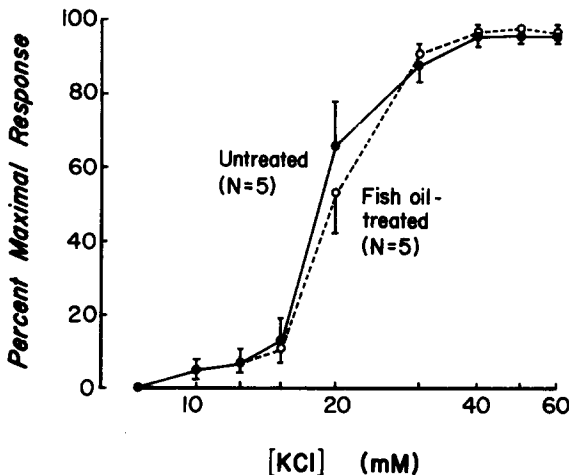

Figure 4. Dose-response to KCl. Aortic strips from untreated and fish oil-treated rats were made to contract in response to increasing concentrations of KCl. Phentolamine (10^{-6}M) was added to the bath to block the effect of norepinephrine released from adrenergic nerve endings. Values are the mean $\pm$ SEM for 5 rats in each group.
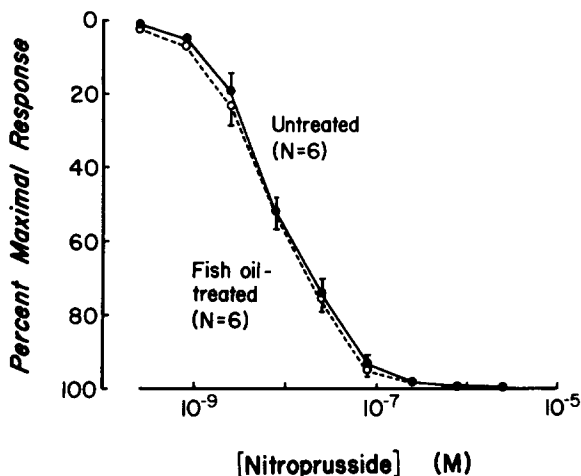
Figure 5. Dose-response to nitroprusside. Aortic strips from untreated and fish oil-treated rats were contracted with 30 mM KCl. After the contractile response had reached a plateau, nitroprusside was added cumulatively to the muscle bath. Values are the mean ± SEM for 6 rats in each group.

Table 1. Fatty acid composition of aortae[a].

FATTY ACID	FISH OIL-TREATED RATS	UNTREATED RATS
14:0	5.3 ± 0.3	2.2 ± 0.1
16:0	24.4 ± 0.6	25.3 ± 0.5
16:1 (n-7)	7.8 ± 0.4	5.6 ± 0.4
17:1 (n-8)	1.1 ± 0.0	0.6 ± 0.1
18:0	8.9 ± 1.9	9.7 ± 0.8
18:1 (n-9)	25.2 ± 0.8*	32.2 ± 1.4
18:2 (n-6)	11.5 ± 0.9*	16.1 ± 1.0
20:1 (n-9)	1.9 ± 0.2	1.3 ± 0.1
20:2 (n-6)	0.8 ± 0.1	0.4 ± 0.1
20:3 (n-6)	0.2 ± 0.1	0.3 ± 0.1
20:4 (n-6)	2.6 ± 0.6*	4.4 ± 0.8
20:5 (n-6)	3.0 ± 0.1*	0.4 ± 0.1
22:4 (n-6)	1.5 ± 0.0	0.7 ± 0.2
22:5 (n-6)	0.2 ± 0.0	0.2 ± 0.0
22:5 (n-3)	1.6 ± 0.1*	0.3 ± 0.1
22:6 (n-3)	2.8 ± 0.3	0.9 ± 0.2

[a] Fatty acids are designated by chain length:number of double bonds with the number in parentheses representing the carbon atom between terminal bond and methyl group. Values expressed as mole% are mean ± SEM, n=4 rats from each group. The asterisks indicate statistically significant differences between fish oil-treated rats and untreated rats ($p < 0.05$, Student's "t" test).

We suggest that treatment with norepinephrine causes a release of arachidonic acid in aortic strips from untreated rats, whereas there is a simultaneous release of arachidonic acid and EPA in response to norepinephrine in aortae from fish oil-treated rats. In the aortae from untreated rats, the arachidonic acid is enzymatically converted to the dienoic prostaglandins which contribute to the contractile responses induced by norepinephrine. In aortae from fish oil-treated rats, the simultaneous release of EPA with arachidonic acid suppresses the conversion of arachidonic acid to the dienoic prostaglandins and thereby abrogates any contributory action of the dienoic prostaglandins on norepinephrine-induced contractions.

Whitaker et al. (5) have shown that arachidonic acid and EPA are released with equal facility in tissue in response to vasoactive stimuli; also, they showed that EPA is a poor substrate for the blood vessel cyclooxygenase. It is suggested that the simultaneous release of EPA and arachidonic acid from aortae effectively blocks the conversion of arachidonic acid to vasoconstrictor metabolites. Furthermore, Whitaker et al. found that incorporation of ^{14}C-EPA into aortic rings did not lead to increased production of PGI$_3$, suggesting that it is not the formation of trienoic prostaglandins that are responsible for the biologic activity of EPA.

Dietary manipulation of EPA intake has been tried to a limited extent in rats (6,7) and humans (8). Seiss et al. (8) placed seven men on a mackerel diet for one week and found a reduction in platelet aggregation and thromboxane synthesis after low dose collagen stimulation. These changes corresponded to a change in the EPA/arachidonic acid ratio in platelet membranes. Seiss' group suggested that the reduction in the conversion of arachidonic acid to thromboxane was the result of competition between EPA and arachidonic acid for platelet cyclooxygenase.

The molecular mechanism by which high dietary EPA alters vascular reactivity is not known. It is not likely a result of changes in the contractile machinery (e.g., contractile proteins), as there were no differences in contractile responses to varying concentrations of KCl or in the relaxing effect of nitroprusside on aortic strips from untreated rats and fish oil-treated rats. It is very likely that the changes in vascular responsiveness to norepinephrine and sodium arachidonate in our study are a result of direct changes in prostaglandin metabolism for the following reasons: 1) cyclooxygenase inhibition resulted in a similar vascular response to norepinephrine in the two groups of rats; 2) indomethacin inhibited vascular responses to sodium arachidonate in both groups of rats; 3) aortae from the fish oil-treated rats contained increased amounts of EPA [20:5 (n-3)] and decreased amounts of arachidonic acid [20:4 (n-6)]; and 4) since vascular responses to PGF$_2\alpha$ and PGE$_2$ were not altered, the difference in responsiveness is not due to a change in events distal to the synthesis of prostaglandins (e.g., at receptors). This does not preclude the possible effect of other changes in cellular metabolism. For example, von Lossanyc, et al. (9) reported that a

(9) reported that a mackerel diet in monks significantly lowered serum cholesterol and serum triglycerides and raised high density lipoprotein cholesterol in addition to changing the EPA/arachidonic acid ratio. It is not known whether these changes in serum lipids were causative or secondary to changes in prostaglandin metabolism.

Whatever the molecular mechanisms involved, these findings are significant in that they demostrate a change in the vasculature's responsiveness to vasoactive stimuli due to a change in prostaglandin metabolism. The importance of these observations on isolated aortic strips in relation to the overall cardiovascular system are not clear. Generally, prostaglandins produced locally by the blood vessels reduce vascular responsiveness to norepinephrine in vivo (10). In vitro, the prostaglandins have been shown to attenuate or potentiate vascular responses to norepinephrine depending upon the regional vascular bed and the species studied (4, 10, 11). Kondo and associates (11) reported that PGE_2 potentiated the vascular response to norepinephrine in the isolated mesenteric and hindlimb vascular beds of rats, whereas PGE_2 inhibited the vascular response to norepinephrine in the splenic vascular bed. In all three vascular beds, indomethacin attenuated the vascular response to norepinephrine, and PGE_2 reversed the inhibitory effect of indomethacin in mesenteric and hindlimb beds, but not in the splenic bed. Thus, it is possible that dietary manipulation of the polyunsaturated fatty acids may change vscular responsiveness, and the precise nature of the effect will be dependent upon the vascular bed studied.

Acknowledgements

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