Marine Oils Dose-Dependently Inhibit Vasoconstriction of Forearm Resistance Vessels in Humans

Jaye P.F. Chin, Anthony P. Gust, Paul J. Nestel, and Anthony M. Dart

The effects of dietary supplementation with marine oils on vascular reactivity in human forearm resistance arteries were studied. Healthy male adults (six to nine subjects per group) were given either maxEPA capsules (content: eicosapentaenoic acid, 0.178 g/g; docosahexaenoic acid, 0.116 g/g) at doses of 20, 10, or 5 g/day or placebo capsules at 20 g/day for 28 days. Capsule compliance was confirmed by measurement of platelet membrane incorporation of n-3 fatty acids. Blood pressure was not affected by either maxEPA or placebo. The influence of treatment interventions on forearm vasoconstrictive responses to local infusions of angiotensin II and norepinephrine was examined using venous occlusion plethysmography before and after treatment. Responses to both agonists were significantly suppressed by 20 g/day maxEPA (slopes before and after maxEPA, respectively: angiotensin II, 3.34 and 0.89; norepinephrine, 0.91 and 0.41). When analyzed as difference in area under the dose–response curves, the suppressive effects of maxEPA were clearly dose dependent (angiotensin II: 20 g area reduced by 72%, 10 g by 67%, 5 g by 33%). Similarly, responses to norepinephrine were dose-dependently suppressed by maxEPA (20 g area reduced by 61%, 10 g by 63%, and 5 g by 33%). Placebo had no effect on the responses to either constrictor. The responses to both agonists returned to preoil levels after 2 months' discontinuation of 20 g/day maxEPA. We conclude that the suppressive effects of marine oils on vascular reactivity may, in part, contribute to their cardioprotective influence in humans. (Hypertension 1993;21:22-28)

KEY WORDS • n-3 polyunsaturated fatty acids • forearm resistance arteries • blood pressure • human studies

Dietary supplementation with n-3 polyunsaturated fatty acids of marine origin is widely reported to exert a range of biological effects of relevance to cardiovascular disease. Their protective effects include the ability to lower plasma lipids, in particular triglycerides and very low density lipoproteins1-2; to decrease production of the platelet aggregating factor thromboxane A23 and its product β-thromboglobulin4; and to lower blood pressure in subjects with hypercholesterolemia2-5 and in patients with essential hypertension.6,7 In the present study, we investigated whether dietary supplementation with marine oils induces changes in vascular reactivity that may contribute to their cardioprotective effect. This hypothesis follows reports of attenuated constrictor responses to norepinephrine in vessels isolated from spontaneously hypertensive rats fed marine oils8 as well as reduced constrictor responses to norepinephrine and angiotensin II in isolated perfused rabbit ear artery preparations in the presence of eicosapentaenoic acid, a major constituent of marine oils.9 Similar findings have also been reported in humans, in which a reduction in whole-body pressor responses to angiotensin II was observed after treatment with marine oils.4 In this study, a direct assessment of the influence of marine oils on vascular reactivity in humans is made using forearm venous occlusion plethysmography.

Methods

Subjects

Twenty-nine healthy adult males (18-32 years old) were recruited to participate in the study. Criteria for inclusion in the study included nonsmoking status, absence of alcohol abuse (less than two standard alcohol drinks per day), systolic blood pressure <140 and diastolic blood pressure <90 mm Hg, total serum cholesterol <5.5 mmol/l, and total triglyceride <2.0 mmol/l. In addition, all volunteers had normal findings on physical examination as well as routine hematology and biochemical blood analyses.

The protocol used was approved by the Alfred Group of Hospitals Ethics Committee, and written informed consent was obtained from all subjects.

Marine Oil Supplementation

A single-blind, placebo-controlled study design was used. All subjects were instructed to maintain, during oil supplementation, their preoil supplementation lifestyles. A parallel rather than crossover protocol was used because of our previous demonstration that a long...
Experimental Protocol

Subjects rested supine throughout the course of the experiment in a comfortable, relaxing environment maintained at a constant temperature of 22°C. Measurements of forearm vessel constriction were made both before (day 1) and after (day 29) each treatment intervention. Four subjects on the 20 g/day maxEPA regimen returned in the MOP capsules, as this was inherent in the experiment in a comfortable, relaxing environment maintained at a constant temperature of 22°C. Measurements of forearm vessel constriction were made both before (day 1) and after (day 29) each treatment intervention. Four subjects on the 20 g/day maxEPA regimen returned.

The incorporation of n-3 fatty acids into platelet membranes was analyzed both to monitor volunteer compliance in capsule consumption and to assess the effects of the varying maxEPA doses in changing membrane fatty acid composition. Forty milliliters of blood was taken from each subject after each treatment intervention and was placed into Tris-buffered tubes before separation of platelets by centrifugation. Platelet pellets thus obtained were stored in aqueous form (in 1 ml saline) at -70°C until batched and transported on dry ice to the CSIRO Division of Human Nutrition, Adelaide. Platelet fatty acids were subsequently transesterified and methylated, and the methyl esters were analyzed by gas chromatography with a capillary column. Results are given as percent of total platelet membrane fatty acid pool for eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid.

Statistical Analysis

Results are expressed as mean±SEM or standard error of the difference (SED) when appropriate. Statistical analysis was by Student's t test (paired when required). A value of p<0.05 was used as the criterion for statistical significance.

Lines of best fit were calculated for each group of agonist dose–response curves both before and after each treatment intervention. Comparisons of appropriate regression lines were then made using an analysis of covariance for comparisons of slopes and elevations.

A recommended method of analysis, the method of summary measures, was also undertaken for further analysis of dose–response curves, because there was a strong dependency between measurements within the same patient. A summary measure was obtained by calculation of the area under each individual dose–response (%FVR) curve obtained before and after each treatment intervention. Analysis performed by area under the curve gave the added advantage of a

### Table 1. Oil Capsule Content

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Mixed oil placebo (g)</th>
<th>maxEPA (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total saturated</td>
<td>29.6</td>
<td>25.4</td>
</tr>
<tr>
<td>18:1+16:1</td>
<td>30.9</td>
<td>28.3</td>
</tr>
<tr>
<td>18:2</td>
<td>39.5</td>
<td>...</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td>...</td>
<td>17.8</td>
</tr>
<tr>
<td>22:6 n-3</td>
<td>...</td>
<td>11.6</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>1.34</td>
<td>1.61</td>
</tr>
</tbody>
</table>

P/S ratio, polyunsaturated/saturated ratio. Placebo capsule content is based on the ratio cesatil (palm)/safflower/olive, 4:5:4/1.0. Composition is shown per 100 g.

Because a high within-subject coefficient of variation (range, 0.02–0.47; mean, 0.19±0.02; see Table 3) was observed on basal forearm vascular resistance, the response to each concentration of the agonists used was compared with the basal forearm vascular resistance obtained immediately before the administration of each dose. Similarly, because a high between-subject (pretreatment) variation of basal forearm vascular resistance values (range, 26.9–79.1 FVR units) was observed, responses were analyzed as a percent of basal responses.

### Platelet Membrane Fatty Acids

For statistical significance.

Lines of best fit were calculated for each group of agonist dose–response curves both before and after each treatment intervention. Comparisons of appropriate regression lines were then made using an analysis of covariance for comparisons of slopes and elevations.

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Results

Platelet Membrane Fatty Acids

Platelet membrane incorporation of the n-3 polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid dose-dependently increased with maxEPA supplementation (Table 2). Compared with levels obtained from subjects on the placebo supplementation, combined eicosapentaenoic acid and docosahexaenoic acid levels increased by approximately 230% in those on the 10 g/day maxEPA regimen, and by 103% in subjects on the 5 g/day maxEPA regimen, by 60.6±3.4% (Figure 2A; AUC before versus after 10 g/day maxEPA, 0.91 versus 0.41, \( p < 0.05 \)) after supplementation with maxEPA. Analysis of the slopes of dose–response curves demonstrated a similar attenuated response to norepinephrine after 20 g/day maxEPA treatment (before versus after maxEPA, 0.91 versus 0.41, \( p < 0.05 \)). These responses returned to preoil levels after 2 months’ discontinuation of 20 g/day maxEPA (Figure 1A) and were unaltered by MOP (Figure 1B).

As previously mentioned, basal forearm blood flow decreased after 10 g/day maxEPA. This corresponded to an increase in basal forearm vascular resistance (see Table 3) and complicated interpretation of the results obtained to the vasoconstrictor norepinephrine. Responses to this agonist were attenuated when expressed as percent difference in forearm vascular resistance (see Figure 1A). The area under the dose–response curve (AUC) obtained to norepinephrine decreased by 60.6±3.4% (Figure 2A; AUC before versus after maxEPA, 4,159.9±620.8 versus 1,576.1±116.6 units, \( p < 0.05 \)) after supplementation with maxEPA. Analysis of the slopes of dose–response curves demonstrated a similar attenuation of the responses to norepinephrine when expressed as percent difference in forearm vascular resistance (Figure 1A). The area under the dose–response curve (AUC) obtained to norepinephrine decreased by 60.6±3.4% (Figure 2A; AUC before versus after maxEPA, 4,159.9±620.8 versus 1,576.1±116.6 units, \( p < 0.05 \)) after supplementation with maxEPA. Analysis of the slopes of dose–response curves demonstrated a similar attenuation of the responses to norepinephrine when expressed as percent difference in forearm vascular resistance (Figure 1A). The area under the dose–response curve (AUC) obtained to norepinephrine decreased by 60.6±3.4% (Figure 2A; AUC before versus after maxEPA, 4,159.9±620.8 versus 1,576.1±116.6 units, \( p < 0.05 \)) after supplementation with maxEPA.
TABLE 3. Effects of Treatment Regimens on Responses of Forearm Vascular Resistance to Norepinephrine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Norepinephrine (25 ng/min)</th>
<th>Norepinephrine (50 ng/min)</th>
<th>Norepinephrine (100 ng/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FVR&lt;sub&gt;b&lt;/sub&gt;</td>
<td>FVR&lt;sub&gt;oc&lt;/sub&gt;</td>
<td>% diff</td>
</tr>
<tr>
<td>placebo</td>
<td>Before: 47.8</td>
<td>54.7</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>After: 46.9</td>
<td>50.9</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>SED: 10.6</td>
<td>11.0</td>
<td>2.7</td>
</tr>
<tr>
<td>5 g/day maxEPA</td>
<td>Before: 79.1</td>
<td>86.4</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>After: 79.2</td>
<td>71.7</td>
<td>-7.5</td>
</tr>
<tr>
<td></td>
<td>SED: 19.6</td>
<td>15.2</td>
<td>7.1</td>
</tr>
<tr>
<td>10 g/day maxEPA</td>
<td>Before: 43.6</td>
<td>51.1</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>After: 73.9</td>
<td>74.2</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>SED: 14.1</td>
<td>14.6</td>
<td>3.8</td>
</tr>
<tr>
<td>20 g/day maxEPA</td>
<td>Before: 26.9</td>
<td>36.2</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>After: 40.1</td>
<td>50.5</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>SED: 13.9</td>
<td>18.0</td>
<td>5.1</td>
</tr>
</tbody>
</table>

FVR<sub>b</sub>, mean basal forearm vascular resistance (FVR) obtained just before norepinephrine infusion; FVR<sub>oc</sub>, mean FVR during norepinephrine infusion; a. diff, mean absolute difference between FVR during norepinephrine and at rest; % diff, mean percent difference between FVR during norepinephrine and at rest; SED, standard error of difference. maxEPA treatment at 5, 10, and 20 g/day was for 28 days.

Table 4 shows forearm vascular resistance measurements obtained immediately before and during each infusion of angiotensin II for each treatment regimen. Dietary supplementation with 20 g/day maxEPA for 28 days significantly suppressed the three-point dose-response curve to angiotensin II (Figure 3A; slope before versus after maxEPA, 3.2 versus 0.9, p<0.05). When analyzed as area under the dose-response curves, dietary supplementation with 20 g/day maxEPA suppressed the response to angiotensin II by 71.8±9.0% (Figure 2B; AUC before versus after maxEPA, 1,971.4±253.9 versus 607.2±200.1 units, p<0.05). In the four subjects who returned after a 2-month washout period, responses to angiotensin II returned to preoil levels (Figure 3A). Responses to angiotensin II were also significantly suppressed by maxEPA at the lower doses of 10 g/day (by 67.1±4.0% when analyzed as AUC, p<0.05, Figure 2B) and 5 g/day (by 33.5±17.5%, p<0.05, Figure 2B). MOP supplementation (20 g/day for 28 days, Figure 3B) had no effect on responses to angiotensin II.

Discussion

In the present study, forearm vasoconstrictor responses to the sympathetic nervous system neurotransmitter norepinephrine and the renin-angiotensin system effector angiotensin II were markedly attenuated after dietary supplementation with marine oils. One strength of the study lies in the null effect seen after placebo supplementation, which confirms that the decreased responses to both agonists seen with marine oils were not a result of habituation of the study protocol, changed caloric intake, or differences in polyunsaturated/saturated ratio. The findings are further substantiated by the return to normal (before maxEPA) responses seen with both constrictors after the 2-month washout period. Although the placebo capsules were

**FIGURE 1.** Panel 1A: Line graph shows three-point dose-response curve to norepinephrine is significantly suppressed by dietary supplementation with 20 g/day maxEPA for 28 days. These responses returned to preoil levels after a 2-month washout period. Analysis was by covariance for comparisons of slopes and elevations. *p<0.05, significantly different compared with dose-response curve obtained before treatment. Panel 1B: Line graph shows mixed oil placebo capsules had no effect on responses obtained to norepinephrine. FVR, forearm vascular resistance.
norepinephrine

Hypertension
Vol 21, No 1 January 1993

between the dose of maxEPA and its suppressive effect on decrease in AUC, percentage decrease in area under the vasoconstriction to norepinephrine and angiotensin II. %

FIGURE 2. Bar graphs show clear dose-dependent relation from animals fed marine oils demonstrated similar

performed in animal models,

5 g/day maxEPA

20 g/day maxEPA

Placebo

After

Before

SED

5 g/day maxEPA

Before

After

SED

10 g/day maxEPA

Before

After

SED

20 g/day maxEPA

Before

After

SED

The suppressive effects of marine oils on drug-in-
duced vasoconstriction are in accordance with studies performed in animal models, in which vessels isolated from animals fed marine oils demonstrated similar antagonistic effects. Our findings are also in accord with the observation that marine oils lower the peak pressures induced by the endogenous stressors angiotensin II and norepinephrine, thus protecting against cardiovascular dysfunction related to high levels of these agonists. The modification by marine oils in responses to the effectors of the sympathetic nervous system and the renin-angiotensin system, both of which have been reported to contribute to the development of hypertension, may also, in part, underlie epidemiological reports of lower incidences of hypertension in populations with high fish consumption.

The blood pressure-lowering effect of marine oils, previously reported in several other papers, was not observed in the present study. A closer examination of the literature, however, reveals that a hypotensive effect of marine oils appears to be present only in subjects with high blood pressure or hypercholesterolemia and not in healthy, normocholesterolemic, normotensive subjects or subjects with controlled (treated) hypertension. It has previously been suggested that the blood pressure response to marine oils is dependent on the initial blood pressure of the patient, and it is likely that any changes induced by marine oils on the blood pressure of normotensive subjects is too small to demonstrate significance, particularly in a parallel study design. That the blood pressure-lowering effect of marine oils has also been demonstrated, to date, only in normotensive subjects with hyperlipidemia suggests the interesting possibility that this effect is dependent on changes in circulating lipoproteins. The lack of effect of marine oils on blood pressure in the present study was due to differences in dosage as compared with previous studies. Reported doses effective in lowering blood pressure in hypertensive and hypercholesterolemic patients vary, ranging from approximately 3.0 to 15 g of n-3 polyunsaturated fatty acids per day. In our study, the maximum dose of 20 g/day maxEPA (equivalent to n-3 polyunsaturated levels of 5.88 g/day) was

![Graph 2A: norepinephrine](image)

![Graph 2B: angiotensin II](image)

**TABLE 4. Effects of Treatment Regimens on Responses of Forearm Vascular Resistance to Angiotensin II**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Angiotensin II (8 ng/min)</th>
<th>Angiotensin II (16 ng/min)</th>
<th>Angiotensin II (32 ng/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FVR&lt;sub&gt;r&lt;/sub&gt;</td>
<td>FVR&lt;sub&gt;a&lt;/sub&gt;</td>
<td>a. diff</td>
</tr>
<tr>
<td>Placebo</td>
<td>Before</td>
<td>52.9</td>
<td>66.9</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>45.7</td>
<td>51.6</td>
</tr>
<tr>
<td></td>
<td>SED</td>
<td>12.3</td>
<td>15.2</td>
</tr>
<tr>
<td>5 g/day maxEPA</td>
<td>Before</td>
<td>59.7</td>
<td>67.5</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>48.3</td>
<td>51.9</td>
</tr>
<tr>
<td></td>
<td>SED</td>
<td>6.3</td>
<td>8.1</td>
</tr>
<tr>
<td>10 g/day maxEPA</td>
<td>Before</td>
<td>26.0</td>
<td>37.1</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>53.0</td>
<td>61.1</td>
</tr>
<tr>
<td></td>
<td>SED</td>
<td>8.6</td>
<td>18.9</td>
</tr>
<tr>
<td>20 g/day maxEPA</td>
<td>Before</td>
<td>36.6</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>46.9</td>
<td>56.9</td>
</tr>
<tr>
<td></td>
<td>SED</td>
<td>21.9</td>
<td>28.7</td>
</tr>
</tbody>
</table>

FVR<sub>r</sub>, mean basal forearm vascular resistance (FVR) obtained just before angiotensin II infusion; FVR<sub>a</sub>, mean FVR during angiotensin II infusion; a. diff, mean absolute difference between FVR during angiotensin II and at rest; % diff, mean percent difference between FVR during angiotensin II and at rest; SED, standard error of difference. maxEPA treatment 5, 10, and 20 g/day was for 28 days.
ineffective in reducing blood pressure in normotensive subjects. Because blood pressure was unaltered in these subjects, we suggest that the changes observed with marine oils, seen only on drug-induced vasoconstriction, is consistent with a more efficacious effect of maxEPA on amplified vasoconstrictor responses seen in individuals with high blood pressure. This may underlie the more potent effect of maxEPA as a hypotensive agent in hypertensive patients compared with normotensive subjects.

The finding that 10 g/day maxEPA decreased forearm basal blood flow was unexpected and suggests that at this concentration marine oils may be exerting a chronic vasoconstrictor effect as evidenced by the increase obtained in basal forearm vascular resistance. The change in basal forearm resistance values induced by 10 g/day maxEPA rendered interpretation of the responses obtained to the vasoconstrictors used difficult; however, when results were expressed as a percentage of the basal responses obtained, 10 g/day maxEPA was observed to decrease the slope and area of the concentration-response curves obtained to both these agonists. At this concentration, therefore, vascular reactivity to both norepinephrine and angiotensin II is suppressed, although a potentiation to basal vascular resistance results was obtained. Because alteration of prosta-


9. Juan H, Sametz W: Vasoconstriction induced by norepinephrine and angiotensin II is antagonised by eicosapentaenoic acid independent of formation of trienoic eicosanoids. *Naunyn Schmiedebergs Arch Pharmacol* 1986;332:288–292


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Hypertension. 1993;21:22-28
doi: 10.1161/01.HYP.21.1.22

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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