Journal of the American College of Cardiology
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Published by Elsevier Science Inc.

Vol. 35, No. 2, 2000
ISSN 0735-1097/00/$20.00
PII S0735-1097(99)00548-3

Dietary Supplementation With Marine Omega-3 Fatty Acids Improve Systemic Large Artery Endothelial Function in Subjects With Hypercholesterolemia

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OBJECTIVE This work was undertaken to determine whether dietary supplementation with marine omega-3 fatty acids improve systemic large artery endothelial function in subjects with hypercholesterolemia.

BACKGROUND Marine omega-3 fatty acids improve vascular function, but the underlying mechanism(s) are unclear. We studied the effects of marine omega-3 fatty acids on large artery endothelial function in subjects with hypercholesterolemia.

METHODS Hypercholesterolemic subjects with no other known cause for endothelial dysfunction were recruited to a prospective, placebo-controlled, randomized, double-blind, parallel-group study. Treatment with omega-3 fatty acids at a dose of 4 g/day (n = 15/group) was compared with placebo, at the beginning (day 0) and end (day 120) of a four-month treatment period. Endothelial function was assessed pre- and posttreatment by noninvasive ultrasonic vessel wall tracking of brachial artery flow-mediated dilation (FMD).

RESULTS Treatment with marine omega-3 fatty acids resulted in a significant improvement in FMD (0.05 ± 0.12 to 0.12 ± 0.07 mm, $p < 0.05$) and a significant reduction in triglycerides (2.07 ± 1.13 to 1.73 ± 0.95 mmol/liter, $p < 0.05$), whereas treatment with placebo resulted in no change in FMD (0.03 ± 0.10 to 0.04 ± 0.10 mm) or triglycerides (2.29 ± 2.09 to 2.05 ± 1.36 mmol/liter) (both $p < 0.05$ treated compared with control). Responses to sublingual glyceryl trinitrate were unchanged.

CONCLUSIONS Marine omega-3 fatty acids improve large artery endothelium-dependent dilation in subjects with hypercholesterolemia without affecting endothelium-independent dilation. (J Am Coll Cardiol 2000;35:265–70) © 2000 by the American College of Cardiology

Atherosclerotic coronary artery disease is a major cause of morbidity and mortality in Western civilization. Atherosclerosis may be regarded as the long-term consequence of a chronic inflammatory condition of large arteries (1), in which endothelial dysfunction (2) plays a key role. Endothelial function has been assessed in the coronary circulation by measuring vascular reactivity to intracoronary infusion of endothelium-dependent agonists such as acetylcholine. Patients with atherosclerotic coronary artery disease exhibit paradoxical vasoconstriction (3), as do patients with hypercholesterolemia and angiographically normal coronaries (4). Such tests are invasive, expensive and not without risk. An alternative approach is to study vascular reactivity noninvasively with ultrasonic assessment of brachial artery flow-mediated dilation (FMD) (5). The brachial artery is of a similar size to coronary arteries, and although it is unusual for the brachial artery to have significant atheroma, brachial responses have been shown to correlate well with responses in the coronary circulation for a given individual (6). Endothelial function can be measured noninvasively by "wall tracking" of brachial artery dilation in response to increased flow generated by hyperemia of the hand (7,8). Flow-related endothelial function has been shown to be mediated by NO (9,10) and impaired with all known risk factors for atheroma (11–15). Endothelial dysfunction, thus measured, appears to be representative of generalized endothelial dysfunction and offers a potentially useful measure of the susceptibility to atheroma.

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Manuscript received December 31, 1998; revised manuscript received August 17, 1999, accepted October 18, 1999.

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Abbreviations and Acronyms

DHA = docosahexanoic acid
EPA = eicosapentanoic acid
FMD = flow-mediated dilation
GTN = glyceryl trinitrate
HDL = high-density lipoprotein
LDL = low-density lipoprotein
NO = nitric oxide
RF = radio frequency
VLDL = very low density lipoprotein

Among dietary interventions that might protect against atheroma and its complications are diets rich in fish. The protective effects of diets rich in fish oil are quite strongly supported by experimental (16,17), epidemiological (18–23) and clinical trial data (24). Beneficial effects of fish oil supplementation on endothelial function in resistance arteries in vivo (25) and in vitro (26) have been reported. Accordingly, we carried out a placebo-controlled four-month trial of marine omega-3 fatty acids ("fish oil") in fit subjects with hypercholesterolemia in order to test whether omega-3 fatty acids also improved large artery endothelial function as measured by flow-mediated brachial artery dilation.

METHODS

Subjects. Thirty subjects were recruited from the Lipid Clinic at the University Hospital of Wales, Cardiff. All had confirmed hypercholesterolemia (serum total cholesterol >6.5 mmol/liter) after a low-fat diet for three months. Those already on lipid-lowering agents had stable cholesterol levels, and the dose of lipid-lowering agent remained unaltered for the duration of the trial. Exclusion criteria were active smokers, recent ex-smokers (two years), diabetes, hypertension (including treated hypertension) and a clinical history of coronary, cerebral or peripheral vascular disease. Subjects taking hormone supplements, vasoactive medications or proprietary medications such as vitamins, antioxidants or fish oils were also excluded.

Study design. This was a prospective, placebo-controlled, randomized, double-blind, parallel-group trial. Effects of treatment were compared at the beginning (day 0) and end (day 120) of a four-month treatment period in parallel groups of subjects with hypercholesterolemia. All subjects underwent a full clinical examination. Venous blood samples were obtained, and flow-mediated brachial artery dilation was measured on days 0 and 120.

Marine omega-3 fatty acids. Thirty subjects were recruited and randomly assigned to two groups of 15 subjects to receive: a) marine omega-3 fatty acids (K85; Pronova a.s, Oslo, Norway), or b) placebo (corn oil), each as two 1-g capsules twice daily for 120 days. Baseline characteristics were similar in both groups (Table 1). The K85 capsules used in the study were omega-3 concentrate enriched in eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) and produced from high-quality whole-body fish oil. The concentration of these two fatty acids as esters is about 85%, which is approximately threefold higher than in Maxepa capsules. K85 also contains 4 IU of vitamin E per capsule. The corn oil placebo capsules contained no vitamin E.

Table 1. Baseline Characteristics of Subjects in Omega-3 Fatty Acids Study

	Placebo (n = 15)	Marine Omega-3 Fatty Acid (n = 13)
Age (years)	50 ± 12	56 ± 13
Male/Female (n)	11/4	8/6
Total cholesterol (mmol/liter)	7.45 ± 0.64	7.85 ± 1.64
HDL cholesterol (mmol/liter)	1.34 ± 0.39	1.41 ± 0.38
LDL cholesterol (mmol/liter)	5.31 ± 0.73	5.00 ± 0.73
Triglyceride (mmol/liter)	2.29 ± 2.09	2.07 ± 1.13
Glucose (mmol/liter)	4.95 ± 0.66	4.95 ± 0.59
Body mass index (kg/m^2)	25.18 ± 2.70	26.90 ± 3.04
Smoking status	0	0
Blood pressure	137 ± 10	138 ± 13
mm Hg	86 ± 9	87 ± 9
Statin (n)	3	1
Fibrate (n)	1	2
Nil (n)	12	10

No significant differences between the two groups. Data are given as mean ± SD.

Measurement of endothelial function. Flow-mediated dilation was measured by ultrasonic wall tracking as reported previously (7). The system used comprises a specially adapted duplex color flow echo machine (Diasonics Spectra) with a 7.5-MHz linear phased-array transducer (giving high axial resolution), a personal computer and a 4-Mb high-speed memory. The brachial artery is identified using the ultrasound transducer, and anatomical landmarks are identified to allow repeat studies. A standoff device containing ultrasound-coupling gel prevents compression of the anterior wall of the artery. The transducer is held in a stereotactic clamp, and a two-dimensional longitudinal B-mode image of the brachial artery is obtained. The radio frequency (RF) signals (sampling frequency 1 kHz) from the M-mode output are digitized and relayed to the wall tracking system (Vadirec, Medical Systems Arnhem, Oosterbeek, The Netherlands). On completion of 10-s data acquisition, the RF signal is displayed so that the position of the anterior and posterior vessel walls on the RF signal can be identified and marked. Vessel wall movements are tracked using the stored RF signals to produce displacement waveforms of the anterior and posterior vessel walls together with the distension waveform (diameter change as a function of time). The distension waveform enables measurement of "end-

diastolic" diameter for each beat (theoretical resolution ± 3 μm) (27).

Blood pressure was recorded throughout the study by photo-plethysmography (Finapres) from a finger cuff on the middle finger of the ipsilateral arm. Blood flow was measured throughout the study using an 8-MHz continuous wave Doppler probe mounted at an angle of 60° in a perspex block and positioned over the brachial artery distal to the 7.5-MHz probe. The Doppler signals were analyzed by a spectrum analyser (SciMed Dopstation, Bristol, UK) and stored on metal audiotape using a high-performance recorder (Nakamichi B-100E, Nakamichi Corporation, Japan). Brachial artery blood flow was calculated by multiplying the mean blood velocity (corrected for Doppler angle) by the internal brachial artery diameter measured by wall tracking.

Study protocol. All studies were performed in the morning in a temperature-controlled room (21°C to 23°C) on fasting subjects after a 15-min supine rest, with the arm held outstretched on a pneumatic cushion. Patients were asked to avoid caffeine-containing beverages for 12 h before the study. Measurements were made at baseline, during hand hyperemia and after sublingual glyceryl trinitrate (GTN), an endothelium-independent vasodilator.

Hand hyperemia. A pediatric sphygmomanometer cuff was inflated at the wrist to suprasystolic pressure (systolic pressure ± 50 mm Hg) for 5 min. Blood flow was recorded from 15 s before until 90 s after cuff release, and internal brachial artery diameter was measured for 10 s at 60 to 70 s after cuff release. All measurements were repeated ≥ 15 min later until values reached original baseline levels. Flow-mediated dilation was defined as brachial artery diameter at 60 to 70 s after cuff release minus baseline diameter (expressed in mm).

GTN. Measurements were repeated 3 min after sublingual GTN spray (400 μg).

Serum concentrations. Fasting venous blood samples were obtained at each study for measurement of total serum cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol (calculated), triglyceride and glucose. Urea and electrolytes and liver function were also measured.

Statistics. Data are presented as mean ± SD. Data were tested for normality using the Shapiro-Wilks test. Where normality was established unpaired Student's *t* tests were used to make comparisons between groups and Student's paired *t* test to make comparisons within groups. $p < 0.05$ was regarded as significant.

Ethical approval. Ethical approval for this study was granted by the local research and ethics committee of South Glamorgan Health Authority. All subjects gave written informed consent. The investigation conformed to the principles outlined in the Declaration of Helsinki.

RESULTS

There were no significant differences between the treatment groups at baseline (Table 1). Of the 15 subjects allocated to each group, 28 completed the study. The two subjects who did not return for the second scan were in the fish oil group; no reasons for leaving the study were reported. No adverse side effects were reported. Compliance with treatment was assessed by a count of capsules returned at study end and was considered satisfactory (>95% for placebo and marine omega-3 fatty acids).

Changes in brachial artery blood flow immediately after wrist cuff release (peak flow, 1 min after cuff release and 3 min after sublingual GTN) were similar in all groups before and after treatment (Fig. 1A). Flow-mediated dilation increased significantly after omega-3 fatty acids treatment compared with placebo (Table 2, Fig. 1B). Treatment with omega-3 fatty acids significantly reduced triglyceride levels (Table 2) but had no effect on serum concentration of total cholesterol, VLDL, LDL or HDL cholesterol, whereas treatment with placebo had no significant effect on the lipid profiles (Table 2).

There was no correlation between the improvement in endothelium-dependent FMD and the reduction in triglycerides in the marine omega-3 fatty acids group.

Glyceryl trinitrate-induced dilation was similar pre- and posttreatment in both groups (Table 2, Fig. 1C).

DISCUSSION

The major findings of this double-blind placebo-controlled study are that marine omega-3 fatty acids (fish oils) improve endothelial function in systemic large arteries in patients with hypercholesterolemia. This study also confirms the loss of FMD in the brachial artery, reflecting impaired endothelium-derived nitric oxide (NO) activity in hypercholesterolemic patients as previously reported (28,29). It confirms also that vascular smooth muscle dilator responsiveness to NO is preserved, as evidenced by the normal dilator response to GTN.

Dietary supplementation with marine omega-3 fatty acids for four months resulted in a significant improvement in endothelium-dependent FMD of the brachial artery. This artery is of a similar size to the coronary arteries, and brachial responses have been shown to correlate well with responses in the coronary circulation for a given individual (6). There was a significant decrease in serum triglycerides with omega-3 fatty acids supplementation, which has previously been reported (30). In this study, the improvement in endothelial function did not correlate with the reduction in triglycerides, which is not unexpected, as our subjects had hypercholesterolemia with significantly elevated total and LDL cholesterol levels, with only modestly elevated triglyceride levels. Hypertriglyceridemia is not as strongly associ-

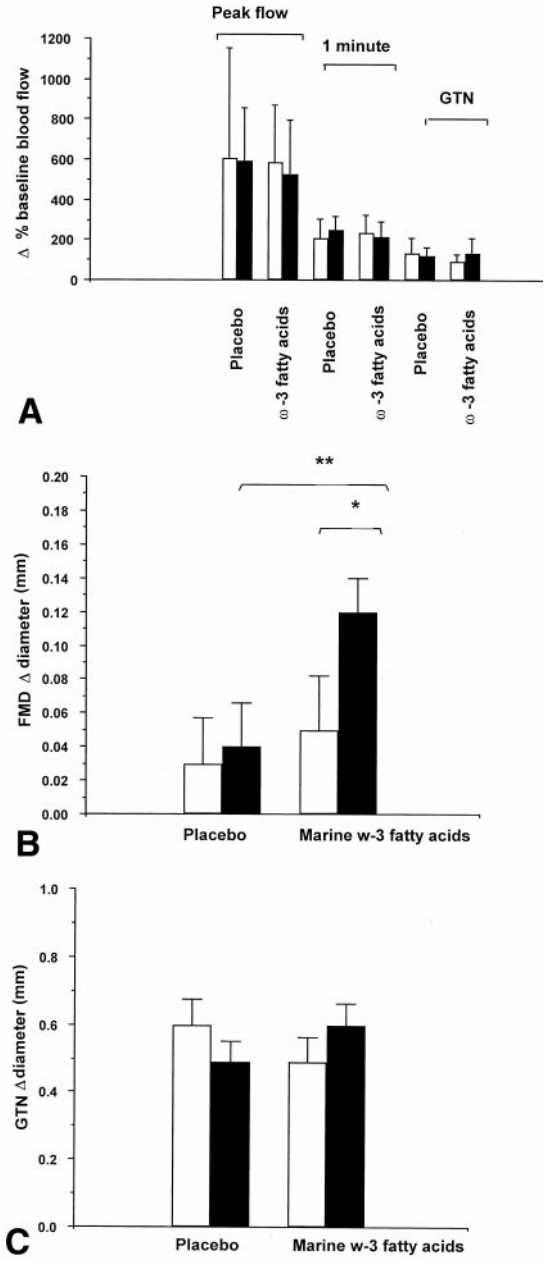

Figure 1. The effect of four months of treatment with placebo and omega-3 fatty acids on the following. **A**, Brachial artery blood flow (expressed as percent change from baseline) at: peak flow immediately after wrist cuff deflated, 1 min after cuff deflated and 3 min after 400 μg sublingual GTN. There were no significant differences between groups. **B**, Flow-mediated dilation expressed as absolute change (mm) from baseline diameter. There is a significant improvement within the omega-3 fatty acids group posttreatment (*$p < 0.05$) and when compared with placebo posttreatment (**$p < 0.05$). **C**, Glyceryl trinitrate-mediated dilation expressed as absolute change (mm) from baseline diameter. There were no significant differences between groups. Data are presented as mean ± SEM.
Solid bars = posttreatment; **Open bars** = pretreatment.

ated with coronary atherosclerosis (31) as elevated LDL cholesterol (32), and when multivariate analysis is used to correct for LDL and HDL cholesterol, much of the association with hypertriglyceridemia disappears (33). Further support for a lack of association between hypertriglyceridemia and endothelial function comes from a recent study that demonstrated that severe hypertriglyceridemia was not associated with significant dysfunction of the L-arginine/NO pathway in forearm resistance vessels (34).

Possible mechanism(s) for improvement in endothelial function. The mechanism underlying the improvement in endothelial function in patients treated with marine omega-3 fatty acids in this study is unclear. We did not give indomethacin, therefore, we cannot exclude the possibility that vasodilator prostaglandins played a role. However, based on animal studies (16) and a recent clinical study (26), this would appear to be unlikely. Recent evidence suggests that the mechanism(s) responsible for the benefit seen with a fish oil-rich diet is likely to relate to changes in membrane bilipid layer composition with multiple potential effects on endothelial function. The recent in vitro small artery study by Goode et al. (26) demonstrated that the greatest improvement in endothelial function occurred in those patients who had the greatest increase in membrane EPA and DHA, as reflected by increases in these fatty acids in the red cell membrane. Hence, it is possible that dietary supplementation with marine omega-3 fatty acids may change the membrane fluidity of endothelial cells, promoting increased synthesis and/or release of NO. The marine omega-3 fatty acids preparation we used contains a small amount of antioxidant vitamin E (equal to approximately 16 IU vitamin E/day), which theoretically may be expected to have an effect. In a study of similar design, we have shown dietary supplementation with 20 IU vitamin E/day for four months had no benefit in hypercholesterolemic subjects (unpublished data, J.G.), as have others (35). Feeding humans fish oils has been shown to reduce oxygen-derived free radical formation in neutrophils and monocytes, and to enhance NO production by cultured human endothelial cells (36). Speculatively, it is also possible that a reduction in the formation of oxygen derived free radicals by endothelial cells and thus increased bioavailability of NO contributed to the effects observed in this study.

Clinical implications. This study adds evidence relevant to the complex but important issue of dietary reduction of atherogenesis, given the key-initiating role of endothelial dysfunction. Marine omega-3 fatty acids offer attractive potential, which has recently been supported by evidence of clinical benefit (37). Clinical evidence is necessarily weighted towards changes in the end-stage complications of coronary artery disease, and this may be susceptible to measures that alter plaque cap inflammation and vulnerability to fissuring and thrombosis as well as the intrinsic process of atherogenesis. The latter changes may be longer term and less readily detectable. This study, through the measurement of endothelial function indices, supports a possible future role for omega-3 fatty acids and may help to

Table 2. Effects of Four Months of Treatment With Placebo and Omega-3 Fatty Acids

	Placebo (n = 15)		Omega-3 Fatty Acids (n = 13)	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
Baseline diameter (mm)	4.17 ± 0.65	4.08 ± 0.68	4.12 ± 0.84	4.02 ± 0.77
Absolute change in baseline diameter				
FMD (mm)	0.03 ± 0.10	0.04 ± 0.10	0.05 ± 0.12	0.12 ± 0.07*
GTN 400 μg (mm)	0.60 ± 0.29	0.49 ± 0.23	0.49 ± 0.25	0.60 ± 0.22
Total cholesterol (mmol/liter)	7.45 ± 0.64	7.20 ± 0.71	7.85 ± 1.64	7.69 ± 1.25
HDL cholesterol (mmol/liter)	1.34 ± 0.39	1.28 ± 0.29	1.41 ± 0.38	1.34 ± 0.38
LDL cholesterol (mmol/liter)	5.31 ± 0.73	5.25 ± 0.71	5.00 ± 0.73	5.35 ± 1.02
Triglyceride (mmol/liter)	2.29 ± 2.09	2.05 ± 1.36	2.07 ± 1.13	1.73 ± 0.95*
Glucose (mmol/liter)	4.95 ± 0.66	4.82 ± 0.40	4.95 ± 0.59	5.05 ± 0.35

Flow-mediated dilation (FMD) and GTN-induced dilation are shown as absolute change compared with baseline diameter. Flow-mediated dilation was significantly increased and triglyceride level decreased in the omega-3 fatty acids group compared with placebo (both $^*p < 0.05$).

explain the benefits shown in epidemiological studies of populations consuming a fish-rich diet.

Acknowledgment

We thank our colleagues Dr. J. A. E. Rees and Dr. M. A. Mir for permission to recruit their patients; research nurses L. A. Luddington and M. Brownlee for help with organizing patient recruitment and data collection; S. T. Gorman for help in vascular scanning; Dr. F. Dunstan for statistical advice; and Professor A. H. Henderson for advice and comments during the preparation of this manuscript.

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