

Fish Oil and Endothelial Function

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Eicosapentaenoic acid and docosahexaenoic acid modulate mitogen-activated protein kinase activity in endothelium.

Xue H, Wan M, Song D, Li Y, Li J

Omega-3 polyunsaturated fatty acids (PUFA) regulate inflammation and immunoreaction partially via affecting endothelial functions. However, the intracellular signaling mechanisms for inhibiting endothelial activation by omega-3 PUFA remain unclear. We investigated the effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on mitogen-activated protein kinases (MAPK) of endothelium. We analyzed the expression of extracellular signal-related kinases (ERK1/2), Jun amino-terminal kinases (JNK), and p38 mRNA by real-time RT-PCR and the kinases activity by western blotting in tumor necrosis factor-alpha (TNF-alpha)-activated human umbilical vein endothelial cells (HUVEC). We observed that EPA or DHA alone significantly reduced the TNF-alpha-induced activation of p38 and JNK kinases at a concentration of 20µM, but EPA is a more potent inhibitor than DHA. In contrast, both EPA and DHA significantly counteracted the TNF-alpha-mediated deactivation of ERK1/2 kinases. Meanwhile, both EPA and DHA significantly attenuated the TNF-alpha-induced expression of p38 and ERK1/2 mRNA, and DHA but not EPA also reduced the TNF-alpha-induced JNK mRNA expression. We present data show that both EPA and DHA alone diminish activation of p38 and JNK kinases, while maintaining the activation of ERK1/2 kinases of TNF-alpha-stimulated HUVEC. This may contribute to the inhibiting effects of omega-3 PUFA on endothelial activation by proinflammatory stimuli.

Am J Cardiol. 2006 Feb 15;97(4):547-51. Epub 2006 Jan 4.

Effects of dietary omega-3 fatty acid supplementation on endothelium-dependent vasodilation in patients with chronic heart failure.

Morgan DR, Dixon LJ, Hanratty CG, El-Sherbeeny N, Hamilton PB, McGrath LT, Leahey WJ, Johnston GD, McVeigh GE.

We investigated the effects of omega-3 fatty acids administration on endothelium-dependent vasodilation in patients ≥ 65 years old who received treatment for chronic heart failure (CHF). Twenty patients (mean age 73 years; 15 men) with grade II and III CHF who were on maximal medical management were recruited. Patients were randomized in a double-blind, crossover fashion to 6 weeks of omega-3 fatty acid (1.8 g eicosapentaenoic acid and 1.2 g docosahexaenoic acid) or olive oil. Forearm blood flow (FBF) responses to incremental doses of intra-arterial sodium nitroprusside, acetylcholine (ACH), angiotensin-II, and N(g)-nitro-L-arginine methyl ester were assessed by venous occlusion strain gauge plethysmography. The endothelium-dependent increase in FBF was greater in response in ACH infusion after omega-3 fatty acid administration (7.9, 95% confidence interval [CI] 4.81 to 11.08 to 11.3, 95% CI 7.31 to 15.23 arbitrary units ($p < 0.05$) compared with baseline (7.95, 95% CI 4.8 to 11.08 arbitrary units) and olive oil administration (7.27, 95% CI 4.66 to 9.88 arbitrary units) ($p = \text{NS}$ for both). Neither omega-3 fatty acid nor olive oil altered endothelium-independent vasodilation in response to infusion of sodium nitroprusside, nor did they influence vasoconstrictor responses to angiotensin-II or N(g)-nitro-L-arginine methyl ester. Dietary omega-3 fatty acid supplementation was accompanied by an increase in FBF response to ACH, which represents enhanced endothelium-dependent vasodilation in CHF. Further studies are warranted to assess the mechanism responsible for the beneficial actions of omega-3 fatty acids in CHF.

Nutr Metab (Lond). 2006 Jan 5;3:4.

Effect of diet and omega-3 fatty acid intervention on asymmetric dimethylarginine.

Eid HM, Arnesen H, Hjerkin EM, Lyberg T, Ellingsen I, Seljeflot I.

ABSTRACT : BACKGROUND AND AIM : Impaired vasodilatation has been suggested to be caused by inhibition of nitric oxide generation by the recently described asymmetric dimethylarginine (ADMA). In the present study we wanted to explore whether n-3 polyunsaturated fatty acid (PUFA) supplementation and/or diet intervention have beneficial influence on endothelial function assessed as plasma levels of ADMA and L-arginine. METHODS : A male population ($n = 563$, age 70 ± 6 yrs) with long-standing hyperlipidemia, characterized as high risk individuals in 1970-72, was included, randomly allocated to receive

placebo n-3 PUFA capsules (corn oil) and no dietary advice (control group), dietary advice (Mediterranean type), n-3 PUFA capsules, or dietary advice and n-3 PUFA combined and followed for 3 years. Fasting blood samples were drawn at baseline and the end of the study. RESULTS : Compliance with both intervention regimens were demonstrated by changes in serum fatty acids and by recordings from a food frequency questionnaire. No influence of either regimens on ADMA levels were obtained. However, n-3 PUFA supplementation was accompanied by a significant increase in L-arginine levels, different from the decrease observed in the placebo group ($p < 0.05$). In individuals with low body mass index ($< 26 \text{ kg/m}^2$), the decrease in L-arginine on placebo was strengthened ($p = 0.01$), and the L-arginine/ADMA ratio was also significantly reduced ($p = 0.04$). CONCLUSION : In this rather large randomized intervention study, ADMA levels were not influenced by n-3 PUFA supplementation or dietary counselling. n-3 PUFA did, however, counteract the age-related reduction in L-arginine seen on placebo, especially in lean individuals, which might be discussed as an improvement of endothelial function.

Eur J Nutr. 2005 Sep;44(6):327-33. Epub 2004 Sep 14.

n-3 polyunsaturated fatty acids supplementation decreases asymmetric dimethyl arginine and arachidonate accumulation in aging spontaneously hypertensive rats.

Raimondi L, Lodovici M, Visioli F, Sartiani L, Cioni L, Alfarano C, Banchelli G, Pirisino R, Cecchi E, Cerbai E, Mugelli A.

BACKGROUND: Plasma accumulation of asymmetric dimethyl arginine (ADMA) is considered as a risk factor for endothelial dysfunction and a strong predictor for coronary heart diseases. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) increasing plasma levels have been positively associated with reduced cardiovascular mortality with a mechanism(s) yet unclear. We hypothesised that ADMA reduction might be a part of EPA and DHA beneficial effects on the cardiovascular system. AIM: To verify this hypothesis we measured ADMA plasma levels in aged spontaneously hypertensive rats (SHR) supplemented for 8 weeks with EPA and DHA. METHODS: 16-month-old SHR were supplemented with EPA and DHA (EPA-DHA) or with olive oil (1 g/kg/day; OLIVE). At the end of the treatments, the plasma of each animal was analysed for 1) the total fatty acid composition, by gas-chromatography, 2) ADMA levels, by high pressure liquid chromatography, 3) nitrite and homocysteine concentration by chemiluminescence and by polarisation immunoassay respectively. Moreover, the activity of dimethyl arginine dimethyl amino hydrolase, the main enzyme involved in ADMA metabolism, was measured spectrophotometrically in the kidney from each rat. RESULTS: Animals supplemented with EPA and DHA showed: 1) lower ADMA and arachidonate plasma levels ($587.4 \pm 113.7 \text{ nM}$ and $0.49 \pm 0.11 \text{ mM}$ respectively) than the values found in OLIVE rats ($1365 \pm 399 \text{ nM}$ and $1.07 \pm 0.07 \text{ mM}$ respectively) 2) higher nitrite content ($0.73 \pm 0.05 \text{ microM}$) than OLIVE ($0.23 \pm 0.08 \text{ microM}$). CONCLUSIONS: EPA and DHA supplementation reduced ADMA accumulation in SHR in parallel with a decrease of arachidonate availability. This finding suggests that the control of the inflammatory ground of endothelium might play an important role in EPA and DHA effect on this novel and highly predictive cardiovascular risk factor.

J Cell Physiol. 2005 Sep;204(3):881-8.

Docosahexaenoic acid induces apoptosis in proliferating human endothelial cells.

Kim HJ, Vosseler CA, Weber PC, Erl W.

n-3 polyunsaturated fatty acids (PUFAs) have been shown to exert beneficial effects in the prevention of cardiovascular disease, inflammation, and on tumor growth. To investigate effects of PUFAs on proliferation and apoptosis in endothelial cells, we tested the n-3 PUFA docosahexaenoic acid (DHA) and the n-6 PUFA arachidonic acid (AA) in human umbilical vein endothelial cells (HUVEC). The mitochondrial membrane potential (MMP) and the production of reactive oxygen species were examined by flow cytometry. Phosphorylation of p53 or p38 MAP kinase, and total levels of p53 were measured by Western blot. DNA binding activity of p53 was analyzed with a TransAM transcription factor assay kit. Tube formation was assessed on Matrigel. In proliferating HUVEC, but not in confluent cells, DHA reduced cell viability and induced apoptosis, as demonstrated by increases in membrane leakage (propidium iodide (PI) staining), Annexin-V binding, sub G(1) phase in the cell cycle, and TUNEL-positive cells. AA had no effect on these parameters. In addition to a reduced MMP and increased reactive oxygen species, phosphorylation of p38 and p53 (serine 15) and impaired DNA binding of p53 were observed. There was no change in total levels of p53. The p38 inhibitor SB203580 had no effect on Annexin V binding. DHA also attenuated HUVEC tube formation. Taken together, DHA induces apoptosis in proliferating, but not in resting HUVEC, potentially via the phosphorylation of p53, resulting in decreased p53 DNA binding. The results suggest that anti-angiogenic effects of DHA may be due to induction of apoptosis in proliferating endothelial cells. Copyright 2005 Wiley-Liss, Inc.

J Membr Biol. 2005 Jul;206(2):103-16.

Omega-3 fatty acids and the regulation of expression of endothelial pro-atherogenic and pro-inflammatory genes.

De Caterina R, Massaro M.

By partially replacing the corresponding omega-6 analogues in membrane phospholipids, omega-3 fatty acids have been shown to decrease the transcriptional activation of genes--e.g., adhesion molecules, chemoattractants, inflammatory cytokines--involved in endothelial activation in response to inflammatory and pro-atherogenic stimuli. This regulation occurs, at least in part, through a decreased activation of the nuclear factor-kappaB system of transcription factors, secondary to decreased generation of intracellular hydrogen peroxide. Such regulation by omega-3 fatty acids is likely linked to the presence of a higher number of double bonds in the fatty acid chain in omega-3 compared with omega-6 fatty acids. By similar mechanisms, omega-3 fatty acids have been recently shown to reduce gene expression of cyclooxygenase-2, an inflammatory gene involved, through the activation of some metalloproteinases, in plaque angiogenesis and plaque rupture. The quenching of gene expression of pro-inflammatory pro-atherogenic genes by omega-3 fatty acids has consequences on the extent of leukocyte adhesion to vascular endothelium, early atherogenesis and later stages of plaque development and plaque rupture, ultimately yielding a plausible comprehensive explanation for the vasculoprotective effects of these nutrients.

Am J Clin Nutr. 2005 Mar;81(3):583-9.

Influence of long-term intervention with dietary counseling, long-chain n-3 fatty acid supplements, or both on circulating markers of endothelial activation in men with long-standing hyperlipidemia.

Hjerkinn EM, Seljeflot I, Ellingsen I, Berstad P, Hjerermann I, Sandvik L, Arnesen H.

BACKGROUND: Dietary factors and very-long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs) may influence the atherothrombotic process. Elevated concentrations of circulating cell adhesion molecules, thrombomodulin (TM), von Willebrand factor (vWF), and tissue-type plasminogen activator antigen (tPAag) are related to atherothrombotic cardiovascular disease. **OBJECTIVE:** The randomized Diet and Omega-3 Intervention Trial (DOIT) targeted a comparison of the effect of 3-y dietary counseling, n-3 PUFA supplementation (2.4 g/d), or both on circulating markers of endothelial activation. **DESIGN:** The study included 563 elderly men with long-standing hyperlipidemia. The men were randomly assigned by factorial design into 4 groups: control (no dietary counseling and placebo capsules), dietary counseling (and placebo capsules), n-3 PUFA supplementation (no dietary counseling), and dietary counseling and n-3 PUFA supplementation. **RESULTS:** Serum concentrations of fatty acids reflected good compliance. Dietary counseling was followed by significantly reduced concentrations of soluble intercellular adhesion molecule 1 (sICAM-1; $P < 0.001$), sTM ($P = 0.004$), and tPAag ($P < 0.001$) than in subjects without dietary counseling. After n-3 PUFA supplementation, significantly reduced concentrations of sICAM-1 ($P < 0.001$) and sTM ($P = 0.006$) were observed when compared with subjects receiving placebo capsules. An increase in tPAag was not significantly different from that observed in subjects receiving placebo capsules. For sICAM-1, a significant effect was observed for both interventions combined. **CONCLUSIONS:** Each intervention (dietary counseling or n-3 PUFA supplements) reduced sTM and sICAM-1 concentrations, indicating decreased endothelial activation. The tPAag increase in the groups not receiving dietary counseling (pooled), which indicates progression of atherosclerosis, was significantly counteracted by dietary counseling.

Int J Clin Pharmacol Ther. 2004 Dec;42(12):672-9.

Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: results from the EARLY study.

Engler MM, Engler MB, Malloy M, Chiu E, Besio D, Paul S, Stuehlinger M, Morrow J, Ridker P, Rifai N, Mietus-Snyder M.

OBJECTIVE: The primary objective of this study was to determine whether the National Cholesterol Education Program Step II (NCEP-II) diet or supplementation with docosahexaenoic acid (DHA) with the diet, affects endothelial function in children with familial hypercholesterolemia (FH) or the phenotype of familial combined hyperlipidemia (FCH). As secondary endpoints, the influence of diet and DHA supplementation on lipid profiles as well as biomarkers for oxidative stress and inflammation, and asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, were all evaluated. **METHODS:** In a double-blind, placebo-controlled, randomized, crossover study design, 20 children (ages 9-19 years) with FH ($n = 12$) and FCH ($n = 8$) received nutritional counseling based on the National Cholesterol Education Program Step II (NCEP-II) and food guide pyramid dietary guidelines for 6 weeks. They were then randomly assigned to supplementation with docosahexaenoic acid (DHA 1.2 g/d) or placebo for 6 weeks, followed by a washout phase of 6 weeks and crossover phase of 6 weeks while continuing the NCEP-II diet. Endothelium-dependent flow-mediated dilation (FMD) of the brachial artery was determined by high-resolution ultrasound. Plasma levels of total cholesterol, triglycerides and lipoprotein classes (LDL, HDL, VLDL) were measured by ultracentrifugation and enzymatic methods, plasma F2 isoprostanes by gas chromatography/mass spectrometry, urinary 8-OH-2' deoxyguanosine by liquid chromatography, high sensitivity C-reactive protein by immunonephelometry and ADMA by liquid chromatography. **RESULTS:** FMD increased significantly after DHA supplementation compared

to baseline ($p < 0.001$), diet alone ($p < 0.002$), placebo ($p < 0.012$) and washout ($p < 0.001$) phases of the study without affecting biomarkers for oxidative stress, inflammation or ADMA. DHA supplementation was associated with increased levels of total cholesterol ($p < 0.01$), LDL- and HDL cholesterol concentrations ($p < 0.001$) compared to the NCEP-II diet. CONCLUSION: This study demonstrates that DHA supplementation restores endothelial-dependent FMD in hyperlipidemic children. The endothelium may thus be a therapeutic target for DHA. This is consistent with a hypothesis of increasing NO bioavailability, with the potential for preventing the progression of early coronary heart disease in high-risk children.

J Nutr. 2004 Jul;134(7):1806-11.

Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women.

Lopez-Garcia E, Schulze MB, Manson JE, Meigs JB, Albert CM, Rifai N, Willett WC, Hu FB.

We evaluated the hypothesis that intake of (n-3) fatty acids is inversely associated with biomarkers of inflammation and endothelial activation. We conducted a cross-sectional study of 727 women from the Nurses' Health Study I cohort, aged 43-69 y, apparently healthy at time of a blood draw in 1990. Dietary intake was assessed by a validated FFQ in 1986 and 1990. C-reactive protein (CRP) levels were 29% lower among those in the highest quintile of total (n-3) fatty acids, compared with the lowest quintile; interleukin-6 (IL-6) levels were 23% lower, E-selectin levels 10% lower, soluble intracellular adhesion molecule (sICAM-1) levels 7% lower, and soluble vascular adhesion molecule (sVCAM-1) levels 8% lower. The intake of alpha-linolenic acid was inversely related to plasma concentrations of CRP (beta = -0.55, $P = 0.02$), IL-6 (beta = -0.36, $P = 0.01$), and E-selectin (beta = -0.24, $P = 0.008$) after controlling for age, BMI, physical activity, smoking status, alcohol consumption, and intake of linoleic acid (n-6) and saturated fat. Long-chain (n-3) fatty acids (eicosapentaenoic and docosahexaenoic) were inversely related to sICAM-1 (beta = -0.11, $P = 0.03$) and sVCAM-1 (beta = -0.17, $P = 0.003$). Total (n-3) fatty acids had an inverse relation with CRP (beta = -0.44, $P = 0.007$), IL-6 (beta = -0.26, $P = 0.009$), E-selectin (beta = -0.17, $P = 0.004$), sICAM-1 (beta = -0.07, $P = 0.02$), and sVCAM-1 (beta = -0.10, $P = 0.004$). These associations were not modified by intake of vitamin E, dietary fiber, trans fatty acids, or by the use of postmenopausal hormone therapy. In conclusion, this study suggests that dietary (n-3) fatty acids are associated with levels of these biomarkers reflecting lower levels of inflammation and endothelial activation, which might explain in part the effect of these fatty acids in preventing cardiovascular disease.

J Hypertens. 2002 Aug;20(8):1493-9.

Blood pressure response to fish oil supplementation: meta-regression analysis of randomized trials.

Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR, Kok FJ.

OBJECTIVE : The antihypertensive effect of fish oil was estimated from randomized trials using meta-regression analysis. Modification of the blood pressure (BP) effect by age, gender, blood pressure, and body mass index was examined. **METHODS :** A total of 90 randomized trials of fish oil and BP were identified through MEDLINE (1966-March 2001). Trials with co-interventions, patient populations, non-placebo controls, or duration of < 2 weeks were excluded. A total of 36 trials (50 strata) were included, 22 of which had a double-blind design. Original reports were retrieved for data collection on sample size, study design, duration, fish oil dose, BP changes and baseline characteristics of trial populations. Pooled BP estimates were obtained by meta-regression analysis, weighted for trial sample sizes. Stratified analyses according to population characteristics were performed. **RESULTS :** Intake of fish oil was high in most trials (median dose: 3.7 g/day). Fish oil reduced systolic BP by 2.1 mmHg [95% confidence interval (CI): 1.0, 3.2; $P < 0.01$] and diastolic BP by 1.6 mmHg (95% CI: 1.0, 2.2; $P < 0.01$). Restricting the analysis to double-blind trials yielded BP reductions of 1.7 mmHg (95% CI: 0.3, 3.1) and 1.5 mmHg (95% CI: 0.6, 2.3), respectively. BP effects tended to be larger in populations that were older (> 45 years) and in hypertensive populations (BP \geq 140/90 mmHg). **CONCLUSIONS :** High intake of fish oil may lower BP, especially in older and hypertensive subjects. The antihypertensive effect of lower doses of fish oil (< 0.5 g/day) however, remains to be established.

Am J Clin Nutr. 2002 Aug;76(2):326-30.

The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans.

Nestel P, Shige H, Pomeroy S, Cehun M, Abbey M, Raederstorff D.

BACKGROUND: n-3 Fatty acids influence vascular function, but the effect of individual fatty acids on systemic arterial compliance (SAC) has not been reported. SAC, which reflects arterial elasticity, is emerging as a new cardiovascular risk factor and appears to predict future cardiovascular events. **OBJECTIVE:** We tested whether the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) improve SAC in dyslipidemic subjects. **DESIGN:** Thirty-eight dyslipidemic subjects were randomly assigned to receive 3 g EPA/d ($n = 12$), 3 g DHA/d ($n = 12$), or a placebo ($n = 14$) in a 7-wk parallel, double-blind

trial. Arterial functions were measured at the beginning and end of the interventions. Plasma lipids and plasma fatty acids were also measured. RESULTS: Consumption of the n-3 fatty acids significantly increased SAC, whereas consumption of the placebo did not ($P = 0.043$; repeated-measures analysis of variance across the 3 groups); the increase was 36% with EPA and 27% with DHA. The major components contributing to the increase in SAC (systolic and pulse pressures and total vascular resistance) tended to decrease but not significantly. Plasma total and VLDL triacylglycerol were significantly lower in the n-3 fatty acid groups ($P = 0.026$ and 0.006 , respectively; repeated-measures analysis of variance) than in the placebo group. CONCLUSION: EPA and DHA increase SAC and tend to reduce pulse pressure and total vascular resistance, effects that may reduce the risk of adverse cardiovascular events.

Eur Heart J. 2002 Feb;23(3):216-22.

Relationship between circulating n-3 fatty acid concentrations and endothelial function in early adulthood.

Leeson CP, Mann A, Kattenhorn M, Deanfield JE, Lucas A, Muller DP.

AIMS: Fish consumption is inversely associated with cardiovascular mortality, presumably because of n-3 fatty acids in fish. Whether the protection of n-3 fatty acids extends beyond clinical coronary disease to influence the early vascular biology of atherosclerosis remains unclear. This study determined whether circulating levels of n-3 fatty acids are associated with vascular endothelial function in early adulthood. METHODS AND RESULTS: Three hundred and twenty-six adults (157 males, 169 females, aged 20 to 28 years) had high-resolution ultrasound measurements of flow-mediated brachial artery dilatation (FMD) (endothelium-dependent) and arterial response to glyceryl trinitrate (endothelium-independent). Levels of the n-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid in plasma and erythrocyte membranes of subjects were measured. n-3 Fatty acid levels were not related to vascular function in the whole group. In smokers, however, n-3 fatty acids were positively related to flow-mediated dilatation (plasma DHA vs. FMD: 0.045 mm. %(-1), 95% CI 0.011 to 0.079, $P=0.01$). Flow-mediated dilatation was also associated with n-3 fatty acid levels in subjects in the top third of the insulin, glucose and triglyceride distributions. CONCLUSION: In young smokers and those with higher fasting insulin, glucose or triglyceride concentrations (factors associated with endothelial dysfunction), n-3 fatty acid levels were positively associated with flow-mediated dilatation. This raises the possibility that physiological levels of circulating n-3 fatty acids may protect the endothelium from early adulthood.

Eur Heart J. 2001 Mar;22(5):428-36.

Omega-3 fatty acids improve blood pressure control and preserve renal function in hypertensive heart transplant recipients.

Holm T, Andreassen AK, Aukrust P, Andersen K, Geiran OR, Kjekshus J, Simonsen S, Gullestad L.

BACKGROUND: Hypertension and cyclosporine-induced nephrotoxicity are common complications in heart transplant recipients. Omega-3 fatty acids may prevent blood pressure rise early, but have not been studied long-term after heart transplantation. METHODS AND RESULTS: Forty-five clinically stable hypertensive heart transplant recipients were studied 1-12 years after transplantation and randomized in a double-blind fashion to receive either 3.4 g of omega-3 fatty acids daily or placebo for 1 year. Ambulatory 24 h blood pressure monitoring and haemodynamic studies were performed before randomization and at the end of the study. Systolic blood pressure increased by $8\pm/3$ mmHg ($P<0.01$) in the placebo group, with a non-significant increase in diastolic blood pressure of $3\pm/2$ mmHg ($P=0.10$), accompanied by a 14% increase in systemic vascular resistance ($P<0.05$). In contrast, no change in blood pressure or systemic vascular resistance was recorded in the omega-3 group. Plasma creatinine increased ($P<0.01$) and glomerular filtration rate decreased ($P<0.05$) in the placebo group, while no changes were observed in the omega-3 group. The antihypertensive effect was related to an increase in serum eicosapentaenoic and docosahexaenoic acid. CONCLUSION: Treatment with omega-3 fatty acids may reduce the long-term continuous rise in blood pressure after heart transplantation and may offer a direct or indirect renoprotective effect, making these fatty acids a potentially attractive treatment for post-transplant hypertension.

Circulation. 2000 Sep 12;102(11):1264-9.

Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men.

Mori TA, Watts GF, Burke V, Hilme E, Puddey IB, Beilin LJ.

BACKGROUND: Recent evidence supports differential effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the 2 major omega3 fatty acids of marine origin, on blood pressure in humans and vascular reactivity in adult spontaneously hypertensive rats. We investigated possible differences in the effects of purified EPA or DHA on forearm vascular reactivity in overweight hyperlipidemic men that might contribute to the blood pressure-lowering effects of fish oils. METHODS AND

RESULTS: With a double-blind, placebo-controlled trial of parallel design, 59 overweight, mildly hyperlipidemic men were randomized to receive 4 g/d purified EPA, DHA, or olive oil (placebo) capsules while continuing their usual diets for 6 weeks. Forearm blood flow (FBF) was measured with venous occlusion, strain-gauge plethysmography during the sequential intra-arterial administration of acetylcholine (7.5, 15, and 30 microg/min), sodium nitroprusside (1.5, 3, and 10 microg/min), norepinephrine (10, 20, and 40 ng/min), a single-dose infusion of N:(G)-monomethyl-L-arginine (L-NMMA) (1 mg/min), and coinfusion of acetylcholine (7.5, 15, and 30 microg/min) and L-NMMA. Forty of the 56 subjects who completed the study underwent FBF measurements. Plasma phospholipid EPA levels increased significantly ($P<0.0001$) after supplementation with EPA, and DHA composition increased with DHA supplementation ($P<0.0001$). Relative to placebo, DHA, but not EPA, supplementation significantly improved FBF in response to acetylcholine infusion ($P=0.040$) and coinfusion of acetylcholine with L-NMMA ($P=0.040$). Infusion of L-NMMA alone showed no group differences. DHA significantly enhanced dilatory responses to sodium nitroprusside ($P<0.0001$) and attenuated constrictor responses to norepinephrine ($P=0.017$).

CONCLUSIONS: Relative to placebo, DHA, but not EPA, enhances vasodilator mechanisms and attenuates constrictor responses in the forearm microcirculation. Improvements in endothelium-independent mechanisms appear to be predominant and may contribute to the selective blood pressure-lowering effect observed with DHA compared with EPA in humans.

Hypertension. 1999 Aug;34(2):253-60.

Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans.

Mori TA, Bao DQ, Burke V, Puddey IB, Beilin LJ.

Animal studies suggest that the 2 major omega3 fatty acids found in fish, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may have differential effects on blood pressure (BP) and heart rate (HR). The aim of this study was to determine whether there were significant differences in the effects of purified EPA or DHA on ambulatory BP and HR in humans. In a double-blind, placebo-controlled trial of parallel design, 59 overweight, mildly hyperlipidemic men were randomized to 4 g/d of purified EPA, DHA, or olive oil (placebo) capsules and continued their usual diets for 6 weeks. Fifty-six subjects completed the study. Only DHA reduced 24-hour and daytime (awake) ambulatory BP ($P<0.05$). Relative to the placebo group, 24-hour BP fell 5.8/3.3 (systolic/diastolic) mm Hg and daytime BP fell 3.5/2.0 mm Hg with DHA. DHA also significantly reduced 24-hour, daytime, and nighttime (asleep) ambulatory HRs ($P=0.001$). Relative to the placebo group, DHA reduced 24-hour HR by 3.5 \pm 0.8 bpm, daytime HR by 3.7 \pm 1.2 bpm, and nighttime HR by 2.8 \pm 1.2. EPA had no significant effect on ambulatory BP or HR. Supplementation with EPA increased plasma phospholipid EPA from 1.66 \pm 0.07% to 9.83 \pm 0.06% ($P<0.0001$) but did not change DHA levels. Purified DHA capsules increased plasma phospholipid DHA levels from 4.00 \pm 0.27% to 10.93 \pm 0.62% ($P<0.0001$) and led to a small, nonsignificant increase in EPA (1.52 \pm 0.12% to 2.26 \pm 0.16%). Purified DHA but not EPA reduced ambulatory BP and HR in mildly hyperlipidemic men. The results of this study suggest that DHA is the principal omega3 fatty acid in fish and fish oils that is responsible for their BP- and HR-lowering effects in humans. These results have important implications for human nutrition and the food industry.

Arterioscler Thromb Vasc Biol. 1999 Jul;19(7):1681-6.

The effect of supplementation with omega-3 fatty acids on soluble markers of endothelial function in patients with coronary heart disease.

Johansen O, Seljeflot I, Hostmark AT, Arnesen H.

During progression of atherosclerosis the overlying endothelial cells alter their expression of some surface molecules. Circulating levels of such molecules may be quantified. We investigated the effect of omega-3 fatty acids (n-3 FA) on the levels of tissue plasminogen activator antigen, von Willebrand factor, and the soluble forms of thrombomodulin, P-selectin, E-selectin, and vascular cell adhesion molecule-1 in 54 patients with coronary heart disease. Twenty-three of the patients had taken 5.1 g/d n-3 FA for 6 months (group I) and 31 were given corn oil as placebo (group II). For another 4 weeks ("the study period") they all got 5.1 g/d of n-3 FA. Compliance was confirmed by demonstration of changes in relevant fatty acids in serum phospholipids. At baseline, significant differences between the groups were found with lower median values of von Willebrand factor (128% versus 147%) and soluble thrombomodulin (24.9 versus 32.5 ng/mL) and higher median values of soluble E-selectin (41.4 versus 35.5 ng/mL) and soluble vascular cell adhesion molecule-1 (573 versus 473 ng/mL) in group I. During the study period differences in changes between the groups were found; tissue plasminogen activator antigen and soluble thrombomodulin decreased (P for difference between the groups 0.001 and 0.015, respectively), whereas soluble E-selectin and soluble vascular cell adhesion molecule-1 increased (P for difference between the groups <0.01 for both) in group II relative to group I. Our results indicate that n-3 FA supplementation decreases hemostatic markers of atherosclerosis, whereas markers of inflammation may be increased. The latter may be the result of lipid peroxidation as a simultaneous decrease of vitamin E and increase in thiobarbituric acid-reactive substances were observed.

J Cardiovasc Pharmacol. 1999 Apr;33(4):633-40.

Long-term treatment with eicosapentaenoic acid augments both nitric oxide-mediated and non-nitric oxide-mediated endothelium-dependent forearm vasodilatation in patients with coronary artery disease.

Tagawa H, Shimokawa H, Tagawa T, Kuroiwa-Matsumoto M, Hirooka Y, Takeshita A.

Long-term treatment with eicosapentaenoic acid (EPA) is known to improve impaired endothelium-dependent relaxations of atherosclerotic blood vessels in animals and humans. However, it remains to be determined which mechanisms are involved in this beneficial effect of EPA. In this study, we investigated our hypothesis that EPA improves both nitric oxide (NO)-mediated and non-NO-mediated endothelium-dependent vasodilatation in patients with coronary artery disease. The study included eight patients with documented coronary artery disease. The forearm vascular responses to the endothelium-dependent vasodilator acetylcholine and substance P were examined before and after intraarterial infusion of NG-monomethyl-L-arginine (L-NMMA). Same measurements were repeated after the treatment with EPA (1,800 mg/day) for 6 weeks. The long-term treatment with EPA augmented forearm blood-flow response to both acetylcholine and substance P. Furthermore, acute administration of L-NMMA significantly inhibited the EPA-induced augmented response to acetylcholine but not that to substance P. The forearm vascular response to sodium nitroprusside was unchanged by the EPA treatment. These results indicate that long-term treatment with EPA augments both NO-dependent and non-NO-dependent endothelium-dependent forearm vasodilatation in patients with coronary artery disease. Thus the beneficial effects of EPA appear to extend to non-NO-dependent mechanism(s).

Circulation. 1997 Nov 4;96(9):2802-7.

Dietary supplementation with marine fish oil improves in vitro small artery endothelial function in hypercholesterolemic patients: a double-blind placebo-controlled study.

Goode GK, Garcia S, Heagerty AM.

BACKGROUND: Marine fish oils improve vascular function, but the mechanism of benefit is unclear. We conducted a study to examine the effects of fish oils given to hypercholesterolemic patients on small artery function in vitro. **METHODS AND RESULTS:** In a randomized, double-blind, placebo-controlled trial, subcutaneous gluteal fat biopsies were taken from 16 hypercholesterolemic patients (serum total cholesterol, 7.97 \pm 0.16 mmol/L [mean \pm SEM]) and 12 age- and sex-matched control subjects (mean cholesterol, 5.11 \pm 0.34 mmol/L). Small arteries were mounted on a wire myograph for isometric tension experiments. Patients and control subjects were randomized to receive fish oil (Maxepa 5 capsules BID) or placebo for 3 months. A second biopsy was taken and the studies were repeated. Relaxation to acetylcholine was significantly improved in the hypercholesterolemic group given Maxepa but not in the placebo group (mean maximum relaxation before and after, 48 \pm 6.2% and 68.83 \pm 2.19%, $P=$.0054). The dysfunction was not restored to control values (84.3 \pm 5.2%, $P=$.0002). There was also a smaller but significant impairment in endothelium-independent relaxation provoked by sodium nitroprusside ($P<$.01). A good correlation between the increase in eicosapentaenoic acid ($n=3$) in red cell membrane and improvement in relaxation in the hypercholesterolemic group given fish oils was seen ($r=$.781, $P<$.02). **CONCLUSIONS:** Marine fish oil significantly improved endothelial function in peripheral small arteries in hypercholesterolemia patients. This may provide a mechanism for the beneficial effects of these fatty acids in coronary heart disease.

Arq Bras Cardiol. 1997 Jul;69(1):13-8.

[Effects of omega-3 acids on endothelium-dependent relaxation in hypercholesterolemic rabbits]

[Article in Portuguese]

Jorge PA, Neyra LC, Ozaki RM, de Almeida E.

PURPOSE: To study the effect of omega-3 fatty acid on endothelium-dependent relaxation, total plasma cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides levels as well as, the malondialdehyde (MDA) content of the LDL particles and arterial wall. **METHODS:** Fourteen male rabbits were randomly assigned to hypercholesterolemic and omega-3 groups. The dose of omega-3 fatty acid utilized was 300g/kg/day during 15 days. All rabbits were fed a diet supplemented with cholesterol (0.5%) and coconut oil (2%) for four weeks. At the end of the experiment the animals were killed and the aorta removed for measurement of MDA content and the endothelium-dependent relaxation studies. Total plasma cholesterol, VLDL-cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides was measured by enzymatic kits. MDA was also measured in natives and oxidized LDL and arterial wall. **RESULTS:** Cholesterol and VLDL-cholesterol were increased significantly in the omega-3 treated animals. The triglyceride level was significantly reduced ($p <$ 0.05). The MDA content was increased in the LDL particles and in the arterial wall ($p <$ 0.05). Endothelium-dependent relaxation was significantly reduced ($p <$ 0.05). **CONCLUSION:** Omega-3 fatty acid impairs the endothelium-dependent relaxation when administered to cholesterol fed rabbits, increases the cholesterol and reduces the triglycerides plasma levels. The lipid peroxidation of the LDL particles and arterial wall was increased.

Circulation. 1996 Jan 15;93(2):365-71.

Omega-3 lipid infusion in a heart allotransplant model. Shift in fatty acid and lipid mediator profiles and prolongation of transplant survival.

Grimminger F, Grimm H, Fuhrer D, Papavassilis C, Lindemann G, Blecher C, Mayer K, Tabesch F, Kramer HJ, Stevens J, Seeger W.

BACKGROUND: omega-3 Fatty acids may have a major impact on immune responses involved in heart transplant rejection. We compared the effects of posttransplant intravenous supplementation with omega-3-rich versus omega-6-rich lipid emulsions on graft survival, plasma fatty acid profiles, and levels of arachidonic acid versus eicosapentaenoic acid-derived lipid mediators. **METHODS AND RESULTS:** Inbred PVG and Wistar-Kyoto rats were used as donors and recipients, respectively, in a model of heterotopic heart transplantation. Animals received 9 g/kg body wt per day of either fish oil-derived (n = 8) or soybean oil-derived fat (n = 7) in the form of a continuously infused lipid emulsion; controls were sham-infused with saline (n = 8). Graft rejection was assessed by loss of activity of the transplant. The fish oil-derived preparation but not that originating from soybean oil caused an increase in total and free plasma fatty acids. Substantial quantities of eicosapentaenoic acid and docosahexaenoic acid appeared in the free fatty acid fraction, surpassing those of arachidonic acid. Ex vivo stimulation of neutrophils with the Ca²⁺ ionophore A23187 demonstrated an increase in 5-series leukotriene (LT) generation in animals undergoing omega-3 lipid infusion (LTB₅, omega-oxidation products of LTB₅, LTA₅ secretion), with 5-series/4-series LT ratios ranging between 0.08 and 0.36. Ratios of TX B₃/B₂ liberated from ex vivo stimulated platelets even approached 1:1 in omega-3 supplemented rats. Graft survival was 7.6 +/- 0.3 (mean +/- SEM) days in saline-infused, 10.4 +/- 0.7 in omega-6 lipid-infused, and 12.9 +/- 0.4 in omega-3 lipid-infused animals. **CONCLUSIONS:** Posttransplant intravenous alimentation with fish oil-derived lipid emulsions prolongs heart transplant survival in excess to omega-6 lipids. Profound changes in fatty acid profiles and lipid mediator generation may underlie this finding.

Clin Exp Pharmacol Physiol. 1995 Feb;22(2):71-81.

How do fish oils affect vascular function?

Chin JP, Dart AM.

1. This is a review on the mechanisms by which fish oils affect vascular function and how such changes contribute to their documented cardioprotective effects. 2. Evidence that fish oils depress vascular responses to contractile agents will be examined. It is concluded that this effect of fish oils is mediated predominantly by alterations in prostanoid profile. 3. Effects of fish oils on arterial relaxation are discussed with particular emphasis on endothelium dependent relaxation. It is suggested that the functional impairment of endothelium dependent relaxation documented in a number of cardiovascular disease states can be reversed by dietary fish oils. 4. In addition, possible effects of fish oils on growth factors, inositol trisphosphate and lipid metabolism, the sympathetic nervous system, rheological and membrane properties and inducible nitric oxide are reviewed.

Arterioscler Thromb. 1994 Sep;14(9):1425-9.

Fish oil improves arterial compliance in non-insulin-dependent diabetes mellitus.

McVeigh GE, Brennan GM, Cohn JN, Finkelstein SM, Hayes RJ, Johnston GD.

In a double-blind, placebo-controlled study we investigated the effects of dietary fish oil supplementation on arterial wall characteristics in 20 patients with non-insulin-dependent diabetes mellitus. Estimates reflecting compliance values in the large arteries and more peripheral vasculature, as measured by pulse-contour analysis, improved significantly after 6 weeks of fish oil therapy compared with values recorded at baseline and after 6 weeks' administration of olive oil. The large-artery compliance estimate increased from 1.50 (confidence interval [CI], 1.31 to 1.69) mL/mm Hg at baseline to 1.68 (CI, 1.52 to 1.84) mL/mm Hg after fish oil administration (P < .01). The oscillatory compliance value increased from 0.015 (CI, 0.011 to 0.019) mL/mm Hg at baseline to 0.022 (CI, 0.016 to 0.028) mL/mm Hg after fish oil ingestion (P < .05). No changes occurred in arterial blood pressure, cardiac output, stroke volume, or systemic vascular resistance with either intervention. The improved compliance estimates with fish oil ingestion occurred without altering fasting blood glucose and cholesterol concentrations. These results support the hypothesis that fish oils alter vascular reactivity and favorably influence arterial wall characteristics in patients with non-insulin-dependent diabetes mellitus. These direct vascular effects, expressed at the level of the vessel wall, may contribute to the cardioprotective actions of fish oil in humans.

J Am Coll Cardiol. 1993 Mar 15;21(4):982-9.

Fish oil improves endothelium-dependent coronary vasodilation in heart transplant recipients.

Fleischhauer FJ, Yan WD, Fischell TA.

OBJECTIVES. The purpose of this study was to determine whether dietary fish oil supplementation enhances endothelium-mediated vasodilator responses in human heart transplant recipients, a group known to have coronary artery disease and endothelial dysfunction. **BACKGROUND.** Omega-3 fatty acid supplementation has been shown to enhance endothelium-dependent coronary vasodilation in animal models of atherosclerosis. **METHODS.** Endothelium-dependent vasodilator responses to intracoronary acetylcholine infusion and endothelium-independent responses to nitroglycerin were evaluated in heart transplant recipients who received a high dose of dietary supplementation with omega-3 fatty acids for 3 weeks (5 g of eicosapentaenoic acid plus docosahexaenoic acid/day, n = 7) and compared with responses in a group of matched heart transplant recipients who did not receive fish oil (control patients, n = 7). Acetylcholine was selectively infused into the midportion of the left anterior descending or left circumflex coronary artery, with the noninfused left coronary artery serving as a control vessel. Serial coronary angiograms were performed after infusion with increasing doses of acetylcholine (infused concentrations 10(-6) to 10(-3) mol/liter) and after intracoronary nitroglycerin administration. **RESULTS.** The patients treated with fish oil showed a normal vasodilator response to acetylcholine with 14 +/- 2.5% and 15 +/- 7% vasodilation (vs. baseline diameter) at infused acetylcholine concentrations of 10(-5) and 10(-4) mol/liter, respectively. In contrast, the control patients demonstrated vasoconstrictor responses (-1 +/- 1% and -9 +/- 4%) to acetylcholine at these same doses (p < 0.05 and < 0.005, respectively, for treated vs. control patients). There were no differences in the response to nitroglycerin between the control and treated patients. **CONCLUSIONS.** Dietary supplementation with fish oil significantly alters endothelium-dependent coronary vasodilation in heart transplant recipients without alteration of the responses to endothelium-independent vasodilation. Whether this enhancement of endothelial function can beneficially alter the natural history of heart transplant atherosclerosis warrants further study.

Diabetologia. 1993 Jan;36(1):33-8.

Dietary fish oil augments nitric oxide production or release in patients with type 2 (non-insulin-dependent) diabetes mellitus.

McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, Andrews JW, Hayes JR.

Decreased release of nitric oxide from damaged endothelium is responsible for the impaired endothelium-dependent vasodilator responses found in animal models of vascular disease. Dietary supplementation with fish oils has been shown to augment endothelium-dependent relaxations, principally by improving the release of nitric oxide from injured endothelium. Using forearm venous occlusion plethysmography we studied vascular responses to 60, 120, 180 and 240 nmol/min of acetylcholine (an endothelium-dependent vasodilator) and 3, 6 and 9 nmol/min of glyceryl trinitrate (an endothelium-independent vasodilator) infused into the brachial artery in 23 patients with Type 2 (non-insulin-dependent) diabetes mellitus. NG monomethyl-L-arginine was employed to inhibit stimulated and basal release of nitric oxide from the endothelium. On completion of the baseline studies patients randomly received either fish oil or matching olive oil capsules in a double-blind crossover fashion for 6 weeks followed by a 6-week washout period and a final 6-week treatment phase. Studies, identical to the initial baseline studies, were performed at the end of the active treatment periods at 6 and 18 weeks. Fish oil supplementation significantly improved forearm blood flow responses to each dose of acetylcholine when compared to the vasodilator responses recorded at baseline and after olive oil administration (p < 0.01). Neither fish oil nor olive oil supplementation produced any significant changes in forearm blood flow to the incremental infusions of glyceryl trinitrate when compared with responses recorded during the baseline studies.

Br J Pharmacol. 1992 Jun;106(2):435-42.

Study of mechanisms of glucocorticoid hypertension in rats: endothelial related changes and their amelioration by dietary fish oils.

Yin K, Chu ZM, Beilin LJ.

1. To investigate possible mechanisms of increased systolic blood pressure after 1 weeks treatment with dexamethasone and its amelioration by fish oil feeding, we have examined the reactivity of aortic rings and perfused mesenteric resistance vessels.
2. Thirty six Sprague-Dawley rats were initially divided into two groups and fed a semisynthetic diet containing either (10% by weight) hydrogenated coconut oil and safflower oil mixture (HCO/S) (24 rats) or fish oil (12 rats) for 5 weeks. From the end of the fourth week, dexamethasone (1.25 mg ml⁻¹) in drinking water, was given to half the rats on hydrogenated coconut oil (HCO/S+Dex) and to the fish oil-fed group (fish oil+Dex).
3. One week of dexamethasone treatment raised systolic blood pressure in the HCO/S+Dex rats but not in the fish oil+Dex group.
4. Endothelium-dependent relaxation to acetylcholine (ACh) was decreased in aortic rings taken from HCO/S+Dex rats compared to rats on HCO/S alone. Relaxant responses to ACh of aortic rings from rats given fish oil+Dex were intermediate between the three groups. Aortic endothelium-independent responses to sodium nitroprusside (SNP) were unchanged between the groups, while aortic contractile responses to noradrenaline

were similar in all the groups. 5. In the perfused mesenteric resistance artery, sensitivity to noradrenaline was decreased in rats given fish oil and dexamethasone compared to the other two groups. There were no differences in resistance vessel relaxation to ACh or SNP between groups. 6. Serum corticosterone levels, used as a marker of dexamethasone absorption, were substantially suppressed in dexamethasone-treated rats but levels were higher in rats on fish oil than on HCO/S diets.(ABSTRACT TRUNCATED AT 250 WORDS)

Stroke. 1992 Mar;23(3):407-13.

Dietary omega-3 fatty acids and endothelium-dependent responses in porcine cerebral arteries.

Kim P, Shimokawa H, Vanhoutte PM.

PURPOSE: We sought to determine the effect of dietary omega-3 polyunsaturated fatty acids on cerebrovascular endothelium-dependent responses in studies performed on isolated porcine basilar arteries. **METHODS:** Male Yorkshire pigs (6-8 weeks old) were kept for 4 weeks on a standard diet (control group, n = 12) or on chow supplemented with polyunsaturated fatty acids (eicosapentaenoic acid, 3.5 g/day, or docosahexaenoic acid, 1.5 g/day; treated group, n = 12). Isometric tension recording of the basilar artery was carried out and responses were compared between the two groups. **RESULTS:** The regimen resulted in a decrease in the plasma arachidonic acid level and an increase in eicosapentaenoic acid. Endothelium-dependent relaxations induced by bradykinin and adenosine diphosphate were augmented in the basilar arteries of the treated group. Incubation with indomethacin (10(-5) M) prevented the augmentation of the relaxations induced by bradykinin, but not those caused by adenosine diphosphate. The indomethacin-sensitive, endothelium-dependent contractions to arachidonic acid remained comparable in the two groups, indicating that the activity of cyclooxygenase was not affected by the diet. **CONCLUSIONS:** Dietary supplementation with omega-3 polyunsaturated fatty acids enhances endothelium-dependent relaxations in the basilar artery by two mechanisms: 1) replacement of endogenous arachidonic acid and suppression of the concomitant release of vasoconstrictor prostaglandins from the endothelium, and 2) enhancement of the release of endothelium-derived relaxing factor.

Eicosanoids. 1991;4(4):217-23.

Omega-3 polyunsaturated fatty acids augment endothelium-dependent vasorelaxation by enhanced release of EDRF and vasodilator prostaglandins.

Lawson DL, Mehta JL, Saldeen K, Mehta P, Saldeen TG.

Dietary supplementation with fish oil results in augmentation of endothelium-dependent vasorelaxation in experimental animals. The present study was designed to evaluate the direct in vitro effects of omega-3 polyunsaturated fatty acids (omega-3 PUFAs) on vascular reactivity in isolated rat aortic rings. Aortic rings were incubated with the omega-6PUFA arachidonic acid (AA, 10(-7) M) or the omega-3 PUFAs eicosapentaenoic acid (EPA, 10(-7) M) and docosahexaenoic acid (DHA, 10(-7) M) in an organ bath at 37 degrees C. Following contraction with norepinephrine, changes in isometric force were measured in response to the endothelium-dependent vasodilators acetylcholine (ACh, 10(-10) to 10(-5) M) or the calcium ionophore A23187 (10(-10) to 10(-5) M). Parallel sets of vascular rings were pretreated with the cyclooxygenase inhibitor indomethacin (10(-5) M) or the inhibitor of nitric oxide synthesis NG-monomethyl L-arginine (L-NMMA 5 x 10(-5) M) prior to treatment with AA or EPA. Treatment of rings with EPA resulted in an increase (P less than 0.05) in ACh-mediated vasorelaxation compared both to AA-treated and buffer-treated rings (maximum relaxation 83 +/- 5% vs 46 +/- 5% and 63 +/- 4%, respectively). A similar augmentation was observed in DHA-treated rings. Pretreatment of rings with indomethacin or L-NMMA decreased (P less than 0.05) the ACh-mediated vasorelaxation, although EPA-treated rings showed less (P less than 0.05) attenuation of ACh response compared to AA-treated or untreated control rings.

J Thorac Cardiovasc Surg. 1989 Jun;97(6):841-54; discussion 854-5.

Inhibition of accelerated cardiac allograft arteriosclerosis by fish oil.

Sarris GE, Mitchell RS, Billingham ME, Glasson JR, Cahill PD, Miller DC.

Accelerated coronary arteriosclerosis remains the most important factor limiting long-term survival of heart transplant recipients, and dietary fish oil supplementation with omega-3 polyunsaturated fatty acids has been suggested to have a protective effect against coronary disease in epidemiologic studies and to inhibit arteriosclerosis in animal experiments. Therefore we tested the hypothesis that fish oil administration inhibits the development of allograft coronary arteriosclerosis by using a heterotopic heart transplant model. Three groups of Lewis rats (n = 10 each) received heterotopic heart transplants from Brown-Norway donors and were treated with cyclosporine intraperitoneally on a tapering schedule. Group 1 received fish oil daily by gavage (2 ml/kg/day; Emulsified Super MaxEpa, Twin Labs, Ronkonkoma, N.Y.). Group 2 received an equal amount of safflower oil, as well as aspirin (1 mg/kg/day) and dipyridamole (3 mg/kg/day). Group 3 received safflower oil only. All rats were put to death 110 days later, at which time there was no statistically significant difference in graft function as assessed by palpation (scale 0 to 4, mean = 3.7 +/- 0.5 [+/- standard deviation]; analysis of variance: p = 0.72) or in microscopic grade of rejection (scale, 0

= none to 3 = severe, mean 2.1 +/- 0.6; analysis of variance: $p = 0.68$) between any of the groups. The coronary arteries were histologically scored for the degree of arteriosclerosis (scale, 0 = normal to 3 = occluded), and a mean grade of coronary disease was calculated for each heart. The fish oil-treated group had significantly less severe allograft coronary arteriosclerosis (analysis of variance: $p = 0.005$) than did groups 2 and 3 (mean grade 0.23 +/- 0.22 versus 1.04 +/- 0.75 and 0.96 +/- 0.55 (p less than 0.05, Scheffe F test), whereas groups 2 and 3 had similar degrees of coronary disease (p = no significant difference). These data demonstrate that fish oil supplementation inhibited accelerated coronary arteriosclerosis in this cyclosporine-treated heart allograft rat model, whereas antiplatelet agents in these doses were ineffective. Although the mechanism of this protective effect remains incompletely understood, it does not appear to involve enhanced immunosuppression. Fish oil and specific omega-3 polyunsaturated fatty acids should be further investigated as potentially useful agents to ameliorate accelerated allograft coronary arteriosclerosis in other animal species and perhaps eventually in man.

Am J Physiol. 1989 Apr;256(4 Pt 2):H968-73.

Dietary omega 3 fatty acids and endothelium-dependent relaxations in porcine coronary arteries.

Shimokawa H, Vanhoutte PM.

Dietary supplementation with cod-liver oil significantly augments endothelium-dependent relaxations in porcine coronary arteries. The present study was designed to examine the effect of dietary administration of omega 3 polyunsaturated fatty acids (mainly eicosapentaenoic acid, the major component of fish oil) on endothelium-dependent relaxations in porcine coronary arteries. Male Yorkshire pigs were maintained 4 wk on a regular diet with or without supplementation with purified eicosapentaenoic acid (3.5 g/day) and docosahexaenoic acid (1.5 g/day). Endothelium-dependent relaxations were examined in vitro. In rings from the treated group, endothelium-dependent relaxations were augmented in response to bradykinin, serotonin, and ADP, but not to the calcium ionophore A23187. These augmentations were not altered by indomethacin but were significantly inhibited by methylene blue, an inhibitor of guanylate cyclase. In the treated group, endothelium-dependent relaxations to aggregating platelets also were significantly augmented; platelet-induced contractions of quiescent rings were inhibited more by the presence of the endothelium than in arteries from the control group. Bioassay experiments demonstrated that the release of endothelium-derived relaxing factor(s) by bradykinin and relaxations of the vascular smooth muscle to the factor(s) were greater in arteries from the treated group. These observations indicate that dietary omega 3 polyunsaturated fatty acids augment receptor-operated endothelium-dependent relaxations, partly due to the augmented release of endothelium-derived relaxing factor(s) and partly due to the augmented relaxation of the vascular smooth muscle to the factor(s).

Br J Pharmacol. 1988 Dec;95(4):1191-6.

Dietary omega 3 polyunsaturated fatty acids augment endothelium-dependent relaxation to bradykinin in coronary microvessels of the pig.

Shimokawa H, Aarhus LL, Vanhoutte PM.

1. The effects of chronic dietary supplementation with omega 3 polyunsaturated fatty acids on endothelium-dependent relaxations were examined in isolated coronary microvessels of the pig. 2. Animals were maintained for four weeks with or without dietary supplementation of purified eicosapentaenoic acid (3.5 g daily) and docosahexaenoic acid (1.5 g daily). Fatty acid profiles of plasma lipids showed that only the fraction of eicosapentaenoic acid increased by the treatment, together with a decrease of that of arachidonic acid. 3. In the treated group, endothelium-dependent relaxations to bradykinin were significantly augmented, while contractions to acetylcholine or relaxations to nitroprusside were unaltered. 4. These results indicate that dietary omega 3 polyunsaturated fatty acids (mainly eicosapentaenoic acid) augment endothelium-dependent relaxations in coronary microvessels of the pig, without changing the ability of vascular smooth muscle to contract or relax.

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