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Omega-3 Fatty Acids in Cardiac Biopsies From Heart Transplantation Patients

Correlation With Erythrocytes and Response to Supplementation

William S. Harris, PhD; Scott A. Sands, PhD; Sheryl L. Windsor, MT; Hakim A. Ali, MD; Tracy L. Stevens, MD; Anthony Magalski, MD, Charles B. Porter, MD; A. Michael Borkon, MD

Background—Omega-3 fatty acids (FAs) appear to reduce the risk of sudden death from myocardial infarction. This reduction is believed to occur via the incorporation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) into the myocardium itself, altering the dynamics of sodium and calcium channel function. The extent of incorporation has not been determined in humans.

Methods and Results—We first determined the correlation between red blood cell (RBC) and cardiac omega-3 FA levels in 20 heart transplant recipients. We then examined the effects of 6 months of omega-3 FA supplementation (1 g/d) on the FA composition of human cardiac and buccal tissue, RBCs, and plasma lipids in 25 other patients. Cardiac and RBC EPA+DHA levels were highly correlated ($r=0.82$, $P<0.001$). Supplementation increased EPA+DHA levels in cardiac tissue by 110%, in RBCs by 101%, in plasma by 139%, and in cheek cells by 73% ($P<0.005$ versus baseline for all; responses among tissues were not significantly different).

Conclusions—Although any of the tissues examined could serve as a surrogate for cardiac omega-3 FA content, RBC EPA+DHA was highly correlated with cardiac EPA+DHA; the RBC omega-3 response to supplementation was similar to that of the heart; RBCs are easily collected and analyzed; and they have a less variable FA composition than plasma. Therefore, RBC EPA+DHA (also called the Omega-3 Index) may be the preferred surrogate for cardiac omega-3 FA status. (*Circulation*. 2004;110:1645-1649.)

Key Words: biopsy ■ fatty acids, omega-3 ■ fish oils ■ myocardium

A link between omega-3 fatty acids (FAs) and mortality from coronary heart disease (CHD) was first observed in Greenland Inuits.¹ The higher levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in the plasma² and platelets³ of Inuits compared with Danes were inversely related to population rates of acute myocardial infarction. Experimental and clinical trial evidence has continued to accumulate, further supporting a cardioprotective effect for omega-3 FAs. Higher blood levels of EPA and DHA were associated with reduced risk for primary cardiac arrest,⁴ sudden cardiac death,⁵ and fatal ischemic heart disease.⁶ Total mortality was reduced in CHD patients advised to eat oily fish⁷ or given omega-3 FA supplements.⁸ The latter also reduced risk of sudden cardiac death by 45%. The American Heart Association has recently recommended that patients with known CHD consume ≈ 1 g/d of EPA+DHA.⁹

Early efforts to understand the mechanism(s) by which omega-3 FAs reduce risk of death from CHD focused on their effects on classic CHD risk factors,⁹ but lowering of serum lipids and blood pressure does not appear to explain the

observed reductions in risk for sudden cardiac death.¹⁰ Possible effects of <1 g EPA and DHA on platelet aggregation, endothelial function, heart rate variability, and inflammatory markers are poorly understood. Original observations in experimental myocardial infarction¹¹ and later studies by McLennan et al¹²⁻¹⁴ in rats and monkeys suggested that omega-3 FAs had a direct protective effect on the heart itself. In a dog model of ventricular tachyarrhythmia,¹⁵ infused omega-3 FAs reduced the number of potentially fatal arrhythmias. Investigations using isolated cardiac myocytes revealed that EPA and DHA could prolong the refractory state of these cells by interaction with fast-acting sodium channels and L-type calcium channels.^{16,17} This antiarrhythmic effect is believed to be due to the release of EPA and DHA from myocardial membrane phospholipids by ischemia-activated phospholipase A₂, and the subsequent interaction of the free acids with ion channels.¹⁰ It has been demonstrated that omega-3 FA feeding increases the amount of EPA and DHA incorporated into the heart tissue of experimental animals, but the extent to which intakes of these FAs that are known to

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reduce risk for sudden cardiac death alter human cardiac FA composition is not known.

The present studies had 2 purposes. The first was to determine the EPA and DHA content of human myocardial tissues under normal and supplemented conditions. The second was to determine whether a readily available tissue [plasma, cheek, or red blood cells (RBCs)] could serve as a surrogate marker for cardiac EPA and DHA levels.

Methods

Protocols

This report describes the results of 2 separate but related studies conducted in stable cardiac transplant recipients. In the cross-sectional study, we examined the correlation between the EPA+DHA content of cardiac tissue and RBCs and how each of these tissues correlated with self-reported omega-3 FA intakes. For this study, all patients who were undergoing routine cardiac biopsies during the 2-month study period were invited to participate, regardless of their dietary omega-3 FA status. They were asked to consent to the harvesting of 1 extra tissue sample for FA analysis, and they provided information about background fish and fish oil consumption. Patients taking any dose of fish oil supplements or eating AHA-recommended amounts of fish (at least 2 fish meals per week) were considered high consumers of omega-3 FAs; those not supplementing or eating <2 fish meals per week were classified as low consumers.

In the second study (the supplementation study), we determined the effects of 6 months of supplemental omega-3 FAs on EPA and DHA levels in 4 human tissues: heart, RBCs, plasma, and cheek cells. Patients were required to meet the following criteria: be at least 3 months after transplantation; have no hospitalizations for transplant-associated infections or rejection episodes in the previous 3 months; be off fish oil supplements for at least 6 months; have a serum creatinine level ≤ 2.0 mg/dL; be on stable doses of medications, including steroids and immunosuppressive drugs; and be on a biopsy cycle of at least 2 per year. Patients were asked to take 2 capsules per day of a supplement (30/20 TG Fish Oil, Ocean Nutrition Canada, Ltd) containing 500 mg EPA+DHA per capsule (30% EPA and 20% DHA) as triglyceride.

Informed consent was obtained before initiation. The Saint Luke's Hospital Institutional Review Board approved both studies.

Laboratory Methods

Tissue Collection Procedures

Heart biopsies were collected during right heart catheterization via the internal jugular vein. Tissue was obtained from the interventricular septum, placed immediately into cold saline, and frozen at -70°C .

Cheek cells were harvested by scraping the buccal membranes with a plastic spoon after 3 preliminary rinses with distilled water. The sample was briefly centrifuged; the supernatant was decanted; and the cells were frozen at -70°C .

RBCs and plasma were obtained from fasting blood collected into EDTA. The cells were sedimented by centrifugation; the plasma was removed and frozen; and the buffy coat was discarded. The packed RBCs were stored at -70°C . (Washing of RBCs was shown in preliminary experiments not to be necessary because it did not alter FA composition.)

Materials

Isopropanol, methanol, methylene chloride, hexane, 14% boron trifluoride-methanol (BF_3), butylated hydroxytoluene, and L- α -phosphatidylcholine diheptadecanoate (internal standard) were obtained from Sigma-Aldrich. Butylated hydroxytoluene was added to all organic solvents at 50 mg/L.

Tissue Preparation/Lipid Extraction

Heart and cheek tissues were first lyophilized (Savant Speed-Vac Plus) overnight. Heart biopsies were further pulverized by grinding between 2 ground-glass slides. After lyophilization, all samples were resuspended in saline and subjected to 10 to 15 seconds of sonication (4710 Ultrasonic Homogenizer, Cole-Parmer). Lipids were then extracted with methanol (containing the internal standard) and methylene chloride as described,¹⁸ and the solvent was evaporated under nitrogen.

Thawed RBCs were extracted with isopropanol (containing the internal standard) and methylene chloride (1:30:14.4). After centrifugation of the stroma, the solvent was transferred and evaporated under nitrogen.

Plasma lipids were extracted with methanol, saline, and methylene chloride (1:25:75:50). After centrifugation, the aqueous layer was discarded, and the organic layer was transferred and evaporated under nitrogen.

Lipids extracted from heart, cheek, and RBC samples were methylated with BF_3 at 100°C for 10 minutes. These conditions transmethylated glycerophospholipid but not sphingolipid FAs.¹⁹ Plasma lipid samples were heated with BF_3 , methanol, and benzene at 100°C for 45 minutes, conditions that transmethylate all FAs.¹⁹ After cooling, all samples were extracted with hexane and water (1:2:2). The hexane layer was removed and evaporated under nitrogen, and the FA methyl esters were reconstituted in hexane for analysis by flame ionization gas chromatography as previously described.²⁰ FAs were identified by comparison with known standards, and FA composition is reported as weight percent of total FAs. The coefficient of variation for the RBC EPA+DHA assay was 10% to 12%; for whole-plasma EPA+DHA, 13% to 17%.

Plasma Lipids and Lipoproteins

Fasting serum from all 25 subjects in study 2 was analyzed before and after supplementation for total cholesterol, triglycerides, and HDL cholesterol (LDL-C) on a Cobas Fara II (Roche) with enzymatic reagents. HDL-C was determined in the serum supernatant after precipitation of VLDL and LDL as described by Warnick et al²¹ LDL-C was determined by the Friedewald equation.²²

Statistical Analysis

The Microsoft Excel 97 Data Analysis Package was used to calculate correlation coefficients between tissues according to Pearson's method. To test the hypothesis that 2 independent correlation coefficients differed significantly, each was first converted into a z score, and then a probability value was computed from a normal z table. Paired t tests were used to compare baseline and end-of-study values in the supplementation study, and unpaired t tests were used for comparisons between those in the cross-sectional study consuming high versus low amounts of omega-3 FAs. Differences between means were considered significant at $P < 0.05$. Unless otherwise noted, results are presented as mean \pm SEM.

Results

Cross-Sectional Study

Cardiac and RBC samples were obtained from 20 consecutive cardiac transplantation patients. The average age of this group was 45 ± 14 years; 15 were men. The average time from transplantation was 1.8 years (range, 0.3 to 9.7 years). There was a highly significant correlation between heart and RBC omega-3 levels (Figure 1) for all patients combined ($r = 0.82$; $P < 0.001$). Of the 20 patients, 13 were considered high omega-3 consumers, and 7 were considered low consumers. Although the correlation coefficients were virtually identical within each group ($r = 0.84$, $P < 0.01$), the relationships between the 2 tissues differed. The slope of the regression line was steeper in the low group than in the high group (Figure 1). The EPA+DHA as a percent of total RBC FA was

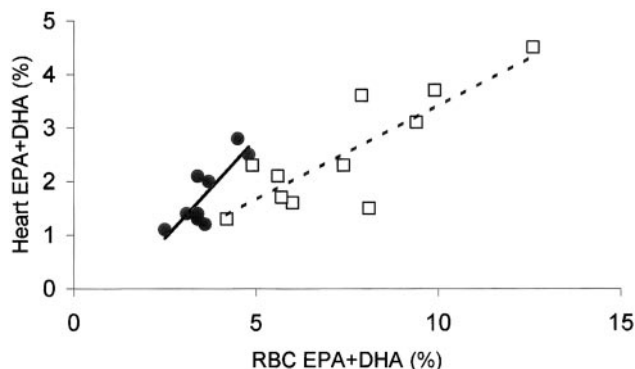


Figure 1. Correlation of omega-3 levels in cardiac tissue and RBCs in study 1 (n=20). Correlation between RBCs and cardiac EPA+DHA levels in group as a whole was 0.82 ($P<0.001$). When patients are separated according to estimated omega-3 intake (● and solid line, low intake; □ and dashed line, high intake), correlation between RBCs and cardiac EPA+DHA levels remained essentially the same ($r=0.84$ in both groups; $P<0.01$). However, slope was greater in low than in high consumers (0.74 vs 0.35, respectively).

7.0±0.70% in the latter compared with 3.3±0.15% in the former ($P=0.002$). The values for cardiac tissue were 2.5±0.27% and 1.5±0.14%, respectively ($P=0.003$).

Supplementation Study

Twenty-five patients participated in the supplementation study, and samples from all 4 tissues were available from 21. The average age of this group was 55±9 years; 16 were men. The average time from transplantation was 5.2 years (range, 0.5 to 12.5 years). In all 4 tissues examined, a statistically significant increase in EPA+DHA content was observed after 6 months of fish oil supplementation (the Table). The average increase in cardiac EPA+DHA was 110%, whereas that in RBC, cheek, and plasma was 101%, 73%, and 139%, respectively (Figure 2). There was no statistically significant difference among these responses. The mean percent increases in EPA and DHA individually were similar in all 4 tissues, although the rise in the former was generally much greater than in the latter. In the heart, EPA increased by 272±44% and DHA by 94±27%; in the cheek, 124±36%

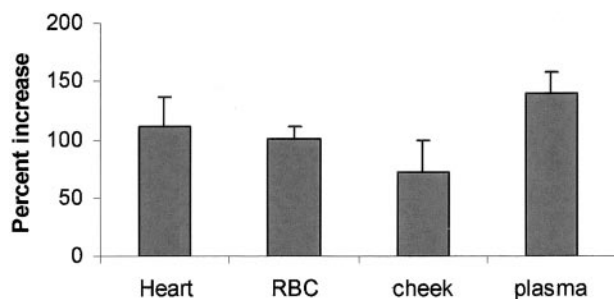


Figure 2. Mean percent increases in proportion of total FAs present as EPA+DHA in 4 tissues (heart, RBC, cheek, and plasma) examined in study 2. There were no significant differences in observed responses. Values were calculated as average of each individual's percent change in EPA+DHA in each tissue. Calculation of percent change in mean values (from the Table) will produce different numbers.

and 95±48%; in plasma, 365±52% and 104±16%; and in RBCs, 279±33% and 84±9%, respectively.

At baseline, correlations between cardiac tissue and the 3 potential surrogates were as follows: RBCs, $r=0.47$ and $P=0.031$; cheek, $r=0.49$ and $P=0.023$; and plasma, $r=0.22$ and $P=NS$. For RBCs (the only surrogate examined in both the supplementation and cross-sectional studies), the correlation tended ($P=0.06$) to be lower in the former ($r=0.47$) than in the latter ($r=0.82$).

In the 25 subjects in the supplementation study, there was no significant effect of 1 g/d EPA+DHA on serum lipids and lipoproteins: total cholesterol, 179±9 versus 179±8 mg/dL; triglycerides, 232±42 versus 197±30 mg/dL; HDL-C, 47±4 versus 46±4 mg/dL; and LDL-C, 87±6 versus 91±6 mg/dL.

Discussion

Current evidence suggests that the cardioprotective mechanism of omega-3 FAs depends on their presence in myocardial cell membranes.¹⁰ Thus, knowledge of cardiac omega-3 FA status may have clinical significance. Because it cannot be measured routinely, a surrogate tissue is needed that is both easily sampled and highly reflective of cardiac omega-3 FA levels.

Surrogates for Cardiac Omega-3 FA Content

Three tissues were examined as possible surrogates: RBCs, plasma, and cheek cells. An examination of baseline correlations between RBCs and cardiac tissue (study 1) and the responses of all 4 tissues to supplementation (Study 2), as well as practical and analytical issues, were taken into consideration to determine which of these 3 tissues could best serve as a surrogate for the heart.

Cheek Cells

Cheek cells have been used for noninvasive assessment of tissue omega-3 FA levels in infants²³ and adults.²⁴ In breast-fed infants, EPA+DHA accounted for 1.1% of total cheek cell phospholipid FAs; in adults, 0.9%. These values compare favorably with our presupplementation value of 1.6% (Table).

On a percentage basis, the cheek cell response was not statistically different from that seen in the other tissues; however, it was, on average, less than that seen in the heart (73% versus 110%). From a practical point of view, obtaining cheek cells is actually more time consuming and complicated than obtaining blood. It requires abstaining from food, tooth brushing, and using lipstick or lip balm on the morning of the test. It also involves three 30-second rinses and 2 episodes of buccal scrapping, followed by further rinses. In addition, much less tissue is obtained by buccal scrapping than by phlebotomy, making analysis more challenging.

Plasma

On a percentage basis, the plasma response was not statistically different from that seen in the other tissues; however, it was, on average, more than that seen in the heart (139% versus 110%). Plasma FAs are carried in several different lipid classes: cholesteryl esters, triacylglycerols, phospholipids, and nonesterified FAs. Because each of these classes has a unique FA composition, changes in plasma levels of a

Selected FA Content of 4 Tissues in Cardiac Transplantation Patients Before and After 6 Months of Supplementation With 1 g EPA+DHA

FA	Cardiac		Plasma		RBC		Cheek	
	Before	After	Before	After	Before	After	Before	After
Palmitic	18.3±1.5	18.9±2.7	27.0±1.8	27.7±2.0	19.8±3.5	19.8±3	16.6±2.9	16.0±2.4
Stearic	23.3±6.6	25.9±8.2	14.4±1.7	14.6±1.6	16.5±2.7	16.0±2.3	22.6±5.4	21.1±4.5
Oleic	14.2±5.1	12.7±4.5	9.1±1.1	9.0±1.3	17.3±2.8	16.8±2.3	17.8±4.4	19.2±3.6
Linoleic	9.1±2.8	7.8±3.3	21.9±2.6	20.2±3.2	10.5±2.6	9.4±2.6	8.2±2.9	8.3±2
Linolenic	0.3±0.2	0.4±0.5	0.1±0.04	0.2±0.05	0.1±0.2	0.1±0.2	0.6±0.7	0.4±0.3
Arachidonic	9.1±3.6	7.7±2.7	12.3±2.4	11.0±2.6	17.3±2.7	14.6±1.5†	2.4±0.7	2.6±0.8
DTA n-6	0.6±0.4	0.6±0.6	0.4±0.2	0.3±0.1†	5.1±1.3	3.0±0.7†	0.4±0.2	0.4±0.3
DPA n-6	0.3±0.2	0.2±0.1	0.3±0.1	0.2±0.1†	0.9±0.2	0.5±0.1†	0.1±0.1	0.1±0.2
EPA	0.18±0.10	0.6±0.3†	0.52±0.35	1.8±0.8†	0.42±0.11	1.5±0.5†	0.27±0.25	0.41±0.21*
DPA n-3	0.81±0.46	0.84±0.37	0.71±0.20	0.97±0.32†	2.7±0.5	3.9±0.7†	0.33±0.35	0.51±0.38
DHA	1.5±0.8	2.3±1.0†	2.5±1	4.7±1.1†	4.2±1.1	7.5±1.3†	1.3±0.6	1.8±0.7*
EPA+DHA	1.7±0.9	2.9±1.2†	3.1±1.2	6.5±1.6†	4.7±1.1	9.0±1.7†	1.6±0.6	2.2±0.8†
Total n-6 HUFA	10.0±3.7	8.4±2.6	13.0±2.4	11.5±2.7†	23.3±4.0	18.1±2.0†	2.9±0.7	3.2±0.8
Total n-3 HUFA	2.5±1.2	3.7±1.5†	3.8±1.3	7.5±1.8†	7.3±1.4	12.9±2.2†	1.9±0.6	2.7±1.0†
n-6:n-3 HUFA	4.4±1.4	2.5±0.7†	3.8±1.3	1.7±0.7†	3.3±0.7	1.4±0.3†	1.6±0.5	1.3±0.5†

DTA indicates docosatetraenoic acid (C22:4); DPA, docosapentaenoic acid; and HUFA, highly unsaturated fatty acids (ie, those with ≥ 20 carbons and ≥ 3 double bonds). Values are mean \pm SD; n=21.

* $P < 0.05$, † $P < 0.01$ vs presupplementation within each tissue.

specific class (eg, triacylglycerol) can alter total plasma FA composition independently of FA intake. The coefficients of variation for baseline EPA+DHA levels were 23% for RBCs and 39% for plasma (Table), reflecting the higher interindividual variability in EPA+DHA content in the latter versus the former. FAs esterified in cardiac tissue are virtually all in phospholipids, so it is not surprising that plasma phospholipid FAs are a better reflection of tissue FA composition than whole plasma is.²⁵ Thus, if only plasma is available (as in a clinical trial), then measuring the FA composition of the phospholipid fraction would be preferred over measuring that of whole plasma.

Red Blood Cells

There was a high correlation ($r=0.82$) between cardiac and RBC EPA+DHA content in the cross-sectional study, but a lower correlation was found in the supplementation study ($r=0.47$). There are several possible reasons for this difference. First, because the supplementation study excluded subjects taking omega-3 FA supplements and the cross-sectional study did not, there was a greater spread in EPA+DHA values in the latter (5-fold in RBCs, 10-fold in the heart) than in the former (2.6-fold and 7-fold, respectively). This made correlations more readily discernable. Alternatively, at low intakes, tissue uptake may be more variable.

In addition, the mean percent increase in the RBC EPA+DHA with supplementation (101%) more closely approximated that seen in cardiac tissue (110%) than did the change in plasma or cheek cells. RBCs may also be preferred over plasma because the EPA+DHA content of the former is not affected by recent food consumption. We found that RBC EPA+DHA was altered in postprandial blood from 10

healthy volunteers (-5% ; $P=NS$), whereas plasma EPA+DHA decreased by -24% ($P=0.006$; unpublished observation). Thus, the RBC EPA+DHA, also called the Omega-3 Index,²⁵ may be analogous to HbA_{1c} and reflect average tissue exposure to omega-3 FAs. These and other²⁵ factors suggest that the EPA+DHA content of RBCs may be the most appropriate surrogate for cardiac omega-3 FA content of the tissues examined.

How rapidly omega-3 FAs become incorporated into various tissues is not known. However, if cardiac myocytes, incubated for only 3 to 5 days, incorporate sufficient membrane EPA+DHA to measurably alter electrophysiological properties,¹⁶ then it seems likely that supplementation might enrich the myocardium with omega-3 FAs in relatively short order. The observed rapidity with which supplementation reduced cardiac events in the GISSI study²⁶ supports this hypothesis.

Effects of Supplementation on Serum Lipid and Lipoprotein Concentrations

Consistent with many past studies, supplementation with ≈ 1 g EPA+DHA did not materially alter serum lipid or lipoprotein levels.²⁷ Most notably, in the GISSI-Prevenzione study,²⁶ 850 mg EPA+DHA had no effect on total or lipoprotein cholesterol levels, lowered triglycerides by only 6%, but decreased CHD events substantially. The reduction in triglycerides observed here was 15% ($P=0.07$).

Conclusions

RBC EPA+DHA content is highly correlated with and responds to omega-3 supplementation in a very similar manner as cardiac omega-3 FA levels. RBCs are easily isolated and may be analyzed in the fasted or fed state, and

their EPA+DHA content reflects intakes over the past several weeks. We conclude that the Omega-3 Index may serve as a surrogate for cardiac omega-3 FA content.

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References

- Bang HO, Dyerberg J, Hjorne N. The composition of food consumed by Greenland Eskimos. *Acta Med Scand.* 1976;200:69–73.
- Dyerberg J, Bang HO, Hjorne N. Fatty acid composition of the plasma lipids in Greenland Eskimos. *Am J Clin Nutr.* 1975;28:958–966.
- Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. *Lancet.* 1979;433–435.
- Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA.* 1995;274:1363–1367.
- Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med.* 2002;346:1113–1118.
- Lemaitre RN, King IB, Mozaffarian D, et al. N-3 polyunsaturated fatty acids, fatal ischemic heart disease and non-fatal myocardial infarction in older adults: the Cardiovascular Health Study. *Am J Clin Nutr.* 2002;76:319–325.
- Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and Reinfarction Trial (DART). *Lancet.* 1989;2:757–761.
- GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E in 11,324 patients with myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet.* 1999;354:447–455.
- Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation.* 2002;106:2747–2757.
- Leaf A, Kang JX, Xiao YF, et al. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. *Circulation.* 2003;107:2646–2652.
- Culp BR, Lands WEM, Lucchesi BR, et al. The effect of dietary supplementation of fish oil on experimental myocardial infarction. *Prostaglandins.* 1980;20:1021–1031.
- McLennan PL, Bridle TM, Abeywardena MY, et al. Comparative efficacy of n-3 and n-6 polyunsaturated fatty acids in modulating ventricular fibrillation threshold in marmoset monkeys. *Am J Clin Nutr.* 1993;58:666–669.
- McLennan PL, Abeywardena MY, Charnock JS. Dietary fish oil prevents ventricular fibrillation following coronary artery occlusion and reperfusion. *Am Heart J.* 1988;116:709–717.
- McLennan PL. Relative effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on cardiac arrhythmias in rats. *Am J Clin Nutr.* 1993;57:207–212.
- Billman GE, Kang JX, Leaf A. Prevention of sudden cardiac death by dietary pure w-3 polyunsaturated fatty acids in dogs. *Circulation.* 1999;99:2452–2457.
- Kang JX, Xiao YF, Leaf A. Free, long-chain, polyunsaturated fatty acids reduce membrane electrical excitability in neonatal rat cardiac myocytes. *Proc Natl Acad Sci U S A.* 1995;92:3997–4001.
- Leaf A. The electrophysiologic basis for the antiarrhythmic and anticonvulsant effects of n-3 polyunsaturated fatty acids: heart and brain. *Lipids.* 2001;36(suppl):S107–S110.
- Carlson LA. Extraction of lipids from human whole serum and lipoproteins and from rat liver tissue with methylene chloride-methanol: a comparison with extraction with chloroform-methanol. *Clin Chim Acta.* 1985;149:89–93.
- Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J Lipid Res.* 1964;5:600–608.
- Lu G, Windsor SL, Harris WS. Omega-3 fatty acids alter lipoprotein subfraction distributions and the in vitro conversion of very low density lipoproteins to low density lipoproteins. *J Nutr Biochem.* 1999;10:151–158.
- Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg⁺² precipitation procedure for quantitation of high density lipoprotein cholesterol. *Clin Chem.* 1982;28:1379–1388.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;19:499–502.
- Connor SL, Zhu N, Anderson GJ, et al. Cheek cell phospholipids in human infants: a marker of docosahexaenoic and arachidonic acids in the diet, plasma, and red blood cells. *Am J Clin Nutr.* 2000;71:21–27.
- Lund EK, Harvey LJ, Ladha S, et al. Effects of dietary fish oil supplementation on the phospholipid composition and fluidity of cell membranes from human volunteers. *Ann Nutr Metab.* 1999;43:290–300.
- Harris WS, von Schacky C. The Omega-3 Index: a new risk factor for sudden cardiac death. *Prev Med.* 2004;39:212–220.
- Marchioli R, Barzi F, Bomba E, et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation.* 2002;105:1897–1903.
- Harris WS. n-3 Fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr.* 1997;65(suppl):1645S–1654S.

Corrections

In the article “Omega-3 Fatty Acids in Cardiac Biopsies From Heart Transplantation Patients: Correlation With Erythrocytes and Response to Supplementation,” by Harris et al, which appeared in the September 21, 2004, issue of the journal (*Circulation*. 2004;110:1645–1649), the authors have identified 2 errors. On page 1646, under the heading “Plasma Lipids and Lipoproteins,” the abbreviation in parentheses on the third line should be HDL-C, not LDL-C. Also, in the final line in column 1 of page 1648, the word “not” was omitted. The sentence should read, “We found that RBC EPA+DHA was not altered in postprandial blood from 10 healthy volunteers. . .” The authors regret these errors.

DOI: 10.1161/01.CIR.0000148060.90440.08

Regarding the Correspondence page “Dietary Intervention Combined With Exercise Improves Vascular Dysfunction but Also Obstructive Sleep Apnea in Obese Children,” which appeared in the September 21, 2004 issue of the journal (*Circulation*. 2004;110:e314), it has been brought to our attention that this title is misleading. The 2 letters on this page pertained to an article published in the April 27, 2004, issue of the journal by Woo et al (*Circulation*. 2004;109:1981–1986) titled “Effects of Diet and Exercise on Obesity-Related Vascular Dysfunction in Children.” The second letter (available at <http://circ.ahajournals.org/cgi/content/full/110/12/e314>), written by Tsung O. Cheng, should have been titled “Childhood Obesity Among the Chinese.” We regret this error.

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The article “Antibodies From Preeclamptic Patients Stimulate Increased Intracellular Ca²⁺ Mobilization Through Angiotensin Receptor Activation” by Thway et al, which appeared in the September 21, 2004, issue of the journal (*Circulation*. 2004;110:1612–1619), was inadvertently published without the authors’ corrections. The corrected version is available online at <http://circ.ahajournals.org/cgi/content/full/110/12/1612>. We regret these errors.

DOI: 10.1161/01.CIR.0000149087.81831.11

In the article “Critical Role of Macrophage 12/15-Lipoxygenase for Atherosclerosis in Apolipoprotein E-Deficient Mice” by Huo et al that appeared in the October 5, 2004, issue of the journal (*Circulation*. 2004;110:2024–2031), an error appeared in the legend of Figure 4. The following sentences should have been deleted: d, Serum-free cell medium conditioned by 12/15-LO^{+/+} macrophages slightly increased endothelial VCAM-1 expression. e, In presence of LDL, 12/15-LO^{+/+} macrophages cocultured with endothelial cells significantly increased expression of VCAM-1 on endothelial cells. n=4.

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