Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on hemodynamics in humans\textsuperscript{1–3}

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ABSTRACT The hemodynamic effects of highly purified eicosapentaenoic acid (EPA, 20:5n–3) and docosahexaenoic acid (DHA, 22:6n–3) have not been evaluated in humans. We therefore conducted a randomized, double-blind, parallel-design intervention study to assess possible separate effects of EPA and DHA on blood pressure, heart rate, and cardiac mechanics. Healthy, nonsmoking men aged 36–56 y (n = 224) were randomly assigned to dietary supplementation with 4 g/d of ethyl ester concentrates of DHA or EPA or 4 g corn oil/d (control). Mean blood pressure at baseline was 122/77 mm Hg and was positively associated with concentrations of serum phospholipid saturated fatty acids. Blood pressure did not change during the intervention. Mean heart rate at baseline was 63.4 beats/min; it decreased 2.2 beats/min in the DHA group (P = 0.006 compared with control), increased 1.9 beats/min in the EPA group (P = 0.04 compared with control), and remained practically unchanged in the control group. In a pooled analysis, changes in heart rate were independent of baseline heart rate and were associated with changes in concentrations of serum phospholipid DHA and docosahexaenoic acid (22:5n–3). Echocardiography in a subsample of 52 men showed improved left ventricular diastolic filling in the marine oil groups compared with the corn oil group (P = 0.02). In contrast, an increase in plasma concentrations of saturated fatty acids was associated with delayed diastolic filling. We conclude that dietary DHA and EPA influence heart rate and that the fatty acid composition of plasma phospholipids may affect cardiac mechanics in humans. \textit{Am J Clin Nutr} 1998;68:52–9.

KEY WORDS Eicosapentaenoic acid, EPA, docosahexaenoic acid, DHA, phospholipids, heart rate, blood pressure, echocardiography, clinical trials, humans, saturated fatty acids, n–3 fatty acids, marine oil

INTRODUCTION Dietary supplementation with n–3 polyunsaturated fatty acids of marine origin is reported to exert a wide range of biological effects of relevance to cardiovascular disease. The protective effects of these fatty acids include the ability to lower serum triacylglycerols and decrease the ability of platelets to aggregate (1, 2). The effects of marine oils on hemodynamics are less clear. Several reports indicate that marine oils may lower blood pressure in subjects with hypercholesterolemia and in patients with essential hypertension (3); however, these findings are not corroborated by other investigators (4). Marine oils have been reported to lower resting heart rate (5), to reduce ventricular extrasystoles in humans (6), and to possibly prevent ventricular fibrillation (7). Incorporation of dietary fat into cell membranes may alter the physiochemical properties of the membrane and thereby influence vascular tone (8) and myocardial relaxation (9). Dietary saturated fat lowered the left ventricular ejection fraction in nonhuman primates, whereas marine oils increased the left ventricular ejection fraction by enhancing ventricular filling (9). Whether n–3 fatty acids modify cardiac function and diastolic filling in healthy humans has to our knowledge not been examined.

Docosahexaenoic acid (DHA, 22:6n–3) and eicosapentaenoic acid (EPA, 20:5n–3) are considered to be the biologically active fatty acids in marine oils. The amount and proportion of DHA and EPA in different marine sources vary considerably. Earlier studies of hemodynamic effects of n–3 fatty acids in humans used oils differing both in total dose and in relative content of DHA and EPA. Animal studies have shown that DHA and EPA accumulate in different compartments in the body and may be metabolized differently and have different functions (10). Studies in rats indicate that DHA, which in contrast with EPA normally accumulates in mammalian heart cells, prevents ischemia-induced arrhythmias more effectively than EPA (11). It is unknown whether some of the inconsistent findings on hemodynamic effects of marine oils in humans are due to separate effects of DHA and EPA.

We therefore designed a randomized, controlled intervention trial to extend previous findings by comparing the effects of dietary supplements containing highly purified DHA and EPA on cardiovascular function. We also examined the relations between blood pressure, heart rate, left ventricular mechanics, and concentrations of serum phospholipid fatty acids.

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SUBJECTS AND METHODS

Subjects and study design

The subjects and experimental design were described in detail previously (12). In 1993 we recruited 234 nonsmoking, healthy men aged 36–56 y from a population study. The men met the following criteria at 2 visits during a 4-mo observation period: mean systolic blood pressure <170 mm Hg, mean diastolic blood pressure <100 mm Hg, and the difference between the diastolic blood pressures measured on the 2 visits <15 mm Hg. The study was approved by the regional board of research ethics and each subject gave his written, informed consent.

The study was a double-blind, placebo-controlled intervention trial performed according to Good Clinical Trial Practice requirements (13). At baseline, computer-generated random numbers were used to assign subjects to dietary supplementation with 4 g 90% pure ethyl ester DHA/d, 4 g 95% pure ethyl ester EPA/d, or 4 g corn oil/d (control) for 7 wk. The supplements were administered in indistinguishable soft gelatin capsules that each contained 1 g oil and 4–6 IU vitamin E as the antioxidant (Pronova Biocare A/S, Oslo). Each subject was asked to ingest 4 capsules daily. In comparison, 500 g fatty fish contains ~3.4 g EPA and 5 g DHA (14).

Blood pressure and echocardiographic measurements

Participants were examined after an overnight fast on 2 separate occasions within an interval of 3–5 d both at baseline and after 7 wk of supplementation. A trained assistant measured blood pressure and heart rate with an automatic instrument (Dinamap; Critikon, Tampa, FL) as described previously (15). The instrument measured pressure by the oscillometric method and calculated the mean arterial pressure automatically as the area under the pressure wave form divided by the time during which the area was measured (16). Heart rate was derived from the median pulse-to-pulse interval during the time that blood pressure was measured (17). After the subjects had been seated for 5 min, 3 recordings were made at 2-min intervals. The mean of the 2 final recordings was used in the analyses. Calibration of the instrument before and after the study showed that there was no drift during the study period.

M-mode echocardiography was used as a noninvasive tool to measure left ventricular function at baseline and at the end of intervention. One blinded investigator (ESPM) studied 20 randomly selected subjects in each intervention group. With the subject in a supine, left lateral position, a transverse section of the left ventricle was obtained at the level of the tips of the mitral valve tips, showing the movements of the left ventricle (LVS) and left ventricular posterior wall (LVPW) during the cardiac cycle. ECG, electrocardiogram; a, left ventricular end-diastolic diameter (LVEDD); b, left ventricular end-systolic diameter (LVESD); c, left ventricular diameter at the end of fast expansion (LVEFED).

Clinical and laboratory measurements

Diets were assessed by a certified clinical nutritionist using the diet history method as described previously (12). Body mass index (BMI) was calculated as body weight divided by the square of the height (kg/m²). We assessed physical activity (sedentary, moderate, or active) with a questionnaire at baseline and at the end of the intervention.

The collection and preparation of blood samples have been described in detail (12). Blood samples were analyzed after the completion of the intervention and before the randomization code was broken. Fatty acids in serum phospholipids were measured by gas-liquid chromatography as described previously (12). Serum total cholesterol and triacylglycerol were analyzed by enzymatic colorimetric methods with commercial kits (CHOD-PAP for cholesterol and GPO-PAP for triacylglycerols; Boehringer Mannheim, Mannheim, Germany). HDL cholesterol was measured after precipitation of the LDLs with heparin and manganese chloride. Serum lipids and serum creatinine, sodium, and potassium were analyzed on a Hitachi 737 Automatic Analyzer (Boehringer Mannheim) with reagents from the manufacturer. Plasma active renin was measured quantitatively by immunoradiometric assay (Nichols Institute BV, Wijchen, Netherlands) with reagents from the manufacturer.

Statistical analysis

The primary endpoint was a change in diastolic blood pressure between the beginning (baseline) and the end of the 7-wk intervention trial. The study was designed to detect a difference of 3 mm Hg in diastolic blood pressure at a two-sided level of significance of 0.05, with a power of 0.90. Blood pressure and heart rate at baseline and at the end of the intervention were calculated as the average of the values obtained on 2 separate occasions within an interval of 3–5 d. All variables were normally distributed except baseline serum phospholipid EPA. Log transformation normalized the distribution of EPA but did not influence the analyses; hence, untransformed data were used. Because of missing values, analyses of plasma active renin; all
fatty acids; and serum creatinine, sodium, and potassium were based on 216, 219, and 222 subjects, respectively. Change was calculated as the value obtained after the intervention minus the value obtained at baseline. Percentage change was calculated as the groupwise percentage change from baseline. One-sample t tests were used to assess within-group change. Results were considered statistically significant when the two-sided P value was < 0.05. One-way analysis of variance was used for between-group comparisons of change by contrasting group in the SAS general linear models procedure when the overall F test was significant at P < 0.05 (18).

We did not adjust for multiple comparisons (19) and thus caution should be applied when interpreting P values in the present study because 3 contrasts were tested. When Tukey’s multiple comparison procedure was used (20), the 95% CI included the null value of no effect for those contrasts for which the unadjusted P value was > 0.03. In subgroup analyses, two-sample t tests were used to compare change in the marine oil groups (pooled) with change in the corn oil group. Linear relations were examined by computing crude and adjusted Pearson correlation coefficients and by developing multiple linear regression models.

RESULTS

Three of the 234 men who were randomly assigned to a study group did not complete the study. Seven men were excluded because of either renal disease (n = 1), poor compliance with the study protocol (n = 1), initiation of a vasoactive drug (n = 1), cancer surgery (n = 1), or change in level of physical activity during the intervention (n = 3), thus leaving 224 men for the present analysis. The DHA, EPA, and corn oil groups were well balanced at baseline (Table 1) (12). Compliance was satisfactory; the subjects in each of the 3 groups took > 90% of the prescribed number of capsules. There were no important side effects.

In the DHA group, mean serum phospholipid DHA and EPA concentrations increased by 69% and 29%, respectively, whereas docosapentaenoic acid (DPA, 22:5–n–3) decreased by 33%. In the EPA group, mean serum EPA and DPA concentrations increased by 297% and 130%, respectively, whereas serum DHA decreased by 15%. We reported previously that in both the DHA and EPA groups, serum n–3 fatty acid concentrations increased at the expense of saturated, monounsaturated, and n–6 fatty acid concentrations (12).

There were minor changes in concentrations of serum sodium, whereas creatinine, potassium, and plasma active renin concentrations did not change during supplementation with DHA, EPA, or corn oil (Table 2). Baseline plasma active renin concentrations were inversely correlated with baseline systolic (r = −0.24, P = 0.0004) and diastolic (r = −0.18, P = 0.0006) blood pressure, whereas serum total cholesterol concentrations were positively correlated with baseline systolic (r = 0.22, P = 0.001) and diastolic (r = 0.34, P = 0.001) blood pressure (pooled analysis of all participants).

Effect of marine oil supplementation on blood pressure and heart rate

Systolic and diastolic blood pressure did not change in any treatment group during the intervention (Table 2). Neither were there changes in blood pressure in subgroup analyses when the participants were stratified according to baseline values for systolic blood pressure, diastolic blood pressure, plasma active renin, serum cholesterol, serum saturated fatty acids, serum n–3 fatty acids, dietary intake of saturated fat, or dietary intake of salt (data not shown).

Heart rate decreased by 2.2 ± 4.6 beats/min in the DHA group, increased by 1.9 ± 5.1 beats/min in the EPA group, and remained practically unchanged in the corn oil group (Table 2). The change in the DHA group was significantly different from that in the EPA and corn oil groups (DHA compared with EPA, P = 0.0001; DHA compared with corn oil, P = 0.006; EPA compared with corn oil, P = 0.04). The predictors of baseline heart rate were analyzed in a multiple regression model that included baseline blood pressure and baseline concentrations of phospholipid fatty acids. Baseline heart rate was associated with baseline diastolic blood pressure (β = 0.38, P = 0.0001) and baseline DHA (β = −0.02, P = 0.002; adjusted model R² = 0.12, P = 0.0001). The model predicted a reduction in heart rate of 2.6 beats/min (95% CI: −4.4, −0.8 beats/min) if diastolic blood pressure increased by 0.74 mm Hg and serum phospholipid DHA increased by 128 μmol/L (the mean changes in diastolic blood pressure and serum phospholipid DHA in the DHA group). This predicted value was similar to the observed reduction in heart rate during supplementation with DHA (Table 2). Compared with that in the control group, heart rate in the DHA group decreased and that in the EPA group increased across all values of baseline heart rate (Figure 2). The slopes of the changes in the DHA and EPA groups were parallel and noncoincident when tested in multiple regression analysis (20), indicating that EPA and DHA have different effects on change in heart rate independent of baseline heart rate. In pooled analyses of all subjects, change in heart rate was inversely correlated with change in serum phospholipid DHA (r = −0.25, P = 0.0002) and positively correlated with DPA (r = 0.26, P = 0.0001), but was not correlated with change in EPA.

### Table 1

Baseline characteristics of subjects randomly assigned to dietary supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DHA (n = 72)</th>
<th>EPA (n = 75)</th>
<th>Corn oil (n = 77)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>43.2 ± 5.1</td>
<td>44.3 ± 5.2</td>
<td>45.1 ± 5.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9 ± 2.6</td>
<td>25.6 ± 2.9</td>
<td>24.6 ± 2.7</td>
</tr>
<tr>
<td>Serum lipids (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>6.0 ± 0.94</td>
<td>5.98 ± 0.94</td>
<td>6.02 ± 1.08</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.36 ± 0.30</td>
<td>1.33 ± 0.31</td>
<td>1.41 ± 0.28</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>1.24 ± 0.58</td>
<td>1.23 ± 0.57</td>
<td>1.22 ± 0.55</td>
</tr>
<tr>
<td>Diet (daily intake)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>10 370 ± 2561</td>
<td>10 223 ± 2170</td>
<td>10 877 ± 2455</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>29.0 ± 5.7</td>
<td>30.0 ± 4.6</td>
<td>29.7 ± 5.6</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>33.6 ± 14.4</td>
<td>34.3 ± 10.9</td>
<td>35.4 ± 11.4</td>
</tr>
<tr>
<td>P:S</td>
<td>0.40 ± 0.15</td>
<td>0.39 ± 0.13</td>
<td>0.40 ± 0.13</td>
</tr>
<tr>
<td>EPA (g)</td>
<td>0.18 ± 0.20</td>
<td>0.19 ± 0.18</td>
<td>0.19 ± 0.21</td>
</tr>
<tr>
<td>DHA (g)</td>
<td>0.34 ± 0.32</td>
<td>0.35 ± 0.28</td>
<td>0.36 ± 0.32</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>3497 ± 913</td>
<td>3501 ± 772</td>
<td>3679 ± 968</td>
</tr>
<tr>
<td>Serum phospholipid fatty acids (μmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of fatty acids</td>
<td>4090 ± 676</td>
<td>4106 ± 592</td>
<td>4257 ± 708</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>2131 ± 310</td>
<td>2119 ± 300</td>
<td>2192 ± 380</td>
</tr>
<tr>
<td>EPA</td>
<td>59.8 ± 53.7</td>
<td>61.4 ± 41.0</td>
<td>64.0 ± 40.3</td>
</tr>
<tr>
<td>DHA</td>
<td>185 ± 88</td>
<td>184 ± 65</td>
<td>203 ± 69</td>
</tr>
</tbody>
</table>

1 x ± SD. P:S, ratio of polyunsaturated to saturated fatty acids.
HEMODYNAMIC EFFECTS OF DHA AND EPA

TABLE 2
Blood pressure; heart rate; serum concentrations of creatinine, sodium and potassium; and concentrations of plasma active renin at baseline and change after 7 wk of supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DHA (n = 72)</th>
<th>EPA (n = 75)</th>
<th>Corn oil (n = 77)</th>
<th>F test:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Change</td>
<td>Baseline</td>
<td>Change</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>121.3 ± 9.3</td>
<td>0.5 ± 5.4</td>
<td>123.2 ± 9.8</td>
<td>−0.5 ± 5.7</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>76.1 ± 6.9</td>
<td>0.7 ± 4.6</td>
<td>78.1 ± 7.3</td>
<td>0.5 ± 4.2</td>
</tr>
<tr>
<td>Mean arterial BP (mm Hg)</td>
<td>90.6 ± 7.3</td>
<td>1.2 ± 5.9</td>
<td>2.9 ± 8.0</td>
<td>0.4 ± 4.8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>63.1 ± 8.3</td>
<td>−2.2 ± 4.6&lt;sup&gt;6,7&lt;/sup&gt;</td>
<td>63.7 ± 7.6</td>
<td>1.9 ± 5.1&lt;sup&gt;2,6,7&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>90.4 ± 10.5</td>
<td>1.1 ± 5.4</td>
<td>89.4 ± 10.4</td>
<td>0.0 ± 5.0</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>142.3 ± 1.7</td>
<td>−0.4 ± 1.4&lt;sup&gt;2,4&lt;/sup&gt;</td>
<td>141.9 ± 1.5</td>
<td>0.5 ± 7.7&lt;sup&gt;9&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.5 ± 0.3</td>
<td>0.0 ± 0.4</td>
<td>4.5 ± 0.3</td>
<td>0.0 ± 0.3</td>
</tr>
<tr>
<td>Plasma active renin (mU/L)</td>
<td>27.4 ± 16.8</td>
<td>1.6 ± 9.3</td>
<td>24.7 ± 9.8</td>
<td>0.3 ± 8.2</td>
</tr>
</tbody>
</table>

1<sup>1</sup> ± SD. BP, blood pressure.
2<sup>1</sup> ANOVA for between-group comparisons of change.
3<sup>1,4</sup> Significantly different from baseline (one-sample t test); 6<sup>6</sup> P < 0.05, 7<sup>7</sup> P < 0.01, 9<sup>9</sup> P < 0.01.
4<sup>2</sup> Significantly different from EPA, P = 0.0001.
5<sup>6,8</sup> Significantly different from corn oil; 6<sup>6</sup> P = 0.006, 8<sup>8</sup> P = 0.04, 9<sup>9</sup> P = 0.009.

Effect of marine oil supplementation on left ventricular function

Echocardiography was performed in a random subsample and the analysis suffered from lack of statistical power. Two of 60 men undergoing echocardiography dropped out of the study during the intervention. Six men were excluded because high-quality echocardiographic measurements could not be obtained (n = 4) or because they changed their level of physical activity during the intervention (n = 2), leaving 52 men in the analysis. Left ventricular dimensions as measured by LVEDD, LVESD, LVEFEd, and FEE tended to increase after supplementation with DHA and EPA (Table 3). Because the effects of DHA and EPA were similar, we pooled the data from the DHA and EPA groups. Changes in LVEFEd (Figure 3) and FEE (Figure 4) were significantly different from changes in the corn oil group (P = 0.02 and P = 0.03, respectively), indicating improved early left ventricular filling after marine oil supplementation.

Serum phospholipid fatty acids and left ventricular function

In a pooled analysis of all men undergoing echocardiography, there was a strong inverse association between change in LVEDD, LVESD, and LVEFEd and change in serum saturated fatty acids (r = −0.36, r = −0.38, and r = −0.41, respectively; all P < 0.01; Table 4). The association was strongest for change in serum phospholipid palmitic acid (16:0) (r = −0.45, P = 0.0008, Figure 5). The relation between plasma fatty acids and left ventricular function was independent of changes in blood pressure, heart rate, blood lipids, serum n−3 fatty acids, and the ratio of polyunsaturated to saturated fatty acids (data not shown).

Serum phospholipid fatty acids and blood pressure at baseline

Mean baseline fatty acid concentrations were reported previously (12). Fatty acids of all main classes were positively correlated with blood pressure in univariate and age-adjusted analyses (Table 5). The associations between saturated and monounsaturated fatty acids and systolic blood pressure were not significant after adjustment for BMI. Adjustment for serum total cholesterol weakened the association between saturated fatty acids and diastolic blood pressure (data not shown). In multiple regression analysis, the statistically significant predictors of systolic blood pressure were BMI, physical activity, plasma active renin, α-linolenic acid (18:3n−3), and DPA (adjusted model R<sup>2</sup> = 0.33, P = 0.0001), whereas diastolic blood pressure was predicted by BMI, physical activity, serum total cholesterol, and DPA (adjusted model R<sup>2</sup> = 0.28, P = 0.0001). There were no statistically significant associations between serum fatty acids and heart rate in univariate or age-adjusted analyses.

DISCUSSION

This study showed that the fatty acid composition of serum phospholipids can affect heart rate and possibly left ventricular diastolic function in humans. Several lines of evidence support a causal relation between increased dietary intake of DHA and decreased heart rate. First, we compared the effects of purified DHA and EPA in a randomized, double-blind intervention trial. Although sufficient blinding is a problem in dietary supplementation trials with n−3 fatty acids, the participants in the present study were unable to discriminate between the DHA and EPA.
TABLE 3
Left ventricular dimensions at baseline and change after 7 wk of supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil\(^1\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>DHA ((n = 16))</th>
<th>EPA ((n = 18))</th>
<th>Corn oil ((n = 18))</th>
<th>(F) test:</th>
<th>(p) (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD (cm)</td>
<td>5.67 ± 0.51</td>
<td>5.34 ± 0.74</td>
<td>5.81 ± 0.81</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>LVEDD (cm)</td>
<td>3.32 ± 0.29</td>
<td>3.16 ± 0.57</td>
<td>3.51 ± 0.56</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>FS (%)</td>
<td>41.3 ± 4.4</td>
<td>40.6 ± 5.7</td>
<td>39.7 ± 4.3</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>LVEFED (cm)</td>
<td>4.98 ± 0.37</td>
<td>4.75 ± 0.73</td>
<td>5.31 ± 0.64</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>FEE (%)</td>
<td>71.3 ± 9.7</td>
<td>72.5 ± 10.6</td>
<td>78.9 ± 9.5</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>238 ± 56</td>
<td>205 ± 94</td>
<td>266 ± 91</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)\(x ± SD\). LVEDD, left ventricular transverse end-diastolic diameter; LVEDS, left ventricular transverse end-systolic diameter; FS, fractional shortening; FEE, fractional early expansion; LV mass, left ventricular mass.

\(^2\)ANOV A for between-group comparisons of change.

supplements. Next, the difference between change in the DHA group and that in the EPA and corn oil groups was highly significant (Table 2), which reduces the possibility of a chance finding. Third, the effect was consistent across baseline heart rate values (Figure 2) and, finally, the change in heart rate was correlated with change in serum phospholipid DHA in a pooled analysis of all participants. Whether the increase in heart rate in the EPA group was due to EPA, DPA, or a relative deficit of DHA remains unknown.

A decrease in heart rate after marine oil supplementation has been observed both in animals (7, 9, 21) and in humans (5, 22). Free DHA and EPA reduced both contraction rate and fibrillation after arrhythmogenic stimuli in neonatal rat cardiac myocytes (23). Reduced electrical excitability by inhibition of myocyte voltage-gated sodium channels has been suggested as the primary underlying mechanism. Marine oils reduced the susceptibility to ischemia-induced and reperfusion-induced arrhythmias in animal feeding studies (7, 24, 25). In humans, dietary marine oils reduced the incidence of ventricular premature complexes (6) and increased electrocardiogram R-R variability (26), indicating possible antiarrhythmic effects. Dietary intake of DHA and EPA from seafood was associated with a reduced risk of primary cardiac arrest in a population-based case-control study (27). Finally, 2 secondary prevention trials of myocardial infarction survivors hypothesized that high intakes of fish (28) and \(\alpha\)-linolenic acid (18:3n-3) (29) reduce the incidence of fatal cardiac arrhythmias. DHA may be more effective than EPA in preventing ischemia-induced arrhythmias in rats (11). On the basis of our data, we can speculate that DHA is responsible for the postulated antiarrhythmic effect of n-3 fatty acids.

Early left ventricular diastolic filling is mainly due to the myocardial relaxation rate and elastic recoil, and a delayed fall of intraventricular pressure in the diastole is an early sign of left ventricular ischemia and hypertrophy (30). The present study suggests that highly unsaturated n-3 fatty acids improve early left ventricular diastolic filling. In contrast, an increase in serum saturated fatty acids was associated with delayed diastolic filling. Our findings are supported by results obtained in human cardiac transplant patients showing that dietary marine oils improved left ventricular diastolic function, as measured by reduced deceleration time (31).

\(\text{FIGURE 3. Change in left ventricular transverse diameter at the end of fast expansion (LVEFED) after 7 wk of dietary supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil (control). Bar with solid circle denotes the mean value and 95% CI for pooled analysis of the DHA and EPA groups. Bar with open square denotes the mean value and 95% CI for the corn oil group. DHA and EPA groups (pooled mean) were significantly different from the corn oil group, } P = 0.02.\)

\(\text{FIGURE 4. Change in left ventricular fractional early expansion (FEE) after 7 wk of dietary supplementation with docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), or corn oil (control). Bar with solid circle denotes the mean value and 95% CI for pooled analysis of the DHA and EPA groups. Bar with open square denotes the mean value and 95% CI for the corn oil group. DHA and EPA groups (pooled mean) were significantly different from the corn oil group, } P = 0.03.\)
Similarly, marmosets fed diets enriched in n-3 and n-6 fatty acids had increased left ventricular ejection fractions due to enhanced diastolic filling compared with marmosets fed a saturated fat diet (9). Taken together, these data support the hypothesis that dietary patterns can affect left ventricular diastolic filling by modifying the fatty acid composition of phospholipids in plasma and cell membranes. We observed that saturated fatty acids were associated with changes in left ventricular filling independent of changes in heart rate. Hence, the underlying mechanism or mechanisms may be other than those responsible for the changes in heart rate. The fatty acid composition of cell membranes may affect heart function by modifying membrane fluidity and elastic properties of myocardial cells. It is also possible that saturated fatty acids and n-3 fatty acids affect diastolic function through separate mechanisms. Nitrogen monoxide has been found to influence left ventricular relaxation (32), and n-3 fatty acids may influence nitrogen monoxide release (33).

Neither DHA nor EPA lowered blood pressure in this group of normotensive men. This is in accordance with recent meta-analyses that concluded that marine oils in relatively high doses decrease blood pressure in persons with hypertension or cardiovascular disease (3, 34). Salt restriction augmented the decrease in blood pressure observed in hypertensive humans after marine oil supplementation (35) and it has been suggested that marine oils affect hypertension when it is mediated through increased activity in the renin-angiotensin system (36). It is also possible that long-term effects of n-3 fatty acids depend on both the amount and type of other fats consumed. However, in the present 7-wk study, we found no effect modification by values for blood pressure, serum cholesterol, plasma active renin, serum concentrations of saturated fatty acids, serum concentrations of n-3 fatty acids, or dietary intake of salt or fat. The study participants were a homogenous group, which may limit the possibility of detecting effect modification.

At baseline, both systolic and diastolic blood pressure were positively associated with concentrations of serum phospholipid DPA, whereas there was no association between EPA or DHA and blood pressure. A previous study in mildly hypertensive humans found a negative association between plasma EPA and blood pressure (37). Absorption or metabolism of n-3 fatty acids may differ between normotensive and hypertensive persons. A potential source of bias in cross-sectional studies is a change in behavior and dietary habits caused by the awareness of high blood pressure and the recent focus on the potential cardio-protective effects of marine oils.

We observed a strong positive association between baseline concentrations of saturated fatty acids in serum and blood pressure. A positive relation between dietary intake of saturated fat

### TABLE 4

Mean change in phospholipid fatty acid concentrations and correlations between change in fatty acid concentrations and change in left ventricular dimensions after dietary supplementation with docosahexaenoic acid, eicosapentaenoic acid, or corn oil.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Change in fatty acid concentration</th>
<th>Pearson correlation coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LVEDD</td>
<td>LVEDD</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>$-8.43 \pm 215$</td>
<td>$-0.36$</td>
</tr>
<tr>
<td>16:0</td>
<td>$-4.21 \pm 144$</td>
<td>$-0.38$</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>$-30.1 \pm 89.9$</td>
<td>$-0.24$</td>
</tr>
<tr>
<td>Polysaturated fatty acids</td>
<td>$-23.0 \pm 191$</td>
<td>$-0.07$</td>
</tr>
<tr>
<td>n-6 fatty acids</td>
<td>$-129 \pm 219$</td>
<td>$0.16$</td>
</tr>
<tr>
<td>n-3 fatty acids</td>
<td>$108 \pm 128$</td>
<td>$0.18$</td>
</tr>
<tr>
<td>All fatty acids</td>
<td>$54.8 \pm 432$</td>
<td>$-0.26$</td>
</tr>
</tbody>
</table>

$^{1} n = 52$, except saturated fatty acids and all fatty acids, for which $n = 51$. LVEDD, left ventricular transverse end-diastolic diameter; LVEDSD, left ventricular transverse end-systolic diameter; FS, fractional shortening; LVEFED, left ventricular transverse diameter at the end of fast expansion; FEE, fractional early expansion; LV mass, left ventricular mass.

$^{2}$ Saturated fatty acids include 16:0, 18:0, 20:0, 22:0, and 24:0.


$^{4}$ Monounsaturated fatty acids include 16:1, 18:1, 20:1, 22:1, and 24:1.

$^{5}$ n-6 fatty acids include 18:2, 20:3, 20:4, and 22:4.


$^{7}$ $P < 0.01$.

$^{8}$ $P < 0.05$.

$^{9}$ $P < 0.001$.

and blood pressure has been shown in population studies (38–40), whereas the relation between saturated fatty acids in blood and blood pressure is less clear (41, 42). Saturated fatty acids may influence blood pressure through direct effects on the vascular system or through the actions of cholesterol as an intermediate promoting atherosclerosis and vascular stiffness. In a dietary intervention trial, blood pressure decreased significantly after an 8-wk diet low in both total fat and saturated fat, thereby suggesting a direct effect of saturated fat on blood pressure (43).

The present study provides evidence for a relation between concentrations of serum fatty acids and hemodynamics in humans. EPA and DHA supplementation had differential effects on resting heart rate, and further studies should examine whether humans. EPA and DHA supplementation had differential effects after an 8-wk diet low in both total fat and saturated fat, thereby mediating promoting atherosclerosis and vascular stiffness. In a vascular system or through the actions of cholesterol as an intermediate promoting atherosclerosis and vascular stiffness. In a dietary intervention trial, blood pressure decreased significantly after an 8-wk diet low in both total fat and saturated fat, thereby suggesting a direct effect of saturated fat on blood pressure (43).

The present study provides evidence for a relation between concentrations of serum fatty acids and hemodynamics in humans. EPA and DHA supplementation had differential effects on resting heart rate, and further studies should examine whether the two n—to fatty acids influence the susceptibility to cardiac arrhythmias differently. A short-term increase in dietary EPA or DHA does not affect blood pressure in normotensive subjects, but may improve left ventricular function and thereby contribute to the postulated cardioprotective effects of marine oils.

REFERENCES


40. Beegom R, Singh RB. Association of higher saturated fat intake with higher risk of hypertension in an urban population of Trivandrum in south India. Int J Cardiol 1997;58:63–70.

