Effects of fish-oil supplementation on myocardial fatty acids in humans\textsuperscript{1–3}

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ABSTRACT

Background: Increased fish or fish-oil consumption is associated with reduced risk of cardiac mortality, especially sudden death. This benefit putatively arises from the incorporation of the long-chain n–3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) into cardiomyocyte phospholipids.

Objective: The study examined the kinetics of incorporation of n–3 fatty acids into human myocardial membrane phospholipids during supplementation with fish oil and \( \alpha \)-linolenic acid–rich flaxseed oil.

Design: Patients with low self-reported fish intake (<1 fish meal/wk and no oil supplements) accepted for elective cardiac surgery involving cardiopulmonary bypass were randomly allocated to 1 of 6 groups: no supplement; fish oil (6 g EPA + DHA/d) for either 7, 14, or 21 d before surgery; flaxseed oil; or olive oil (both 10 mL/d for 21 d before surgery). Right atrial appendage tissue removed during surgery and blood collected at enrollment and before surgery were analyzed for phospholipid fatty acids.

Results: Surgery rescheduling resulted in a range of treatment times from 7 to 118 d. In the fish-oil-treated subjects, accumulation of EPA in n–3 fatty acids was rapid and displaced mainly arachidonic acid. Flaxseed oil supplementation yielded a small increase in atrial EPA but not DHA, whereas olive oil did not significantly change atrial n–3 fatty acids.

Conclusion: The results of the present study show that dietary n–3 fatty acids are rapidly incorporated into human myocardial phospholipids at the expense of arachidonic acid during high-dose fish-oil supplementation. Am J Clin Nutr 2007;85:1222–8.

KEY WORDS Fish oils, fatty acids, n–3 fatty acids, dietary fats, myocardium, humans

INTRODUCTION

Several cohort (1–5), dietary intervention (6–8), and case-control (9, 10) studies have shown that fish and n–3 polyunsaturated fatty acids (PUFAs) confer significant cardiovascular benefit. The cohort studies documented a 42–67\% lower incidence of cardiac death associated with intake of fish or vegetable n–3 fats, and the intervention studies reported a 32–73\% lower risk of cardiac death with or without nonfatal myocardial infarction (1–8). A large intervention study using a fish-oil concentrate reported a 45\% reduction in sudden cardiac death with fish oil (8). In addition, case-control studies have found that, compared with the lowest quartile, erythrocyte or whole blood n–3 PUFAs in the highest quartile are associated with a 90\% reduction in risk of primary cardiac arrest (10) or sudden cardiac death (9).

The mechanism by which eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) confer cardiovascular protection may involve a replacement of arachidonic acid (AA) in vascular and cardiac membrane phospholipids. AA is a precursor of proinflammatory eicosanoids, whereas EPA and DHA have been shown to be anti-thrombotic and anti-arrhythmic, respectively. It has been proposed that the erythrocyte EPA + DHA content (the omega-3 index) could serve as a risk factor for death from coronary artery disease, with EPA + DHA \( \geq \)8% of total erythrocyte fatty acids being proposed as a target to reduce the risk of death from coronary artery disease (11). However, only one small study looked at the incorporation of dietary n–3 fatty acids into human myocardium, and that study used endocardial biopsy specimens from transplanted hearts (12). No studies have examined nontransplanted hearts; in particular, no data exist on the rate of increase in human myocardial EPA and DHA in response to dietary intervention. This information is important for prospective acute dietary interventions, such as for acute coronary syndrome, and is also pertinent in determining the likely time to benefit in longer-term administration of fish oil. Thus, in the present study we examined the rate of incorporation of dietary n–3 PUFAs into human myocardium during fish-oil treatment compared with the effect of flaxseed oil–derived n–3 fatty acids on human myocardial fatty acids.

SUBJECTS AND METHODS

Subjects

Patients accepted for elective on-pump cardiac surgery (coronary artery bypass graft, valve repair or replacement, or both) were recruited if they had not previously had cardiac surgery, were expected to have at least 21 d waiting time before surgery,

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TABLE 1

Fatty acid composition of the dietary oils

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Fish oil</th>
<th>Flaxseed oil</th>
<th>Olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitc (16:0)</td>
<td>0.9</td>
<td>5.2</td>
<td>10.1</td>
</tr>
<tr>
<td>Saturated</td>
<td>2.6</td>
<td>9.2</td>
<td>14.2</td>
</tr>
<tr>
<td>Total monounsaturated</td>
<td>9.4</td>
<td>16.0</td>
<td>79.5</td>
</tr>
<tr>
<td>LA (18:2n-6)</td>
<td>1.7</td>
<td>15.8</td>
<td>5.7</td>
</tr>
<tr>
<td>Total n-6</td>
<td>6.7</td>
<td>16.0</td>
<td>5.8</td>
</tr>
<tr>
<td>ALA (18:3n-3)</td>
<td>1.3</td>
<td>58.7</td>
<td>0.5</td>
</tr>
<tr>
<td>SDA (18:4n-3)</td>
<td>3.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EPA (20:5n-3)</td>
<td>3.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DPA (22:5n-3)</td>
<td>3.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DHA (22:6n-3)</td>
<td>3.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total n-3</td>
<td>75.1</td>
<td>58.8</td>
<td>0.5</td>
</tr>
</tbody>
</table>

1 AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; OA, oleic acid; SDA, stearidonic acid.

and if they reported a low frequency of ingestion of fish (≤1 fish meal/wk) and no fish oil or other oil supplements. The study design was a random, blinded allocation to parallel treatment groups. Randomization was by sealed envelope. Of 6 treatments groups, 5 received a supplement and the other received no test dietary intervention. The dietary treatments were 10 mL/d of either fish-oil concentrate (Berg Lipidtech AS, Aalesund, Norway) for 7, 14, or 21 d before surgery and flaxseed oil or olive oil for 21 d before surgery. The dietary oils contained a total of 1.86% lemon and lime oil flavoring to improve palatability and binding. The oil was provided in bottles, and the subjects were instructed to take it in fruit juice as described previously (13). The fish-oil concentrate was selected because it contained approximately equal proportions of EPA and DHA, providing a daily intake of ~3 g of each fatty acid. The flaxseed oil comparator provided a similar total amount of n-3 PUFA content, but as α-linolenic acid (ALA). The olive oil comparator provided <1% of the n-3 PUFA intake of flaxseed oil, also as ALA. The fatty acid composition of the oils is detailed in Table 1.

Subjects allocated to an intervention were advised of their treatment start date once a surgery date was set. Subjects who had their surgery date rescheduled (earlier or later) after the start of the intervention were advised to continue the allocated oil up to the time of surgery.

Subjects who failed to complete the intervention, or from whom an atrial specimen was not obtained at surgery, were replaced as needed by the next available patient to ensure that a total of 30 atrial specimens were obtained from subjects who completed the fish-oil supplementation and 10 from subjects in each of the other groups. Compliance was assessed by interview and by changes in erythrocyte and plasma fatty acid composition. Informed consent was obtained before participation in the study, which was approved by the Research Ethics Committee of the Royal Adelaide Hospital.

Tissue collection procedures

Nonfasting blood was collected into lithium heparin tubes at enrollment and again at the time of admission to the hospital before surgery. Right atrial appendage tissue that is removed as a standard surgical procedure during cannulation of the heart in preparation for cardiopulmonary bypass was collected and placed immediately into ice-cold saline, transferred to the laboratory, removed from the saline, and stored at −70 °C until analyzed.

Fatty acid extraction and analysis

Erythrocyte and plasma phospholipids were extracted as described (14). Atrial samples were cleaned of adipose tissue and clotted blood. Approximately 0.2 g tissue was homogenized in 2 mL saline before mixing with 3 mL methanol. Chloroform (6 mL) was added, the samples were centrifuged (1560 × g, 10 min, room temperature), and the chloroform phase was transferred to a 20-mL scintillation vial and evaporated to dryness.

Phospholipids were separated by thin-layer chromatography and after methanolysis of the phospholipid fractions. Fatty acid methyl esters were analyzed by gas-liquid chromatography as described (15).

Safety

Postoperative blood loss from mediastinal chest tube drains was recorded at the time of tube removal.

Statistical analysis

Continuous variables were compared by using analysis of variance with post-testing by Tukey-Kramer multiple comparisons test or Kruskal-Wallis with post-testing by Dunn’s test as appropriate. Subjects completing the fish-oil treatment were allocated to tertiles on the basis of duration of fish-oil treatment.

Analyses were conducted by using INSTAT version 3.06 (Graphpad Software, San Diego, CA). Regression analysis was used to explore relations between duration of intervention and fatty acid content of atrium and erythrocytes (PRISM version 4.1; Graphpad Software). Best-fit curves were obtained by using the linear and nonlinear regression analytic features of PRISM. For each data set, linear regression, sigmoidal dose-response (variable slope), and second-order polynomial curves were calculated, with the best-fit curve selected by comparison of the $r^2$ values.

RESULTS

Subjects

To achieve our group size objectives, we enrolled a total of 84 subjects to yield 10 atrial samples in each treatment group as detailed in Figure 1. The required total of 60 subjects was enrolled initially, 24 of whom withdrew for the reasons shown and were replaced.

Alterations to the scheduling of elective surgery were common. As a consequence, the actual duration of oil consumption did not match that specified by the group allocation. Thus, a spread of treatment durations occurred across a range from 7 to 63 d for the fish-oil groups, from 18 to 118 d in the flaxseed oil group, and from 15 to 79 d for the olive oil group. The basic characteristics at baseline of all the subjects from whom atrial tissue was obtained and who completed the intervention did not differ significantly as detailed in Table 2.
Baseline fatty acids

There were no significant differences between any of the groups in erythrocyte or plasma phospholipid n-3 or n-6 fatty acids at baseline. Baseline erythrocyte and plasma fatty acid compositions are provided in the supplemental data section (see Supplemental Table 1 in the current issue online at www.ajcn.org).

Effects of fish-oil consumption on rate of change in tissue fatty acids

The range of days of fish-oil consumption allowed nonlinear regression analysis of EPA, DHA, and EPA/DHA within atrial and erythrocyte phospholipids relative to duration of fish-oil treatment. These relations appeared to be curvilinear and are shown in Figure 2.

To tabulate the fatty acids in groups according to duration of treatment, the subjects allocated to take fish oil were divided into tertiles on the basis of the number of days of actual fish-oil consumption rather than the original group allocation. Details of the phospholipid fatty acid content of atrial myocardium and erythrocytes for all groups are provided in Table 3. Plasma values are provided in the supplemental data section (see Supplemental Table 2 in the current issue online at www.ajcn.org).

In atrial phospholipids, there were no significant differences between the control and the fish-oil groups in the proportions of saturated fatty acids, monounsaturated fatty acids, or the 18-carbon PUFAs linoleic acid (LA) and ALA. Fish-oil supplementation resulted in progressively higher proportions of EPA, DHA, EPA/DHA, and total n-3 PUFAs through each tertile of duration of fish-oil treatment. The progressively higher proportions of n-3 PUFAs in atrial phospholipids were associated with a reciprocal lowering of long-chain n-6 PUFAs, predominantly AA (Figure 3), with no significant differences in the proportion of total long-chain PUFAs between any of the groups (Table 3).

There was also no significant effect of fish-oil supplementation on saturated or monounsaturated fatty acids in erythrocyte phospholipids. The proportions of EPA and DHA matched the pattern seen in atrial phospholipids and were progressively higher than in the control group over the 3 tertiles of duration of fish-oil treatment. These changes were associated with a small

### TABLE 2

Characteristics at enrollment of the subjects from whom atrial specimens were obtained

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Fish-oil group</th>
<th>Flaxseed oil group</th>
<th>Olive oil group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>60.7 ± 14.5²</td>
<td>63.9 ± 7.1</td>
<td>65.2 ± 13.4</td>
<td>63.5 ± 6.7</td>
</tr>
<tr>
<td>Men (n)</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.6 ± 2.0</td>
<td>30.1 ± 3.1</td>
<td>30.2 ± 5.7</td>
<td>28.6 ± 3.7</td>
</tr>
</tbody>
</table>

¹ No significant differences were observed between the groups, \( P > 0.05 \) (ANOVA).
² \( x \pm SD \) (all such values).
but significantly higher proportion of total long-chain PUFAs in the second tertile only compared with the control group, as well as with lower proportions of n−6 PUFAs. Initially, higher n−3 PUFAs were associated with lower LA in erythrocyte phospholipids, with progressively lower long-chain n−6 PUFAs being associated with more sustained treatment (Table 3).

**Effect of fish-oil supplementation on atrial and erythrocyte EPA and DHA during the first 10 d of supplementation**

During the first 10 d of fish-oil treatment, there was a similarity in absolute differences in erythrocyte and atrial phospholipid EPA+DHA content (mean differences of 2.5% and 2.9% of total fatty acids, respectively) compared with that in the control group (Table 3). In erythrocytes, absolute differences in EPA and DHA during the initial period of treatment were almost identical (1.3% and 1.2%, respectively). However, in atrium, the increment in DHA was more than twice that for EPA (2.0% and 0.9%, respectively), which indicated that during the initial stages of supplementation, DHA accumulated in atrial phospholipids significantly more rapidly than did EPA ($P < 0.001$).

**Effects of flaxseed oil treatment on atrial fatty acids**

The flaxseed oil treatment yielded a significantly higher proportion of ALA in atrium relative to a modest untreated comparator level (0.13%; Table 3), and although EPA was ~50% higher in the flaxseed oil group, the difference was not significant. Over the ranges of treatment times observed, regression analysis failed to show an effect of duration of flaxseed oil treatment on any of the phospholipid fatty acids examined (data not shown).

**Effects of olive oil treatment on atrial fatty acids**

No significant differences were found between the olive oil and the control groups for any fatty acid examined (Table 3).

**Safety**

No significant differences were found in total postsurgical blood loss between any of the groups (Figure 4). Two subjects were returned to surgery to control postoperative bleeding, 1 of whom completed the olive oil arm and 1 allocated to flaxseed oil, but who was subsequently withdrawn because an atrial specimen was not obtained. There were 2 deaths in the immediate postoperative period, 1 in the olive oil group on postoperative day 3 of cardiac and respiratory arrest, and 1 in the control group on postoperative day 20 of respiratory failure.

One subject suffered an ischemic stroke due to an embolism related to aortic valve replacement. This subject was originally allocated to fish-oil treatment but was subsequently withdrawn because of noncompliance. There were no cases of postoperative myocardial infarction. No significant differences were found between the groups for length of time in the intensive care unit or total length of stay (data not shown).

**DISCUSSION**

This study describes, for the first time, a time course of the incorporation of long-chain n−3 PUFAs from dietary fish-oil treatment into human myocardial membranes. Even though the American Heart Association recommends a daily intake of ~1 g EPA+DHA for persons with diagnosed heart disease, little information is available on the effects of dietary n−3 PUFA supplementation on myocardial fatty acid amounts in humans. At the
dose of fish oil used in the present study, EPA and DHA accumulated rapidly within atrial phospholipids, and this was at the expense of long-chain n-6 fatty acids, mainly AA. Also, DHA accumulated in atrial phospholipids more rapidly than did EPA early during treatment with fish oil, even though DHA and EPA were present in equal proportions.

Information regarding the complete fatty acid profile of human myocardium is scarce. Early studies on human myocardium focused only on fatty acids that constituted the major proportions (saturates, oleic acid, LA and AA, and DHA) (16–18). Subsequent studies, which provide more comprehensive details of the long-chain PUFA content of human myocardium, used papillary muscle harvested during mitral valve replacement (19, 20), unspeciated myocardial material obtained at autopsy (21), and endocardial biopsy samples from the interventricular septum obtained from heart transplantation recipients (12). Although no published data are available on comparisons of the fatty acid profile between the ventricle and atrial appendage, the fatty acid profile in atrial myocardium of the subjects in our untreated group was similar to that found in papillary muscle (19) and autopsy material (21). The fatty acid profile obtained from heart transplant patients (12) differs from these reports and from the transplant patients (12) differs from these reports and from the transplant patients (12) differs from these reports and from the
The beneficial effects of dietary $n-3$ fatty acids are thought to accrue from their incorporation into cardiomyocyte phospholipids with consequent effects on myocardial membrane function and release as free fatty acids by the action of phospholipase A$_2$ (24). In the time course analysis of the GISSI-P study, the survival curves for total mortality, coronary artery disease mortality, cardiovascular disease mortality, and sudden death began to diverge after $\approx 60$ d from the start of treatment (25), and did not become significantly different until $90$ d for total mortality, $120$ d for sudden death, and $240$ d for coronary artery disease and cardiovascular disease mortality. Gradual accretion of $n-3$ fatty acids into membrane phospholipids (26) may result in a delay in protection afforded by eating fish or taking a fish-oil supplement until an effective level is reached. Incorporation of $n-3$ PUFAs into red blood cells is known to vary between individuals and to be potentially influenced by several factors, including age, body mass index, presence of diabetes (27), and background diet, especially $n-6$ PUFA intake (28). Individual differences may also occur in the incorporation of EPA and DHA into myocardium. The present study provides insight into the likely extent of this variability.

Survivors of myocardial infarction are at greatest risk of sudden death in the first month after the myocardial infarction with an event rate about 3-fold higher than that for the period from 1 to 6 mo after myocardial infarction (29). If the cardioprotective effects of fish oil arise from EPA or DHA within cardiac phospholipids, it follows that initial, high-dose fish-oil supplementation, which rapidly elevates cardiac levels of EPA and DHA, could be helpful during this period of vulnerability to life-threatening ventricular arrhythmias.

The displacement of myocardial AA by EPA and DHA may provide additional benefits to those provided by the $n-3$ fatty acids alone. In isolated neonatal rat cardiomyocytes, addition of AA in the free fatty acid form can be both pro- and anti-arrhythmic. The pro-arrhythmic effect is attributable to oxygenated metabolites of AA, because the addition of cyclooxygenase and lipoxygenase inhibitors ablates this effect, while leaving the anti-arrhythmic effect of free AA intact (30). In an early study reporting the fatty acid profiles of human myocardium obtained from autopsy, it was observed that the AA-to-DHA ratio in cases of sudden cardiac death was substantially higher than in cases of accidental death who had no evidence of coronary artery disease (31).

Dietary flaxseed oil supplementation resulted in atrial EPA being $\approx 50\%$ higher than in controls, although this was a non-significant difference, whereas there was no effect on atrial DHA. This failure of dietary ALA to influence plasma and tissue DHA, while increasing tissue ALA and EPA, has been shown previously (32, 33). It was suggested that the specificity of these effects may be explained through competitive inhibition by ALA of D$^\delta$ desaturase metabolism of 24:5n$-3$ to 24:6n$-3$, which is putatively involved in the multistep conversion of EPA to DHA (34, 35). Although the daily intake of EPA+DHA with fish oil (6.3 g) was quantitatively similar to that of ALA with flaxseed oil (5.8 g), flaxseed oil was $\approx 10\%$ as effective as fish oil in elevating atrial EPA and atrial EPA+DHA. The mean increments in atrial EPA and EPA+DHA with flaxseed oil treatment were 0.26% and 0.6%, respectively, of total atrial phospholipid fatty acids, whereas these values were 2.5% and 6.2%, respectively, with fish oil.

The olive oil–supplemented group was included to determine the suitability of use of olive oil as a placebo oil in future studies.
into the anti-arrhythmic effects of n-3 fatty acids. It was previously reported that oleic acid has no anti-arrhythmic properties (36, 37), and the results of the present study show that olive oil treatment in a dose of 10 mL/d does not affect n-3 fatty acids in atrial tissue. By comparison, healthy volunteers given 7 g corn oil/d (which delivers ~3.5 g LA/d) for 20 wk reduced mean erythrocyte content of EPA+DHA from 5.5% to 4.5% of total phospholipid fatty acids (11). Thus, olive oil appears to be a more suitable comparator oil for studies into the effects of dietary n-3 PUFAs on tissue long-chain n-3 PUFAs.

In conclusion, we showed that EPA and DHA in cardiac phospholipids can be increased substantially within 1 wk through daily consumption of fish oil providing 6 g EPA+DHA/d. The kinetics of incorporation of EPA and DHA into human myocardium observed in this study provides an indication of practically achievable, optimal rates of EPA and DHA incorporation that are not likely to be matched by lower doses of fish oil. Our findings provide a foundation for further studies into the optimal use of fish oil as a preventative against life-threatening arrhythmias that are most prone to occur early after myocardial infarction.

We thank all of the administrative, theater, and ward staff of the Cardio-thoracic Surgical Unit of the Royal Adelaide Hospital for their invaluable assistance in conducting this trial and the staff of the Child Nutrition Research Unit, Flinders Medical Centre, Adelaide, for fatty acid analyses.

The authors’ responsibilities were as follows—RGM, MJJ, LGC, and GDY: designed the study and obtained funding; RGM and KR-T: conducted the study; RAG: responsible for the analyses of fatty acids; RGM: analyzed the data and wrote the first draft of the manuscript; JRME, JS, and RS: contributed to the design of the trial and the staff of the Child Nutrition Research Unit, Flinders Medical Centre, Adelaide, for fatty acid analyses.

REFERENCES