Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other n−3 fatty acids1-3

Michael J James, Virginia M Ursin, and Leslie G Cleland

ABSTRACT
Background: For many persons who wish to obtain the health benefits provided by dietary n−3 fatty acids, daily ingestion of fish or fish oil is not a sustainable long-term approach. To increase the number of sustainable dietary options, a land-based source of n−3 fatty acids that is effective in increasing tissue concentrations of the long-chain n−3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is required.

Objective: The objective of the study was to examine the ability of dietary stearidonic acid (SDA) to increase tissue concentrations of EPA and DHA in healthy human subjects and to compare the effectiveness of SDA with that of the n−3 fatty acids α-linolenic acid (ALA) and EPA.

Design: Encapsulated SDA, ALA, or EPA was ingested daily in doses of 0.75 g and then 1.5 g for periods of 3 wk each by healthy male and postmenopausal female subjects (n = 15/group) in a double-blind, parallel-group design.

Results: Dietary SDA increased EPA and docosapentaenoic acid concentrations but not DHA concentrations in erythrocyte and in plasma phospholipids. The relative effectiveness of the tested dietary fatty acids in increasing tissue EPA was 1.0:3.0:0.07 for EPA:SDA:ALA.

Conclusions: Vegetable oils containing SDA could be a dietary source of n−3 fatty acids that would be more effective in increasing tissue EPA concentrations than are current ALA-containing vegetable oils. The use of SDA-containing oils in food manufacture could provide a wide range of dietary alternatives for increasing tissue EPA concentrations.


KEY WORDS  Stearidonic acid, n−3 fats, healthy volunteers, eicosapentaenoic acid, α-linolenic acid, metabolism

INTRODUCTION
The therapeutic and preventive benefits of dietary n−3 fatty acids with regard to cardiovascular disease and rheumatoid arthritis have been well documented (1, 2). Most evidence for benefits applies to the long-chain n−3 fatty acids eicosapentaenoic acid (EPA; 20:5n−3) and docosahexaenoic acid (DHA; 22:6n−3), which occur in fish and fish oil. However, it is problematic to recommend to patients or to the community that they increase fish intake. Dietary preferences are strong, and, if individuals do not habitually consume fish, a health message is unlikely to alter this habit.

We have argued that the key to increasing n−3 fatty acid intake is to provide a wide range of foods enriched with n−3 fatty acids so that persons can choose foods and ingredients that suit their usual dietary habits. By using foods such as milk, margarine, and sausage (all of which were enriched with fish oil), as well as canned and fresh fish, we demonstrated that subjects can have a dietary EPA+DHA intake of 1.5−1.8 g/d without altering their dietary habits (3, 4). This intake is equivalent to that supplied by ≥1 fish meal/d.

However, the modification of foods with oil that will efficiently increase tissue concentrations of EPA and DHA requires enrichment with fish oils. With increasing applications, this is likely to become problematic because of pressure on global fish stocks, and aquaculture is unlikely to be a sustainable solution because the industry relies heavily on wild fish stocks for feed (5).

These issues could be resolved by the provision of n−3 fatty acids via the terrestrial food chain. Currently, α-linolenic acid (ALA; 18:3n−3) is the main n−3 fatty acid available in vegetable oils. Although there may be cardiovascular benefits associated directly with ALA (6), there is poor conversion of ingested ALA to the longer-chain n−3 fatty acids EPA and DHA (7). A possible explanation for the low conversion of ALA to EPA, a 3-enzyme process, is that the initial enzyme, Δ6-desaturase (EC 1.14.99.25), is rate limiting in humans, as it was shown to be in rodents (8, 9). Thus, the ingestion of vegetable oils enriched in stearidonic acid (SDA; 18:4n−3) may be an efficient means of increasing tissue EPA concentrations.

This study examined whether dietary SDA can increase tissue EPA and DHA concentrations in healthy human volunteers and compared its efficacy with that of dietary ALA and EPA. The comparative abilities of these dietary n−3 fatty acids to inhibit inflammatory cytokine production were also examined.

SUBJECTS AND METHODS
Subjects
Forty-five healthy subjects, men and postmenopausal women aged 18–65 y, who were normolipidemic and whose body mass

1 From the Rheumatology Unit, Royal Adelaide Hospital, Adelaide, Australia (MJJ and LGC), and Calgene LLC, Davis, California (VMU).
2 Supported by the Monsanto Company; the study was initiated by the investigators.
3 Reprints not available. Address correspondence to MJ James, Rheumatology Unit, Royal Adelaide Hospital, North Terrace, Adelaide, SA, Australia 5000. E-mail: michael.james@adelaide.edu.au.
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index (in kg/m²) was in the range of 20–30 were recruited. Exclusions were men > 2 times/wk, use of dietary supplements rich in n-6 fatty acids (± 4.9 g/d), low-dose aspirin, consumption of restaurant or carry-out evening meals, > 3 fatty acids, habitual consumption of > 1 fish meal/mo, and plasma phospholipid EPA+DHA concentrations > 7.9% of total fatty acids (± SD concentration recorded in recruited subjects: 4.9 ± 1.0% of total fatty acids).

Subjects were randomly allocated (in blocks of 3) to 1 of 3 groups for the administration of ALA, SDA, or EPA on a double-blind basis. After unblinding at the study conclusion, the numbers of men and women, respectively, in each group were 10 and 5 in the EPA group, 9 and 6 in the SDA group, and 10 and 5 in the ALA group.

Written informed consent was obtained from all subjects. The study was approved by the Royal Adelaide Hospital Research Ethics Committee.

### Study design

After a 3-wk run-in period, subjects ingested either ALA, SDA, or EPA supplied as ethyl esters in capsules. Vanillin was added to all oils to mask odors. Intakes were 0.75 g/d for 3 wk and then 1.5 g/d for 3 wk. At –3, 0, 3, and 6 wk, 36 mL blood was taken by venipuncture after an overnight fast. Blood was aliquotted into various tubes for laboratory procedures.

### Cell separations and fatty acid analysis

Blood (20 mL) was added to tubes containing 4 mL 4.5% (wt by vol) EDTA in water and 4 mL 6% (wt by vol) dextran in normal saline at a pH of 7.0. Erythrocytes were allowed to sediment under gravity at 37 °C for 30 min. The leukocyte-rich plasma was layered onto Lymphoprep, density 1.077 (Nycomed, Australia). All organic solvents contained butylated hydroxy anisole (0.005%) as an antioxidant.

### Cytokine synthesis

Blood (4 mL) was added to heparinized tubes, bacterial lipopolysaccharide (200 ng/mL) was added, and the mixture was incubated at 37 °C in 5% CO₂ for 24 h. Plasma was collected and assayed for interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) by enzyme-linked immunosorbent assay, as described elsewhere (3).

### Measurement of plasma lipids

Blood (4 mL) was collected in heparinized tubes, and plasma total, LDL, and HDL cholesterol and plasma triacylglycerol were measured in the clinical diagnostic laboratories of the Royal Adelaide Hospital, Institute of Medical and Veterinary Science.

### Diet

Subjects were instructed in avoiding dietary n-6 fatty acids and substituting monounsaturated (n-9) fatty acids where possible. To facilitate this pattern of intake, they were provided with salad dressing, cooking oil, and margarine that were low in n-6 fatty acids and high in n-9 fatty acids. Subjects were provided with diet diaries and instructed in keeping weighed food records on designated days (weekdays and weekend days).

### Dietary intake

Diet diaries were analyzed with the use of DIET/1 software (Xyris Software, Brisbane, Australia) to obtain data on macronutrient (carbohydrate, protein, fat, alcohol, and the ratio of polyunsaturated fat to saturated fat) intake. This software uses the Australian Nutrient Data Table database (NUT-TAB95; Australian Government Publishing Service, Canberra). The analysis also provides intake data on n-6 and n-9 fatty acids.

### Statistical analysis

Two-way repeated-measures analysis of variance (ANOVA), followed by Tukey’s post hoc test of significance, was conducted with SAS software, version 8.2 (SAS Institute Inc, Cary, NC).

### RESULTS

#### Baseline body mass index and weight change during trial

The average body mass index for all subjects was 26.4, and there was no significant difference in baseline body mass index between the groups. Subjects were weighed at each visit, and there was no significant change in weight during the trial (data not shown). One subject in the EPA group withdrew from the study.

#### Energy and macronutrient intake

The mean (± SD) energy intake was 9.1 ± 0.2 MJ. Summary data for the macronutrient intakes are shown in Table 1. There

### Table 1

<table>
<thead>
<tr>
<th>Intake</th>
<th>g/d (% of dietary energy)</th>
</tr>
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<tbody>
<tr>
<td>Protein</td>
<td>96.7 ± 5.4 (17.2 ± 0.9)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>260 ± 11.8 (48.6 ± 1.6)</td>
</tr>
<tr>
<td>Total fat</td>
<td>76.7 ± 4.8 (31.1 ± 1.4)</td>
</tr>
<tr>
<td>n-6 Fat</td>
<td>7.1 ± 0.8 (2.9 ± 0.3)</td>
</tr>
<tr>
<td>Monounsaturated fat</td>
<td>28.2 ± 1.6 (11.4 ± 0.4)</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>34.4 ± 2.8 (14.0 ± 0.9)</td>
</tr>
</tbody>
</table>

1. ± SD; n = 44.
was no significant difference in energy or macronutrient intake between any group at baseline, and there were no statistically significant changes during the study in any group.

Compliance

A count of returned capsules was used to assess compliance in capsule intake. Compliance among all groups and at all doses was 99.5 ± 0.77%, and there was no significant difference between the dietary groups in either of the dietary periods.

Fatty acids

Eicosapentaenoic acid

At baseline, there was no significant difference between the dietary groups in EPA concentrations in erythrocyte or plasma phospholipids. The ingestion of EPA or SDA at 0.75 g/d (0–3 wk) or 1.5 g/d (3–6 wk) significantly (P < 0.0001) increased EPA concentrations in the phospholipid fractions of erythrocytes and plasma (Tables 2 and 3). The ingestion of ALA at either dose did not significantly increase EPA concentrations. Similar results were observed for EPA concentrations in platelets and mononuclear cell phospholipids, plasma cholesterol esters, and plasma triacylglycerol (data not shown).

Docosapentaenoic acid

The ingestion of EPA at 0.75 g/d or 1.5 g/d significantly (P < 0.0001) increased the concentration of docosapentaenoic acid (DPA; 22:5n−3) in the phospholipid fractions of erythrocytes and plasma (Tables 2 and 3). The ingestion of SDA at 1.5 g/d significantly (P < 0.0001) increased DPA concentrations. Similar results were observed for DPA concentrations in platelets and mononuclear cell phospholipids, plasma cholesterol esters, and plasma triacylglycerol (data not shown).

Docosahexaenoic acid

None of the dietary test fatty acids at either dose resulted in a numerical increase in DHA concentrations in the phospholipids of either plasma or erythrocytes (Tables 2 and 3). Whereas there were slight decreases in DHA concentrations, particularly at the higher doses of the test fatty acids, the interaction term for dietary group × time effects was not statistically significant (two-way ANOVA), and therefore, analyses of the time effects for each diet were not performed.

Numerical comparison of test fatty acids in increasing tissue EPA concentrations

With the use of the data in Tables 2 and 3, the increases in erythrocyte and plasma phospholipid EPA can be calculated, and this allows a comparison of the relative effectiveness of the dietary fatty acids in increasing tissue EPA in erythrocyte and plasma phospholipids.

At the 0.75-g daily dose (0–3 wk), the effectiveness of SDA was 3.1- to 5.0-fold that of ALA, and the effectiveness of EPA was 3.1- to 5.9-fold that of SDA. At the 1.5-g daily dose (3–6 wk), the effectiveness of SDA was 3.7- to 4.1-fold that of ALA, and the effectiveness of EPA was 3.1- to 3.6-fold that of SDA.

Cytokine and eicosanoid synthesis

Whereas there were significant time effects for the synthesis of both TNF-α and IL-1β, the diet-by-week interaction term was not significant (two-way ANOVA) (Tables 4 and 5). Therefore, subgroup analyses for the time effects for each diet were not performed.

The test fatty acids had no consistent effect on the lipopolysaccharide-stimulated synthesis of prostaglandin E2, and thromboxane A2 synthesis during blood clotting was not affected by any of the test fatty acids (data not shown).

Plasma lipids

There were no significant differences between groups at any visit or between visits for any group in concentrations of fasting triacylglycerol or of total, LDL, or HDL cholesterol (data not shown).

DISCUSSION

The principal finding of this study is that dietary SDA was metabolized to EPA in humans. The relative effectiveness of each
of the fatty acid supplements in increasing EPA concentrations in plasma or erythrocyte phospholipids was EPA > SDA > ALA. These relativities support the proposition that the activity of Δ^6-desaturase is rate limiting in the conversion of ALA to EPA in humans, as it was shown to be in rodents (8, 9). In the SDA dietary group, neither SDA nor its immediate metabolite 20:4n-3 accumulated in detectable amounts (data not shown), which suggests that elongase and Δ^5-desaturase activities were sufficient to prevent tissue accumulation with the ingestion of ≤ 1.5 g SDA/d. Dietary SDA and EPA were qualitatively similar with respect to their ability to increase tissue EPA and DPA, but they did not increase DHA. Whereas SDA is metabolized to EPA and DPA by the actions of elongase and Δ^5-desaturase, further metabolism to DHA depends on Δ^6-desaturase activity followed by β-oxidation (10). Given the rate-limiting activity of Δ^6-desaturase in the conversion of ALA to EPA, its activity may limit the conversion of DPA to DHA also. Whereas a study of > 6 wk may result in increased DHA concentrations, the lack of increase in DHA with the ingestion of ALA or EPA was reported previously (11, 12).

The test fatty acids—ALA, SDA, and EPA—were provided as encapsulated dietary supplements. Although it is not known whether dietary linoleic acid (LA; 18:2n-6) alters SDA metabolism to EPA, it has been shown in rats and humans that dietary LA decreases ALA metabolism to EPA (13–15). Therefore, it was considered prudent to control the type of dietary fat in the background diet as much as is practical in free-living humans. This was achieved by providing advice on avoiding unnecessary LA intake and by providing margarine, salad dressing, and cooking oil that were low in n-6 fatty acids and high in n-9 fatty acids. As a consequence, the LA intake of = 3% of dietary energy in this study is probably lower than the community average.

The inhibition of the synthesis of TNF-α or IL-1β, or both, measured ex vivo after the ingestion of fish oil has been reported in healthy males and females and in rheumatoid arthritis patients (3, 16–19). Therefore, the comparative effect of the dietary fatty acids on the production of these inflammatory cytokines was examined. Whereas the synthesis of both TNF-α and IL-1β decreased during the course of the study, the initial statistical analysis by two-way ANOVA did not support further analysis of the effect of the individual fatty acid supplements. The mechanism for inhibition of cytokine synthesis by long-chain n-3 fatty acids in not known, but it has been shown that polymorphisms in the promoter regions of the TNF-α and TNF-β genes are involved in determining not only the levels of synthesis but also the response to fish oil (20).

An important outcome from this study is provided by quantifying the relative effectiveness of the test fatty acids in increasing tissue EPA concentrations. If the ability of dietary EPA to increase tissue EPA is set to unity, the numerical relative effectiveness of

**TABLE 4**

Tumor necrosis factor α synthesis in lipopolysaccharide-treated whole blood

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Week</th>
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<tbody>
<tr>
<td></td>
<td>ng/mL</td>
</tr>
<tr>
<td></td>
<td>-3</td>
</tr>
<tr>
<td>EPA (n = 14)</td>
<td>36.6 ± 16.5</td>
</tr>
<tr>
<td>SDA (n = 15)</td>
<td>37.6 ± 14.6</td>
</tr>
<tr>
<td>ALA (n = 15)</td>
<td>30.9 ± 13.1</td>
</tr>
</tbody>
</table>

1 ± SD. EPA, eicosapentaenoic acid; SDA, stearidonic acid; ALA, a-linolenic acid. There was a significant effect of week, P = 0.01 (two-way repeated-measures ANOVA).

2 From −3 to 0 wk, run-in period; from 0 to 3 wk, 0.75 g fatty acid/d; from 3 to 6 wk, 1.5 g fatty acid/d.
EPA:SDA:ALA was ≈1:0.3:0.07 with the use of erythrocyte and plasma phospholipid EPA values. Thus, 1 g dietary SDA is approximately equivalent to 300 mg dietary EPA in terms of increasing tissue concentrations of EPA.

Although reports on the cardiovascular benefits of dietary n−3 fats generally cite the intakes of total long-chain n−3 fats, it is useful to examine these in the context of the potential contribution of dietary SDA. The study by the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico (GISSI) showed that daily intake of a fish oil concentrate containing ≈300 mg EPA + 600 mg DHA by survivors of a myocardial infarction was associated with a 30% lower risk of cardiovascular death and, in particular, a 45% lower risk of sudden cardiac death (21). Because it has been shown in a canine model that EPA or DHA was similarly effective in preventing fatal ventricular fibrillation, it can be inferred that, in the GISSI study, the supplemental EPA was one of the protective agents (22). The intake of EPA in the GISSI study would be equivalent to an intake of 1 g SDA/d in terms of increasing tissue concentrations of EPA.

Similar calculations can be made in relation to various recommendations for population intake of n−3 fatty acids. Recommendations by groups in the United Kingdom, Europe, and the United States, respectively, include intakes of 0.5% of dietary energy (23), ≥200 mg EPA+DHA/d (24), and ≥220 mg EPA and 220 mg DHA/d (25). These recommended EPA intakes would be equivalent to ≈300 mg to 1.5 g SDA/d in terms of ability to increase tissue EPA.

If a vegetable oil that contained 10% SDA were available for use in the manufacture of margarine, salad dressing, mayonnaise, and cooking oil, it could be calculated that the use of those products for domestic food preparation would readily result in SDA intakes > 1.5 g/d. Thus, the use of SDA-containing oils could provide SDA intakes that, in terms of ability to increase tissue EPA, are equivalent to dietary EPA intakes associated with cardiovascular benefits in the GISSI study or are in the recommended intake range for populations. There are naturally occurring seeds of the Boraginaceae family with SDA content ≤17% of total fatty acids, although currently these are wildflower seeds and not cultivated oilseeds (26). The overexpression of \( \Delta^{6} \)-desaturase in oilseeds by genetic modification represents an additional source of SDA-enriched oils (27).

Thus, SDA-rich oils have the potential to provide a useful land-based dietary resource for increasing tissue concentrations of long-chain n−3 fatty acids in humans. If used in the food industry, these oils can provide a wide range of dietary alternatives to fish and encapsulated fish oil supplements. As we have argued here, this type of approach, which allows persons to increase tissue concentrations of long-chain n−3 fatty acids without altering their usual dietary preferences, is the key to long-term compliance with health messages to increase daily intakes of n−3 fatty acids (3).

The efficient fatty acid analyses provided under the management of Mark Neumann, Department of Paediatrics and Child Health, Flinders Medical Centre, and the subject recruitment, screening, and dietetic input under the management of Peter Clifton are acknowledged. Thanks go to Julie Lindenmann and Mark Obukowicz for their roles in facilitating the commencement of the project and to Maryanne Demasi, Gillian Caughey, and Robert Metcalf for assistance in planning and managing the logistics of the trial conduct and data collection and analysis.

MJJ and LGC were responsible for the concept of the study, experimental design, collation, and analysis and interpretation of data. VU was responsible for purification and encapsulation of test fatty acids and the coding and randomization protocols and provided input into experimental design and data interpretation. VU is an employee of Monsanto Company. MJJ and LGC have no association with Monsanto Company.

REFERENCES