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# Influence of an algal triacylglycerol containing docosahexaenoic acid (22 : 6*n*-3) and docosapentaenoic acid (22 : 5*n*-6) on cardiovascular risk factors in healthy men and women

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The intake of long-chain *n*-3 PUFA, including DHA (22 : 6*n*-3), is associated with a reduced risk of CVD. *Schizochytrium sp*. are an important primary source of DHA in the marine food chain but they also provide substantial quantities of the *n*-6 PUFA docosapentaenoic acid (22 : 5*n*-6; DPA). The effect of this oil on cardiovascular risk factors was evaluated using a double-blind randomised placebo-controlled parallel-design trial in thirty-nine men and forty women. Subjects received 4 g oil/d for 4 weeks; the active treatment provided 1·5 g DHA and 0·6 g DPA. Active treatment increased plasma concentrations of arachidonic acid, adrenic acid, DPA and DHA by 21, 11, 11 and 88 mg/l respectively and the proportions of DPA and DHA in erythrocyte phospholipids by 78 and 27 % respectively. Serum total, LDL- and HDL-cholesterol increased by 0·33 mmol/l (7·3 %), 0·26 mmol/l (10·4 %) and 0·14 mmol/l (9·0 %) compared with placebo (all $P \leq 0{\cdot}001$). Factor VII (FVII) coagulant activity increased by 12 % following active treatment ($P = 0{\cdot}006$). There were no significant differences between treatments in LDL size, blood pressure, plasma glucose, serum C-reactive protein, plasma FVII antigen, FVII activated, fibrinogen, von Willebrand factor, tocopherol or carotenoid concentrations, plasminogen activator inhibitor-1, creatine kinase or troponin-I activities, haematology or liver function tests or self-reported adverse effects. Overall, the oil was well tolerated and did not adversely affect cardiovascular risk.

**Docosahexaenoic acid: Docosapentaenoic acid: Lipids: Haemostasis: C-reactive protein**

Prospective cohort studies indicate that the intake of EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) derived from the consumption of oily fish is associated with a lower risk of fatal IHD (Albert *et al.* 1998; Hu *et al.* 2002) and stroke (Mozaffarian *et al.* 2005). A meta-analysis of secondary prevention trials of IHD concluded that an intake in the range of 0·7–1 g/d of a mixture of EPA and DHA decreased the incidence of sudden cardiac death (Bucher *et al.* 2002). This beneficial effect appeared to occur rapidly following dietary intervention (Marchioli *et al.* 2002), implying that it was mediated by a process that was amenable to short-term influences such as a decreased susceptibility of an atherosclerotic plaque to rupture or an amelioration of the consequences of plaque rupture such as thrombosis or ventricular fibrillation. It is currently uncertain whether EPA or DHA or both together are responsible for this protective effect. Besides elevated blood pressure and dyslipidaemia, several pro-inflammatory and haemostatic factors are known to predict the risk of cardiovascular events such as elevated C-reactive protein, fibrinogen and von Willebrand factor concentrations, and plasminogen activator type 1 (PAI-1) and factor VII coagulant (FVIIc) activities (Meade *et al.* 1993; Thompson *et al.* 1995; Cooper *et al.* 2000; Ridker *et al.* 2000). However, the effects of DHA in isolation from EPA on these risk factors are uncertain.

DHA can be synthesised from linolenic acid (18 : 3*n*-3) in mammalian tissues but its formation is limited by feedback inhibition and by competition from linoleic acid (18 : 2*n*-6) (Makrides *et al.* 2000; Rosell *et al.* 2005). Preformed DHA appears to be a major determinant of the proportion of DHA in membrane lipids. The richest dietary source of DHA is oily fish, but small amounts are also provided by eggs and meat (British Nutrition Foundation, 1992). The high proportions of EPA and DHA in oily fish result from the accumulation of lipids synthesised by marine algae. Unlike DHA from land animals which is derived by synthesis from linolenic acid, DHA in algae has been shown to be derived *via* a novel metabolic pathway that does not involve linolenic acid as substrate (Metz *et al.* 2001). Although DHA from *Crypthecodinium cohnii* is widely used in the formulation of breast-milk substitutes, there have been few thorough investigations of DHA from algal sources on cardiovascular risk factors. Theobald *et al.* (2004) reported a daily intake of 0·7 g DHA provided as a triacylglycerol derived from *C. cohnii* taken for 3 months resulted in a 7 % increase in LDL-cholesterol. Oil from *Schizochytrium sp*. is also rich in DHA, but unlike that derived from *C. cohnii*, it contains about 15 % docosapentaenoic acid (22 : 5*n*-6; DPA). The aim of the present study was to undertake a comprehensive evaluation of a purified

**Abbreviations:** CRP, C-reactive protein; DHA-S, DHA-rich triacylglycerol derived from *Schizochytrium sp.*, DPA, docosapentaenoic acid; FVIIa, factor VII activated; FVIIag, factor VII antigen; FVIIc, factor VII coagulant; PAI-1, plasminogen activator inhibitor-1; TAG, triacylglycerol.

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triacylglycerol (TAG) derived from *Schizochytrium sp*. on cardiovascular risk factors in healthy men and women.

## Subjects, materials and methods

### *Design*

A randomised double-blind placebo-controlled parallel design was selected because of the long carry-over effect of DHA in membrane lipids. Equal numbers of males and females were allocated to each treatment by stratified randomisation, which was conducted by an independent company (Rho Inc., Chapel Hill, NC, USA). Treatment allocation was withheld from the investigators until the data analysis was complete (except for the *post hoc* analyses). Subjects were asked to consume four gelatin capsules daily; each capsule contained 1 g oil. Both active and placebo capsules were manufactured to contain similar amounts of antioxidants and vitamin E. The active treatment consisted of 4 g refined DHA-rich TAG derived from *Schizochytrium sp*. (DHA-S) and the placebo consisted of 4 g refined olive oil. The fatty acid composition of the supplements is shown in Table 1. Both treatments provided 148 kJ and 4 g fat. The active treatment provided 1·5 g DHA, 0·6 g DPA, 0·04 g MUFA and 1·2 g saturated fatty acids (mainly myristic and palmitic acid). The placebo provided 0·6 g PUFA (mainly linoleic acid), 2·7 g MUFA (mainly oleic acid) and 0·5 g saturated fatty acids (mainly palmitic acid). Responses were assessed by measurements made on two occasions at baseline and on days 28 and 29 of each treatment.

### *Subjects*

Subjects were recruited by a circular email from within the staff population of King's College London, St Thomas', Guy's and King's College Hospitals, all located in London. Exclusion criteria were: current tobacco use; history of myocardial infarction or diabetes mellitus; current pregnancy; current use of lipid-lowering or blood pressure-lowering or immunosuppressive drugs; hormone replacement therapy; use of systemic corticosteroids, androgens, phenytoin or erythromycin, thyroid hormone, drugs for regulating haemostasis but excluding aspirin; self-reported alcohol intake $>21$ units/week for women and $>28$ units/week for men (1 unit $=$ 10 ml ethanol); BMI $<18$ or $>35$ kg/m$^2$; serum cholesterol $>7{\cdot}8$ mmol/l or fasting serum TAG $>3{\cdot}0$ mmol/l; systolic blood pressure $>140$ mmHg or diastolic blood pressure $>90$ mmHg; abnormal haematology or liver function test. Subjects were also excluded if they showed unwillingness to abstain from the consumption of oily fish, fish oil supplements, algal oil supplements, or flaxseed (linseed) oil supplements throughout the course of the study. Subjects received a small financial incentive for their cooperation in the study. The protocol was reviewed and approved by the Research Ethics Committee of King's College London and participants gave informed written consent before commencing the study.

Subjects attended a screening clinic where a small fasting venous blood sample (17 ml) was obtained for measurement of serum lipid profile, blood counts, blood glucose and liver function tests. A urinary pregnancy test was conducted on premenopausal women to confirm non-pregnant status. Habitual dietary intake was assessed before the intervention and during intervention by a 3 d dietary record which was converted to nutrient intake by computer from food tables based on data using software from CompEat (version 4; Nutrition Systems, Grantham, Lincolnshire, UK).

**Table 1.** Fatty acid content (mg/g) of docosahexaenoic acid-rich oil derived from *Schizochytrium sp.* (DHA-S) and placebo

| Fatty acid | DHA-S | Placebo |
|---|---|---|
| 14 : 0 | 75·1 | 0 |
| 16 : 0 | 203·9 | 124·0 |
| 16 : 1 | 4·1 | 13·7 |
| 18 : 0 | 5·1 | 24·7 |
| 18 : 1 | 5·6 | 652·9 |
| 18 : 2*n*-6 | 4·5 | 119·8 |
| 20 : 4*n*-6 | 7·3 | 0 |
| 20 : 4*n*-3 | 9·1 | 0 |
| 20 : 5*n*-3 | 17·5 | 0 |
| 22 : 5*n*-6 | 155·4 | 0 |
| 22 : 6*n*-3 | 380·0 | 0 |

### *Measurements*

Seated blood pressure and body weight in minimum indoor clothing were recorded on two consecutive days at baseline and on days 28 and 29 of treatment. Height (to 0·1 cm) without shoes and weight (to 0·1 kg) in minimum indoor clothing were measured using a stadiometer and beam balance respectively. Seated blood pressure was measured using an automated sphygmomanometer (Omron HEM 705-CP; Omron Healthcare Inc., Bannockburn, IL, USA); three blood pressure measurements were made at intervals of 1 min and the average taken. The subjects recorded in diaries any signs of illness, medication used, menstrual phase and any deviations from the protocol. Subjects were asked to return any unused capsules and a capsule count was made to check compliance. At the end of the study, subjects were requested to complete a questionnaire about their appreciation of the supplements and about any experienced side effects (headache, stomach complaints, nausea, bloated feeling, flatulence, diarrhoea, constipation, itching, eruptions or rashes, fatigue, dizziness, and heavy menstrual period).

The study was conducted double blind except for the measurements of factor VII antigen (FVIIag) and factor VII activated (FVIIa) concentrations and LDL size which were conducted post hoc, but the technicians who made the measurements were blind to the allocation of treatment.

### *Blood sampling*

Venous blood samples were obtained using the vacutainer technique (Becton Dickinson Vacutainer Systems, Plymouth, Devon, UK) following an overnight fast on two occasions at baseline and on days 28 and 29 of treatment. Blood for serum lipids, creatine kinase, troponin-I and liver function tests was collected in a vacutainer containing no anti-coagulant (vacutainer 17 490), serum separated by centrifuging at 1500 ***g*** for 15 min and kept at 4°C until analysed within 3 d. For full blood counts, blood was collected into 2 ml potassium EDTA vacutainer tubes (vacutainer 368047) and kept at room temperature until counted on the same day. Blood for fatty acid and fat-soluble vitamin analyses was collected into an EDTA tube (vacutainer 17 644), chilled to 4°C, centrifuged at 1500 ***g*** for 15 min, and samples of plasma were frozen at $-80$°C until analysed. Packed erythrocytes were washed three times with 5 volumes of ice-cold saline (8·9 NaCl/l) containing 40 mg EDTA/l) and lipid extracts were prepared using isopropanol–chloroform (11:7, v/v)

containing 50 mg butylated hydroxytoluene/l within 2 d of blood collection. The lipid extracts were stored at −20°C until analysed. Blood (4·5 ml) for determination of fibrinogen, von Willebrand factor antigen, PAI-1 activity and FVII assays was collected into a vacutainer containing 0·5 ml 0·105 M-trisodium citrate (vacutainer 367691) and kept at room temperature until completion of centrifugation (1500 ***g*** for 15 min). Plasma was carefully separated avoiding contamination with platelets within 2 h of blood collection and 0·25 ml samples were snap-frozen in liquid $N_2$ and stored at −80°C until analysed.

*Analytical methods*

Total cholesterol, HDL and TAG concentrations were measured using fully enzymic procedures with reagents from Wako (Neuss, Germany) and assays were conducted on a Technicon DAX48 automated chemistry analyser (Bayer Diagnostics, Newbury, Berkshire, UK). LDL-cholesterol concentration was calculated using the Friedewald formula (Friedewald *et al.* 1972). LDL size was determined on pre-stained plasma on an iodixanol gradient using a Beckman NVT 65 near-vertical rotor (Davies *et al.* 2003). Erythrocyte fatty acid composition and plasma fatty acids were determined by capillary GLC (Sanders *et al.* 1997) and plasma retinol, tocopherol and carotenoid concentrations were determined by HPLC (Thurnham *et al.* 1988). Liver function tests, troponin-I, plasma glucose concentrations and serum creatine kinase activity were determined using standard procedures at King's College Hospital (Clinical Pathology Accreditation UK Ltd, reference 0396; Sheffield, UK). Sensitive C-reactive protein (CRP) was determined on a COBAS analyser using reagents supplied by Wako Chemicals (ultrasensitive C-reactive protein method). Full blood counts were conducted at King's College Hospital on a Sysmex counter (Sysmex UK Ltd, Milton Keynes, UK). Plasma fibrinogen and von Willebrand factor antigen concentration, FVIIc activity, FVIIag and FVIIa concentrations and PAI-1 activity were determined by the Medical Research Council coagulation laboratory at the Wolfson Institute of Preventive Medicine as previously described (Sanders *et al.* 2001).

*Statistical analysis*

Sample-size estimates were based on thirty-two subjects to give power to detect a 0·7 SD unit change in mean values with 80 % power for $P<0·05$. In order to increase the statistical power for the lipid assays to 0·5 SD units, analyses were made on blood samples collected on two consecutive days at baseline and at the end of each treatment. Data for serum TAG, CRP and fibrinogen were log-transformed before statistical analysis. Data were analysed by analysis of covariance using the baseline value as a covariate using SPSS/PC version 10 (SPSS Inc., Chicago, IL, USA) with adjustments for age, BMI and sex. Sex × treatment interactions were tested in order to determine whether there were differences in response between sexes. Where appropriate, comparisons with baseline values were made using a paired t test.

## Results

A total of eighty subjects were recruited but one male subject withdrew from the study for personal reasons (their details are shown in Table 2); there were no statistically significant differences between groups at baseline. The supplements were well tolerated and no significant side effects were reported. The dietary intakes of the seventy-nine subjects who completed the study are shown in Table 3 and did not change on treatment except for the contribution made by the supplement. Liver function tests, haematology, plasma glucose, retinol and carotenoid concentrations, creatine kinase and troponin-I activities (markers of muscle damage and cardiac muscle damage respectively) were not affected by treatment (data not shown).

Table 4 shows the plasma concentrations of stearic acid (18 : 0), arachidonic acid (20 : 4*n*-6), EPA (20 : 5*n*-3), adrenic acid (22 : 4*n*-6), DPA*n*-6 (22 : 5*n*-6) and DHA (22 : 6*n*-3) were significantly greater and that of palmitoleic acid (16 : 1) was lower following the DHA treatment compared with the placebo. Table 5 shows the changes in erythrocyte lipids where the proportions of DHA (22 : 6*n*-3), EPA (20 : 5*n*-3) and DPA (22 : 5*n*-6) increased and those of linoleic acid (18 : 2*n*-6), dihomo-γ-linolenic acid (20 : 3*n*-6) and DPA*n*-3 (22 : 5*n*-3) fell. Plasma concentrations of α-tocopherol adjusted for plasma

**Table 2.** Details of the study subjects at screening*

(Mean values and standard deviations)

| | Women | | | | Men | | | |
|---|---|---|---|---|---|---|---|---|
| | DHA-S (*n* 20) | | Placebo (*n* 20) | | DHA-S (*n* 20) | | Placebo (*n* 19) | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Age (years) | 31·6 | 13·2 | 35·2 | 14·5 | 29·8 | 11·5 | 33·4 | 14·7 |
| Height (cm) | 164·9 | 5·6 | 166·1 | 6·9 | 178·7 | 6·2 | 180·1 | 8·6 |
| Weight (kg) | 63·8 | 9·4 | 63·8 | 10·2 | 78·1 | 10·1 | 79·0 | 14·8 |
| BMI (kg/m$^2$) | 23·4 | 2·8 | 23·0 | 2·4 | 24·4 | 2·7 | 24·3 | 3·5 |
| Alcohol intake (10 ml units/week) | 7·9 | 5·7 | 5·6 | 5·9 | 10·7 | 9·5 | 10·3 | 9·5 |
| Fasting plasma glucose (mmol/l) | 4·46 | 0·40 | 4·57 | 0·53 | 4·61 | 0·54 | 4·74 | 0·37 |
| Serum cholesterol (mmol/l) | 4·59 | 0·97 | 4·73 | 0·86 | 4·42 | 0·8 | 3·99 | 0·80 |
| Serum HDL-cholesterol (mmol/l) | 1·75 | 0·47 | 1·69 | 0·46 | 1·3 | 0·3 | 1·4 | 0·2 |
| Serum triacylglycerols (mmol/l) | 1·07 | 0·57 | 1·04 | 0·61 | 1·4 | 0·9 | 1·2 | 0·6 |
| Systolic blood pressure (mmHg) | 116·6 | 12·5 | 117·0 | 13·1 | 126·7 | 11·8 | 125·4 | 11·1 |
| Diastolic blood pressure (mmHg) | 67·8 | 9·4 | 72·6 | 9·2 | 75·0 | 7·7 | 75·3 | 9·7 |

* There were no statistically significant differences between treatment groups (two-sample *t* test).

**Table 3.** Nutrient intakes and body weights in subjects before and during treatment with docosahexaenoic acid-rich oil derived from *Schizochytrium sp.* (DHA-S) or placebo*

(Mean values and standard deviations)

| | DHA-S (*n* 39) | | | | Placebo (*n* 39) | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | | End of study | | Baseline | | End of study | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Energy (MJ/d) | 7·4 | 2·5 | 7·8 | 2·5 | 8·0 | 2·5 | 7·5 | 2·00 |
| Protein (% energy) | 14·4 | 2·5 | 15·3 | 4·6 | 14·9 | 2·8 | 14·4 | 2·4 |
| Fat (% energy) | 32·2 | 3·4 | 34·4 | 8·2 | 35·0 | 7·6 | 34·8 | 6·7 |
| Saturated fatty acids (% energy) | 11·2 | 3·4 | 11·3 | 4·5 | 12·6 | 4 | 12·4 | 4·4 |
| PUFA (% energy) | 4·7 | 2·1 | 5·4 | 2·1 | 4·9 | 2·2 | 5·3 | 2·2 |
| Cholesterol (mg/d) | 210 | 90 | 195 | 109 | 227 | 139 | 217 | 127 |
| Carbohydrate (% energy) | 49·2 | 7·6 | 46·1 | 8·4 | 44·9 | 8·5 | 46·1 | 7·6 |
| Sugar (% energy) | 19·8 | 7·5 | 18·7 | 8·2 | 17·8 | 7·7 | 19·3 | 7·5 |
| Starch (% energy) | 25·0 | 6·2 | 23·3 | 7·7 | 23·2 | 6·2 | 22·8 | 6·3 |
| NSP (g/d) | 16·1 | 6·7 | 13·1 | 5·7 | 15·7 | 6·3 | 11·6 | 4·1 |
| Body weight (kg) | 70·9 | 12·0 | 71·6 | 2·1 | 71·2 | 14·6 | 71·6 | 14·7 |

* There were no statistically significant differences between treatments and no statistically significant sex × treatment interactions (analysis of covariance).

cholesterol concentrations were not affected by the treatment: 5·8 (SD 1·1; *n* 38) *v.* 5·8 (SD 1·2) μmol/mmol cholesterol before and after DHA treatment compared with 6·0 (SD 1·1) *v.* 5·9 (SD 0·9) μmol/mmol cholesterol on placebo. Table 6 shows the results for serum lipids; there were no sex × treatment interactions so the data for both sexes have been combined. Total serum cholesterol, LDL- and HDL-cholesterol concentrations increased following the DHA-S supplement (all $P \leq 0{\cdot}001$). Serum TAG concentrations fell slightly following DHA-S compared with baseline (paired *t* test; $P = 0{\cdot}002$), but not on placebo; the difference between treatments did not quite achieve statistical significance. Out of the seventy-seven on whom LDL size was determined, only ten subjects (three in the placebo group and seven in the DHA-treated group) showed a predominance of heavy LDL. There were no significant changes in LDL size between treatments. Table 7 shows the results for blood pressure, CRP and haemostatic variables. There was a trend for systolic blood pressure to fall on the DHA-S treatment (paired t test compared with baseline; $P = 0{\cdot}0004$), but this did not achieve statistical significance ($P = 0{\cdot}103$) when compared with the placebo. Compared with the baseline, FVIIc increased following the DHA-S treatment compared with the placebo ($P = 0{\cdot}006$). At baseline, FVIIc was correlated with serum HDL- ($r$ 0·366; $P = 0{\cdot}001$) and total cholesterol concentrations ($r$ 0·289; $P = 0{\cdot}01$). However, post hoc analyses of FVIIag or FVIIa concentrations failed to reveal any differences between treatments.

## Discussion

The purpose of the present study was to evaluate the effect of an intake of 1·5 g DHA/d provided as DHA-S on cardiovascular risk factors in healthy men and women. DHA-S was well tolerated by the subjects and did not result in any self-reported adverse effects or any pathological changes in haematology or other biochemical

**Table 4.** Plasma fatty acid concentrations (mg/l) in subjects before and after treatment with docosahexaenoic acid-rich oil derived from *Schizochytrium sp.* (DHA-S) or placebo

(Mean values, standard deviations and 95 % confidence intervals)

| | DHA-S (*n* 40) | | | | Placebo (*n* 39) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | | End of study | | Baseline | | End of study | | | Treatment effect* | |
| Fatty acids | Mean | SD | Mean | SD | Mean | SD | Mean | SD | *P* value | Mean | 95 % CI |
| 16 : 0 | 689 | 236 | 698 | 231 | 576 | 248 | 571 | 261 | NS | 53 | −36, 142 |
| 16 : 1 | 21 | 25 | 18 | 21 | 22 | 20 | 25 | 24 | NS | −6 | −14, 1 |
| 18 : 0 | 217 | 72 | 235 | 74 | 198 | 70 | 198 | 65 | <0·05 | 27 | 1, 52 |
| 18 : 1*n*-9 | 656 | 322 | 541 | 338 | 485 | 322 | 566 | 339 | NS | −122 | −257, 13 |
| 18 : 2*n*-6 | 938 | 282 | 951 | 289 | 839 | 253 | 828 | 222 | NS | 64 | −130, 57 |
| 18 : 3*n*-3 | 23 | 11 | 24 | 14 | 20 | 10 | 22 | 14 | NS | −1 | −6, 5 |
| 20 : 3*n*-6 | 50 | 23 | 44 | 25 | 44 | 16 | 47 | 25 | NS | −7 | −17, 3 |
| 20 : 4*n*-6 | 202 | 60 | 223 | 66 | 181 | 62 | 177 | 60 | <0·01 | 31 | 10, 53 |
| 20 : 5*n*-3 | 31 | 25 | 44 | 24 | 37 | 33 | 30 | 20 | <0·01 | 15 | 6, 24 |
| 22 : 4*n*-6 | 6 | 8 | 17 | 15 | 4 | 5 | 4 | 4 | <0·0001 | 13 | 8, 18 |
| 22 : 5*n*-6 | 13 | 13 | 25 | 17 | 9 | 13 | 11 | 12 | <0·001 | 11 | 4, 16 |
| 22 : 5*n*-3 | 35 | 17 | 42 | 24 | 30 | 18 | 33 | 17 | NS | 7 | −2, 15 |
| 22 : 6*n*-3 | 77 | 36 | 165 | 72 | 76 | 48 | 72 | 39 | <0·0001 | 92 | 69, 115 |

* Treatment effect was analysed by analysis of covariance using the baseline value as a covariate; there were no statistically significant sex × treatment interactions.

**Table 5.** Erythrocyte phospholipid fatty acid composition (weight %) in subjects before and after treatment with docosahexaenoic acid-rich oil derived from *Schizochytrium sp.* (DHA-S) or placebo

(Mean values, standard deviations and 95 % confidence intervals)

| | DHA-S (*n* 40) | | | | Placebo (*n* 39) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | | End of study | | Baseline | | End of study | | | Treatment effect* | |
| Fatty acids | Mean | SD | Mean | SD | Mean | SD | Mean | SD | *P* value | Mean | 95 % CI |
| 16 : 0 | 19·9 | 1·5 | 20·2 | 1·1 | 19·7 | 0·9 | 19·6 | 0·9 | < 0·05 | 0·5 | 0·1, 1·0 |
| 16 : 1 | 0·3 | 0·1 | 0·2 | 0·1 | 0·3 | 0·1 | 0·2 | 0·1 | NS | 0 | −0·1, 0 |
| 18 : 0 | 16·4 | 0·9 | 16·4 | 1·0 | 16·6 | 0·9 | 16·3 | 2·9 | NS | −0·1 | −0·4, 0·2 |
| 18 : 1*n*-9 | 13·9 | 2·5 | 14·1 | 1·4 | 14·3 | 1·2 | 13·9 | 2·5 | NS | −0·2 | −0·6, 0·2 |
| 18 : 2n-6 | 11·3 | 1·0 | 10·6 | 1·1 | 11·3 | 1·1 | 11·1 | 2·1 | < 0·001 | −0·7 | −1·1, −0·4 |
| 20 : 3*n*-6 | 1·8 | 0·4 | 1·7 | 0·4 | 1·8 | 0·3 | 1·8 | 0·4 | < 0·01 | −0·2 | −0·3, −0·1 |
| 20 : 4*n*-6 | 16·7 | 1·4 | 15·8 | 1·6 | 16·0 | 1·7 | 15·4 | 3·0 | NS | −0·2 | −0·7, 0·2 |
| 20 : 5*n*-3 | 1·1 | 0·4 | 1·3 | 0·4 | 1·3 | 0·7 | 1·2 | 0·6 | < 0·05 | 0·2 | 0, 0·3 |
| 22 : 4n-6 | 3·1 | 0·9 | 2·9 | 0·9 | 2·9 | 0·9 | 2·7 | 1·2 | NS | −0·2 | −0·4, 0 |
| 22 : 5*n*-6 | 0·5 | 0·2 | 0·8 | 0·3 | 0·4 | 0·2 | 0·4 | 0·2 | < 0·0001 | 0·4 | 0·3, 0·5 |
| 22 : 5*n*-3 | 2·8 | 0·8 | 2·5 | 0·8 | 2·9 | 1·0 | 2·8 | 1·1 | < 0·0001 | −0·4 | −0·5, −0·3 |
| 22 : 6*n*-3 | 6·1 | 1·1 | 7·6 | 1·5 | 6·5 | 1·7 | 6·1 | 2·1 | < 0·0001 | 1·7 | 1·1, 2·2 |

* Treatment effect was analysed by analysis of covariance using the baseline value as a covariate; there were no statistically significant sex × treatment interactions.

indices. Although the present study was not specifically designed to compare DHA-S with DHA-derived *C. cohnii*, we did note a different effect on plasma and erythrocyte lipids compared with our previous studies on *C. cohnii* and fish oil (Sanders *et al.* 1981, 1997; Sanders & Hinds, 1992). With TAG derived from *C. cohnii*, a dose–response decline in arachidonic acid of 11 % with 0·7 g DHA/d and 18 % with 2·6 g of DHA/d was observed (T. A. B. Sanders and H. E. Theobald, unpublished results). In the present study, supplementation with DHA-S did not result in a decline in the proportion of arachidonic acid (20 : 4*n*-6) in plasma or erythrocyte lipids; indeed there was a significant increase in the plasma concentration. Furthermore, DHA-S resulted in a significant increase in DPA*n*-6 (22 : 5*n*-6) in both plasma and erythrocyte lipids, a phenomenon hitherto not previously observed in our laboratory, which has experience of over twenty studies in this area. One likely explanation is that DPAn-6 (22 : 5*n*-6) is retroconverted to arachidonic acid in a similar manner in which DHA is retroconverted to EPA (Schlenk *et al.* 1969).

DHA has been shown to lower plasma TAG in subjects with hypertriacylglycerolaemia using intakes considerably greater than in the present study (Davidson *et al.* 1997; Mori *et al.* 2000). The DHA-S supplement increased LDL-cholesterol concentrations by 11 %, which is consistent with a previous report in moderately hypercholesterolaemic subjects where an intake of 0·7 g DHA derived from *C. cohnii* resulted in a 7 % increase in LDL-cholesterol (Theobald *et al.* 2004). Some studies with fish oil containing DHA have reported increases in LDL-cholesterol or LDL apo B concentrations, particularly in hypertriacylglycerolaemic subjects (Sullivan *et al.* 1986; Harris, 1997). It has been suggested that LDL particles may be lighter following an increased intake of long-chain *n*-3 fatty acids, particularly in subjects with elevated plasma TAG concentrations and a predominance of dense LDL (Minihane *et al.* 2000). In order to address this question, measurements of LDL size were undertaken post hoc but we were unable to demonstrate any evidence of a change in the proportion of dense LDL or in the peak

**Table 6.** Serum lipid concentrations in subjects before and after treatment with docosahexaenoic acid-rich oil derived from *Schizochytrium sp.* (DHA-S) or placebo

(Mean values, standard deviations and 95 % confidence intervals)

| | DHA-S (*n* 40) | | | | Placebo (*n* 39) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | | End of study | | Baseline | | End of study | | | Treatment effect* | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | *P* value | Mean | 95 % CI |
| Serum cholesterol (mmol/l) | 4·58 | 0·92 | 4·77 | 0·91 | 4·45 | 1·03 | 4·33 | 0·91 | 0·0005 | 0·35 | 0·19, 0·50 |
| Serum LDL-cholesterol (mmol/l) | 2·52 | 0·91 | 2·69 | 0·96 | 2·47 | 0·79 | 2·39 | 0·72 | 0·0002 | 0·27 | 0·13, 0·40 |
| Serum HDL-cholesterol (mmol/l) | 1·52 | 0·40 | 1·62 | 0·40 | 1·53 | 0·39 | 1·50 | 0·34 | 0·001 | 0·14 | 0·05, 0·21 |
| LDL:HDL ratio | 1·81 | 0·89 | 1·83 | 0·92 | 1·68 | 0·59 | 1·67 | 0·59 | 0·42 | 0·05 | −0·08, 0·18 |
| LDL-I + II (%) | 72·1 | 14·7 | 72·1 | 14·1 | 78·3 | 10·8 | 77·6 | 12·6 | 0·846 | −0·3 | −3·6, 2·9 |
| LDL-III (%) | 27·9 | 14·7 | 27·9 | 13·1 | 21·6 | 10·8 | 22·4 | 12·6 | 0·846 | 0·3 | −2·9, 3·6 |
| Serum triacylglycerols (mmol/l)† | 1·08 | 0·50 | 0·93 | 0·41 | 0·92 | 0·43 | 0·90 | 0·44 | 0·152 | −0·09 | −0·21, 0·03 |

* Treatment effect was analysed by analysis of covariance using the baseline value as a covariate with adjustments for age, sex and BMI; there were no statistically significant sex × treatment interactions.

† Geometric mean.

**Table 7.** Haemostatic and inflammatory cardiovascular risk factors in subjects before and after treatment with docosahexaenoic acid-rich oil derived from *Schizochytrium sp.* (DHA-S) or placebo (Mean values, standard deviations and 95 % confidence intervals)

| | DHA-S (*n* 40) | | | | Placebo (*n* 39) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | | End of study | | Baseline | | End of study | | | Treatment effect* |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | *P* value | Mean | 95 % CI |
| Systolic blood pressure (mmHg) | 121·3 | 12·2 | 116·0 | 11·2 | 120·4 | 11·4 | 118·6 | 10·7 | 0·103 | −3·1 | −6·7, 0·6 |
| Diastolic blood pressure (mmHg) | 72·1 | 7·8 | 71·7 | 7·0 | 73·9 | 8·5 | 72·3 | 6·9 | 0·595 | 0·6 | −1·7, 2·9 |
| Pulse pressure (mmHg) | 49·1 | 7·8 | 44·3 | 7·5 | 46·5 | 6·9 | 46·5 | 7·6 | 0·03 | 3·0 | −5·9, −0·1 |
| C-reactive protein (mg/l) | 2·4 | 4·4 | 2·5 | 2·8 | 2·0 | 3·9 | 2·1 | 3·2 | 0·649 | 0·2 | −1·1, 2·5 |
| Plasma fibrinogen (mg/l) | 2·82 | 0·62 | 2·87 | 0·52 | 2·66 | 0·56 | 2·68 | 0·70 | 0·557 | 0·07 | −0·16, 31 |
| Factor VII coagulant (%) | 104 | 36 | 114 | 35 | 108 | 30 | 106 | 29 | 0·006 | 12 | 4, 20 |
| Factor VII antigen (%) | 131 | 41 | 134 | 42 | 121 | 42 | 122 | 46 | 0·81 | 1 | −7, 9 |
| Factor VII activated (units/l) | 42 | 31 | 42 | 24 | 39 | 27 | 37 | 21 | 0·236 | 4 | −3, 12 |
| Plasminogen activator inhibitor-1 activity (units × $10^3$/l) | 9·9 | 6·9 | 12·0 | 7·8 | 10·3 | 9·2 | 9·9 | 7·8 | 0·165 | 2·2 | −9·0, 5·4 |
| von Willebrand factor antigen (%) | 68 | 32 | 70 | 31 | 72 | 26 | 78 | 26 | 0·175 | −6 | −15, 3 |

*Treatment effect was analysed by analysis of covariance using the baseline value as a covariate with adjustments for age, sex and BMI; there were no statistically significant sex × treatment interactions.

density of LDL. The increase in LDL-cholesterol observed with algal oils rich in DHA appears not to occur in studies using fish oil at equivalent dosages in normolipidaemic subjects (Sanders *et al.* 1997; Finnegan *et al.* 2003). This raises the intriguing question of why DHA-S and *C. cohnii* possess this LDL-raising effect. The amount of saturated fatty acids (1·2 g/d) and cholesterol (32 mg/d) provided by the *Schizochytrium sp.* supplement was low (less than the amounts present in fish oil) and so cannot explain this LDL-cholesterol-raising effect. Further research into the mechanisms involved is warranted.

The results of studies with fish oil have been variable with regard to the effects of long-chain *n*-3 fatty acids on HDL-cholesterol. Harris (1997) concluded that there is a trend for a 3 % increase in HDL-cholesterol concentration in normolipidaemic subjects, which is in agreement with a controlled metabolic feeding study (Sanders *et al.* 1997) where a significant increase in $HDL_2$ cholesterol concentration but a non-significant 3 % increase in total HDL-cholesterol was demonstrated with diets providing 1·6 g DHA/d and 3·4 g EPA/d. The 9 % increase in HDL-cholesterol observed in the present study is much greater than changes previously observed in studies with fish oils. One possible reason for this increase in HDL-cholesterol could be DPA*n*-6 in the *Schizochytrium sp.* supplement. The observed effects on lipid metabolism might be a consequence of long-chain PUFA, especially DPA*n*-6, acting as a ligand for the liver X receptor (Yoshikawa *et al.* 2002) which activates the ABC-A1 transporter (Tontonoz & Mangelsdorf, 2003) which stimulates reverse cholesterol transport. Although the supplement increased LDL, which is associated with an increased risk of CVD, it also increased HDL-cholesterol, which is associated with a decreased risk. As neither the LDL:HDL ratio nor LDL size changed this would imply that the lipoprotein changes induced by the *Schizochytrium sp.* supplement have a net neutral effect on cardiovascular risk.

The present study was unable to detect any effect of DHA-S on CRP or fibrinogen concentrations or PAI-1 activity. A striking observation was the 12 % increase in FVIIc on the DHA-S. The increase in FVIIc may be related to the increase in HDL- and LDL-cholesterol concentrations, as FVIIc was correlated with both HDL- and LDL-cholesterol concentrations. In order to ascertain whether the increase in FVIIc was attributable to an increase in zymogen or in FVIIa, we conducted analyses *post hoc* of FVIIag and FVIIa, but these assays were unable to demonstrate any significant changes in either measure.

A systematic review found a significant blood pressure-lowering effect on long-chain *n*-3 fatty acids (Geleijnse *et al.* 2002), particularly in subjects with hypertension. In the present study which was conducted in normotensive subjects there was a trend for systolic blood pressure to be 3 mmHg lower in the subjects treated with the *Schizochytrium sp.* Supplement, but the study lacked sufficient statistically power to detect such a change. Consequently, a much larger study would be needed to test whether intakes of DHA as used in the present study have a blood pressure-lowering effect.

In conclusion, the present study demonstrates that the consumption of oil from *Schizochytrium sp.* does not result in a reduction in the proportions of arachidonic acid in blood lipids. It raises LDL-cholesterol significantly but also HDL-cholesterol concentrations without altering the LDL:HDL ratio or LDL size. Overall, the oil was well tolerated and did not adversely affect cardiovascular risk.

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