European Journal of Clinical Nutrition (2011) 65, 247–254

0954-3007/11
www.nature.com/ejcn

ORIGINAL ARTICLE

Enhanced increase of omega-3 index in response to long-term n-3 fatty acid supplementation from triacylglycerides versus ethyl esters

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Background: There is a debate currently about whether different chemical forms of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are absorbed in an identical way. The objective of this study was to investigate the response of the omega-3 index, the percentage of EPA + DHA in red blood cell membranes, to supplementation with two different omega-3 fatty acid (*n*-3 FA) formulations in humans.
Design: The study was conducted as a double-blinded placebo-controlled trial. A total of 150 volunteers was randomly assigned to one of the three groups: (1) fish oil concentrate with EPA + DHA (1.01 g + 0.67 g) given as reesterified triacylglycerides (rTAG group); (2) corn oil (placebo group) or (3) fish oil concentrate with EPA + DHA (1.01 g + 0.67 g) given as ethyl ester (EE group). Volunteers consumed four gelatine-coated soft capsules daily over a period of six months. The omega-3 index was determined at baseline (t_0) after three months (t_3) and at the end of the intervention period (t_6).
Results: The omega-3 index increased significantly in both groups treated with *n*-3 FAs from baseline to t_3 and t_6 ($P<0.001$). The omega-3 index increased to a greater extent in the rTAG group than in the EE group (t_3: 186 versus 161% ($P<0.001$); t_6: 197 versus 171% ($P<0.01$)).
Conclusion: A six-month supplementation of identical doses of EPA + DHA led to a faster and higher increase in the omega-3 index when consumed as triacylglycerides than when consumed as ethyl esters.
European Journal of Clinical Nutrition (2011) **65,** 247–254; doi:10.1038/ejcn.2010.239; published online 10 November 2010

Keywords: *n*-3 fatty acids; triacylglycerides; ethyl esters; omega-3 index; eicosapentaenoic acid; docosahexaenoic acid

Introduction

Cardiac societies recommend an increased intake of the long-chain omega-3 fatty acids (*n*-3 FAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), especially for patients with known cardiovascular disease or for individuals at high risk of cardiovascular disease (Smith *et al*., 2006; Graham *et al*., 2007; Fruchart *et al*., 2008). Although most of the large intervention studies on which these recommendations are based were conducted with EPA + DHA ethyl esters (EEs), nutrition and cardiac societies recommend increasing the intake of *n*-3 FAs by consuming at least two servings of (preferably oily) fish per week, providing 400–500 mg per day of EPA + DHA, mainly esterified as triacylglycerides (TAGs), but also in membrane phospholipids (PLs). For the portion of the population reluctant to eat fish, the societies recommend concentrated fish oil preparations, which typically contain EPA and DHA as TAGs or EEs. These recommendations presume that different chemical forms of EPA + DHA have an identical efficacy.

The majority of previous studies which compared the bioavailability of EPA + DHA from TAGs or reesterified triacylglycerides (rTAGs) versus EEs (El Boustani *et al*., 1987; Lawson and Hughes, 1988a, b; Beckermann *et al*., 1990; Luley *et al*., 1990; Nordøy *et al*., 1991; Krokan *et al*., 1993; Dyerberg *et al*., 2010) were short term with a maximal

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The study supplements used in this study were a gift from Dr Loges + Co. GmbH, Winsen, Germany.
Received 15 March 2010; revised 21 September 2010; accepted 22 September 2010; published online 10 November 2010

length of seven weeks. The focussed end points in these trials—FA composition of plasma PLs, TAGs, chylomicron TAGs or serum PLs—similarly reflect short-term rather than long-term intake (Katan *et al*., 1997; Cao *et al*., 2006). Moreover, larger doses (>2 g per day EPA and DHA) were used in many of these studies, which is in contrast to the recommendations of the societies mentioned.

We compared the bioavailability of a moderate dose of *n*-3 FA-TAGs versus *n*-3 FA-EEs in comparison with a placebo as part of a six-month randomized controlled study, where we likewise assessed the effects of the two *n*-3 FA formulations on the lipid profile in hyperlipidemic subjects. The omega-3 index, the percentage of EPA + DHA in red blood cell (RBC) membranes, reflects the long-term intake and *n*-3 FA status of an individual (Harris and von Schacky, 2004; Harris, 2007, 2008; Sun *et al*., 2007).

Subjects and methods

Subjects

A total of 150 subjects between 30 and 75 years old were recruited via newspaper advertisements in four different German cities (Munich, Hamburg, Hannover and Goslar) between April and October, 2008. As the study was conducted to assess the effects of the two fish oil formulations on the lipid profile in hyperlipidemic subjects, the inclusion criterion was hyperlipidemia solely treated with HMG-CoA-reductase inhibitors (statins). The exclusion criteria were serious illness (type 1 diabetes, cancer, coronary heart disease, bleeding disorders), body mass index $>35\,kg\,m^{-2}$, gastrointestinal disorders, medications known to affect lipid metabolism, daily fish consumption or ingestion of dietary *n*-3 FAs, and plant sterol supplements. Written informed consent was obtained from all participants. The trial was conducted with respect to Good Clinical Practice (GCP) guidelines and the protocol was approved by an ethics commission.

Study design

Qualified participants were randomly assigned to one of the three groups in a double-masked manner. Randomization was conducted using a computer-generated randomization scheme. Codes were kept in a remote secure location by an independent third party. All participants, as well as medical, laboratory and clinical trial staff and investigators assessing the end points, were blinded to the randomization until all study data had been collected, checked and verified. Each of the study products was given two codes to further facilitate the masking of the capsules. The capsules were provided in numbered containers.

The three different types of gelatine-coated soft capsules contained (1) *n*-3 FAs as rTAGs (rTAG group; $n=52$); (2) corn oil (placebo group; $n=49$) or (3) *n*-3 FA-EEs (EE group; $n=49$), and were provided by Dr Loges and Co. GmbH (Winsen, Germany), a pharmaceutical company. rTAGs are made from fish body oil to concentrate specific FAs in the resulting products. Therefore, FAs from the natural TAGs are transferred to ethanol, forming EEs. After removing undesirable FAs, EEs are enzymatically reconverted into TAGs, now called rTAGs. The FA composition of the two different supplements was identical (Table 1). The daily intake of EPA and DHA in both *n*-3 FA groups was 1008 and 672 mg, respectively. The placebo capsules contained corn oil and were outwardly identical to the *n*-3 FA capsules in all aspects.

Table 1 Composition of the two supplements per capsule and overall daily consumption (four capsules)

	n-3 FAs as TAGs or EEs	
Nutrient	*Per capsule*	*Per day (four capsules)*
Total *n*-3	504 mg	2016 mg
20:5*n*-3 (EPA)	252 mg	1008 mg
22:6*n*-3 (DHA)	168 mg	672 mg
α-Tocopherol	6 mg	24 mg

Abbreviations: DHA, docosahexaenoic acid; EEs, ethyl esters; EPA, eicosapentaenoic acid; n-3 FAs, omega-3 fatty acids; TAGs, triacylglycerides.

Participants were instructed to ingest four capsules of their assigned study supplement daily together with food, two in the morning and two in the evening, and to maintain their usual exercise and dietary habits throughout the intervention time of six months.

The subjects' height and weight were measured, and fasting blood samples were collected by venipuncture into K-EDTA tubes at baseline (t_0) after three months (t_3) and at the end of the intervention period (t_6). Subjects additionally completed a questionnaire to obtain information about lifestyle habits (for example, usual fish intake, physically activity) as well as tolerability of the two different *n*-3 FA formulations.

Volunteers' compliance was assessed by count of leftover capsules between the three investigation dates.

RBC membrane fatty acid analysis

RBC membrane FA composition was analyzed according to the omega-3 index methodology as described previously (Harris and von Schacky, 2004). FA methyl esters were generated from RBCs by acid transesterification and analyzed by gas chromatography using a GC2010 Gas Chromatograph (Shimadzu, Duisburg, Germany) equipped with a SP2560, 100-m column (Supelco, Bellefonte, PA, USA), using hydrogen as the carrier gas. FAs were identified by comparison with a FA standard mixture characteristic for RBCs. Results for the omega-3 index are given as EPA + DHA expressed as a percentage of the total identified FAs after response factor correction. The coefficient of variation for EPA + DHA was 5%. Quality was assured according to DIN ISO 15189.

Statistics

SPSS 17 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The results are presented as mean ± s.d. The sample size ($n = 50$ per group) was calculated for differences in serum TAG levels (primary end point) in order to be sufficient to prove a slightly more than medium-sized superiority (standardized difference of means $(\mu_1 - \mu_2)/\sigma = 0.566$) of verum over placebo with a 5% significance level and a power of 80%. If the test for difference of all the three groups resulted in significance, the three pair-wise tests could be done without adjustment of the significance level following the closed test procedure. The increase of the omega-3 index was a secondary end point; therefore, $P < 0.05$ was considered significant. Statistical comparisons were based on per protocol population, defined as subjects completing all visits and not developing any of the exclusion criteria during the intervention period. An intention to treat analysis would have included non-compliant subjects from both the groups, and would have obscured rather than clarified the effects of *n*-3 FA treatment. Testing for normality (KS test) revealed normal distribution for arachidonic acid (AA), EPA, DHA and omega-3 index at baseline in each intervention group. Baseline markers were compared among groups using one-way analysis of variance. Scheffe tests of contrast were performed where appropriate. Changes in values of the different variables observed at baseline and t_3 or t_6 were evaluated within groups by Student's *t*-test for dependent samples (Table 2). To compare differences of RBC membrane FAs among groups, corresponding baseline values were used as covariates to diminish possible effects caused by diverse t_0 values.

Results

Of the 150 eligible subjects who started the study, 21 were excluded from subsequent statistical analyses. The number of subjects lost during the study and the number available for analysis are summarized in Figure 1. Five subjects failed one or more of the inclusion criteria at baseline visit. Four more subjects decided to discontinue participation before the t_3 visit and three before the t_6 visit. Three subjects failed to attend the t_3 visit because of illness, which was not related to the study product, and two additional subjects had relevant changes in lipid metabolism affecting medication (medical treatment of thyroid dysfunction). Four more participants were excluded from subsequent statistical analysis because of the lack of compliance. Hence, 129 participants completed the study protocol and were included in the analysis.

Subject characteristics

The three groups were normally distributed with respect to age, gender, body weight and body mass index, and did not show any differences between the groups (Table 3).

Table 2 RBC membrane FA composition (percentage of total fatty acids) at baseline (t_0), three (t_3) and six months (t_6)[a]

	rTAG group (n = *41*)	*Placebo group* (n = *43*)	*EE group* (n = *45*)
C14:0			
t_0	0.53 ± 0.58	0.45 ± 0.16	0.44 ± 0.22
t_3	0.43 ± 0.16	0.41 ± 0.13	0.42 ± 0.15
t_6	0.35 ± 0.15	0.42 ± 0.21	0.31 ± 0.11[d]
C16:0			
t_0	21.44 ± 2.54	21.56 ± 1.75	21.36 ± 1.72
t_3	21.69 ± 1.33	21.33 ± 1.51	21.44 ± 1.76
t_6	20.72 ± 1.33[e]	9.99 ± 1.45[d]	20.34 ± 1.37[d]
C16:1n-7t			
t_0	0.90 ± 2.28	0.57 ± 1.61	0.55 ± 1.62
t_3	0.21 ± 0.13	0.22 ± 0.16	0.21 ± 0.09
t_6	0.15 ± 0.14[b]	0.15 ± 0.12	0.12 ± 0.09
C16:1n-7			
t_0	0.65 ± 0.32	0.75 ± 0.33	0.58 ± 0.28
t_3	0.60 ± 0.31	0.72 ± 0.27	0.56 ± 0.31
t_6	0.45 ± 0.22[d,e]	0.60 ± 0.28[d]	0.42 ± 0.19[d,e]
C18:0			
t_0	15.76 ± 1.80	15.01 ± 1.85	15.49 ± 1.78
t_3	14.99 ± 2.26[b]	15.06 ± 1.69	14.97 ± 2.10
t_6	14.72 ± 1.81[c]	14.08 ± 2.05[b]	14.61 ± 1.42[c]
C18:1t			
t_0	0.54 ± 0.30	0.51 ± 0.25	0.49 ± 0.12
t_3	0.49 ± 0.16[e]	0.57 ± 0.25	0.55 ± 0.28
t_6	0.35 ± 0.16[d]	0.41 ± 0.36	0.32 ± 0.17[d]
C18:1n-9			
t_0	15.53 ± 1.38	15.62 ± 1.35	15.61 ± 1.14
t_3	14.62 ± 1.23[d,e]	15.40 ± 1.57	14.73 ± 1.15[d,e]
t_6	14.96 ± 1.16[b,e]	15.56 ± 1.49	15.39 ± 1.17
C18:2n-6tt			
t_0	0.26 ± 0.22	0.23 ± 0.13	0.24 ± 0.12
t_3	0.22 ± 0.09	0.19 ± 0.07	0.24 ± 0.09[e]
t_6	0.19 ± 0.08	0.18 ± 0.09	0.20 ± 0.11
C18:2n-6ct			
t_0	0.17 ± 0.32	0.19 ± 0.35	0.15 ± 0.21
t_3	0.11 ± 0.13	0.07 ± 0.07[b]	0.07 ± 0.08[b]
t_6	0.04 ± 0.03[b]	0.05 ± 0.05[c]	0.04 ± 0.09[c]
C18:2n-6tc			
t_0	0.26 ± 0.34	0.22 ± 0.32	0.21 ± 0.28
t_3	0.19 ± 0.11	0.19 ± 0.09	0.24 ± 0.28
t_6	0.14 ± 0.07[b]	0.18 ± 0.32	0.11 ± 0.04[c]
LA			
C18:2*n*−6			
t_0	11.69 ± 1.62	12.23 ± 2.20	12.41 ± 1.98
t_3	10.95 ± 1.65[b,e]	12.74 ± 1.75	11.58 ± 1.83[c,e]
t_6	10.90 ± 1.66[c,e]	12.96 ± 2.36[b]	11.86 ± 1.98[b,e]
C18:3*n*−6			
t_0	0.20 ± 0.11	0.20 ± 0.10	0.18 ± 0.07
t_3	0.12 ± 0.05[d,e]	0.16 ± 0.06[b]	0.12 ± 0.09[d,e]
t_6	0.09 ± 0.04[d]	0.13 ± 0.06[d]	0.09 ± 0.04[d]
C20:1*n*−9			
t_0	0.24 ± 0.08	0.24 ± 0.08	0.24 ± 0.06
t_3	0.22 ± 0.06	0.22 ± 0.04	0.24 ± 0.09
t_6	0.19 ± 0.05[b]	0.25 ± 0.15	0.21 ± 0.06[c]
ALA			
C18:3*n*−3			
t_0	0.26 ± 0.27	0.22 ± 0.15	0.25 ± 0.20
t_3	0.22 ± 0.09	0.21 ± 0.07	0.22 ± 0.08
t_6	0.20 ± 0.08	0.22 ± 0.08	0.25 ± 0.13

Table 2 *Continued*

	rTAG group (n = *41*)	*Placebo group* (n = *43*)	*EE group* (n = *45*)
C20:2*n*−6			
t_0	0.27 ± 0.16	0.25 ± 0.14	0.27 ± 0.12
t_3	0.20 ± 0.06[b,e]	0.23 ± 0.05	0.24 ± 0.13
t_6	0.20 ± 0.05[c]	0.25 ± 0.10	0.20 ± 0.06[d]
C20:3*n*−6			
t_0	1.62 ± 0.32	1.66 ± 0.34	1.64 ± 0.32
t_3	1.62 ± 0.97	1.87 ± 0.53[c]	1.40 ± 0.36[d,e]
t_6	2.22 ± 1.45[b]	2.63 ± 1.25[d]	2.17 ± 1.48[b]
AA			
C20:4*n*−6			
t_0	15.40 ± 1.92	16.00 ± 1.95	15.39 ± 1.66
t_3	13.62 ± 1.74[d,e]	16.19 ± 1.82	14.14 ± 1.52[d,e]
t_6	13.20 ± 1.99[d,e]	16.89 ± 2.16[b]	13.56 ± 1.68[d,e]
C24:0			
t_0	0.81 ± 0.86	0.62 ± 0.51	0.64 ± 0.50
t_3	0.51 ± 0.22[b]	0.56 ± 0.30	0.49 ± 0.23
t_6	0.80 ± 0.38	0.70 ± 0.34	0.72 ± 0.35
EPA			
C20:5*n*−3			
t_0	1.34 ± 0.63	1.45 ± 0.50	1.45 ± 0.57
t_3	4.36 ± 1.13[d,e,f]	1.20 ± 0.33[d]	3.88 ± 0.98[d,e,f]
t_6	4.52 ± 1.26[d,e,f]	1.29 ± 0.55[b]	4.10 ± 1.30[d,e,f]
C24:1*n*−9			
t_0	0.66 ± 0.29	0.54 ± 0.22	0.65 ± 0.37
t_3	0.55 ± 0.25	0.62 ± 0.31	0.57 ± 0.29
t_6	0.96 ± 0.34[d]	0.91 ± 0.35[d]	0.89 ± 0.42[b]
C22:4*n*−6			
t_0	2.62 ± 0.66	2.42 ± 0.57	2.52 ± 0.56
t_3	1.82 ± 0.50[d,e]	2.59 ± 0.53[b]	1.92 ± 0.46[d,e]
t_6	1.59 ± 0.47[d,e]	2.66 ± 0.64[b]	1.75 ± 0.52[d,e]
C22:5*n*−6			
t_0	0.49 ± 0.17	0.47 ± 0.17	0.44 ± 0.13
t_3	0.33 ± 0.11[d,e]	0.52 ± 0.15[b]	0.39 ± 0.27[e]
t_6	0.31 ± 0.10[d,e]	0.54 ± 0.17[c]	0.34 ± 0.16[c,e]
C22:5*n*−3			
t_0	2.71 ± 0.38	2.79 ± 0.41	2.82 ± 0.47
t_3	3.85 ± 0.44[d,e]	2.87 ± 0.39	3.80 ± 0.37[d,e]
t_6	4.04 ± 0.57[d,e]	2.78 ± 0.45	3.86 ± 0.50[d,e]
DHA			
C22:6*n*−3			
t_0	5.67 ± 1.34	5.79 ± 1.09	5.97 ± 1.28
t_3	8.08 ± 1.04[d,e,f]	5.87 ± 1.05	7.58 ± 0.94[d,e,f]
t_6	8.73 ± 1.32[d,e,f]	6.16 ± 1.13[c]	8.14 ± 1.27[d,e,f]
Omega-3 index			
t_0	7.00 ± 1.90	7.24 ± 1.44	7.42 ± 1.76
t_3	12.44 ± 1.98[d,e,f]	7.06 ± 1.29	11.46 ± 1.74[d,e,f]
t_6	13.25 ± 2.36[d,e,f]	7.45 ± 1.57	12.24 ± 2.33[d,e,f]

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EE group, intervention group that consumed omega-3 fatty acids as ethyl esters; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; omega-3 index, EPA + DHA; RBC, red blood cell; rTAG group, intervention group that consumed omega-3 fatty acids as reesterified triacylglycerides.
[a]Mean ± s.d.
[b]Significant changes between baseline and month three or baseline and month six; $P<0.05$.
[c]Significant changes between baseline and month three or baseline and month six; $P<0.01$.
[d]Significant changes between baseline and month three or baseline and month six; $P<0.001$.
[b,c,d]Calculated within groups by Student's *t* test for dependent samples.
[e]Values are significantly different between rTAG or EE group, respectively, and Placebo group; $P<0.05$.
[f]Values are significantly different between rTAG group and EE group; $P<0.05$.
[e,f]Calculated by analysis of covariance with corresponding baseline value as a covariate.

There were no significant changes in body mass index during the trial (data not shown). A total of 19 volunteers reported side effects occurring during the intervention time. Although eight subjects from both active treatment groups reported eructation, three subjects from the EE group reported flatulence.

RBC membrane fatty acids

RBC membrane FAs for $t_0/t_3/t_6$ are shown in Table 2. Baseline values of AA, EPA and DHA did not differ between groups.

Between t_0, t_3 and t_6, there were significant changes in RBC membrane FAs in both *n*-3 FA-treated groups for AA, EPA and DHA: AA decreased, whereas EPA and DHA increased ($P<0.001$). Significant changes were also seen in the placebo group, showing an increase in AA and DHA between t_0 and t_6, and a decrease in EPA between t_0 and t_3 ($P<0.001$), as well as between t_0 and t_6 ($P<0.05$) (Table 2).

Three months of *n*-3 FA treatment resulted in significant increases of EPA, docosapentaenoic acid and DHA content in RBC membranes compared with the placebo group, whereas AA contents significantly decreased ($P<0.001$). In a similar way, the observed tendencies were constant between t_3 and t_6 with a slight, although not significant, further increase of EPA and DHA. The decrease between t_3 and t_6 for AA was significant in both *n*-3 FA groups compared with the placebo group (rTAG group, −0.43%; EE group, −0.58%; $P<0.001$).

The increase in the omega-3 index was significantly higher in the rTAG group compared with the EE group: performing an analysis of covariance to compare absolute values of the omega-3 index between the three groups revealed that the omega-3 index was significantly higher after three and six months in both *n*-3 FA-treated groups compared with the placebo group ($\Delta t_3\_t_0$: $P<0.001$; $\Delta t_6\_t_0$: $P<0.001$). Similarly, the omega-3 index was significantly higher after three and six months in the rTAG group compared with the EE group ($\Delta t_3\_t_0$: $P<0.001$; $\Delta t_6\_t_0$: $P<0.01$) (Figure 2).

Discussion

To our knowledge, this is the first long-term randomized study comparing EPA + DHA rTAGs with EEs, with regard to their incorporation into RBC membranes, that is, the omega-3 index in humans. EPA + DHA consumed as rTAGs resulted in a higher increase of the omega-3 index than when consumed as EEs over a six-month period.

Previous studies, which compared the effects of *n*-3 FA administration from TAGs or rTAGs versus EEs on incorporation of EPA and DHA into plasma lipids (for example, TAGs, chylomicron TAGs, PLs), revealed heterogeneous results (El Boustani *et al*., 1987; Lawson and Hughes, 1988a, b; Beckermann *et al*., 1990; Luley *et al*., 1990; Reis *et al*., 1990; Nordøy *et al*., 1991; Hansen *et al*., 1993; Krokan *et al*., 1993; Dyerberg *et al*., 2010). Although most trials reported a faster and higher mean relative bioavailability of DHA and EPA

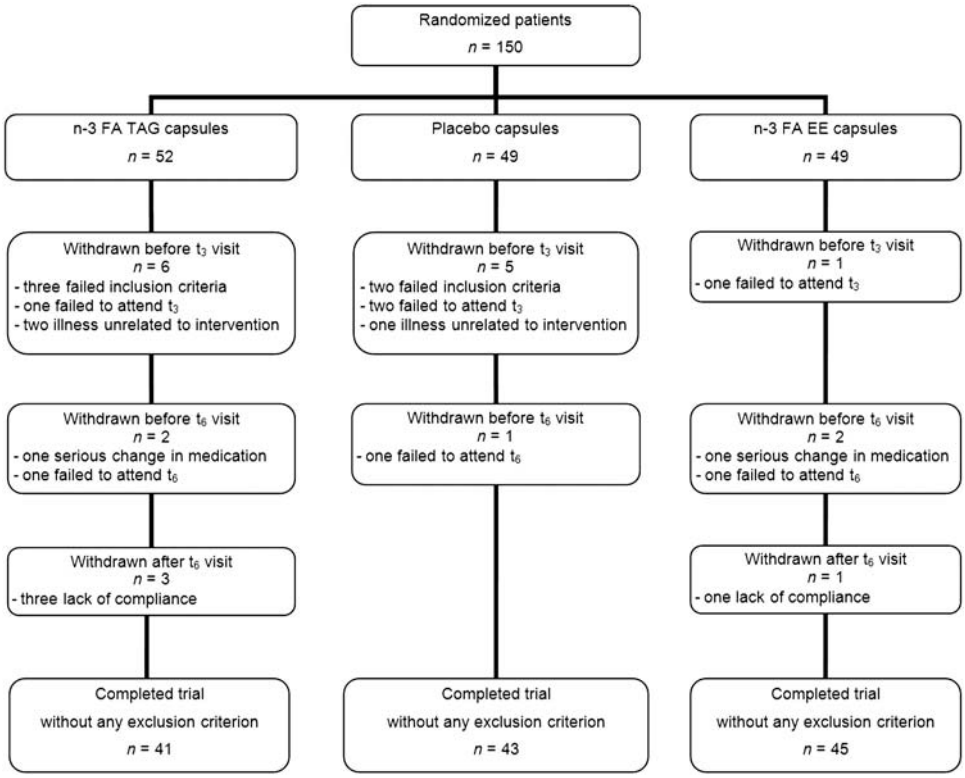

Figure 1 Flow chart for enrollment in and progress through the randomized trial.

Table 3 Characteristics of the 129 participants included in the *per protocol* group[a]

	rTAG group	*Placebo group*	*EE group*
Gender (male/female)	23/18	16/27	25/20
Age, years (mean ± s.d., range)	61.2 ± 9.8 (33–75)	61.7 ± 7.9 (34–73)	59.9 ± 9.0 (34–72)
Body weight (mean ± s.d., range)	73.3 ± 11.9 (51.0–103.0)	76.1 ± 14.2 (55.0–111.5)	77.2 ± 12.9 (53.0–102.0)
BMI, kg m^{-2} (mean ± s.d., range)	25.6 ± 2.8 (20.0–30.2)	26.1 ± 3.4 (19.9–34.4)	25.8 ± 2.9 (19.0–32.2)

Abbreviations: BMI, body mass index; EE group, intervention group that consumed omega-3 fatty acids as ethyl esters; rTAG group, intervention group that consumed omega-3 fatty acids as reesterified triacylglycerides.
[a]Mean ± s.d.

from TAGs or rTAGs compared with EEs (El Boustani *et al*., 1987; Lawson and Hughes, 1988a, b; Beckermann *et al*., 1990; Dyerberg *et al*., 2010), other findings suggest a comparable bioavailability (Luley *et al*., 1990; Nordøy *et al*., 1991; Krokan *et al*., 1993) or were heterogeneous in their outcomes (Reis *et al*., 1990; Hansen *et al*., 1993). In their recent publication, Dyerberg *et al*. (2010) clearly demonstrated in a short-term study over two weeks that EPA + DHA from rTAGs show a superior bioavailability compared with EEs. Harris *et al*. (1988) compared the differences of EPA + DHA incorporation into plasma PLs between *n*-3 FA-TAGs and methyl esters, which are assumed to be equivalent to EEs. Although no differences in EPA incorporation could be detected between methyl esters and TAG, the DHA incorporation was slightly elevated after methyl ester administration.

However, short-term bioavailability—as measured by plasma levels—does not necessarily reflect the incorporation into tissues. We chose the omega-3 index (RBC membranes EPA + DHA) as the end point because it reflects the incorporation of EPA and DHA into tissues, including cardiac tissue (Harris *et al*., 2004; Metcalf *et al*., 2007; Harris, 2008), and is thus a reasonable reflection of a person's EPA + DHA status. After six months, but not after seven weeks, of supplementation, a steady state is reached in RBC membranes (Arterburn *et al*., 2006). Our approach contrasts with previous studies

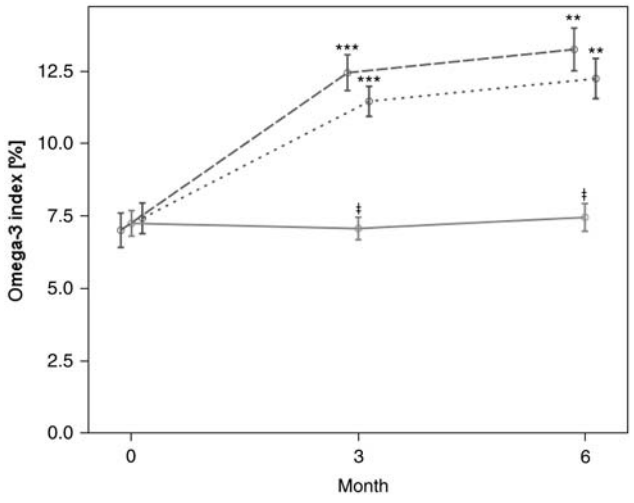

Figure 2 Omega-3 index at baseline (t_0) and after three (t_3) and six (t_6) months of daily supplementation with either *n*-3 FA given as rTAGs, as EEs or with a placebo (—— rTAG group; - - - EE group; — placebo group). Values are given as mean ± s.d. ***Significant difference in the omega-3 index between rTAG and EE group at t_3; $P<0.001$. **Significant difference in the omega-3 index between rTAG and EE group at t_6; $P<0.01$. ‡Significant differences in the omega-3 index between rTAG and EE group, respectively, and placebo group at t_3 and t_6; $P<0.001$.

mentioned in the Introduction, which had a maximum duration of seven weeks, and analyzed FA compartments reflecting short-term rather than long-term intake.

The omega-3 index increase was significantly higher after consumption of *n*-3 FA as rTAGs than after EEs. The mechanisms explaining this observation are unclear at present. Therefore, we discuss the following possible explanations:

- Differences in amount of ingested study medication: Capsule counts did not reveal any differences between the two relevant groups (percentage of ingested capsules was 95% in the rTAG group versus 96% in the EE group).
- Age: In a previous study, older subjects exhibited a stronger response to DHA supplementation than younger subjects (Vandal *et al*., 2008). In the present study, however, age was not different between groups.
- Matrix effects: There are conflicting results in RBC membrane FA composition after administering *n*-3 FA-EEs in capsules or *n*-3 FA-TAGs as fish. Although Visioli *et al*. (2003) observed a higher uptake of *n*-3 FAs in the fish-meal group than in the capsule group, Harris *et al*. (2007) found similar elevations in omega-3 index produced by both products. However, the study conducted by Visioli *et al*. was a small pilot study, including only 4–8 volunteers. Additionally, their study was retrospective, not randomized and, moreover, *n*-3 FA intake from the two sources was not matched. Nevertheless, absorption of *n*-3 FAs from EE was three-fold higher in the presence of a high-fat meal compared with a low-fat meal, but still not as high as from *n*-3 FA-TAGs (Lawson and Hughes, 1988a, b). In another study, no differences were seen in *n*-3 FA-rTAG and -EE absorption after administering these FAs in meals with similar fat contents (Nordøy *et al*., 1991). However, the study was conducted with very high doses of EPA + DHA (~22–24 g) and with five volunteers, a comparatively low number of cases. In regard to the results of Visioli *et al*. (2003) and Lawson and Hughes (1988a, b), matrix effects and different nutritional patterns could be held responsible for different findings.
- Differences in digestion: *n*-3 FA-TAGs, but not *n*-3 FA-EEs, have a glycerol backbone (Carlier *et al*., 1991). In the small intestine, pancreatic lipase hydrolyzes FAs from alcohol backbones and free FAs, and monoacylglycerols are taken up by enterocytes. *In vitro* studies conducted by Yang *et al*. (1990a) showed that the FA–ethanol bond is hydrolyzed 10–50 times more slowly by pancreatic lipase compared with hydrolysis of TAGs. This might result in a delay in, or even in less, *n*-3 FA entering the enterocyte when EPA + DHA are consumed as EEs. However, such *in vitro* experiments cannot mimic the *in vivo* situation; for example, the extreme excess of pancreatic lipase versus substrate in the normal gut is not considered.
- Differences in absorption: Free FAs must be reconverted in the enterocyte to TAGs for further transport. Glycerol molecules or 2-monoacylglycerols are needed for this. *n*-3 FA-TAG, but not *n*-3 FA-EE, has its own glycerol or monoacylglycerol substrate in storage. Yang *et al*. (1990b) suggested that slower absorption of FAs observed in rats fed with EEs might be attributed to a lower efficiency of the phosphatidic acid pathway, which is required in the absence of dietary 2-monoacylglycerols.
- Stereo specificity: As reported by Visioli *et al*. (2003), EPA and DHA are esterified mainly in the sn-2 position in TAG molecules, and this position is to a large extent prevented from hydrolysis during digestion and absorption, which might facilitate digestion and absorption of TAGs. However, recent investigations by Dyerberg *et al*. (2010) showed that the stereochemistry of FAs in acylglycerols has no influence on the bioavailability of EPA and DHA.

Taken together, although we clearly cannot delineate a specific mechanism, we propose that differences in digestion and in absorption between EEs and TAGs, which might partly depend on the nutritional matrix, are the most likely mechanisms to explain our findings.

In keeping with previous publications (Belluzzi *et al*., 1994; Katan *et al*., 1997; Harris *et al*., 2007; Barceló-Coblijn *et al*., 2008), RBC membrane baseline levels of EPA were lower than baseline levels of DHA. Both increased upon supplementation, with increases in EPA being more pronounced, corresponding to the larger amount of EPA than DHA in the capsules. As observed previously (Krokan *et al*., 1993; Belluzzi *et al*., 1994; Arterburn *et al*., 2006), most omega-6 FAs were significantly reduced at t_3 and t_6.

The relative increases in EPA RBC membrane content between t_0 and t_6 is 286% for the rTAG group and 215% for the EE group. These observations are in agreement with findings from Katan *et al*. (1997), presenting a change of around 260% for EPA RBC membrane content in consequence of 1 g EPA per day as TAGs after six and similarly twelve months. Referring to these findings, it can be concluded that EPA values would not rise much higher in the rTAG group of the present study if supplementation were to be continued.

Contrary to EPA, our data shows that DHA is still accumulating in RBC membranes between month three and six in both the rTAG group (Δ-t_6_t_3: 19%) and the EE group (Δ-t_6_t_3: 21%). DHA is located in the inner leaflet of the PL bilayer and becomes integrated in membranes during RBC development (von Schacky and Weber, 1985). As the first investigation time point takes place ~90 days after baseline and RBC lifespan is ~120 days (~four months), this further accumulation of DHA is not surprising. DHA reaches a steady state in RBC membranes after four–six months (Arterburn *et al*., 2006). As also described by Marangoni *et al*. (1993), accumulation of EPA in RBC membranes is faster in comparison with DHA. This observation might be caused by the preferential distribution of EPA in the outer layer of RBC membranes, which allows a more rapid exchange with plasma lipoproteins (Popp-Snijders *et al*., 1984; Cartwright *et al*., 1985). In respect to previous findings (Tynan *et al*., 1995; Katan *et al*., 1997; Arterburn *et al*., 2006), we could expect that differences in the omega-3 index will persist after the six-month intervention, provided the supplementation dosage remains stable.

Additionally, the daily intake of four capsules of both *n*-3 FA study supplements for six months (1.01 g EPA + 0.67 g DHA per day) caused a boost in the mean omega-3 index up to 13.2 and 12.2% for the rTAG group and EE group, respectively. We calculated the hypothetical number of *n*-3 FA-EE capsules, which are needed to yield the same rise in the omega-3 index obtained from four *n*-3 FA-rTAG capsules intake. Hence, subjects would have to consume at least five capsules of *n*-3 FA-EEs daily to achieve the same effect in the omega-3 index. Nevertheless, the goal for treatment with EPA + DHA—an omega-3 index of >8%—is highly exceeded after consumption of four or five capsules of our study medication. In any case, with respect to the strength of different increases in the omega-3 index between *n*-3 FA given as rTAGs or as EEs, we expect higher increases in RBC membrane EPA + DHA content, even for lower dosages of our tested rTAG capsules as compared with EE capsules.

Omega-3 index baseline levels of our study participants were slightly higher than expected (rTAG group: 7.00 ± 1.90%; placebo group: 7.24 ± 1.44%; EE group: 7.42 ± 1.76%). Previously, in Germany, we found a mean omega-3 index 6.14 ± 1.83% in 1000 unselected, non-supplementers, whereas it was 5.94 ± 1.41% in 190 atherosclerotic subjects (von Schacky, 2010). Preliminary data indicated slight differences between the participating centers: Hannover, Hamburg, Goslar and Munich (not shown). This should be evaluated more systematically. However, our trial aimed at comparing the magnitude of change in the omega-3 index in the three study groups, not at measuring absolute levels. Therefore, we consider it more important that baseline omega-3 index levels in our trial were comparable among the three groups.

In conclusion, our findings underscore that the intake of both *n*-3 FA study supplements resulted in an enhanced incorporation of EPA and DHA into tissues, as evidenced by changes in RBC membrane FA content. As the resulting omega-3 index was significantly higher after *n*-3 FA-rTAG administration compared with *n*-3 FA-EE, the results indicate that *n*-3 FA-rTAG is superior to *n*-3 FA-EE in view of the EPA + DHA tissues incorporation following a long-term administration. However, whether this difference would result in differences in clinical outcomes (that is, reduction in serum TAG levels, reductions in coronary heart disease events) is unclear and needs further investigations. Nevertheless, these obvious differences between rTAGs and EEs should be considered in the *n*-3 FA intake recommendations.

Conflict of interest

C von Schacky received a speaker's honoraria from Solvay, and grant support from Sanofi-Aventis and Smartfish. He founded Omegametrix, a company offering FA analyses. A Hahn and JP Schuchardt worked as consultants for companies, which also produce and merchandise FA supplements. The authors are solely responsible for the design and conduct of the study; collection, management, analysis and interpretation of the data; as well as preparation of the manuscript.

Acknowledgements

Gratuitous supply of the study supplements from Dr Loges + Co. GmbH, Winsen, Germany is gratefully acknowledged. We specially thank Heike Kohrs for her technical assistance in the laboratory.

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