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

Running title: Omega-3 index: superiority of n-3 fatty acid triacylglycerides vs. ethyl-esters

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The study supplements used in this study were a gift from Dr. Loges + Co. GmbH, Winsen, Germany.

47 **ABSTRACT**

48 **Background:** There is a debate currently about whether different chemical forms of
49 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are absorbed in an identical
50 way. The objective of this study was to investigate the response of the omega-3 index, the
51 percentage of EPA + DHA in red blood cell membranes, to supplementation with two
52 different n-3 FA formulations in humans.

53 **Design:** The study was conducted as a double-blind placebo-controlled trial. 150 volunteers
54 were randomly assigned to one of three groups: 1) fish oil concentrate with EPA + DHA
55 (1.01g + 0.67g) given as re-esterified triacylglycerides (rTAG-group); 2) corn oil (placebo-
56 group); or 3) fish oil concentrate with EPA + DHA (1.01g + 0.67g) given as ethyl-esters
57 (EE-group). Volunteers consumed four gelatine-coated soft capsules daily over a period of
58 six months. The omega-3 index was determined at baseline (t_0), after three months (t_3) and
59 at the end of the intervention period (t_6).

60 **Results:** The omega-3 index increased significantly in both groups treated with n-3 FA
61 from the baseline to t_3 and t_6 ($P < 0.001$). The omega-3 index increased to a greater extent in
62 the rTAG-group than in the EE-group (t_3 : 186% vs. 161% [$P < 0.001$]; t_6 : 197% vs. 171%
63 [$P < 0.01$]).

64 **Conclusion:** A six month supplementation of identical doses of EPA + DHA led to a faster
65 and higher increase in the omega-3 index when consumed as triacylglycerides than when
66 consumed as ethyl-esters.

67 **KEYWORDS**

68 **n-3 fatty acids, triacylglycerides, ethyl-esters, omega-3 index, eicosapentaenoic acid,**
69 **docosahexaenoic acid**

70

71 **INTRODUCTION**

72 Cardiac societies recommend an increased intake of the long-chain n-3 fatty acids (FAs)
73 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), especially for patients with
74 known cardiovascular disease (CVD) or for individuals at high risk of CVD (Fruchart *et al.*,
75 2008; Graham *et al.*, 2007; Smith *et al.*, 2006). Although most of the large intervention
76 studies on which these recommendations are based were conducted with EPA + DHA ethyl-
77 ester (EEs), nutrition and cardiac societies recommend increasing the intake of n-3 FAs by
78 consuming at least two servings of (preferably oily) fish per week, providing 400-500 mg/d of
79 EPA + DHA, mainly esterified as triacylglycerides (TAGs), but also in membrane
80 phospholipids (PLs). For the portion of the population reluctant to eat fish, the societies
81 recommend concentrated fish oil preparations which typically contain EPA and DHA as
82 TAGs or EEs. These recommendations presume that different chemical forms of EPA + DHA
83 have an identical efficacy.

84 The majority of previous studies which compared the bioavailability of EPA + DHA from
85 TAGs or rTAGs vs. EEs (El Boustani *et al.*, 1987; Lawson & Hughes, 1988 [a & b];
86 Beckermann *et al.*, 1990; Dyerberg *et al.*, 2010; Luley *et al.*, 1990; Nordøy *et al.*, 1991;
87 Krokan *et al.*, 1993) were short-term with a maximal length of seven weeks. The focussed
88 endpoints in these trials – FA composition of plasma PLs, TAGs, chylomicron TAGs, or
89 serum PLs – similarly reflect short-term rather than long-term intake (Katan *et al.*, 1997; Cao
90 *et al.*, 2006). Moreover, larger doses (> 2 g/d EPA and DHA) were used in many of these
91 studies, which is in contrast to the recommendations of the societies mentioned.

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

99 **SUBJECTS AND METHODS**

100 **Subjects**

101 150 subjects between 30 and 75 years old were recruited via newspaper advertisements in
102 four different German cities (Munich, Hamburg, Hannover, and Goslar) between April and
103 October, 2008. Since the study was conducted to assess the effects of the two fish oil
104 formulations on the lipid profile in hyperlipidemic subjects, the inclusion criterion was
105 hyperlipidemia solely treated with HMG-CoA-reductase inhibitors (statins). The exclusion
106 criteria were serious illness (type 1 diabetes, cancer, coronary heart disease, bleeding
107 disorders); body mass index $> 35 \text{ kg/m}^2$; gastrointestinal disorders; medications known to
108 affect lipid metabolism; daily fish consumption or ingestion of dietary n-3 FA; and plant
109 sterol supplements. Written informed consent was obtained from all participants. The trial
110 was conducted with respect to GCP Guidelines and the protocol was approved by an ethics
111 commission.

112

113 **Study design**

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

121 The three different types of gelatine-coated soft capsules contained (1) n-3 FAs as
122 re-esterified TAGs (rTAG-group; n=52); (2) corn oil (placebo-group; n=49); or (3) n-3 FA-
123 EEs (EE-group; n=49), and were provided by Dr. Loges and Co. GmbH (Winsen, Germany),

a pharmaceutical company. Re-esterified TAGs 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 re-esterified TAGs (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 mg, and 672 mg, respectively. The placebo capsules contained corn oil and were outwardly identical to the n-3 FA-capsules in all aspects.

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 the 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 (e.g. usual fish intake, physically activity) as well as tolerability of the two different n-3 FA formulations.

Volunteers' compliance was assessed by count of left-over 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 & 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), 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±SD. The sample size (n = 50 per group) was calculated for differences in serum TAG levels (primary endpoint) 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 three groups resulted in significance, the three pairwise 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 endpoint, 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 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 the baseline in each intervention group. Baseline markers were compared among groups using one way ANOVA. 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 the 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 (BMI), and did not show any differences between the groups (**Table 3**). There were no significant changes in BMI during the trial (data not shown). 19 Volunteers reported side effects occurring during intervention time. While 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**. The 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, while 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, DPA (docosapentaenoic acid) and DHA content in RBC membranes compared with the placebo-group, whereas the 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 for 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 to the EE-group: performing an ANCOVA 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 to 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 to 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, i.e. 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 vs. EEs on incorporation of EPA and DHA into plasma lipids (e.g. TAGs, chylomicron TAGs, PLs), revealed heterogeneous results (El Boustani *et al.*, 1987; Lawson & Hughes,

1988 [a & b]; Beckermann *et al.*, 1990; Dyerberg *et al.*, 2010; Luley *et al.*, 1990; Nordøy *et al.*, 1991; Krokan *et al.*, 1993; Hansen *et al.*, 1993; Reis *et al.*, 1990). While most trials reported a faster and higher mean relative bioavailability of DHA and EPA from TAGs or rTAGs compared to EEs (El Boustani *et al.*, 1987; Lawson & Hughes, 1988 [a & 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 (Hansen *et al.*, 1993; Reis *et al.*, 1990). 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 to EEs. Harris *et al.* (1988) compared the differences of EPA + DHA incorporation into plasma PLs between n-3 FA-TAGs and methyl-esters (ME), which are assumed to be equivalent to EEs. While no differences in EPA incorporation could be detected between ME and TAG, the DHA incorporation was slightly elevated after ME 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 endpoint because it reflects the incorporation of EPA and DHA into tissues, including cardiac tissue (Metcalf *et al.*, 2007; Harris *et al.*, 2004; 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 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 vs. 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. While 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 to a low-fat meal, but still not as high as from n-3 FA-TAGs (Lawson & Hughes, 1988 [a & 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 (~22-24g) and with five volunteers a comparatively low number of cases. In regard to the results of Visioli *et al.* (2003) and Lawson & Hughes (1988 [a & 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.* (1990 [a]) showed that the FA-ethanol bond is hydrolysed 10 to 50 times more slowly by pancreatic lipase compared to 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; e.g. the extreme excess of pancreatic lipase vs. 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.* (1990 [b]) 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 (Katan *et al.*, 1997; Belluzzi *et al.*, 1994; 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 (Arterburn *et al.*, 2006; Belluzzi *et al.*, 1994; Krokan *et al.*, 1993), 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 1g EPA/d 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 ($\Delta-t_6-t_3$: 19%) and the EE-group ($\Delta-t_6-t_3$: 21%). DHA is located in the inner leaflet of the PL bilayer and becomes integrated in membranes during RBC development (von Schacky & Weber, 1985). Since the first investigation time-point takes place approximately 90 days after the baseline, and RBC lifespan is approximately 120 days (~four months), this further accumulation of DHA is not surprising. DHA reaches a steady state in RBC membranes after four to six months (Arterburn *et al.*, 2006). As also described by Marangoni *et al.* (1993), accumulation of EPA in RBC membranes is faster in comparison to 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 (Cartwright *et al.*, 1985; Popp-Snijders *et al.*, 1984). 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: $7.00 \pm 1.90\%$; placebo-group: $7.24 \pm 1.44\%$, EE-group: $7.42 \pm 1.76\%$). Previously, in Germany, we found a mean omega-3 index $6.14 \pm 1.83\%$ in 1000 unselected, non-Supplementers, whereas it was $5.94 \pm 1.41\%$ 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. Since the resulting omega-3 index was significantly higher after n-3 FA-rTAG administration compared to 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 (i.e. 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.

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Declaration

C. von Schacky received a speaker's honoraria from Solvay, and grant support from Sanofi-Aventis and Smartfish. He founded Omegametrix, a company offering fatty acid analyses. A. Hahn and J. P. Schuchardt worked as consultants for companies which also produce and merchandise fatty acid 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.

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479

480

481 **TABLES**

482 **Table 1**

483 Composition of the two supplements per capsule and overall daily consumption (four
484 capsules)

Nutrient	n-3 fatty acids as triacylglycerides or as ethyl-esters	
	per capsule	per day (four capsules)
total n-3	504 mg	2016 mg
20:5n-3 (EPA)	252 mg	1008 mg
22:6n-3 (DHA)	168 mg	672 mg
α -Tocopherol	6 mg	24 mg

485

486 **Table 2**

487 Red blood cell membrane fatty acid composition (% of total fatty acids)* at baseline (t₀), three
 488 (t₃) and six months (t₆)*

		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 ³
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 ^a	9.99±1.45 ³	20.34±1.37 ³
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 ¹	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 ^{3a}	0.60±0.28 ³	0.42±0.19 ^{3a}
C18:0	t ₀	15.76±1.80	15.01±1.85	15.49±1.78
	t ₃	14.99±2.26 ¹	15.06±1.69	14.97±2.10
	t ₆	14.72±1.81 ²	14.08±2.05 ¹	14.61±1.42 ²
C18:1t	t ₀	0.54±0.30	0.51±0.25	0.49±0.12
	t ₃	0.49±0.16 ^a	0.57±0.25	0.55±0.28
	t ₆	0.35±0.16 ³	0.41±0.36	0.32±0.17 ³
C18:1n-9	t ₀	15.53±1.38	15.62±1.35	15.61±1.14
	t ₃	14.62±1.23 ^{3a}	15.40±1.57	14.73±1.15 ^{3a}
	t ₆	14.96±1.16 ^{1a}	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 ^a
	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 ¹	0.07±0.08 ¹
	t ₆	0.04±0.03 ¹	0.05±0.05 ²	0.04±0.09 ²
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 ¹	0.18±0.32	0.11±0.04 ²
LA C18:2n-6	t ₀	11.69±1.62	12.23±2.20	12.41±1.98
	t ₃	10.95±1.65 ^{1a}	12.74±1.75	11.58±1.83 ^{2a}
	t ₆	10.90±1.66 ^{2a}	12.96±2.36 ¹	11.86±1.98 ^{1a}
C18:3n-6	t ₀	0.20±0.11	0.20±0.10	0.18±0.07
	t ₃	0.12±0.05 ^{3a}	0.16±0.06 ¹	0.12±0.09 ^{3a}
	t ₆	0.09±0.04 ³	0.13±0.06 ³	0.09±0.04 ³
C20:1n-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 ¹	0.25±0.15	0.21±0.06 ²
ALA C18:3n-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
C20:2n-6	t ₀	0.27±0.16	0.25±0.14	0.27±0.12
	t ₃	0.20±0.06 ^{1a}	0.23±0.05	0.24±0.13

	t ₆	0.20±0.05 ²	0.25±0.10	0.20±0.06 ³
C20:3n-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 ²	1.40±0.36 ^{3 a}
	t ₆	2.22±1.45 ¹	2.63±1.25 ³	2.17±1.48 ¹
AA	t ₀	15.40±1.92	16.00±1.95	15.39±1.66
	t ₃	13.62±1.74 ^{3 a}	16.19±1.82	14.14±1.52 ^{3 a}
	t ₆	13.20±1.99 ^{3 a}	16.89±2.16 ¹	13.56±1.68 ^{3 a}
C24:0	t ₀	0.81±0.86	0.62±0.51	0.64±0.50
	t ₃	0.51±0.22 ¹	0.56±0.30	0.49±0.23
	t ₆	0.80±0.38	0.70±0.34	0.72±0.35
EPA	t ₀	1.34±0.63	1.45±0.50	1.45±0.57
	t ₃	4.36±1.13 ^{3 a b}	1.20±0.33 ³	3.88±0.98 ^{3 a b}
	t ₆	4.52±1.26 ^{3 a b}	1.29±0.55 ¹	4.10±1.30 ^{3 a b}
C24:1n-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 ³	0.91±0.35 ³	0.89±0.42 ¹
C22:4n-6	t ₀	2.62±0.66	2.42±0.57	2.52±0.56
	t ₃	1.82±0.50 ^{3 a}	2.59±0.53 ¹	1.92±0.46 ^{3 a}
	t ₆	1.59±0.47 ^{3 a}	2.66±0.64 ¹	1.75±0.52 ^{3 a}
C22:5n-6	t ₀	0.49±0.17	0.47±0.17	0.44±0.13
	t ₃	0.33±0.11 ^{3 a}	0.52±0.15 ¹	0.39±0.27 ^a
	t ₆	0.31±0.10 ^{3 a}	0.54±0.17 ²	0.34±0.16 ^{2 a}
C22:5n-3	t ₀	2.71±0.38	2.79±0.41	2.82±0.47
	t ₃	3.85±0.44 ^{3 a}	2.87±0.39	3.80±0.37 ^{3 a}
	t ₆	4.04±0.57 ^{3 a}	2.78±0.45	3.86±0.50 ^{3 a}
DHA	t ₀	5.67±1.34	5.79±1.09	5.97±1.28
	t ₃	8.08±1.04 ^{3 a b}	5.87±1.05	7.58±0.94 ^{3 a b}
	t ₆	8.73±1.32 ^{3 a b}	6.16±1.13 ²	8.14±1.27 ^{3 a b}
omega-3 index	t ₀	7.00±1.90	7.24±1.44	7.42±1.76
	t ₃	12.44±1.98 ^{3 a b}	7.06±1.29	11.46±1.74 ^{3 a b}
	t ₆	13.25±2.36 ^{3 a b}	7.45±1.57	12.24±2.33 ^{3 a b}

Abbreviations: rTAG-group, intervention group which consumed omega-3 fatty acids as re-esterified triacylglycerides; EE-group, intervention group which consumed omega-3 fatty acids as ethyl-esters; LA, linoleic acid; ALA, α -linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; omega-3 index, EPA + DHA

*mean \pm s.d.

¹: Significant changes between baseline and month three or baseline and month six; $P < 0.05$

²: Significant changes between baseline and month three or baseline and month six; $P < 0.01$

³: Significant changes s between baseline and month three or baseline and month six; $P < 0.001$

^{1, 2, 3} Calculated within groups by Student's t test for dependent samples.

^a Values are significantly different between rTAG- or EE-group, respectively, and Placebo-group; $P < 0.05$

^b Values are significantly different between rTAG-group and EE-group; $P < 0.05$.

^{a, b} Calculated by ANCOVA with corresponding baseline value as a covariate

Table 3

Characteristics of the 129 participants included in the *per protocol* group *

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

Abbreviations: rTAG-group, intervention group which consumed omega-3 fatty acids as re-esterified triacylglycerides; EE-group, intervention group which consumed omega-3 fatty acids as ethyl-esters.

*mean ± s.d.

FIGURES

Figure 1:

Flow chart for enrolment in and progress through the randomized trial.

Figure 2:

Omega-3 index at baseline (t_0) and after three (t_3) and six (t_6) months of daily supplementation with either omega-3 fatty acids (n-3 FA) given as re-esterified triacylglycerides (rTAGs), as ethyl-esters (EEs) or with a placebo (— rTAG-group; --- EE-group; — placebo-group). Values are given as mean \pm s.d.

*** Significant difference in 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 ; $P < 0.001$.

Randomized patients
 $n = 150$

n-3 FA TAG capsules
 $n = 52$

Withdrawn before t_3 visit
 $n = 6$

- three failed inclusion criteria
- one failed to attend t_3
- two illness unrelated to intervention

Withdrawn before t_6 visit
 $n = 2$

- one serious change in medication
- one failed to attend t_6

Withdrawn after t_6 visit
 $n = 3$

- three lack of compliance

Completed trial
without any exclusion criterion
 $n = 41$

Placebo capsules
 $n = 49$

Withdrawn before t_3 visit
 $n = 5$

- two failed inclusion criteria
- two failed to attend t_3
- one illness unrelated to intervention

Withdrawn before t_6 visit
 $n = 1$

- one failed to attend t_6

Completed trial
without any exclusion criterion
 $n = 43$

n-3 FA EE capsules
 $n = 49$

Withdrawn before t_3 visit
 $n = 1$

- one failed to attend t_3

Withdrawn before t_6 visit
 $n = 2$

- one serious change in medication
- one failed to attend t_6

Withdrawn after t_6 visit
 $n = 1$

- one lack of compliance

Completed trial
without any exclusion criterion
 $n = 45$

