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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

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

3 Running title: Omega-3 index: superiority of n-3 fatty acid triacylglycerides vs. ethyl-esters
4

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

46

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 kg/m²; 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),

124 a pharmaceutical company. Re-esterified TAGs are made from fish body oil to concentrate
125 specific FAs in the resulting products. Therefore, FAs from the natural TAGs are transferred
126 to ethanol, forming EEs. After removing undesirable FAs, EEs are enzymatically reconverted
127 into TAGs, now called re-esterified TAGs (rTAGs). The FA composition of the two different
128 supplements was identical (**Table 1**). The daily intake of EPA and DHA in both n-3 FA-
129 groups was 1008 mg, and 672 mg, respectively. The placebo capsules contained corn oil and
130 were outwardly identical to the n-3 FA-capsules in all aspects.

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

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

139 Volunteers' compliance was assessed by count of left-over capsules between the three
140 investigation dates.

141

142 **RBC membrane fatty acid analysis**

143 RBC membrane FA composition was analyzed according to the omega-3 index methodology
144 as described previously (Harris & von Schacky, 2004). FA methyl-esters were generated from
145 RBCs by acid transesterification and analyzed by gas chromatography using a GC2010 Gas
146 Chromatograph (Shimadzu, Duisburg, Germany) equipped with a SP2560, 100-m column
147 (Supelco, Bellefonte, PA), using hydrogen as the carrier gas. FAs were identified by
148 comparison with a FA standard mixture characteristic for RBCs. Results for the omega-3
149 index are given as EPA + DHA expressed as a percentage of the total identified FAs after

150 response factor correction. The coefficient of variation for EPA + DHA was 5%. Quality was
151 assured according to DIN ISO 15189.

152

153 **Statistics**

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

173

174 **RESULTS**

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

185

186 **Subject characteristics**

187 The three groups were normally distributed with respect to age, gender, body weight, and
188 body mass index (BMI), and did not show any differences between the groups (**Table 3**).
189 There were no significant changes in BMI during the trial (data not shown). 19 Volunteers
190 reported side effects occurring during intervention time. While eight subjects from both active
191 treatment-groups reported eructation, three subjects from the EE-group reported flatulence.

192

193 **RBC membrane fatty acids**

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

196 Between t_0 , t_3 and t_6 , there were significant changes in RBC membrane FAs in both n-3 FA
197 treated groups for AA, EPA and DHA: AA decreased, while EPA and DHA increased
198 ($P<0.001$). Significant changes were also seen in the placebo-group, showing an increase in

199 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
200 between t_0 and t_6 ($P<0.05$) (**Table 2**).

201 Three months of n-3 FA treatment resulted in significant increases of EPA, DPA
202 (docosapentaenoic acid) and DHA content in RBC membranes compared with the placebo-
203 group, whereas the AA contents significantly decreased ($P<0.001$). In a similar way, the
204 observed tendencies were constant between t_3 and t_6 with a slight, although not significant,
205 further increase for EPA and DHA. The decrease between t_3 and t_6 for AA was significant in
206 both n-3 FA-groups compared with the placebo-group (rTAG-group, -0.43%; EE-group, -
207 0.58%; $P<0.001$).

208 The increase in the omega-3 index was significantly higher in the rTAG-group compared to
209 the EE-group: performing an ANCOVA to compare absolute values of the omega-3 index
210 between the three groups revealed that the omega-3 index was significantly higher after
211 three and six months in both n-3 FA treated groups compared to the placebo-group ($\Delta t_{3_t_0}$:
212 $P<0.001$; $\Delta t_{6_t_0}$: $P<0.001$). Similarly, the omega-3 index was significantly higher after
213 three and six months in the rTAG-group compared to the EE-group ($\Delta t_{3_t_0}$: $P<0.001$; $\Delta t_{6_t_0}$:
214 $P<0.01$) (**Figure 2**).

215

216 **DISCUSSION**

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

221 Previous studies, which compared the effects of n-3 FA-administration from TAGs or rTAGs
222 vs. EEs on incorporation of EPA and DHA into plasma lipids (e.g. TAGs, chylomicron
223 TAGs, PLs), revealed heterogeneous results (El Boustani *et al.*, 1987; Lawson & Hughes,

224 1988 [a & b]; Beckermann *et al.*, 1990; Dyerberg *et al.*, 2010; Luley *et al.*, 1990; Nordøy *et*
225 *al.*, 1991; Krokan *et al.*, 1993; Hansen *et al.*, 1993; Reis *et al.*, 1990). While most trials
226 reported a faster and higher mean relative bioavailability of DHA and EPA from TAGs or
227 rTAGs compared to EEs (El Boustani *et al.*, 1987; Lawson & Hughes, 1988 [a & b];
228 Beckermann *et al.*, 1990; Dyerberg *et al.*, 2010), other findings suggest a comparable
229 bioavailability (Luley *et al.*, 1990; Nordøy *et al.*, 1991; Krokan *et al.*, 1993) or were
230 heterogeneous in their outcomes (Hansen *et al.*, 1993; Reis *et al.*, 1990). In their recent
231 publication, Dyerberg *et al.* (2010) clearly demonstrated in a short-term study over two weeks
232 that EPA + DHA from rTAGs show a superior bioavailability compared to EEs. Harris *et al.*
233 (1988) compared the differences of EPA + DHA incorporation into plasma PLs between n-3
234 FA-TAGs and methyl-esters (ME), which are assumed to be equivalent to EEs. While no
235 differences in EPA incorporation could be detected between ME and TAG, the DHA
236 incorporation was slightly elevated after ME administration.

237 However, short-term bioavailability – as measured by plasma levels – does not necessarily
238 reflect the incorporation into tissues. We chose the omega-3 index (RBC membranes EPA +
239 DHA) as the endpoint because it reflects the incorporation of EPA and DHA into tissues,
240 including cardiac tissue (Metcalf *et al.*, 2007; Harris *et al.*, 2004; Harris, 2008), and is thus a
241 reasonable reflection of a person's EPA + DHA status. After six months, but not after seven
242 weeks, of supplementation, a steady state is reached in RBC membranes (Arterburn *et al.*,
243 2006). Our approach contrasts with previous studies mentioned in the INTRODUCTION which
244 had a maximum duration of seven weeks, and analyzed FA compartments reflecting short-
245 term rather than long-term intake.

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

249 - Differences in amount of ingested study medication: Capsule counts did not reveal any
250 differences between the two relevant groups (percentage of ingested capsules was 95% in the
251 rTAG-group vs. 96% in the EE-group).

252 - Age: In a previous study, older subjects exhibited a stronger response to DHA
253 supplementation than younger subjects (Vandal *et al.*, 2008). In the present study, however,
254 age was not different between groups.

255 - Matrix effects: There are conflicting results in RBC membrane FA composition after
256 administering n-3 FA-EEs in capsules or n-3 FA-TAGs as fish. While Visioli *et al.* (2003)
257 observed a higher uptake of n-3 FAs in the fish-meal group than in the capsule group, Harris
258 *et al.* (2007) found similar elevations in omega-3 index produced by both products. However,
259 the study conducted by Visioli *et al.* was a small pilot study, including only 4-8 volunteers.
260 Additionally, their study was retrospective, not randomized and, moreover, n-3 FA-intake
261 from the two sources was not matched. Nevertheless, absorption of n-3 FAs from EE was
262 three-fold higher in the presence of a high-fat meal compared to a low-fat meal, but still not as
263 high as from n-3 FA-TAGs (Lawson & Hughes, 1988 [a & b]). In another study, no
264 differences were seen in n-3 FA-rTAG and -EE absorption after administering these FAs in
265 meals with similar fat contents (Nordøy *et al.*, 1991). However, the study was conducted with
266 very high doses of EPA + DHA (~22-24g) and with five volunteers a comparatively low
267 number of cases. In regard to the results of Visioli *et al.* (2003) and Lawson & Hughes (1988
268 [a & b]), matrix effects and different nutritional patterns could be held responsible for
269 different findings.

270 - Differences in digestion: n-3 FA-TAGs, but not n-3 FA-EEs, have a glycerol backbone
271 (Carlier *et al.*, 1991). In the small intestine, pancreatic lipase hydrolyzes FAs from alcohol
272 backbones and free FAs, and monoacylglycerols are taken up by enterocytes. *In vitro* studies
273 conducted by Yang *et al.* (1990 [a]) showed that the FA-ethanol bond is hydrolysed 10 to 50
274 times more slowly by pancreatic lipase compared to hydrolysis of TAGs. This might result in

275 a delay in, or even in less, n-3 FA entering the enterocyte when EPA + DHA are consumed as
276 EEs. However, such *in vitro* experiments cannot mimic the *in vivo* situation; e.g. the extreme
277 excess of pancreatic lipase vs. substrate in the normal gut is not considered.

278 - Differences in absorption: Free FAs must be reconverted in the enterocyte to TAGs for
279 further transport. Glycerol molecules or 2-monoacylglycerols are needed for this. n-3 FA-
280 TAG, but not n-3 FA-EE, has its own glycerol or monoacylglycerol substrate in storage. Yang
281 *et al.* (1990 [b]) suggested that slower absorption of FAs observed in rats fed with EEs might
282 be attributed to a lower efficiency of the phosphatidic acid pathway, which is required in the
283 absence of dietary 2-monoacylglycerols.

284 - Stereo specificity: As reported by Visioli *et al.* (2003), EPA and DHA are esterified mainly
285 in the sn-2 position in TAG molecules, and this position is to a large extent prevented from
286 hydrolysis during digestion and absorption, which might facilitate digestion and absorption of
287 TAGs. However, recent investigations by Dyerberg *et al.* (2010) showed that the
288 stereochemistry of FAs in acylglycerols has no influence on the bioavailability of EPA and
289 DHA.

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

293 In keeping with previous publications (Katan *et al.*, 1997; Belluzzi *et al.*, 1994; Harris *et al.*,
294 2007; Barceló-Coblijn *et al.*, 2008), RBC membrane baseline levels of EPA were lower than
295 baseline levels of DHA. Both increased upon supplementation, with increases in EPA being
296 more pronounced, corresponding to the larger amount of EPA than DHA in the capsules. As
297 observed previously (Arterburn *et al.*, 2006; Belluzzi *et al.*, 1994; Krokan *et al.*, 1993), most
298 omega-6 FAs were significantly reduced at t_3 and t_6 .

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

305 Contrary to EPA, our data shows that DHA is still accumulating in RBC membranes between
306 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%).
307 DHA is located in the inner leaflet of the PL bilayer and becomes integrated in membranes
308 during RBC development (von Schacky & Weber, 1985). Since the first investigation time-
309 point takes place approximately 90 days after the baseline, and RBC lifespan is approximately
310 120 days (~four months), this further accumulation of DHA is not surprising. DHA reaches a
311 steady state in RBC membranes after four to six months (Arterburn *et al.*, 2006). As also
312 described by Marangoni *et al.* (1993), accumulation of EPA in RBC membranes is faster in
313 comparison to DHA. This observation might be caused by the preferential distribution of EPA
314 in the outer layer of RBC membranes, which allows a more rapid exchange with plasma
315 lipoproteins (Cartwright *et al.*, 1985; Popp-Snijders *et al.*, 1984). In respect to previous
316 findings (Tynan *et al.*, 1995; Katan *et al.*, 1997; Arterburn *et al.*, 2006), we could expect that
317 differences in the omega-3 index will persist after the six-month intervention, provided the
318 supplementation dosage remains stable.

319 Additionally, the daily intake of four capsules of both n-3 FA study supplements for six
320 months (1.01 g EPA + 0.67 g DHA per day) caused a boost in the mean omega-3 index up to
321 13.2% and 12.2% for the rTAG-group and EE-group, respectively. We calculated the
322 hypothetical number of n-3 FA-EE capsules which are needed to yield the same rise in the
323 omega-3 index obtained from four n-3 FA-rTAG capsules intake. Hence, subjects would have

324 to consume at least five capsules of n-3 FA-EEs daily to achieve the same effect in the
325 omega-3 index. Nevertheless, the goal for treatment with EPA + DHA – an omega-3 index of
326 >8% – is highly exceeded after consumption of four or five capsules of our study medication.
327 In any case, with respect to the strength of different increases in the omega-3 index between
328 n-3 FA given as rTAGs or as EEs, we expect higher increases in RBC membrane EPA +
329 DHA content even for lower dosages of our tested rTAG-capsules as compared with EE-
330 capsules.

331 Omega-3 index baseline levels of our study participants were slightly higher than expected
332 (rTAG-group: $7.00 \pm 1.90\%$; placebo-group: $7.24 \pm 1.44\%$, EE-group: $7.42 \pm 1.76\%$). Previously,
333 in Germany, we found a mean omega-3 index $6.14 \pm 1.83\%$ in 1000 unselected, non-
334 Supplementers, whereas it was $5.94 \pm 1.41\%$ in 190 atherosclerotic subjects (von Schacky,
335 2010). Preliminary data indicated slight differences between the participating centers:
336 Hannover, Hamburg, Goslar, and Munich (not shown). This should be evaluated more
337 systematically. However, our trial aimed at comparing the magnitude of change in the omega-
338 3 index in the three study groups, not at measuring absolute levels. Therefore, we consider it
339 more important that baseline omega-3 index levels in our trial were comparable among the
340 three groups.

341 In conclusion, our findings underscore that the intake of both n-3 FA study supplements
342 resulted in an enhanced incorporation of EPA and DHA into tissues, as evidenced by changes
343 in RBC membrane FA content. Since the resulting omega-3 index was significantly higher
344 after n-3 FA-rTAG administration compared to n-3 FA-EE, the results indicate that n-3 FA-
345 rTAG is superior to n-3 FA-EE in view of the EPA + DHA tissues incorporation following a
346 long-term administration. However, whether this difference would result in differences in
347 clinical outcomes (i.e. reduction in serum TAG-levels, reductions in coronary heart disease

348 events) is unclear and needs further investigations. Nevertheless, these obvious differences
349 between rTAGs and EEs should be considered in the n-3 FA intake recommendations.

350

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355

356 **Declaration**

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

363

364

365

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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

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}

489

490

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

494

*mean \pm s.d.

495

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

496

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

497

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

498

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

499

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

500

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

501

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

502

503

504 **Table 3**505 Characteristics of the 129 participants included in the *per protocol* group *

	rTAG-group	Placebo-group	EE-group
Gender (male/female)	23/18	16/27	25/20
Age, years (mean±SD, range)	61.2±9.8 33 - 75	61.7±7.9 34 - 73	59.9±9.0 34 - 72
Body weight (mean±SD, 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±SD, 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

506

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

510 *mean ± s.d.

511

512

513 **FIGURES**

514

515 **Figure 1:**

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

517

518

519 **Figure 2:**

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

524 *** Significant difference in omega-3 index between rTAG- and EE-group at t_3 ; $P < 0.001$.

525 ** Significant difference in the omega-3 index between rTAG- and EE-group at t_6 ; $P < 0.01$.

526 ‡ Significant differences in the omega-3 index between rTAG- and EE-group, respectively,
527 and placebo-group at t_3 and t_6 ; $P < 0.001$.



