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Moderate doses of EPA and DHA from re-esterified triacylglycerols but not from ethyl-esters lower fasting serum triacylglycerols in statin-treated dyslipidemic subjects: Results from a six month randomized controlled trial

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ABSTRACT

Recently, in a supplementation study over six months, it has been demonstrated that re-esterified omega-3 fatty acid triacylglycerols (n3-FA-rTAGs) led to a higher increase in omega-3-index compared to identical doses of n3-FA ethyl-esters (n3-FA-EEs), suggesting a better long-term bioavailability. The aim of this study was to examine whether differences occur between the two forms in affecting fasting serum lipid levels. 150 dyslipidemic statin-treated participants were randomized to corn oil as a placebo or fish oil either as rTAG or EE in identical doses (1.01 g EPA+0.67 g DHA). No changes in total cholesterol, HDL or LDL levels were observed. In the rTAG-group, but not in the EE-group, fasting serum TAG levels were significantly reduced from baseline after three and six months. There was no significant difference between the two n3-FA-groups. However, serum TAG levels were significantly lowered after six months in the rTAG-group compared to the placebo-group in contrast to the EE-group.

1. Introduction

Fasting or rather non-fasting TAG levels are independent risk factors for cardiovascular events [1–3], and moderately elevated TAG levels (more than 1.7–5.6 mmol/l [150–500 mg/dl]) are common in the adult population. The long-chain PUFAs EPA and DHA reduce both elevated fasting and non-fasting TAG levels [4–9]. The TAG lowering effect is thought to contribute to the reduction in total mortality and cardiovascular morbidity demonstrated for EPA+DHA in patients with cardiovascular disease or with a high risk for cardiovascular disease [10]. In a number of countries, such as the United States or Germany, up to 4 g/d EPA+DHA ethyl-esters (EEs) are licensed drugs in the treatment of hypertriglyceridemia.

In natural fish oils, EPA and DHA are bound in TAG, whereas in many supplements, EPA and DHA occur either as re-esterified TAG (rTAG) or as EE. In a recent randomized six month trial it has been shown that a moderate dose of n3-FA-rTAG (1.68 g/d EPA+DHA) resulted in a faster and greater incorporation of EPA and DHA in red blood cell (RBC) membrane fatty acids (assessed as the omega-3-index) compared to n3-FA-EE [11]. This indicated that the bioavailability of EPA and DHA is greater as rTAG than as EE. It remained unclear whether this difference in bioavailability of EPA+DHA (rTAG vs. EE) is translated into differences in biological effects. Using a subset of subjects from the previous study, the hypothesis that the different forms of n3-FA have different effects on fasting serum lipids with particular attention to TAG levels has been investigated in this study.

2. Patients and methods

A randomized, double-blind, placebo-controlled, parallel design supplementation trial of six months duration was undertaken. This multicentre study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Freiburg ethics commission international (feci code: 08/1294). Written informed consent was obtained from all participants.

2.1. Participants

Participants between 30 und 75 years were recruited via newspaper advertisements in four different German cities (Munich, Hamburg, Hanover, and Goslar). The aim of the study was to investigate the effects of the two n3-FA formulations on serum lipid levels as an adjunct therapy. Therefore, only hyperlipidemic participants solely and stably treated with statins for a minimum of three months were included in the study population.

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In- and exclusion criteria of potential participants were checked via telephone interviews and screening questionnaires sent by post. Participants were included in the study population based on the lipid levels of their latest medical check-ups. Exclusion criteria were serious illness (type 1 diabetes, cancer, coronary heart disease, and bleeding disorders); BMI > 35 kg/m^2; gastrointestinal disorders; medications known to affect lipid metabolism; daily consumption of oily fish; or ingestion of dietary LC n3-FA or plant sterol supplements. Only participants that fulfilled in- and exclusion criteria were included in the study population.

Approximately 300 participants were screened and 150 participants were enrolled in the study population. Participants who experienced changes in statin treatment (type or dose) after baseline investigation or during the intervention period were excluded from the lipid endpoint analysis for the per protocol population. This approach was taken to minimize the effort for the participants, who were not compensated for expenses. The participants were invited for four visits, where they had to appear in a fasting state, which is a very high expenditure, especially for those participants who had long journeys. A screening examination would have meant even more effort.

2.2. Study design

Enrolled 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 and clinical trial staff and investigators assessing the endpoints, were blinded to the randomization until all study data had been collected, checked and verified. To further facilitate the masking of the capsules, each of the study products was given two codes. Capsules were provided in numbered containers. The three different types of gelatine coated soft capsules contained (1) n3-FA as re-esterified TAG (rTAG-group; $n=52$); (2) corn oil (placebo-group; $n=49$); or (3) n3-FA as EE (EE-group; $n=49$). The capsules were provided by Dr. Loges and Co. GmbH, Winsen, Germany, a pharmaceutical company. The EPA and DHA content in both n3-FA supplements (rTAG and EE) was identical (252 mg EPA and 168 mg DHA per capsule). By ingesting four capsules, the daily dose was 1.01 g EPA and 0.67 g DHA, respectively. It should be noted that both supplements contained minor contents (84 mg/capsule) of other LC n3-FA (α-linolenic acid, ALA C18:3; stearidonic acid, SDA C18:4; eicosatetraenoic acid, ETA C20:4; heneicosapentaenoic acid, HPA C21:5; and docosapentaenoic acid, DPA C22:5), however, in identical quantities. The placebo capsules contained corn oil and were identical to the n3-FA capsules in all aspects. Each capsule contained 6 mg α-Tocopherol. 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.

Body height and weight were measured and fasting blood samples were collected by venipuncture at the baseline (t_0), and after three (t_3) and six months (t_6). Additionally, during each visit, participants completed a questionnaire to obtain information about changes in medication, dietary (e.g. weekly fish intake, preferred fish dishes, or species, respectively) and lifestyle habits (e.g. physically activity), as well as the tolerability of the capsules.

The volunteers' compliance was assessed by capsule-intake diaries and a count of left-over capsules between the three investigation dates.

2.3. Assessment of fasting serum lipids and omega-3-index

Blood samples were collected in serum tubes for determination of TAG, total cholesterol (TC), HDL, and LDL serum levels. Duplicate samples were analyzed in an external accredited medical service laboratory (LADR GmbH, MVZ, Hanover) using a spectrophotometric autoanalyser (OLYMPUS, Germany GmbH) with the manufacturer's assay kits, quality controls and reagents. The omega-3-index (RBC membrane EPA+DHA content) was analyzed as previously described [11,12].

2.4. Statistics

PASW Statistics version 18 (SPSS Inc., Chicago IL, USA) was used for the statistical analysis. The results are presented as mean $\pm$ SD. The sample size ($n=50$ per group) was calculated for differences in serum TAG levels after six months of intervention (primary endpoint) in order to be sufficient to prove a slightly more than medium-sized superiority (standardized difference of means $(\mu_1-\mu_2)/d=0.566$) of active treatment over placebo with a 5% level of significance and a power of 80%. Changes in TC, HDL, and LDL serum levels, as well as omega-3-index, were secondary endpoints. Similarly, changes in all parameters after three months of investigation time were defined as secondary endpoints. The significance level was set at 5%, hence $P \leq 0.05$ was considered significant.

Statistical comparisons were based on the *per protocol* (*PP*) *population*, defined as participants being compliant (ingesting $\geq 85\%$ of the given capsules), as well as completing all visits and not developing any of the exclusion criteria (particularly changes in statin treatment and other lipid-lowering medication) during the intervention period. In addition, statistical comparisons based on *modified intent-to-treat* (*MITT*) population were carried out, where any missing values were carried forward to subsequent time points.

Testing for normality (KS-Test) revealed normal distribution for all endpoints at baseline in each intervention group. Baseline levels and differences in serum TAG levels and omega-3 indices were compared among groups using one way ANOVA. Scheffé tests of contrast that were performed where appropriate. Changes in values of the different variables observed between baseline and t_3 or t_6 were evaluated within groups by Student's *t*-test for dependent samples.

3. Results

Of the 150 eligible participants who started the study, 42 were excluded from subsequent statistical analysis. The number of participants lost during the study and the number available for analysis are summarized in Fig. 1. Eighteen participants failed one or more of the inclusion criteria at the baseline visit or had changes in statin therapy (type and/or dose) shortly after. Four more participants decided to discontinue participation before the t_3 visit and four before the t_6 visit. Three participants failed to attend the t_3 visit because of illness, which was not related to the study product, and two additional participants had relevant changes in lipid metabolism affecting medication (medical treatment of thyroid dysfunction). Seven participants had changes in statin therapy (type and/or dose). Four more participants were excluded from the subsequent statistical analysis because of the lack of compliance. Hence, 108 participants completed the study protocol and were included in the PP analysis. Post-hoc power analysis revealed a power (89.4%) for the TAG-difference between rTAG vs. placebo-group (PP population) with an effect size of $d=0.783$, while the power for EE vs. placebo was lower (21.3% with an effect size of $d=0.278$). For the omega-3-index-difference