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Equal bioavailability of omega-3 PUFA from Calanus oil, fish oil and krill oil: A 12-week randomized parallel study

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Calanus AS

Abstract

The bioavailability of long-chain omega-3 polyunsaturated fatty acids (n3 PUFA) can be affected by the form in which they are bound. An alternative source of n3 PUFA is *Calanus finmarchicus* oil (CO), which, unlike fish oil (FO) and krill oil (KO), contains fatty acids primarily bound as wax esters. Recent studies have shown that n3 PUFA from CO are bioavailable to humans, but CO has not been compared to other marine oils such as FO or KO. Therefore, the aim of this study was to investigate the influence of 12 weeks supplementation with CO, FO and KO on the long-term n3 PUFA status in healthy volunteers. The Omega-3 Index (O3I), defined as red blood cell EPA + DHA content as a percentage of total identified fatty acids, was used as a measure to assess n3 PUFA status. Sixty-two participants (mean ± standard deviation [SD] age: 29.7 ± 8.43 years) completed the randomized parallel group study (CO group: $n = 21$, 4 capsules/day, EPA + DHA dose: 242 mg/day; FO group: $n = 22$, 1 capsule/day, EPA + DHA dose: 248 mg/day; KO group: $n = 19$, 2 capsules/day, EPA + DHA dose: 286 mg/day). At baseline, the three groups showed comparable (mean ± SD) O3I values (CO: 5.13 ± 1.12%, FO: 4.90 ± 0.57%, KO: 4.87 ± 0.77%). The post-interventional (mean ± SD) O3I increase was comparable between the three groups (CO: 1.09 ± 0.55%; FO: 1.0 ± 0.53%; KO: 1.15 ± 0.65%, all $p < 0.001$). The study confirms that CO can increase the n3 PUFA status comparable to FO and KO and is therefore an alternative marine source of bioavailable n3 PUFA, especially with regard to sustainability.

KEYWORDS
dietary fat, fatty acid analysis, fatty acid metabolism, fish oil, lipid absorption, n-3 fatty acids, nutrition, physiology, specific lipids

INTRODUCTION

Oils rich in omega-3 polyunsaturated fatty acids (n3 PUFA) are mainly of marine origin such as cold-water fish or krill. An alternative source of n3 PUFA is *Calanus finmarchicus*, a lipid-rich copepod from the North Atlantic Ocean (Payton et al., 2022). The bonding form of n3 PUFA in CO is fundamentally different from conventional

Abbreviations: ADA, adrenic acid (22:4n6); aLNA, alpha-linolenic acid (18:3n3); ANOVA, analysis of variance; ARA, arachidonic acid (20:4n6); CO, *Calanus finmarchicus* oil; COG, Calanus oil group; DBP, diastolic blood pressure; DGLA, dihomo-γ-linolenic acid (20:3n6); DHA, docosahexaenoic acid (22:6n3); DPAn3, docosapentaenoic acid (22:5n3); DPAn6, docosapentaenoic acid (22:5n6); EE, ethyl ester(s); EPA, eicosapentaenoic acid (20:5n3); FA, fatty acids; FFA, free fatty acids; FO, fish oil; FOG, fish oil group; gLNA, γ-linolenic acid (18:3n3); HDL, high-density lipoprotein; KO, krill oil; KOG, krill oil group; LA, linoleic acid (18:2n6); LDL, low-density lipoprotein; MUFA, monounsaturated fatty acid(s); N3 PUFA, omega-3 polyunsaturated fatty acid(s); N6 PUFA, omega-6 polyunsaturated fatty acid(s); O3I, Omega-3-Index; PUFA, polyunsaturated fatty acid(s); PL, phospholipides; RBC, red blood cell(s); rTAG, re-esterified triacylglyceride(s); SBP, systolic blood pressure; SD, standard deviation; SDA, stearidonic acid (18:4n3); SFA, saturated fatty acid(s); TAG, triacylglyceride(s); TC, total cholesterol; TFA, trans fatty acid(s); WE, wax ester(s).

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marine n3 oils. The dry mass of CO contains up to 60% lipids. 80%–90% of all fatty acids contained in CO are bound as wax esters (WE), while triacylglycerides (TAG) and phospholipids (PL) play a minor role with about 1%–10% and 5%–10%, respectively (Falk-Petersen et al., 1987; Schots et al., 2020). In WEs, fatty acids are esterified with mostly unsaturated, fatty alcohols (Kiauw de Munck-Khoe, 2018; Pedersen, Vang, & Olsen, 2014). Approximately 20%–30% of the fatty acids in WEs are n3 PUFA, of which the main components are eicosapentaenoic acid (20:5, EPA), docosahexaenoic acid (22:6, DHA), and stearidonic acid (18:4, SDA) (Pedersen, Vang, & Olsen, 2014).

In order to exert their physiological effects, it is a crucial precondition that n3 PUFA from different oils are bioavailable (Schuchardt & Hahn, 2013). The bioavailability of n3 PUFA depends on several exogenous and endogenous factors, such as (food) matrix effects or individual physiological conditions (Cholewski et al., 2018; Li et al., 2021; von Schacky, 2019). The chemical binding form of n3 PUFA has the greatest influence on bioavailability. N3 PUFA are present in different binding forms in different sources (Lane & Derbyshire, 2018; Zhang et al., 2019). For example, in crude fish oil (FO) n3 PUFA are mainly present as TAG and non-nesterified fatty acids (FFA), whereas in krill oil (KO) n3 PUFA are mainly bound as PL. In most refined fish oils, n3 PUFA are bound as re-esterified (re-constructed) TAG (rTAG) or ethyl-esters (EE). From various bioavailability studies, a ranking of the bioavailability of the different forms of binding is shown as follows: PL > rTAG > TAG > FFA > EE (Cholewski et al., 2018; Schuchardt & Hahn, 2013; von Schacky, 2014; Zhang et al., 2019).

There are several sample types for assessing n3 PUFA levels in the blood, including whole plasma, whole blood, platelets, leukocytes, and plasma lipid classes (i.e., phospholipids, cholesteryl esters, triglycerides, and free fatty acids). The Omega-3 Index (O3I)—defined as the EPA + DHA content of red blood cells (RBCs) as a percentage of total identified fatty acids—is the preferred biomarker for assessing the long-term n3 PUFA status in clinical practice and research (Schuchardt et al., 2022; von Schacky, 2020). RBC cell membranes consist almost exclusively of phospholipids esterified with EPA and DHA and are pre-analytically stable. In addition, the O3I reflects the EPA + DHA composition of other peripheral tissues (gastrointestinal tract, liver, myocardium, and kidney; Cholewski et al., 2018; Harris, 2010; von Schacky, 2014).

A recent study showed that the short-term bioavailability (72 h) of CO, and thus, WE-bound fatty acids, is comparable to that of EE-bound n3 PUFA from fish oil (Cook et al., 2016). The bioavailability of n3 PUFA from CO over 12 weeks has also been confirmed in recent studies (Burhop et al., 2022; Wasserfurth et al., 2020). However, comparative bioavailability studies with CO and other conventional n3 PUFA oils are lacking. Therefore, this study aims to evaluate the influence of CO on the O3I compared to FO with rTAG-bound n3 PUFA and KO with n3 PUFA mainly bound as PL. We hypothesized that the increase in O3I after 12 weeks of CO supplementation would be equivalent to the increase in O3I after FO and KO supplementation.

MATERIALS AND METHODS

Study design

The present study was conducted as a monocentric, open-label, randomized intervention trial with a parallel group design. It was conducted at the Institute of Food Science and Human Nutrition, Leibniz University of Hannover, Germany (hereinafter referred to as the "Institute"). The study consisted of a screening phase and a 12-week intervention phase. For data collection, one examination day was carried out at the beginning (t0) and at the end of the intervention (t12). Ethical approval was granted by the Ethics Committee of the Medical Association of Lower Saxony (Hannover, Germany). Written informed consent was obtained from all participants prior to their participation in the study, in accordance with the guidelines of the Declaration of Helsinki. This trial is registered in the German Clinical Trials Register (DRKS00025685).

Study participants

Participants in the study came from the greater Hannover area and the surrounding districts. They were recruited by Institute staff between June 2021 and June 2022 via press releases and advertisements in newspapers, social media, doctors' surgeries, and pharmacies, as well as via notices at the Leibniz University of Hannover. The main inclusion criteria were age ≥20 to ≤50 years, an omnivorous diet, and willingness to take capsules for 12 weeks.

The following were defined as exclusion criteria: severe chronic diseases, treatment with lipid-lowering agents or other drugs affecting lipid metabolism (e.g., statins, fibrates, bile acid exchange resins, phytosterols, etc.), use of n3 PUFA supplements, regular consumption of fatty fish >250 g (1 portion)/week, diagnosed blood coagulation disorders and use of anticoagulant drugs (except ASS), known allergy or intolerance to components of the test products. Both inclusion and exclusion criteria were established using a screening questionnaire and a screening examination. Each participant had to fulfill all of the inclusion and exclusion criteria.

Randomization

Participants were randomly allocated to the three study groups in a six-block system by a third person (Institute staff member) who was not involved in the study.

Allocation to the three groups was based on the covariates of sex, and age (in descending order). Group allocation was decided and communicated to participants by Institute's staff members only after the intervention phase and after the completion and evaluation of all questionnaires and laboratory analyses. The three groups were named as follows: Calanus oil group (COG), fish oil group (FOG) and krill oil group (KOG). The subjects and the Institute staff conducting the study were blinded to the allocation of the subjects to the different treatment groups.

Composition of supplements

The CO (Zooca® Lipids), FO (EPAX-Seamega 3424 TAG-XO Ultra), and KO (Seamega Krill Oil) supplements used varied in their composition (Table 1). In order to achieve approximately similar concentrations of EPA and DHA in the different groups, the daily intake of capsules was set as follows: 4x in the COG, 2x in the KOG and 1x in the FOG. With this number of capsules, the EPA + DHA intake was 242 mg in the COG, 248 mg in the FOG, and 286 mg in the KOG. In addition to EPA and DHA, CO also contained significant amounts of SDA and small amounts of alpha-linolenic acid (aLNA, 18:3n3) and other n3 PUFA. In FO and KO, the proportion of n3 PUFA other than EPA and DHA was 14% and 16%, respectively. Subjects were asked to take the capsules with mealtimes (breakfast, lunch, or dinner) with plenty of liquid (at least 200 mL).

Subjects were considered compliant only if they consumed at least 80% of the required capsules. At the end of the intervention period, the non-consumed capsules were used for the calculation.

Intervention phase and blood sampling

During the intervention phase from June 2021 to September 2022, participants were invited to the Institute for two visits: the baseline visit (t0) and the final visit after 12 weeks (t12). Blood samples were collected by a licensed physician after an overnight fast (≥12 h) between 6:00 am and 10:00 h (for each participant at the same time at each examination visit, if possible) by venipuncture of an arm vein. After collection, blood samples were treated pre-analytically processed and stored appropriately for analysis.

Analysis of serum lipids

Serum lipid analyses for total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and TAG were performed in an accredited and certified laboratory (Laborärztliche Arbeitsgemeinschaft für Diagnostik und Rationalisierung e.V., Hannover, Germany) to which the samples were delivered by courier.

Analysis of fatty acids in red blood cell membranes and the Omega-3 Index

Fatty acid levels in RBC membranes including the O3I were analyzed using dry blood spots (Harris & Polreis, 2016) and gas chromatography (DeFina et al., 2016) in a certified laboratory (OmegaQuant Analytics, Sioux Falls, SD, USA). Briefly, EDTA blood tubes were spun for 15 min at 2000 rpm for 15 min to isolate the RBC fraction. After removal of the plasma and buffycoat, 100 μL of packed RBC were mixed with 100 μL of an "Erythrosolve" solution (3 mg EDTA per mL of normal saline) to increase the adhesion of the RBCs on the filter cards. 50 μL of the dissolved RBCs were spotted onto OmegaQuant oxidant-pre-treated filter cards. The filter cards were sent to OmegaQuant Analytics (Sioux Falls, SD, USA) for fatty acid analysis.

Sample size and power calculation

A preliminary study (Wasserfurth et al., 2020), in which a 12-week administration of CO (2 g/day) resulted in a

TABLE 1 Dose and composition of the used Calanus oil (CO), fish oil (FO) and krill oil (KO) supplements

	CO		FO		KO	
Fatty acids	**mg/capsule**	**mg/day (4 capsules)**	**mg/ capsule**	**mg/day (1 capsules)**	**mg/capsule**	**mg/day (2 capsules)**
18:3n3 (aLNA)	7.0	28.0	3.0	3.0	7.08	14.2
18:4n3 FA (SDA)	45.0	180	11.5	11.5	17.7	35.4
18:5n3 FA	ND	-	1.0	1.0	ND	-
20:3n3 FA	0.50	2.0	1.0	1.0	ND	-
20:4n3 FA	4.50	18.0	6.50	6.50	2.95	5.90
20:5n3 FA (EPA)	31.5	126	128	128	93.2	186
22:5n3 FA (DPA)	1.50	6.0	15.0	15.0	ND	-
22:6n3 FA (DHA)	29.0	116	104	104	50.15	100
EPA + DHA	62.0	242	248	248	143	286
Total n3 PUFA	119	476	270	270	171	342

change in the O3I from t0: 6.0 ± 1.25 to t12: 7.3 ± 1.25%, that is, by 1.3%, was used as a basis for assuming the expected effects (scatter 1.25%). The tolerance limit was set at 10% of the observed value, that is, 0.13%. If an increase in the O3I of 1.3% was observed in the COG, increases in the FOG and KOG between 1.17% and 1.43% were considered comparable. To verify this statistically, the mean difference between the COG and FOG/KOG was determined. Assuming that the degrees of freedom are approximately 20% of the individual variation (20% of 1.25 = 0.25), $n = 17$/group would be sufficient for a 97.5% confidence interval of length ± 0.13%. Based on $n = 17$, with a maximum drop-out rate of 15%, the recommended number of cases was $n = 20$/group.

Statistical analyses

Statistical analysis was performed immediately after the end of the survey period using the SPSS statistical package (IBM SPSS Statistics 28.0; Chicago, IL, USA). The O3I was defined as the primary outcome. All other parameters were defined as secondary outcomes.

To test for normal distribution, all data were tested using the Kolmogorov–Smirnov test and visual inspection. If the data were normally distributed, one-dimensional analysis of variance (ANOVA) was chosen for the overall comparison. When statistical significance was found between two groups, a post hoc test followed by Bonferroni correction was also used. The Kruskal–Wallis *H* test was used When the data distribution was shewed. In addition, a *t*-test was used to test whether the changes between the results of the two examination days were significant. For skewed distributed data, the sign test was used instead.

An error of $p \leq 0.05$ was used as the basis for evaluation. In order to exclude the influence of slightly different doses of EPA + DHA in the different oils, a calculation of the dose-adjusted O3I delta value was performed after the intervention phase. The mean dose of EPA + DHA was set to 250 mg and the individual delta values of the groups were calculated as a function of dose using the rule of three. All other outcomes were exploratory and evaluated descriptively.

Data were analyzed by protocol. This means that only the data from participants who completed the study were analyzed. All measurements are presented as mean ± SD. All numbers are presented with three significant digits.

RESULTS

Baseline characteristics

Seventy-eight participants were screened for eligibility. Sixty-eight of the participants met the eligibility criteria and were randomized to one of the three study groups (Figure 1). Sixty-two subjects completed the study and the data were statistically analyzed. There were no differences in anthropometric parameters and blood lipids between the three groups (Table 2). The mean age of the study subjects was 29.7 ± 8.43 years. With a mean BMI of 24.3 ± 3.90 kg/m^2, the population can be classified as normal weight. Serum lipids indicated that the subjects were normolipidemic (Table 2).

Compliance of the participants

No adverse effects of the supplements were reported. Average compliance with capsule intake was 89%, with no significant differences between the groups (CO: 88%, FO: 87%, KO: 92%, $p = 0.192$).

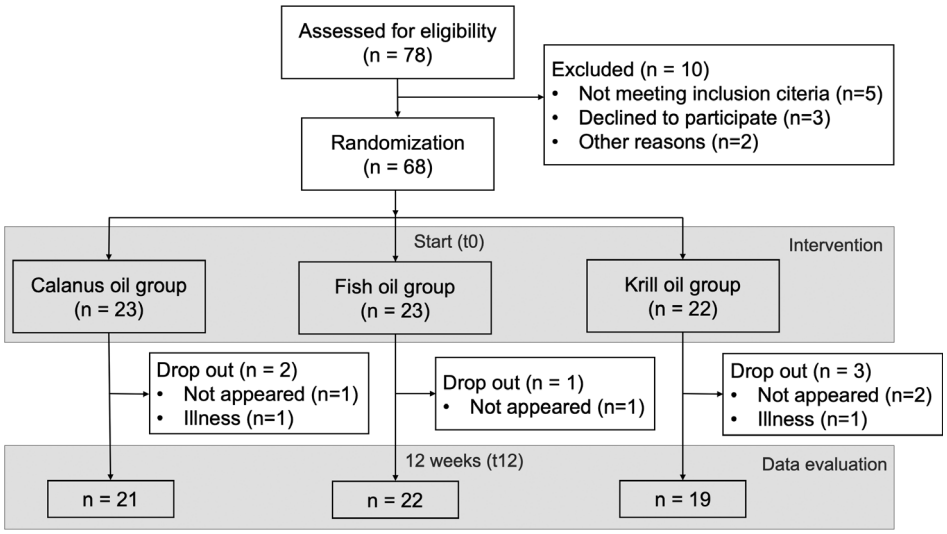

FIGURE 1 Flowchart describing recruitment and group allocation of study subjects

TABLE 2 Baseline demographic and clinical characteristics of the study population

	COG ($n = 21$) 14 women 7 men Mean ± SD	FOG ($n = 22$) 13 women 8 men Mean ± SD	KOG ($n = 19$) 11 women 8 men Mean ± SD	*p*-Value
Age (years)	29.7 ± 8.40	30.7 ± 9.40	29.4 ± 8.30	0.942
Body weight (kg)	74.6 ± 16.1	77.4 ± 19.3	72.7 ± 10.9	0.866
Height (cm)	175 ± 9.20	175 ± 9.90	172 ± 10.7	0.771
BMI (kg/m^2)	24.3 ± 3.90	24.9 ± 4.30	24.8 ± 5.50	0.777
SBP (mmHg)	115 ± 13.7	114 ± 11.5	112 ± 13.9	0.509
DBP (mmHg)	71.5 ± 9.30	72.8 ± 8.60	72.0 ± 9.80	0.633
TC (mmol/L)	4.78 ± 0.86	4.81 ± 0.64	4.91 ± 0.91	0.489
HDL (mmol/L)	1.53 ± 0.32	1.59 ± 0.34	1.45 ± 0.28	0.217
LDL (mmol/L)	2.87 ± 0.64	2.89 ± 0.47	3.03 ± 0.71	0.391
TAG (mmol/L)	1.02 ± 0.45	1.01 ± 0.37	1.24 ± 0.79	0.231

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TAG, triacylglycerides; TC, total cholesterol.

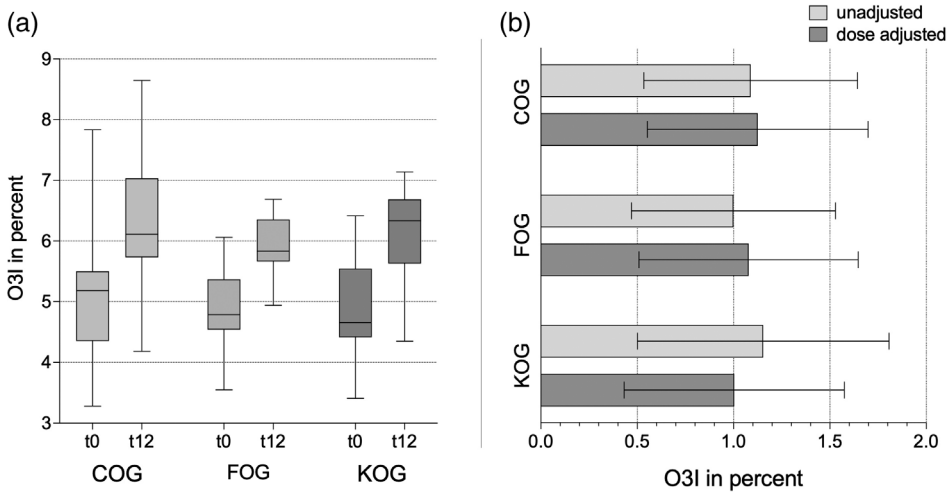

FIGURE 2 (a) Omega-3 Index (O3I) at baseline (t0) and after 12 weeks of intervention (t12). (b) Comparison of unadjusted and dose adjusted (increase in relation to EPA + DHA dose) O3I increase

Omega-3 Index

Baseline O3I values were slightly higher in the COG compared to the FOG and the KOG, but the differences between groups were not significant (Figure 2a, Table 3). After 12 weeks of intervention, the O3I increased significantly to a similar extent (all <0.001) in the COG (+1.09%), the FOG (+1.0%) and the KOG (+1.15%). Accordingly, the increase in the KOG was the highest, followed by the COG and the FOG. When adjusting for the different doses of EPA + DHA in the respective groups (dose-adjusted), the increase in O3I was highest in the COG, followed by the FOG and the COG (Figure 2b). However, none of these differences were significant. The high variability of the O3I values at t0 and t12 in the COG is striking (Figure 2a, Table 3). An individual in-person inspection shows that the initial O3I values of the participants was highly variable (Figure 3), but the O3I response was equally strong between individuals.

Other fatty acids in red blood cell membranes

In all three groups, a shift in the n6/n3 ratio was observed, as the levels of several n6 PUFA decreased. The decrease in n6 PUFA was significant for arachidonic acid (ARA, 20:4n6), adrenic acid (ADA, 22:4n6) and docosapentaenoic acid n6 (DPAn6, 22:5n6). Minor changes in linoleic acid (LA, 18:2n6), γ-linolenic acid (gLNA, 18:3n6), and dihomo-γ-linolenic acid (DGLA, 20:3n6) were not significantly different. Apart from EPA and DHA, no relevant changes were observed in the levels of other n3 PUFA. Remarkably, stearidonic acid (SDA, 18:4n3) levels in the COG were unchanged after the intervention. While the small increase in docosapentaenoic acid (22:5n3, DPAn3) levels in the COG was not significant, DPAn3 levels increased significantly in the FOG and the KOG.

While no significant changes were observed in the total amount of saturated fatty acids (SFA) and PUFA,

TABLE 3 Fatty acid levels of red blood cells (given as a percentage of total fatty acids) at the beginning (t0) and the end (t12) of the study

			COG ($n = 21$)			FOG ($n = 22$)			KOG ($n = 19$)			
Fatty acids		***t***	**Mean ± SD**	**△ t12-t0**	***p*-Value t0 to t12**	**Mean ± SD**	**△ t12-t0**	***p*-Value t0 to t12**	**Mean ± SD**	**△ t12-t0**	***p*-Value t0 to t12**	***p*-Value (ANOVA)**
n6-FA	18:2n6	t0	10.6 ± 0.90	0.11	0.228	10.6 ± 1.46	0.47	**0.011**	10.6 ± 1.05	−0.17	0.603	0.831
	"LA"	t12	10.7 ± 0.89			11.1 ± 1.24			10.4 ± 0.96			0.129
	18:3n6	t0	0.15 ± 0.07	0.02	0.473	0.14 ± 0.08	0.01	0.902	0.12 ± 0.06	0.00	0.908	0.265
	"gLNA"	t12	0.17 ± 0.10			0.15 ± 0.10			0.12 ± 0.05			0.387
	20:2n6	t0	0.30 ± 0.11	0.07	0.124	0.33 ± 0.14	0.01	0.922	0.30 ± 0.10	0.05	0.342	0.604
		t12	0.37 ± 0.11			0.34 ± 0.09			0.35 ± 0.15			0.689
	20:3n6	t0	1.79 ± 0.45	−0.14	0.144	1.68 ± 0.32	−0.10	0.116	1.86 ± 0.51	−0.14	0.139	0.522
	"DGLA"	t12	1.65 ± 0.37			1.58 ± 0.39			1.72 ± 0.37			0.540
	20:4n6	t0	13.9 ± 1.00	−0.48	**<0.001**	14.2 ± 1.38	−0.51	**0.015**	14.1 ± 1.08	−0.36	**0.012**	0.643
	"ARA"	t12	13.4 ± 0.99			13.7 ± 1.04			13.8 ± 0.98			0.447
	22:4n6	t0	2.94 ± 0.43	−0.20	**0.001**	2.99 ± 0.48	−0.32	**0.001**	2.90 ± 0.44	−0.29	**<0.001**	0.809
	"ADA"	t12	2.74 ± 0.41			2.67 ± 0.38			2.61 ± 0.44			0.588
	22:5n6	t0	0.57 ± 0.19	−0.07	**<0.001**	0.64 ± 0.18	−0.12	**<0.001**	0.62 ± 0.15	−0.08	**0.010**	0.440
	"DPAn6"	t12	0.50 ± 0.17			0.52 ± 0.15			0.53 ± 0.12			0.792
n3-FA	18:3n3	t0	0.30 ± 0.13	−0.05	0.151	0.26 ± 0.11	−0.07	**0.043**	0.26 ± 0.14	−0.02	0.723	0.522
	"aLNA"	t12	0.24 ± 0.09			0.19 ± 0.10			0.25 ± 0.08			0.116
	18:4n3	t0	0.10 ± 0.05	0.01	0.582	0.10 ± 0.04	0.02	0.186	0.12 ± 0.07	−0.01	0.744	0.868
	"SDA"	t12	0.11 ± 0.05			0.12 ± 0.07			0.10 ± 0.05			0.679
	20:5n3	t0	0.98 ± 0.25	0.42	**<0.001**	0.94 ± 0.23	0.46	**<0.001**	0.92 ± 0.22	0.53	**<0.001**	0.698
	"EPA"	t12	1.39 ± 0.38			1.40 ± 0.26			1.45 ± 0.30			0.846
	22:5n3	t0	2.35 ± 0.50	0.11	0.155	2.30 ± 0.42	0.18	**0.027**	2.41 ± 0.38	0.20	**0.003**	0.757
	"DPAn3"	t12	2.46 ± 0.46			2.48 ± 0.44			2.61 ± 0.40			0.525
	22:6n3	t0	4.15 ± 0.98	0.73	**<0.001**	3.96 ± 0.55	0.55	**<0.001**	3.95 ± 0.72	0.63	**<0.001**	0.640
	"DHA"	t12	4.88 ± 0.88			4.51 ± 0.52			4.59 ± 0.78			0.232
Sum for-mula	O3I	t0	5.13 ± 1.12	1.09	**<0.001**	4.90 ± 0.57	1.00	**<0.001**	4.87 ± 0.77	1.15	**<0.001**	0.558
		t12	6.27 ± 1.06			5.91 ± 0.05			6.03 ± 0.86			0.358
	SFA	t0	41.9 ± 1.30	0.19	0.286	42.0 ± 1.27	0.03	0.846	41.9 ± 1.07	0.30	0.269	0.926
		t12	42.1 ± 1.13			42.1 ± 0.92			42.2 ± 0.77			0.928
	MUFA	t0	18.3 ± 1.24	−0.48	0.054	18.2 ± 1.05	−0.46	**0.005**	18.3 ± 0.78	−0.60	**0.031**	0.898
		t12	17.8 ± 0.82			17.7 ± 1.02			17.7 ± 1.06			0.934
	PUFA	t0	39.8 ± 1.38	0.28	0.524	39.8 ± 1.62	0.44	0.138	39.7 ± 1.23	0.30	0.392	0.994
		t12	40.1 ± 1.03			40.2 ± 1.21			40.0 ± 1.14			0.888

(Continues)

TABLE 3 (Continued)

Fatty acids	t	COG ($n = 21$)			FOG ($n = 22$)			KOG ($n = 19$)			p-Value (ANOVA)
		Mean ± SD	△ t12-t0	p-Value t0 to t12	Mean ± SD	△ t12-t0	p-Value t0 to t12	Mean ± SD	△ t12-t0	p-Value t0 to t12	
n3:PUFA	t0	7.87 ± 1.10	1.22	**<0.001**	7.56 ± 0.65	1.15	**<0.001**	7.65 ± 0.97	1.34	**<0.001**	0.425
	t12	9.09 ± 1.10			8.71 ± 0.65			8.99 ± 0.99			0.391
n6:PUFA	t0	31.9 ± 1.57	−0.93	**<0.001**	32.2 ± 1.71	−0.71	**0.032**	32.1 ± 1.05	−1.04	**0.003**	0.811
	t12	31.0 ± 1.51			31.5 ± 1.20			31.0 ± 1.19			0.397
n6:n3	t0	4.14 ± 0.04	−0.67	**<0.001**	4.29 ± 0.47	−0.65	**<0.001**	4.26 ± 0.62	−0.76	**<0.001**	0.688
Quotient	t12	3.47 ± 0.05			3.64 ± 0.34			3.50 ± 0.49			0.442
TFA	t0	2.55 ± 0.49	−0.31	**0.009**	2.59 ± 0.59	−0.29	0.070	2.46 ± 0.56	−0.06	0.668	0.724
	t12	2.24 ± 0.40			2.30 ± 0.38			2.40 ± 0.37			0.415

Note: Bold indicates significant value.

the amount of monounsaturated fatty acids (MUFA) decreased slightly in all three groups ($p = 0.054$ in COG, $p = 0.005$ in FOG, and $p = 0.031$ in KOG). In the case of trans-fatty acids (TFA), the levels decreased significantly in the COG ($p = 0.009$), while a trend toward decreasing levels was observed in the FOG ($p = 0.070$). No changes in TFA levels were found in the KOG.

DISCUSSION

Only a few studies have been taken out that investigated the bioavailability of CO. Cook et al. focused on the short-term bioavailability of CO in plasma over 72 h (Cook et al., 2016). Two long-term studies focused on the metabolic effects of CO, but also found that CO, supplemented over 12-weeks, significantly increased the O3I (Burhop et al., 2022; Wasserfurth et al., 2020). The present study also aimed to investigate the influence of CO on the O3I, but in comparison to conventional n3 PUFA sources such as FO and KO.

All three marine oils were consistently well tolerated. Compliance was 89%, well above the average for a 12-week intervention study. The n3 oils, although at very low doses, significantly increased the O3I by approximately 1%. This O3I increase is in the range of O3I increase expected after supplementation with a relatively low dose of ∼250 mg EPA + DHA/day and a baseline O3I of ∼5%. Based on the predictive model of the O3I response to EPA + DHA supplementation from FO (rTAG) in healthy adults (Flock et al., 2013), 269 mg EPA + DHA/day would have to be given to increase the O3I from 5% to 6%. No significant differences were found between the three oils. This again allows the conclusion that CO, with mostly WE-bound fatty acids, has a comparably good effect on the O3I as FO and KO.

The study population was relatively homogeneous. Both age and sex structure as well as baseline levels of the O3I and other fatty acids were similar between the three groups were similar. The O3I of the entire study population is comparable to the German adult population, where an O3I of 4%–6% has been found in several studies (Schuchardt et al., 2022; Stark et al., 2016). Only the basal O3I values of the COG were somewhat more widely distributed than those of the other groups. However, this does not seem to have influenced either the individual O3I response or the average increase in the COG. Taken together, an influence of these factors or a difference between the groups with regard to these factors can be ruled out.

In agreement with the literature (Cholewski et al., 2018; Taha et al., 2014; Wasserfurth et al., 2020), we observed that the increase in EPA + DHA in all groups was accompanied by a decrease in n6 PUFA in all groups, resulting in a significant decrease in the n6:n3

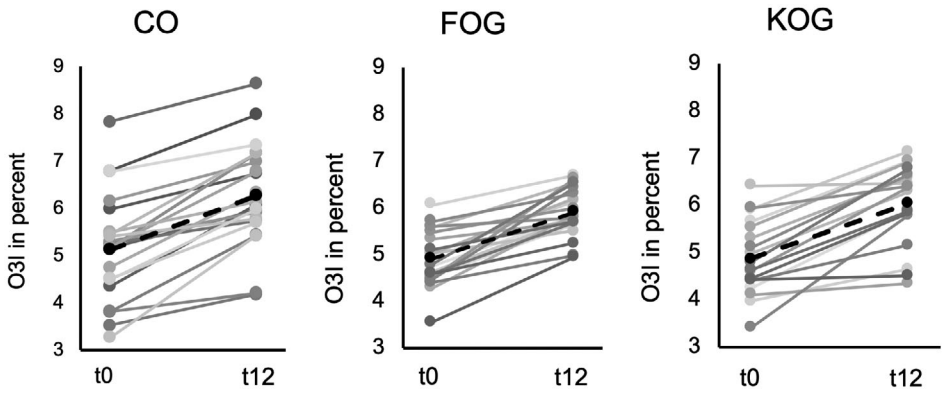

FIGURE 3 Omega-3 Index (O3I) values of individual participants at baseline (t0) and after 12 weeks of intervention (t12). The black, dotted line shows the mean of the group

ratio. As the total PUFA levels remained unchanged in all three groups, it can be concluded that the n6 PUFA in the RBC membrane are replaced by EPA and DHA. While LA levels remain largely unchanged, the decline in n6 PUFA levels is particularly pronounced for ARA and ADA. Again, the changes were similar between the COG, FOG, and the KOG.

To ensure that all groups consumed similar amounts of EPA + DHA, different numbers of capsules had to be taken by the subjects in the three groups. In order to eliminate the remaining differences in the EPA + DHA dosage between the groups, the dose-adjusted effect on the O3I was calculated and showed that the increase in O3I induced by CO is slightly higher compared to FO and KO. However, the difference is minimal and not significant.

Although, we did not observe differences in the O3I response, the bioavailability of n3 PUFA from the three oils may differ. The chemical bond form is the most important factor influencing the bioavailability of n3 PUFA (Schuchardt & Hahn, 2013). WE has not yet been considered important because conventional n3 oils contain TAG-, rTAG-, EE-, and PL-bound n3 PUFA in addition to FFA (Cholewski et al., 2018). WE-bound n3 PUFA have long been thought to be poorly digestible by mammals, including humans (Bogevik, 2011). Accordingly, most bioavailability studies have focused on the digestion and absorption of TAG, EE, or PL (Ahmmed et al., 2020). The few studies on WE suggest that intact WEs are not absorbable (Hargrove et al., 2004), leading to the assumption that the WE-bonds can be effectively cleaved by lipases (Hargrove et al., 2004). Yang et al. showed in an in vitro study that the hydrolysis of WE by porcine pancreatic lipase was 10–50 times slower than that of TAG (Yang et al., 1990). The hydrolysis rate is therefore comparable to that of EE-bounds, where the enzyme efficiency is approximately 2% of that of TAG. The hydrolysis rate is an important factor for the desorption rate of the fatty acids and is therefore an indication of the bioavailability of n3 PUFA (Cook et al., 2016; Savary, 1971). The reason for the low hydrolysis rate may be due to the hydrophobicity of intact WE, which makes emulsification difficult, and the inhibition of lipase by the hydrolysis products (Gouni-Berthold & Berthold, 2002; Place, 1992). It is suggested that bile acids, colipase, and a carboxyl esterase would be required in order to release the fatty acids from the fatty alcohols more rapidly (Gouni-Berthold & Berthold, 2002; Hargrove et al., 2004; Place, 1992). The slower hydrolysis of WE results in a longer residence time in the gastrointestinal tract compared to TAG or PL, which are already absorbed in the upper part of the gastrointestinal tract (Carey et al., 1983; Schots et al., 2020). Whether this "time-effect" ultimately improves the absorption of n3 PUFA from CO in the end remains unknown. Goretta showed that hydrolysed fatty acids from WE are well absorbed in vivo, at least in rats (Gorreta et al., 2002). Taken together, these findings support the notion that it is the hydrolysis itself, rather than the absorption of the WE hydrolysis products, that appears to be the limiting step (Hargrove et al., 2004). In view of the previous results showing a limited digestion of WE, it is noteworthy that CO increases the O3I comparable to FO and KO.

The O3I is determined not only by the amount of EPA + DHA in the respective oil, but also by other n3 PUFA that can be converted to EPA and DHA. There are differences in the n3 PUFA composition between the oils. In contrast to FO, the amount of n3 PUFA other than EPA and DHA is higher in KO and especially in CO. All three oils also contain SDA, but the SDA levels in CO are significantly higher than in FO and KO, resulting in higher total n3 PUFA levels in CO. SDA may contribute to the increase in O3I as already suggested by Wasserfurth et al. (Wasserfurth et al., 2020). SDA is found to a high extent in CO (15% of total FA; Pedersen, Salma, et al., 2014; Pedersen, Vang, & Olsen, 2014). The 18:4 n3 PUFA SDA is an important precursor in the metabolic pathway of EPA and DHA. In a recent study, we showed that acute intake of echium oil, another SDA-rich source (12% of total FA), significantly increased plasma EPA + DHA concentrations (Greupner et al., 2019). Harris et al. showed that SDA-enriched soybean oil (20% of total FA) significantly increased the O3I (Harris et al., 2008). It is likely that SDA can be more easily converted to EPA compared to aLNA, as the metabolic pathway from SDA to EPA bypasses the delta 6 desaturase, which is the rate-limiting enzyme in the conversion process (Saini et al., 2021). It has been suggested that SDA could be

considered as an alternative source of the cardioprotective n3 PUFA EPA & DHA. The significantly higher dose of SDA in the COG compared to the FOG and the KOG may induce a minimal difference in the O3I response. The fact that the RBC SDA levels in the COG remain constant from t0 to t12 (and similarly in the FOG and the KOG), supports this thesis. However, the daily amount of SDA in the COG (180 mg) is considerably higher than the amount of EPA ingested (126 mg/day), so it can be assumed that only a very small proportion of the SDA is converted to EPA or even DHA. What happens to the rest of the SDA is uncertain. To a minor extent, the low aLNA content in CO also contributed to the O3I increase. However, several studies (Brenna et al., 2009; Plourde & Cunnane, 2007) suggest that the conversion rate from aLNA to EPA (∼5%) and especially to DHA is low (∼1%).

Limitations

The study has a number of potential limitations.

Due to the high SDA content in the COG, the total daily amount of n3 PUFA was higher compared to the FOG and the KOG. In order to study the influence of the different n3 PUFA from CO on the EPA or DHA content in RBC, it would be necessary to compare oils that containing the similar levels of EPA, DHA, SDA and other n3 PUFA, which are not available. However, this is a general limitation of comparative n3 PUFA bioavailability studies.

The total fat content is also higher in the COG than in the KOG and significantly higher than in the FOG. It is known that a sufficient amount of fat is required for the digestion of bound n3 PUFA (Davidson et al., 2012; Lawson & Hughes, 1988). Whether the amount of fat in four CO capsules also affects the fat digestion and improves the digestion of WE-bound n3 PUFA compared to the relatively lower total fat content of one FO capsule or two KO capsules cannot be determined.

Due to the small number of cases and the limited age range of the subjects, the results cannot be extrapolated to the general population.

CONCLUSION

Our results show that the long-term effect of CO on the O3I increase is comparable to FO and KO and could serve as a new source marine source for cardioprotective EPA and DHA. Further studies are needed to investigate the fatty acid kinetics after a single dose of CO compared to FO and KO.

AUTHOR CONTRIBUTIONS

Franziska Vosskötter: Data evaluation and curation, writing-original draft preparation. **Milena Burhop:** Data acquisition, curation and evaluation. **Andreas Hahn:** Conceptualization and study design, methodology, reviewing and editing. **Jan Philipp Schuchardt:** Conceptualization and study design, methodology, writing, reviewing and editing, supervision. All authors have read and agreed to the submitted version of the manuscript.

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ETHICS STATEMENT

The study received ethical approval from the Ethics Committee of the Medical Association of Lower Saxony (Hannover, Germany) and written informed consent was obtained from all study participants. The assessment and processing of the data were completed following the Lower Saxony Data Protection Act, adhering to the Declaration of Helsinki and the principles of Good Clinical Practice.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

Ahmmed MK, Ahmmed F, Tian H, Carne A, Bekhit AE. Marine omega-3 (n-3) phospholipids: A comprehensive review of their properties, sources, bioavailability, and relation to brain health. Compr Rev Food Sci Food Saf. 2020;19:64–123. https://doi.org/10.1111/1541-4337.12510

Bogevik AS. Marine wax ester digestion in salmonid fish: a review: wax ester digestion. Aquac Res. 2011;42:1577–93. https://doi.org/10.1111/j.1365-2109.2010.02766.x

Brenna JT, Salem N, Sinclair AJ, Cunnane SC. Alpha-linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. Prostaglandins Leukot Ess Fat Acids. 2009;80:85–91. https://doi.org/10.1016/j.plefa.2009.01.004

Burhop M, Schuchardt JP, Nebl J, Müller M, Lichtinghagen R, Hahn A. Marine oil from C. finmarchicus enhances glucose homeostasis and liver insulin resistance in obese prediabetic individuals. Nutrients. 2022;14:396. https://doi.org/10.3390/nu14020396

Carey MC, Small DM, Bliss CM. Lipid digestion and absorption. Annu Rev Physiol. 1983;45:651–77. https://doi.org/10.1146/annurev.ph.45.030183.003251

Cholewski M, Tomczykowa M, Tomczyk M. A comprehensive review of chemistry, sources and bioavailability of Omega-3 fatty acids. Nutrients. 2018;10:1662. https://doi.org/10.3390/nu10111662

Cook CM, Larsen TS, Derrig LD, Kelly KM, Tande KS. Wax ester rich oil from the marine crustacean, *Calanus finmarchicus*, is a bioavailable source of EPA and DHA for human consumption. Lipids. 2016;51:1137–44. https://doi.org/10.1007/s11745-016-4189-y

Davidson MH, Johnson J, Rooney MW, Kyle ML, Kling DF. A novel omega-3 free fatty acid formulation has dramatically improved

bioavailability during a low-fat diet compared with omega-3-acid ethyl esters: the ECLIPSE (Epanova® compared to Lovaza® in a pharmacokinetic single-dose evaluation) study. J Clin Lipidol. 2012;6:573–84. https://doi.org/10.1016/j.jacl.2012.01.002

DeFina LF, Bassett MH, Finley CE, Barlow CE, Willis BL, Cooper T, et al. Association between omega-3 fatty acids and serum prostate-specific antigen. Nutr Cancer. 2016;68:58–62. https://doi.org/10.1080/01635581.2016.1115102

Falk-Petersen S, Sargent JR, Tande KS. Lipid composition of zooplankton in relation to the sub-arctic food web. Polar Biol. 1987;8:115–20. https://doi.org/10.1007/BF00297065

Flock MR, Skulas-Ray AC, Harris WS, Etherton TD, Fleming JA, Kris-Etherton PM. Determinants of erythrocyte Omega-3 fatty acid content in response to fish oil supplementation: A dose–response randomized controlled trial. J Am Heart Assoc. 2013;2:e000513. https://doi.org/10.1161/JAHA.113.000513

Gorreta F, Bernasconi R, Galliani G, Salmona M, Tacconi MT, Bianchi R. Wax esters of n 3 polyunsaturated fatty acids: A new stable formulation as a potential food supplement. 1—digestion and absorption in rats. LWT-Food Sci Technol. 2002;35:458–65. https://doi.org/10.1006/fstl.2001.0888

Gouni-Berthold I, Berthold HK. Policosanol: clinical pharmacology and therapeutic significance of a new lipid-lowering agent. Am Heart J. 2002;143:356–65. https://doi.org/10.1067/mhj.2002.119997

Greupner T, Koch E, Kutzner L, Hahn A, Schebb NH, Schuchardt JP. Single-dose SDA-rich echium oil increases plasma EPA, DPAn3, and DHA concentrations. Nutrients. 2019;11:2346. https://doi.org/10.3390/nu11102346

Hargrove JL, Greenspan P, Hartle DK. Nutritional significance and metabolism of very long chain fatty alcohols and acids from dietary waxes. Exp Biol Med. 2004;229:215–26. https://doi.org/10.1177/153537020422900301

Harris WS. The Omega-3 index: clinical utility for therapeutic intervention. Curr Cardiol Rep. 2010;12:503–8. https://doi.org/10.1007/s11886-010-0141-6

Harris WS, Lemke SL, Hansen SN, Goldstein DA, DiRienzo MA, Su H, et al. Stearidonic acid-enriched soybean oil increased the Omega-3 index, an emerging cardiovascular risk marker. Lipids. 2008;43:805–11. https://doi.org/10.1007/s11745-008-3215-0

Harris WS, Polreis J. Measurement of the Omega-3 index in dried blood spots. Ann Clin Lab Res. 2016;4:137. https://doi.org/10.21767/2386-5180.1000137

Kiauw de Munck-Khoe L. Omega-3-Wachsester aus *Calanus finmarchicus*. OM & Ernährung. 2018;163:66–8.

Lane KE, Derbyshire EJ. Omega-3 fatty acids—a review of existing and innovative delivery methods. Crit Rev Food Sci Nutr. 2018;58:62–9. https://doi.org/10.1080/10408398.2014.994699

Lawson LD, Hughes BG. Human absorption of fish oil fatty acids as triacylglycerols, free acids, or ethyl esters. Biochem Biophys Res Commun. 1988;152:328–35. https://doi.org/10.1016/S0006-291X(88)80718-6

Li J, Pora BLR, Dong K, Hasjim J. Health benefits of docosahexaenoic acid and its bioavailability: A review. Food Sci Nutr. 2021;9:5229–43. https://doi.org/10.1002/fsn3.2299

Payton L, Noirot C, Last KS, Grigor J, Hüppe L, Conway DVP, et al. Annual transcriptome of a key zooplankton species, the copepod *Calanus finmarchicus*. Ecol Evol. 2022;12:e8605. https://doi.org/10.1002/ece3.8605

Pedersen AM, Salma W, Höper AC, Larsen TS, Olsen RL. Lipid profile of mice fed a high-fat diet supplemented with a wax ester-rich marine oil. Eur J Lipid Sci Technol. 2014;116:1718–26. https://doi.org/10.1002/ejlt.201400052

Pedersen AM, Vang B, Olsen RL. Oil from *Calanus finmarchicus*—composition and possible use: a review. J Aquat Food Prod Technol. 2014;23:633–46. https://doi.org/10.1080/10498850.2012.741662

Place AR. Comparative aspects of lipid digestion and absorption: physiological correlates of wax ester digestion. Am J Physiol-Regul Integr Comp Physiol. 1992;263:R464–71. https://doi.org/10.1152/ajpregu.1992.263.3.R464

Plourde M, Cunnane SC. Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. Appl Physiol Nutr Metab. 2007;32:619–34. https://doi.org/10.1139/H07-034

Saini RK, Prasad P, Sreedhar RV, Akhilender Naidu K, Shang X, Keum Y-S. Omega−3 polyunsaturated fatty acids (PUFAs): emerging plant and microbial sources, oxidative stability, bioavailability, and health benefits—a review. Antioxidants. 2021;10:1627. https://doi.org/10.3390/antiox10101627

Savary P. The action of pure pig pancreatic lipase upon esters of long-chain fatty acids and short-chain primary alcohols. Biochim Biophys Acta BBA-Lipids Lipid Metab. 1971;248:149–55. https://doi.org/10.1016/0005-2760(71)90001-4

Schots PC, Pedersen AM, Eilertsen K-E, Olsen RL, Larsen TS. Possible health effects of a wax ester rich marine oil. Front Pharmacol. 2020;11:961. https://doi.org/10.3389/fphar.2020.00961

Schuchardt JP, Cerrato M, Ceseri M, DeFina LF, Delgado GE, Gellert S, et al. Red blood cell fatty acid patterns from 7 countries: focus on the Omega-3 index. Prostaglandins Leukot Essent Fatty Acids. 2022;179:102418. https://doi.org/10.1016/j.plefa.2022.102418

Schuchardt JP, Hahn A. Bioavailability of long-chain omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids. 2013;89:1–8. https://doi.org/10.1016/j.plefa.2013.03.010

Stark KD, Van Elswyk ME, Higgins MR, Weatherford CA, Salem N. Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. Prog Lipid Res. 2016;63:132–52. https://doi.org/10.1016/j.plipres.2016.05.001

Taha AY, Cheon Y, Faurot KF, MacIntosh B, Majchrzak-Hong SF, Mann JD, et al. Dietary omega-6 fatty acid lowering increases bioavailability of omega-3 polyunsaturated fatty acids in human plasma lipid pools. Prostaglandins Leukot Essent Fatty Acids. 2014;90:151–7. https://doi.org/10.1016/j.plefa.2014.02.003

von Schacky C. Omega-3 index and cardiovascular health. Nutrients. 2014;6:799–814. https://doi.org/10.3390/nu6020799

von Schacky C. Verwirrung um die Wirkung von Omega-3-Fettsäuren: Betrachtung von Studiendaten unter Berücksichtigung des Omega-3-Index. Internist. 2019;60:1319–27. https://doi.org/10.1007/s00108-019-00687-x

von Schacky C. Omega-3 index in 2018/19. Proc Nutr Soc. 2020;79:381–7. https://doi.org/10.1017/S0029665120006989

Wasserfurth P, Nebl J, Boßlau TK, Krüger K, Hahn A, Schuchardt JP. Intake of *Calanus finmarchicus* oil for 12 weeks improves omega-3 index in healthy older subjects engaging in an exercise programme. Br J Nutr. 2020;125:432–9. https://doi.org/10.1017/S0007114520002809

Yang L, Kuksis A, Myher J. Lipolysis of menhaden oil triacylglycerols and the corresponding fatty acid alkyl esters by pancreatic lipase in vitro: a reexamination. J Lipid Res. 1990;31:137–47. https://doi.org/10.1016/S0022-2275(20)42768-3

Zhang T-T, Xu J, Wang Y-M, Xue C-H. Health benefits of dietary marine DHA/EPA-enriched glycerophospholipids. Prog Lipid Res. 2019;75:100997. https://doi.org/10.1016/j.plipres.2019.100997

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