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Pharmacokinetics of Supplemental Omega-3 Fatty Acids Esterified in Monoglycerides, Ethyl Esters, or Triglycerides in Adults in a Randomized Crossover Trial

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ABSTRACT

Background: Omega-3 (n–3) fatty acid (FA) supplements increase blood concentrations of EPA and DHA. Most of the supplements on the market are esterified in triglycerides (TGs) or ethyl esters (EEs), which limits their absorption and may cause gastrointestinal side effects.

Objective: The objective of this study was to compare the 24-h AUC of the plasma concentrations of EPA, DHA, and EPA+DHA when provided esterified in monoglycerides (MAGs), EEs, or TGs, (primary outcomes) and evaluate their side effects over 24 h (secondary outcome).

Methods: This was a randomized, triple-blind, crossover, controlled clinical trial. Eleven women and 11 men between 18 and 50 y of age ingested, in random order, a single oral dose of ∼1.2 g of EPA and DHA esterified in MAGs, EEs, and TGs with low-fat meals provided during the 24-h follow-up. Eleven blood samples over 24 h were collected from each participant, and the plasma n–3 FAs were quantified. Friedman's paired ANOVA statistical rank test was used for the pharmacokinetic parameters and a chi-square statistical test was used for the side effects.

Results: The 24-h AUC of plasma EPA was ∼2 times and ∼1 time higher after the MAG compared with the EE and TG forms of n–3 FAs, respectively ($P \leq 0.0027$). Effects of the EE and TG treatments did not differ. The 3 supplements had similar eructation, dysgeusia, abdominal discomfort, nausea, and bloating side effects.

Conclusions: The plasma n–3 FA concentration in adults is greater after acute supplementation with n–3 FAs esterified in MAGs rather than in EEs or TGs, suggesting that with a lower dose of MAG n–3 FAs, the plasma n–3 FA concentrations attained are similar to those after higher doses of n–3 FAs esterified in EEs or TGs. This trial is registered at www.clinicaltrials.gov as NCT03897660. *J Nutr* 2021;151:1111–1118.

Keywords: pharmacokinetics, omega-3 fatty acids, monoglycerides, triglycerides, ethyl esters, supplement, human

Introduction

The n–3 α-linolenic acid (ALA; 18:3n−3) is considered essential because it cannot be synthesized by the human body, although it, and its metabolites, play a fundamental role in human physiology (1). A certain amount of long-chain n–3 fatty acid (FA) EPA and DHA can be synthesized in humans, but the conversion rate of ALA to EPA and DHA, respectively, is only ∼5% and ∼0.5% (2). Various organizations recommend consuming 2 servings of fatty fish per week or ∼500 mg/d of EPA and DHA (3, 4). This recommendation is based on the idea that n–3 FAs are involved in many physiological functions, such as membrane fluidity (5), anti-inflammatory response (6–8), maintenance of the cardiovascular system (9–13), and cognitive functions (14–19).

Despite the health benefits of consuming n–3 FAs and having higher blood concentrations of n–3 FAs, both are low in North America (20). n–3 FA supplementation is an alternative to consuming fatty fish to increase blood concentrations of EPA and DHA. However, 1 study reported that, when taking the supplement, the fat content of the meal can modify how effective the supplementation is (21). For instance, with a low-fat meal, EPA and DHA in a free fatty acid (FFA) form achieves higher blood concentrations compared with EPA and DHA esterified in the ethyl ester (EE) and triglyceride (TG) forms (22, 23). When n–3 FA supplements esterified in the EE and TG form were consumed with a high-fat meal, it was more effective in increasing their blood concentrations (21). However, with a high-fat meal, there is no consensus as to whether the TG form of EPA+DHA increases their blood concentrations more

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compared with the EE EPA+DHA esterified form (21), or if the 2 forms are equivalent (24). Therefore, how EPA+DHA are esterified and the fat content of the meal taken with the supplement might change the extent to which blood EPA and DHA increase.

Because of their chemical structure, FFA supplements are absorbed into the blood better than EE and TG supplements (22, 23, 25). However, FFAs are not commercialized because they are highly susceptible to oxidation (26), which limits their shelf life. Therefore, most of the n–3 FAs currently on the market are esterified in TGs or EEs. However, side effects are frequently reported with those forms (9, 27, 28).

In a previous study evaluating the pharmacokinetics of n–3 FA esterified in monoglycerides (MAGs), we found higher n–3 FA plasma concentrations than when n–3 FAs were provided esterified in EEs (29). MAGs are directly absorbed by the enterocytes without needing to be hydrolyzed by pancreatic lipases (30, 31). However, in our previous study, MAGs were compared with EEs only, the less well-absorbed esterified form of n–3 FA. Therefore, the objective of this study was to compare the plasma concentration of n–3 FAs and side effects of MAG supplements with EE and TG supplements in healthy men and women. Our hypothesis was that n–3 FAs esterified in MAGs would produce higher plasma concentrations over a 24-h follow-up period than an EE or TG esterified form of n–3 FA.

Methods

Study design and participants

This study was a randomized, crossover clinical trial conducted at the Research Center on Aging, Centre Intégré Universitaire de Santé et des Services Sociaux de l'Estrie–Centre Hospitalier Universitaire de Sherbrooke (CIUSSSE–CHUS), in Sherbrooke (Quebec, Canada). The Research Ethics Board of the CIUSSSE–CHUS approved this trial (reference number 2019-2954). This study was conducted in accordance with the Declaration of Helsinki. Interested individuals were informed of the requested involvement and risks. They were required to read the information and consent form and could ask questions before signing. All participants provided written informed consent prior to starting the trial. This study is registered at clinicaltrials.gov under number NCT03897660.

Recruitment took place from May to August 2019. A total of 33 adults aged 18 to 50 y old contacted us and underwent an initial phone screen. Anyone following a special/restrictive diet and/or currently taking n–3 FA supplements or who had taken them daily in the previous 6 mo and/or consuming fatty fish more than twice a week was excluded. Also excluded were those currently or previously (in the last 6 mo) smoking tobacco or marijuana and/or consuming >10 (female) or 14 (male) alcoholic drinks/wk. Other exclusion criteria assessed during the phone call were as follows: >1 h/d of high-intensity physical activity; blood donation in the past 2 mo; presence of systemic, gastrointestinal, hepatic, renal, cardiac, thyroid, or hormonal problems; or a diagnosis of schizophrenia, psychotic disorder, bipolarity, major depression (<5 y), panic disorder, and/or obsessive-compulsive disorder. Also, women who were pregnant or lactating or going through menopause were excluded. Phone-screened participants were then invited to the Research Center for further screening, which involved a blood draw after a 12-h overnight fast. The blood was collected in tubes containing EDTA as an anticoagulant agent. Blood lipemia (HDL cholesterol >1 mmol/L, LDL cholesterol <4.1 mmol/L, TGs ≤2.25 mmol/L) and fasting blood glucose concentration (3.5 to 6.1 mmol/L) were analyzed at the Centre Hospitalier Universitaire de Sherbrooke clinical laboratory using validated protocols/approaches for the medical clinics. Briefly, HDL-cholesterol, TG, and glucose concentrations were analyzed on a Cobas 8000 from Roche and using reagents and kits from Roche. HDL cholesterol was analyzed by an enzymatic colorimetric test with a homogenous phase, TG was measured by an enzymatic colorimetric test without a glycerol blank, and glucose was quantified with the hexokinase enzymatic reference. LDL cholesterol was calculated with the Friedewald equation. Glycated hemoglobin was measured by a fully automated D100 HPLC instrument-reagent system (Bio-Rad). Individuals with values outside the reference range for any of these biomarkers were excluded. Females of childbearing age were required to use a contraceptive method to avoid getting pregnant during the trial. To limit the influence of sex hormones on n–3 FA blood concentrations (32) when evaluating the different treatments, females had the pharmacokinetics day exclusively in the first 2 wk of their menstrual cycle (i.e., the follicular phase, during which female sex hormones are at a lower level).

Randomization and blinding

A simple randomization was performed to randomly allocate participants to 3 different treatment groups. Participants and research staff were blinded to treatment allocation. The capsules provided were identical in size, shape, and taste. During blood collection, each plasma sample had a random number between 1 and 726 to avoid knowing the participant's number, treatment code, and the time at which the sample was collected.

Procedures

The different treatments tested were n–3 FA supplements in MAGs, EEs, and TGs. Each capsule provided ~440 mg EPA + ~166 mg DHA (**Table 1**). To have matched EPA and DHA concentrations in each treatment, MAG and TG oils were generated from enzymatic esterification of the EE oil before being encapsulated and this procedure was performed by Neptune Wellness Solutions. A minimum 1-wk washout period between treatments was mandatory. Treatments were randomly assigned (**Figure 1**). Typically, a pharmacokinetic day started with collecting a 12-h fasting blood sample followed by oral intake of 2 capsules (~880 mg EPA + ~332 mg DHA) of 1 of the 3 esterified forms of n–3 FA supplements with breakfast. A typical breakfast consisted of 2 slices of bread, 60 mL of jam, 1 banana, and low-fat high-protein chocolate milk. Breakfast provided up to 587 kcal (66.8% carbohydrates, 25.5% proteins, 7.7% lipids). Over the course of the day, other blood samples were collected at 1, 2, 4, 5, 6, 8, 9, 10, 12, and 24 h after the n–3 FA dose intake. Lunch and dinner were provided after blood samples were collected 4 and 9 h after the n–3 FA dose intake. The lunch and dinner provided up to 780 kcal and 818 kcal, respectively,

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Supplemental Tables 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: ALA, α-linolenic acid; CIUSSSE–CHUS, Centre Intégré Universitaire de Santé et des Services Sociaux de l'Estrie–Centre Hospitalier Universitaire de Sherbrooke; C_{max}, maximum peak concentration reached in the blood; EE, ethyl ester; FA, fatty acid; FFA, free fatty acid; MAG, monoglyceride; TG, triglyceride; T_{max}, time required to reach C_{max}; T24h, concentration of n–3 remaining in the plasma 24 h after the single n–3 FA dose intake; %24h, the relative percentage of n–3 FAs to other FAs remaining in the plasma 24 h after the single n–3 FA dose intake; C24h, concentration of n–3FA remaining in the plasma 24h after the single n–3 FA dose intake.

TABLE 1 FA composition of the MAG, EE, and TG supplements[1]

FA	Type of FA	Supplement			CV
		MAG, mg	EE, mg	TG, mg	
14:0	Saturated	2.45 ± 0.20	0.14 ± 0.04	0.34 ± 0.09	1.31
16:0	Saturated	3.62 ± 0.37	1.62 ± 0.05	4.44 ± 0.16	0.450
18:0	Saturated	10.4 ± 0.34	5.37 ± 0.04	4.82 ± 0.29	0.446
16:1n–7	Monounsaturated	1.12 ± 0.04	0.22 ± 0.03	3.13 ± 0.14	1.00
18:1n–9	Monounsaturated	10.8 ± 0.10	15.00 ± 0.06	22.70 ± 0.41	0.373
18:1n–7	Monounsaturated	4.36 ± 0.05	4.67 ± 0.03	5.91 ± 0.13	0.165
18:2n–6	n–6	4.15 ± 0.06	4.24 ± 0.04	5.31 ± 0.11	0.142
20:3n–6	n–6	4.52 ± 0.02	4.00 ± 0.01	3.39 ± 0.02	0.143
20:4n–6	n–6	24.9 ± 0.07	30.5 ± 0.09	29.30 ± 0.43	0.104
18:3n–3	n–3	1.43 ± 0.02	2.17 ± 0.02	3.05 ± 0.02	0.366
20:5n–3 (EPA)	n–3	438 ± 0.75	447 ± 0.65	438 ± 4.43	0.012
22:5n–3	n–3	28.70 ± 0.12	20.20 ± 0.05	24.20 ± 0.44	0.173
22:6n–3 (DHA)	n–3	176 ± 0.76	161 ± 0.69	161 ± 1.69	0.052

[1] Values are mean ± SDs, $n = 4$ capsules/supplement forms. EE, ethyl ester; FA, fatty acid; MAG, monoglyceride; TG, triglyceride.

and a 12-h postdose snack provided 140 kcal. Total meals contained between 1800 and 2300 kcal and consisted of 72% carbohydrates, 17% proteins, and 11% lipids. Participants could remove some item of the menu if they were no longer hungry, but they had to keep the exact same menu for the next visits. Blood samples were centrifuged at 1700 × *g* for 10 min at 4°C and the plasma was stored at −80°C until further analysis.

FA extraction and analysis

FAs were extracted from the plasma samples using the Folch et al. method (33). For each sample, 11.5 mg of triheptadecanoin (17: 0 in TG form) was added to 100 μL of plasma as an internal standard. The FAs were saponified using KOH-methanol, protonated with HCl, and methylated with BF_3-methanol (14%), as previously described in Chevalier and colleagues (29, 34).

The FA composition of the plasma samples was analyzed by GC equipped with a flame ionization detector (model 6890; Agilent). One microliter of the sample was injected in splitless mode at 250°C into a 50-m BPX-70 fused capillary column (0.22-mm inner diameter, 0.25-μm film thickness; SGE). The methylated FAs were then carried by helium at a pressure of 107 kPa. A temperature gradient was applied as follows: 50°C maintained for 2 min, followed by an increase of 20°C/min up to 170°C. The temperature was maintained at 170°C for 10 min and thereafter increased by 10°C/min to 195°C. After 35 min at 195°C, the temperature was again raised to 220°C at a rate of 20°C/min and the column was kept at this temperature for 5 min. FAs coming out of the column were detected by the flame ionization detector at 260°C. The total run time was 61.75 min. Nu-Chek-Prep standards were used to identify peaks. The chromatogram analysis was performed using the OpenLab CDS ChemStation.

Pharmacokinetic parameters

n–3 FA pharmacokinetic metrics were calculated in both the plasma relative percentage to other FAs and the absolute concentration in the plasma. Data are represented as change (Δ) over baseline to evaluate the increase in n–3 FAs after taking the single-dose supplement. The AUC of the concentration and the relative percentage of n–3 FAs over 24 h were calculated using GraphPad Prism 7.03 software. C_{max} was defined as the peak concentration of EPA, DHA, and EPA+DHA for each participant. T_{max} was defined as the time required to reach C_{max}. Finally, T24h and %24h are, respectively, the concentration and the relative percentage of n–3 FAs to other FAs remaining in the plasma 24 h after the n–3 FA dose intake.

Assessment of side effects

In this clinical trial, side effects occurring throughout the day were monitored with a questionnaire. The side-effects questionnaire was used when each blood sample was taken—that is, at 0, 1, 2, 4, 5, 6, 8, 9, 10, 12, and 24 h post–dose intakes. The questionnaire collected data on dysgeusia, belching, nausea, abdominal pain, flatulence, and bloating. When a side effect was reported, the participant had to grade its intensity as low, moderate, or high. The side-effect frequency throughout the day and the number of participants reporting the side effect were quantified.

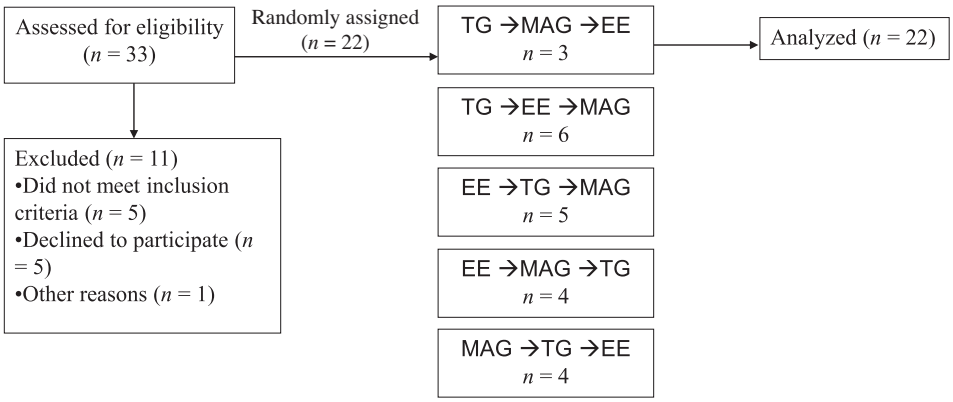

FIGURE 1 Clinical trial flowchart and distribution of participants to the monoglyceride, ethyl ester, and triglyceride n–3 FA supplementation groups. In the randomly assigned participants ($n = 22$), the "*n*" in each treatment order box represents the number of participants who were randomly assigned into that treatment order. EE, ethyl ester; FA, fatty acid; MAG, monoglyceride; TG, triglyceride.

TABLE 2 Participants' anthropometric characteristics[1]

Anthropometric characteristics	Total cohort ($n = 22$)
Age, y	27.9 ± 6.2
Male:female, *n*	11:11
BMI, kg/m^2	24.5 ± 4.0
Plasma TG, mmol/L	0.96 ± 0.54
Plasma HDL-C, mmol/L	1.37 ± 0.28
Plasma LDL-C, mmol/L	2.52 ± 0.82
Plasma glucose, mmol/L	4.46 ± 0.47
HbA1c, %	5.03 ± 0.37

[1]Values are means ± SDs, $n = 22$. HbA1c, glycated hemoglobin; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglyceride.

Statistical analysis

A website sample-size calculator (35) was used to calculate the sample size for this study. Sample size was calculated on the primary outcomes mean and SD of the 24-h AUC of the concentration (milligrams per deciliter) of plasma EPA, DHA, and EPA+DHA when 3 g of n–3 FA were provided in EE versus MAG using data from a previous pharmacokinetic study in our laboratory (29) with an α of 0.05 and a power of 80%. These calculations requested n <10 participants. We hence calculated the sample size with the relative percentage to ensure we had enough statistical power for this analysis, and when using the relative percentage of DHA, a minimum of 22 participants, 11 females and 11 males, was required to have the statistical power necessary to compare the 3 n–3 FA esterified forms in relative percentages. We hence decided to recruit this number of participants. The differences between the supplements have been calculated using the ratio of the SD to the mean of the 3 different forms.

The primary outcome of this study was the 24-h plasma concentrations of EPA, DHA, and EPA+DHA as defined above. Secondary outcomes included C_{max}, $\%_{max}$, T24h, %24h, T_{max}, and side effects. For comparison purposes between our results with other n–3 FA published pharmacokinetics studies, pharmacokinetic curves in relative percentages to other fatty acids were also provided. Statistical analyses were performed using IBM SPSS Statistics 25 and GraphPad Prism 7.03 software. The incremental AUC was calculated with the 24h-curves, by ignoring the areas below baseline. For the pharmacokinetic parameters (AUC, C_{max}, $\%_{max}$, C24h, %24h, T_{max}), we performed the Shapiro-Wilk normality test (α = 0.05) and data were not normally distributed. Therefore, Friedman's ANOVA statistical rank test for paired samples was used. The significant P value was therefore $P = 0.0167$. Dunn's multiple-comparisons test was used for the comparison between treatments (MAG vs. EE, MAG vs. TG, and EE vs. TG). Therefore, the significant P value for multiple comparisons for the primary outcomes was set at $P < 0.0056$.

For side effects, the statistical analyses were performed using IBM SPSS Statistics 25 and Microsoft Office Excel 2007 software. A chi-square statistical test was performed for nominal data for each of the side effects. Phi and Cramer's V values were used to measure the strength of the relation between the nominal variables—namely, side effects and treatments. Phi and Cramer's V values range from 0 (no association between the nominal variables) to 1 (complete association between the nominal variables). A post hoc test was performed to compare the prevalence of the different side effects and their intensities (mild, moderate, high) between treatments (MAG, EE, TG). The significant P value for side effects was set at $P < 0.0056$ after applying a Bonferroni correction to limit the probability of including a type I error. Each value on the pharmacokinetic curves is the mean ± SEM for the 22 participants.

Results

The participants' anthropometric characteristics are presented in Table 2. The participants' mean age was 28 ± 6 y and their BMI (kg/m^2) was 24.5 ± 4.0.

Primary outcomes: AUC of EPA, DHA, and EPA±DHA

Figure 2 presents the pharmacokinetic curves of EPA (left panels), DHA (middle panels), and EPA+DHA (right panels). The AUC of plasma EPA concentrations over 24 h was ∼2 and ∼1 times higher when n–3 FAs were esterified in MAGs vs. EEs or TGs, respectively ($P = 0.0027$, $P < 0.0001$) (Figure 2A, D) and the AUC of the EE form did not differ from that of the TG form. There was no statistically significant difference between the 3 esterified forms for the 24-h AUC of plasma DHA concentration and relative percentage to other FAs (Figure 2B, E). The ANOVA statistical test of the AUC of plasma EPA+DHA concentrations was statistically significant ($P = 0.0062$). After performing the multiple-comparisons test of the AUC of EPA+DHA, it was not possible to determined which treatment differed from the other at the Bonferroni-corrected P < 0.0056. The AUC of plasma EPA+DHA relative percentage to other FAs over 24 h (Figure 2C, F) was ∼1 time higher when provided in MAG versus EE and TG esterified forms ($P = 0.0046$, $P = 0.0009$).

Secondary outcomes: C_{max}, $\%_{max}$, T24h, %24h, T_{max}

The plasma EPA C_{max} was 3.5 and 1.3 times higher with the MAG form versus the EE and TG forms, respectively ($P = 0.0003$, $P < 0.0001$). The plasma EPA $\%_{max}$ was 2.9 and 1.5 times higher with the MAG form versus the EE and TG forms, respectively ($P < 0.0001$, $P < 0.0001$). EPA T_{max} was 5.5 h when provided as MAG and was earlier than the EE and TG forms that reached EPA T_{max} 8.6 and 7.4 h after the dose intake, respectively ($P < 0.0001$, $P = 0.0125$). Finally, T24h was 3 and 1 times higher when provided in MAGs versus EEs and TGs, respectively ($P = 0.0014$, $P = 0.0065$) (Figure 2A, D). For DHA, for all the secondary outcomes expressed as absolute concentration or relative percentage to other FAs, there was no difference between the 3 supplements (Figure 2B, E). The plasma EPA+DHA C_{max} with the MAG supplement was 2.6 and 1.1 times higher than the EE and TG supplements, respectively ($P = 0.0009$, $P = 0.0046$). The plasma EPA+DHA $\%_{max}$ was ∼1.8 times higher with the MAG form versus the EE and TG forms, respectively ($P = 0.0046$, $P = 0.0046$). EPA+DHA T_{max} was 5.6 h when provided as MAGs, 7.5 h when provided as EEs (not significant), and 9.0 h when provided as TGs ($P = 0.0117$). There was no statistical difference in the concentration of EPA+DHA at T24h (Figure 2C, F). There was no difference in the pharmacokinetics between males and females (data not shown).

Secondary outcomes: side effects

The different side effects reported by the participants and their intensity are reported in Table 3. None of the participants reported high-intensity side effects. The result presents the number of times the side effect was reported, out of a possibility of 242, and the number of participants reporting the side effect, out of a possibility of 22. For the number of participants reporting the side effects, there was no significant difference between the 3 forms of treatments for all the side effects measured. For the number of side effects reported, there was a significant and positive association between TG supplement and moderate eructation, moderate dysgeusia, and moderate bloating. Each of these moderate side effects was, however, reported by only 1 participant. The association of eructation, dysgeusia, and bloating with the TG esterified form was significant but with a low magnitude, since the frequency of these side effects was

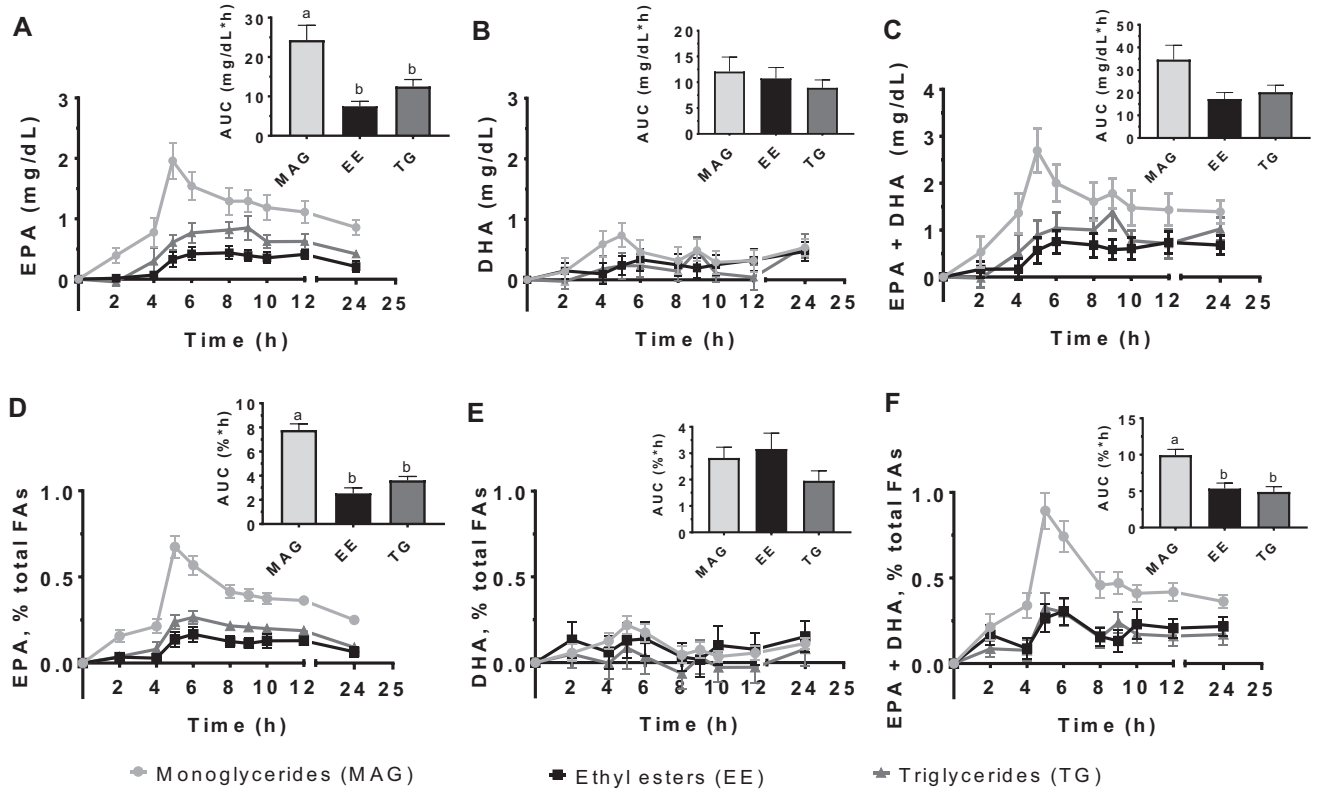

FIGURE 2 Absolute (A–C) and relative (D–F) plasma EPA (A, D), DHA (B, E), and EPA+DHA (C, F) concentrations in adults in the 24 h after taking MAG, EE, and TG n–3 FA supplements. AUCs are shown in the insets. Values are means ± SEMs, $n = 22$. There were 2 missing values: 1 participant 9 h after the intake of the EE supplement and another participant 4 h after the intake of the EE supplement. Since only 1 point was missing per participant, the AUC for these participants were calculated without these missing data. Friedman's ANOVA statistical test for paired samples was performed and the significant P value was set at $P < 0.0056$. Labeled AUC means without a common letter differ, $P < 0.0056$. EE, ethyl ester; FA, fatty acid; MAG, monoglyceride; TG, triglyceride.

low ($P = 0.00006$, Phi = 0.158; $P = 0.0016$, Phi = 0.138; $P = 0.0005$, Phi = 0.146). Mild abdominal pain/discomfort was reported 8 times by 1 participant when taking the MAG esterified form and once with the TG supplement. There was a significant and positive association between mild abdominal pain/discomfort and the MAG supplement, compared with the EE and TG supplements ($P = 0.001$, Phi = 0.122). There were no significant differences for nausea and flatulence between the treatments.

Discussion

In this study, we hypothesized that an MAG form of n–3 FAs would increase the 24-h plasma concentrations of EPA and DHA more than EE and TG esterified forms of n–3 FAs. This study partially confirmed our hypothesis since the plasma concentration over 24 h of EPA when given in the MAG form was 1–2 times higher than with the EE and TG forms. Furthermore, with the MAG form, EPA concentration in the plasma peaked earlier (T_{max}) than with the EE and TG forms. However, it is possible that the T_{max} was modulated by the intake of the meals. The lunch was given 4 h after the single-dose intake and this may have influenced the secretion of the remaining lipids from the enterocytes into the bloodstream (36). This could have contributed to the T_{max} at 5 h reached in many participants. One other explanation of our results is that the structure of the MAG allows direct absorption of the n–3 FAs by enterocytes (31). Unlike the EE and TG forms which require pancreatic lipases to hydrolyze n–3 FAs from the glycerol or the ethyl backbone, the MAG form does not require this hydrolysis step (37). Furthermore, the hydrolysis of EEs and TGs by pancreatic lipases is dependent on meal lipid content. Lawson and Hughes (21) showed that taking an EE n–3 FA supplement with a high-fat meal containing 44 g of lipids increased the plasma concentration of EPA+DHA by 3 times compared with a meal containing 8 g of lipids, while absorption of TG EPA improved by 69–90% with the high-fat meal compared with the low-fat meal. In our study, MAG, TG, and EE n–3 FAs were consumed with a meal containing ~5.5 g fat. Therefore, the higher EPA plasma concentration when provided in MAG compared with the other forms is valid for a low-fat diet, but whether the same result applies under a high-fat diet remains to be established.

Very few studies reported the pharmacokinetics of n–3 FA supplements with the MAG form. The majority of studies in humans used n–3 FA supplements in the form of FFA, EE, and TG, as reviewed by Ghasemifard et al. (25) in 2014. The overall conclusion suggests that n–3 FA plasma concentration was higher when provided in FFA > TG > EE (25), although there are other studies suggesting that the TG form provides higher n–3 FA plasma concentrations than FFA and even than MAG (38, 39). However, according to another study, when pancreatic lipases are inhibited, the MAG form leads to a higher n–3 FA plasma concentration than the TG form (40). The discrepancy between studies may be due to differences in study design and dietary intake, which was not controlled for in the latter studies. Considering that the MAG form should

TABLE 3 Side effects and their intensities reported by the adults in the 24 h after taking MAG, EE, and TG n–3 FA supplements[1]

	Total side effects reported[2]				Number of participants[3]			
Side effects and intensity	MAG	EE	TG	*P*	MAG	EE	TG	*P*
Dysgeusia								
None	227	219	223	0.008	12	12	14	0.728
Mild	15	21	11		10	10	7	
Moderate	0	2	8*		0	1	1	
Eructation								
None	226	220	220	0.001	11	12	13	0.618
Mild	16	22	14		11	10	8	
Moderate	0	0	8*		0	0	1	
Nausea								
None	242	241	238	0.073	22	21	20	0.351
Mild	0	1	4		0	1	2	
Moderate	0	0	0		0	0	0	
Abdominal pain/discomfort								
None	235*	242	241	0.004	21	22	21	0.597
Mild	7*	0	1		1	0	1	
Moderate	0	0	0		0	0	0	
Flatulence								
None	242	239	241	0.091	22	21	21	0.402
Mild	0	0	1		0	0	1	
Moderate	0	3	0		0	1	0	
Bloating								
None	240	240	230*	0.004	20	20	19	0.730
Mild	2	2	6		2	2	2	
Moderate	0	0	6*		0	0	1	

[1] *P* values are derived by a chi-square test followed by a post hoc analysis to determined which treatment group differed from the other. A significant interaction between treatment and intensity of each side effect is identified with the asterisk (*). Significance was set at $P < 0.005$. EE, ethyl ester; FA, fatty acid; MAG, monoglyceride; TG, triglyceride.
[2] Total side effects reported refers to the number of times the side effect was reported after consuming the specific supplement (22 participants × 11 time points, $n = 242$).
[3] Values refer to the number of participants reporting the side effect after consuming the specific supplement ($n = 22$ participants).

be absorbed similarly to the FFA form, our results are similar to several n–3 FA supplementation studies (22, 23, 25, 40, 41). Furthermore, we did not find any significant differences between the EE and TG n–3 FA esterified forms, which is in accordance with previous studies (24, 42) and unlike others (21, 22).

For the primary outcomes, plasma EPA and DHA concentrations 1 h after dose intake fell below the initial fasting concentrations, resulting in a negative AUC for this time point. One potential explanation relates to the increase in blood sugar and insulin secretion after the breakfast since it was rich in carbohydrates. Higher blood carbohydrate concentrations increase the secretion of insulin by the pancreas. This insulin secretion will reduce lipolysis, increase lipogenesis, and reduce blood FA concentrations (43). We hypothesized that FA blood uptake would likely reduce n–3 FA concentration in the bloodstream shortly after consuming the meal, which would explain the drop in EPA and DHA 1 h after the meal.

One of the secondary aims of this study was to evaluate whether MAGs caused fewer side effects than EEs or TGs. The side effects measured were those most frequently reported when consuming n–3 FAs (27, 28, 44). In general, side-effect frequency was low and no participant reported any high-intensity side effects during the study. A total of 13 of the 22 participants reported having at least 1 side effect after consuming MAGs, which is similar to the other esterification forms tested. Therefore, none of the esterified n–3 FA forms had no side effects, which is in line with previous studies (44–46). The present study suggests that side effects were more related to the softgel, which allows the release of fish oil in the stomach, than to the n–3 FA esterification form per se. This idea is supported by a previous study that showed that with the use of a cellulose acetate trimellitate–coated capsule, with a pH of 5.5 and release time of 60 min, there were no side effects, unlike with other materials used for encapsulation (47). This type of material avoids the release of the n–3 FA oil in the stomach and allows the n–3 FA to be released in the upper intestine, which is hypothesized to limit upper gastrointestinal side effects. Hence, the n–3 FA esterification form per se does not seem to be responsible for the side effects, but the fish-oil release in the stomach instead of the small intestine might be responsible for them.

This study had both strengths and limitations. One strength is the crossover design, which was robust enough to detect significant differences between treatments. In this study, every participant was his/her own control, which limits variability within the dataset. Another strength is the control of dietary intakes on the pharmacokinetic days, limiting variability in n–3 FA absorption caused by having a different dietary intake on those days. A major strength of this study is that the results are reported in concentration and relative percentage to other FAs. Several studies only report relative percentage, but the actual concentration in the plasma is what really matters from a physiological standpoint. Side effects were also evaluated using the frequency and intensity questionnaire at each blood draw. In this study, 1 limitation is related to the possibility of a type 1 error since we did not control for multiple testing for secondary outcomes. However, the *P* value for determining a significant

difference has been adjusted for the primary outcomes since there were the 3 primary outcomes (24-h AUC of EPA, DHA, and EPA+DHA). Another limitation of this study is that none of the participants took the supplements in the following order: MAG followed by EE and TG (Figure 1). Finally, the low dose of DHA limited the extent to which plasma DHA increased over 24 h. Moreover, the results of this study were probably biased by the low-fat diet the participants were given and the T_{max} results may be biased by the second meal effect of the 4-h postdose lunch. Since this was a pharmacokinetics study, it does not represent the pharmacodynamics of the 3 different esterified forms, which is another limitation of this study.

According to this study, a single-dose of n–3 FA esterified in MAG produced a higher plasma EPA concentration over 24 h and reached a higher maximum concentration and a higher EPA concentration remaining in the plasma 24 h after the dose intake compared with the EE and TG n–3 FA esterified forms. The number of side effects of the different esterified forms was similar.

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