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ARTICLE

Intestinal bioavailability of n-3 long chain polyunsaturated fatty acids influenced by the supramolecular form of phospholipids

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The aim of this work was to study the bioavailability of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), carried by marine phospholipids (PL) and formulated in different supramolecular forms. Marine PL were administrated in rats either (1) in bulk form, or (2) as an oil-in-water emulsion, or (3) as liposomes. Each dietary formulation was characterized by a similar fatty acid (FA) profile and provided the same n-3 LC-PUFA amount. Intestinal bioavailability of n-3 LC-PUFA was monitored in the lymph compartment in a duct fistula model. On the one hand, the emulsification of plant oils with PL increased the overall intestinal absorption of dietary FA by 84% without affecting the lymph FA profile compared with the bulk form, suggesting that emulsification favoured the absorption of the total dietary FA derived from both triglycerides (TG) and PL. On the other hand, the liposome form did not modify the lymph lipid amount compared with the bulk form, but specifically increased the n-3 LC-PUFA levels. The dietary forms of PL influenced the position of some FA on the glycerol backbone of lymph TG and PL. In conclusion, using marine PL as an emulsifier promoted total FA absorption independently of the dietary lipid carrier (TG or PL) and the FA type. Structuring PL as liposomes specifically increased the intestinal bioavailability of FA esterifed in this lipid class, such as DHA, resulting in a higher incorporation into lymph lipids. Thus, using specific PL supramolecular forms would guide n-3 LC-PUFA towards total lipid absorption or specific FA absorption, according to the dietary needs.

1. Introduction

Today, no one questions the physiological roles of eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) as biological membrane components, modulators of gene expression, or precursors of lipid mediators (*i.e.* oxylipins). However, the mean consumptions of n-3 LC-PUFA in the French adult population, resulting from the latest French epidemiological survey (INCA3)[1] showed that EPA and DHA intakes (117 and 169 mg per day) were at least 32% lower than the guidelines (250 mg per day for each fatty acid (FA)[2]). This could favour the occurrence of some pathologies, including neurological, cardiovascular or inflammatory disorders. In order to cover the physiological needs, two strategies can be envisaged: (1) increasing the consumption of oily fish, seafood and derivative products, as the main natural sources of EPA and DHA, but taking the risk of increasing the total lipid intake, or (2) improving EPA and DHA bioavailability by modulating their molecular and/or supramolecular forms of intake. While in the diet, n-3 LC-PUFA are mainly consumed as triglycerides (TG), the nutritional interest of other molecular lipid species, such as marine phospholipids (PL), has emerged. The positive effects of fish oil consumption (and therefore of TG) on health have been clearly demonstrated[3–5]. Nevertheless, the impact of PL as an n-3 LC-PUFA carrier is still being debated. Several studies compare the metabolic fate of EPA and DHA delivered by TG or PL in oily formulations. Depending on the experimental design used (control group, duration and mode of supplementation, quantity and amount of lipids in the diet, EPA/DHA dietary ratio, tissue analysed), data have suggested variously that PL are more efficient in increasing n-3 LC-PUFA bioavailability[6–12], others studies have been in favour of TG[6,12–16], while still others have pointed out a similar effect of TG and PL[6,8,10,11,16]. Paying particular attention to the lipid formulation to ensure a reliable comparative basis, we have recently showed that n-3 LC-PUFA, provided by TG or PL in bulk phase, were similarly absorbed but differently incorporated and distributed in lymph lipids[17].

Due to their amphiphilic properties, PL exhibit a wide scope of applications in terms of formulation, *i.e.* as an emulsifier in oily systems, and as self-assembled molecules in liposomes. Emulsification is known to increase the intestinal absorption of dietary FA, both in humans[18–20] and animals[21–24]. However, to our knowledge, no data specifically relates to the absorption of FA issued from PL used as an emulsifier and only one study used PL in liposomes to carry dietary FA. In this last case, the DHA level was increased by 66% in lymph lipids compared to fish oil[25]. Nevertheless, in this study, rat groups differed both in terms of molecular lipid species carriers for n-3 LC-PUFA (PL

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vs TG) and supramolecular forms (liposomes for PL *vs* bulk oil for TG), making it difficult to attribute a higher beneficial value to the supramolecular form only.

Hence, the objective of the present study is to provide data on the intestinal absorption of n-3 LC-PUFA from marine PL, according to their supramolecular organisation. Thus, three dietary formulations were compared: PL dispersed in an oily phase, PL used as an emulsifier to stabilise the oil/water interface and PL constituting the bilayers of liposomes. Special attention was paid to ensure that the EPA:DHA ratio and FA profile in the different dietary lipid sources remained the same in order to ensure comparable results. Intestinal absorption of n-3 LC-PUFA was studied *in vivo* in a rat lymph duct fistula model, by analysis of lymph lipids in terms of FA composition and molecular distribution in TG and PL classes.

2. Material and methods

2.1. Lipid formulations

PL were extracted from Lecimarin F50 (Novastell, Etrepagny, France) by solvent fractionation using cold acetone as described by Sehl et al.[17,26]. Polar lipids were recovered by centrifugation (1050 g, 5 min, 4 °C; Sorvall ST-40R, Thermo Fisher Scientific, Waltham, MA, United States of America) and stored at - 20 °C until use. Phospholipid species of the polar lipid extract were characterized by proton nuclear magnetic resonance (^{1}H NMR) according to Cansell et al.[27]: nearly three quarters of PL were represented by phosphatidylcholine (see Supplementary data 1). The glyceride structure of the marine PL was determined enzymatically (see Lipid analysis section). Both EPA and DHA were mainly distributed in the internal (*sn-2)* position (>74%) of the PL molecules (see Supplementary data 2).

The lipid phase was prepared by solubilizing marine PL (13 wt%) in a blend of plant oils (flaxseed/copra/grapeseed/palm/oleic sunflower oils; 1:6:6:30:57, w/w/w/w/w). The lipid mixture was prepared at room temperature under nitrogen in order to prevent PUFA oxidation. The FA composition of the mixture is presented in Table 1.

The oil-in-water (O/W) emulsion was prepared at 4°C under nitrogen flux. The lipid phase was manually dispersed in an aqueous solution (sodium deoxycholate at 0.45% (w/w) in water) to an oil fraction of 45 g/100 g. The coarse O/W emulsion was then sheared using an Ultraturrax Apparatus (IKA, Staufen, Germany) equipped with a generator axis (10 mm S25-N-10G; IKA).

The liposome suspension was obtained by hydration of a marine PL film with distilled water (pH 7.4) (130 mg PL/mL). The suspension was maintained under agitation, at ambient temperature and under nitrogen flux, until a turbid medium without any visual aggregate was obtained.

After preparation, a direct visualization of oil droplets and liposomes was carried out using a phase-contrast microscope (Olympus BX51 X40, Olympus, Germany), equipped with an oil immersion 100X lens. Pictures were taken using a video camera equipped with an analog contrast device (CCD camera, Colorview I, Soft Imaging System, Olympus) and was reprocessed with Analysis software (Olympus). Oil droplets were homogeneously distributed in the aqueous phase with an average droplet size of 75 µm (see Supplementary data 3). The liposome suspension prepared with marine PL exhibited small (< 10 µm), heterogeneous particles (see supplementary data 3). Mean particle diameter, evaluated by the volume-weighted average diameter $d_{4,3}$, and particle size distribution were determined by static light scattering using a Mastersizer 2000 hydro 5M apparatus (Malvern Instruments S.A, United Kingdom) equipped with a helium-neon laser, considering a refractive index of 1.330 for water and 1.460 for lipid particles. The optical observations were confirmed by the particle sizing measurements, especially the heterogeneity of the liposome suspension with two populations centred on 0.3 and 6.3 µm (see Supplementary data 3). Based on the mean diameter measurements, the specific surface area could be evaluated to 0.24 and 20.20 m²/g for oil droplets and liposomes, respectively.

Table 1: Fatty acid composition of the lipid phase based on marine phospholipids and plant oils.

	Lipid phase[1]
	g/100 g of total fatty acids
Σ SFA	31.0
16:0	15.4
18:0	3.3
Σ MUFA	56.7
18:1 n-9	55.1
Σ PUFA	12.3
Σ n-6 PUFA	8.7
18:2 n-6	8.4
20:4 n-6	0.0
Σ n-3 PUFA	3.4
18:3 n-3	0.7
20:5 n-3	0.7
22:6 n-3	1.9
n-6/n-3	2.6

[1]The lipid mixture was composed of marine PL (13 wt.%) dissolved in a blend of plant oils (flaxseed/copra/grapeseed/palm/oleic sunflower oils; 1:6:6:30:57, w/w/w/w/w).

Σ : sum , SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

2.2. Animals

Male Wistar rats (8 weeks-old, body weight: 300-350 g) were obtained from Elevage Janvier (Le Genest-Saint-Isle, France). This study was carried out in strict compliance for the care and use of laboratory animals in accordance with the European Communities Council Guidelines for the Care and Use of Laboratory Animals, according to the European directive 2010/63/EU. All experiments conformed to the Guidelines and were approved by the Ethical committee for animal experimentation (CEEA) under the APAFIS number

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#2017031014448864, and the Ministry of Higher Education, Research and Innovation (MESRI, Paris, France). Rats were housed 4 per cage (480 mm x 375 mm x 210 mm) for at least 7 days before the experiment in a controlled environment, at constant temperature (22°C ± 1°C) and humidity (60%) with free water and food access; light/ dark cycle of 12 h/12 h.

2.3. Experimental design

Twenty-four hours before surgery, rats were fed a fat free diet (SAFE, Epinay, France) with free access to water in order to ensure for all rats, a comparable low lymph lipid baseline, devoid of exogenous fatty acids provided by dietary fats. Each rat was placed under anaesthesia by an intra-peritoneal injection of a ketamine/xylazine mixture (100:10 mg/kg, respectively; Axience, Pantin, France). A polyethylene catheter (0.95 mm x 15 cm; Biotrol, Villeron, France) was inserted into the main mesenteric lymph duct as described by Bollman et al.[28] and Couëdelo et al.[21]. Immediately after surgery, rats were randomly assigned to one of the 3 experimental groups (6 rats/group), corresponding to one of the three experimental dietary intakes of marine PL (bulk phase, emulsion and liposomes). For each group, a volume, equivalent to 1 g of lipids, was orally administrated by gavage, providing 8.6 mg of EPA and 22.3 mg of DHA per rat. In the liposome group, in order to ensure the same intake of FA as the other groups, a blend of plant oils (flaxseed/copra/grapeseed/palm/oleic sunflower oils; 1:6:6:30:57, w/w/w/w/w) was administrated just after liposome administration. Each rat was then placed in an individual restraining cage (rat compartment 228 mm x 89 mm), in a warm environment with freely available water. After gavage, lymph was collected for 6 h in a collection tube stored in ice. To prevent pain, rats received an intra-peritoneal injection of buprenorphine (0.02 mg/kg; Axience) 1 h before and 2 h after surgery. At 6 h post-intubation, rats were euthanised by an intra-peritoneal injection of sodium pentobarbital (Axience) and lidocaine (Ceva, Libourne, France).

2.4. Lipid analysis

Total lipids were extracted from the lymph according to the procedure described by Folch et al.[29]. PL and TG fractions from the lipid extracts were separated by thin layer chromatography (TLC, glass plates 20x20 cm pre-coated with silica gel 60H, Merck Millipore, Burlington, MA, United States of America) using a solvent mixture composed of hexane-diethyl ether-formic acid (75:35:1, v/v/v), extracted from the silica gel[17] and stored at -20 °C until analysis.

The FA composition of the lipid phase and of the lymph samples (*i.e.* total lipids, TG and PL fractions), were submitted to the (trans)methylation procedure adapted from Castro-Gomez[26,30]. The resulting fatty acid methyl esters (FAME) were analysed by gas chromatography (GC) as previously described[17,26]. FA were quantified using an internal standard (1 mg/mL in chloroform/methanol (2/1, v/v)) precisely added in the lipid samples before the (trans)methylation reaction. TG 17:0 (Nu-Chek Prep, Elysian, MN, United States of America) and PC 17:0 (Avanti Polar Lipids, Alabaster, AL, United States of America) were used for TG and PL quantification of the lymph samples, respectively. Lymph total lipids were quantified using TG 17:0.

The proportion of FA esterified in the *sn-2* position of lymph TG was determined using the stereospecificity of lipase B Candida Antarctica (>2000 U/g; 1U = 1µEq FA/h; Sigma-Aldrich) as previously described[17]. Briefly, 2-monoglycerides (2-MG) issued from TG lipolysis were isolated by TLC and directly transmethylated. The proportion of FA esterified in the *sn-2* position of TG was calculated by considering the proportions of each FA from 2-MG compared with those from the three positions of the TG.

The glyceride structure of lymph PL was achieved by phospholipase A_2 (PLA_2) lipolysis adapted from Wolff[17,31], using PLA_2 from bee venom (600 – 2400 U/g; 1U = 1 µEq FA/h; Sigma-Aldrich). Free FA issued from PL lipolysis were isolated by TLC, and directly transmethylated. The proportion of FA esterified in the *sn-2* position of PL was calculated by considering the proportions of each FA released as free FA compared to those from the two positions of the PL.

2.5. Statistical analysis

Data (n=6) were expressed as mean values with standard deviation (mean ± SD). Intergroup comparisons were made on the basis of their respective mean using a one-way ANOVA test followed by a Tukey post-hoc test (R software, version 3.5.1 equipped with Rcommander package). P values lower than 0.05 were considered to be statistically significant.

3. Results

3.1. Influence of the supramolecular form of marine PL on the intestinal absorption of n-3 LC-PUFA

No adverse effect was observed in rats during the six hours following lipid administration. Table 2 presents the FA profiles for marine PL provided to rats in the three different dietary forms (bulk phase, emulsion and liposomes). Irrespective of the supramolecular form used, about half of total FA were monounsaturated FA (MUFA), while saturated FA (SFA) represented over a quarter of FA. PUFA were mainly composed of n-6 PUFA (76-82 % of total PUFA). Among n-3 PUFA, DHA represented more than 50%. The emulsified state of the oil increased the total FA amount in lymph by 84% in comparison with the bulk phase (Table 2) ($p<0.05$). In these two groups, no significant variation of FA proportion was observed, except a 34% decrease in the emulsion group for arachidonic acid (20:4 n-6) ($p<0.01$), due to the dilution of endogenous fatty acids by the more massive absorption of exogenous dietary fatty acids. Conversely, assembly marine PL such as liposomes did not influence the total FA amount in the lymph but significantly increased n-3 PUFA proportion compared with the bulk phase group. More precisely, a significant increase for the EPA level (+ 60%, $p=0.04$) and a tendency for DHA (+ 74%, $p=0.07$) were observed in total lymph lipids of the liposome group. Thus, formulating PL in emulsion and in liposomes increased by a factor of 2 the content of EPA ($p<0.02$) and DHA ($p<0.01$) in total lymph lipids,

in comparison with the bulk form. However, in the emulsion group, this result was linked to a global FA increase while, in the liposome group, it resulted from a specific variation in PUFA proportion.

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Table 2: Effect of the supramolecular form of n-3 long-chain PUFA carried by marine phospholipids on fatty acid composition of total lymph lipids, 6 h after rat intubation[1]

	Bulk phase	Emulsion	Liposomes
		g/100 g of total fatty acids	
Σ SFA	26.3 ± 1.6[2a]	28.2 ± 0.9[a]	27.3 ± 1.2[a]
16:0	17.9 ± 1.0[a]	18.1 ± 0.9[a]	18.3 ± 1.0[a]
18:0	5.0 ± 0.3[ab]	4.6 ± 0.2[a]	5.2 ± 0.2[b]
Σ MUFA	52.5 ± 3.8[ab]	53.3 ± 2.0[a]	49.0 ± 3.2[b]
18:1 n-9	48.8 ± 4.3[a]	50.5 ± 2.4[a]	45.5 ± 3.7[a]
Σ PUFA	21.3 ± 3.0[ab]	18.4 ± 1.3[a]	23.7 ± 2.2[b]
Σ n-6 PUFA	17.5 ± 2.5[a]	14.5 ± 0.8[b]	18.0 ± 1.3[a]
18:2 n-6	14.5 ± 2.0[a]	12.0 ± 0,5[a]	14.2 ± 1.6[a]
20:4 n-6	3.5 ± 0.7[a]	2.3 ± 0.5[b]	3.4 ± 0.3[a]
Σ n-3 PUFA	3.7 ± 0.9[a]	3.8 ± 0.8[ab]	5.6 ± 1.9[b]
18:3 n-3	1.0 ± 0.2[a]	1.0 ± 0.0[a]	0.9 ± 0.1[a]
20:5 n-3	0.5 ± 0.2[a]	0.5 ± 0.2[ab]	0.8 ± 0.3[b]
22:6 n-3	1.9 ± 0.7[a]	2.0 ± 0.6[a]	3.3 ± 1.5[a]
Total FA *(mg/mL of lymph)*	15.3 ± 5.6[a]	28.2 ± 10.8[b]	16.9 ± 1.9[a]
EPA *(µg/mL of lymph)*	57.2 ± 12.6[a]	143.3 ± 53.1[b]	123.1 ± 33.1[b]
DHA *(µg/mL of lymph)*	227.8 ± 73.2[a]	544.0 ± 187.1[b]	497.8 ± 142.2[b]

[1] : Rats received a unique bolus of the lipid mixture in three supramolecular forms : bulk phase, emulsion or liposomes.
[2] : Values are expressed as mean ± SD, n=6. Values with different superscript letters (a, b) in the same line are significantly different, p<0.05; ANOVA followed by Tukey post-hoc test.
Σ : sum ; SFA : saturated fatty acids; MUFA : monounsaturated fatty acids; PUFA : polyunsaturated fatty acids.

3.2. Influence of the supramolecular form of marine PL on the incorporation of n-3 LC-PUFA in lymph lipid fractions

Lymph TG and PL were separated, and their FA compositions were analysed separately for the three groups (Table 3). Lipid emulsification increased by a factor of 2.5 the TG and PL outputs in the lymph (p<0.01), in comparison with the bulk form. For both TG and PL fractions, only the arachidonic acid (20:4 n-6) proportion significantly decreased in the emulsion group compared with the bulk phase group, as a result of its dilution by the dietary fatty acids. In rats fed lipid emulsion, the n-3 LC-PUFA content in lymph TG and PL increased by a factor 2.6 and 2.3, respectively (p<0.01), compared with rats fed bulk phase. In the liposome group, the lymph TG and PL outputs were similar to that of the bulk phase group. The PL structuration in liposomes led to a significant (p<0.04) increase in n-3 PUFA proportions both in TG (+ 60%) and PL (+ 36%) fractions. This increase in proportion concerned both EPA and DHA in lymph TG (+ 75%, p=0.06 and + 83%, p=0.07, respectively) and only EPA in lymph PL (+ 82%, p=0.04). These increases resulted in a tendency to double the EPA (p=0.13) and DHA (p=0.12) contents in lymph TG of the liposome group compared with the bulk phase group. In lymph PL, EPA and DHA contents were similar in the two groups. These results are consistent with the enriched amount in n-3 LC-PUFA observed in total lipids in rats fed liposomes (Table 2) and the fact that TG represent the main lipid species in lymph[25].

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Table 3: Effect of the supramolecular form of n-3 long-chain PUFA carried by marine phospholipids on fatty acid composition of triglycerides (TG) and phospholipids (PL) fractions of lymph, 6 h after rat intubation

	Lymph TG			Lymph PL		
	Bulk phase	**Emulsion**	**Liposomes**	**Bulk phase**	**Emulsion**	**Liposomes**
	g/100 g of total fatty acids					
Σ SFA	25.7 ± 1.9[2a]	26.8 ± 1.2[a]	26.3 ± 1.3[a]	39.2 ± 1.3[a]	41.4 ± 0.9[a]	45.4 ± 2.9[b]
16:0	17.7 ± 0.9[a]	17.7 ± 1.0[a]	18.0 ± 1.0[a]	19.9 ± 1.2[a]	21.3 ± 0.6[ab]	22.7 ± 1.0[b]
18:0	4.0 ± 0.3[a]	3.5 ± 0.2[b]	3.9 ± 0.2[a]	17.8 ± 1.1[a]	18.2 ± 1.1[a]	18.4 ± 1.2[a]
Σ MUFA	55.9 ± 3.8[a]	57.1 ± 1.8[a]	52.8 ± 3.3[a]	14.5 ± 3.2[a]	13.9 ± 0.8[ab]	11.3 ± 1.5[b]
18:1 n-9	52.2 ± 4.0[a]	54.3 ± 2.2[a]	49.4 ± 3.9[a]	8.7 ± 3.5[a]	9.3 ± 1.0[a]	6.5 ± 1.6[a]
Σ PUFA	18.6 ± 2.1[a]	16.0 ± 1.0[a]	20.8 ± 2.2[b]	46.3 ± 2.4[a]	44.4 ± 0.7[a]	45.8 ± 1.6[a]
Σ n-6 PUFA	14.9 ± 1.6[a]	12.1 ± 0.5[b]	15.2 ± 1.4[a]	42.3 ± 2.1[a]	39.8 ± 1.0[b]	40.3 ± 1.6[ab]
18:2 n-6	12.5 ± 1.5[ab]	10.8 ± 0.5[a]	13.0 ± 1.4[b]	24.2 ± 2.3[a]	25.5 ± 2.3[a]	22.2 ± 2.9[a]
20:4 n-6	2.0 ± 0.4[a]	1.1 ± 0.2[b]	1.8 ± 0.2[a]	16.9 ± 1.9[a]	13.4 ± 1.5[b]	17.2 ± 2.1[a]
Σ n-3 PUFA	3.5 ± 0.9[a]	3.8 ± 0.8[ab]	5.6 ± 1.9[b]	3.9 ± 1.1[a]	4.7 ± 0.5[ab]	5.3 ± 1.2[b]
18:3 n-3	1.0 ± 0.1[a]	1.1 ± 0.0[a]	0.9 ± 0.1[a]	0.3 ± 0.0[a]	0.3 ± 0.0[a]	0.3 ± 0.1[a]
20:5 n-3	0.4 ± 0.1[a]	0.5 ± 0.1[a]	0.7 ± 0.3[a]	1.1 ± 0.4[a]	1.4 ± 0.2[ab]	2.0 ± 0.8[b]
22:6 n-3	1.8 ± 0.7[a]	2.0 ± 0.6[a]	3.3 ± 1.7[a]	2.1 ± 0.6[a]	2.4 ± 0.3[a]	2.6 ± 0.4[a]
Total FA (mg/mL of lymph)	10.4 ± 4.9[a]	26.5 ± 9.7[b]	9.7 ± 2.9[a]	0.8 ± 0.4[a]	1.8 ± 0.7[b]	0.9 ± 0.2[a]
EPA (µg/mL of lymph)	35.0 ± 8.5[a]	117.4 ± 43.0[b]	75.1 ± 26.7[ab]	9.8 ± 3.1[a]	26.4 ± 11.2[b]	13.7 ± 4.3[a]
DHA (µg/mL of lymph)	147.7 ± 53.8[a]	517.9 ± 210.2[b]	316.6 ± 134.5[ab]	19.3 ± 5.8[a]	43.8 ± 16.9[b]	21.2 ± 5.7[a]

[1]: Rats received a unique bolus of the lipid mixture in the three supramolecular forms: bulk phase, emulsion or liposomes.
[2]: Values are expressed as mean ± SD, n=6. Values with different superscript letters (a, b) in the same line are significantly different, p<0.05, ANOVA followed by Tukey post-hoc test.
Σ : sum ; SFA : saturated fatty acids; MUFA : monounsaturated fatty acids; PUFA : polyunsaturated fatty acids.

3.3. Influence of the supramolecular form of marine PL on the molecular distribution of n-3 LC-PUFA in lymph TG and PL

Since structuring PL in liposomes seemed to modulate lipid synthesis within the enterocytes, the *sn-2* position of lymph TG and PL was worth investigating (Table 4). In lymph TG, compared with the bulk phase group, lipid emulsification did not influence the distribution of SFA and MUFA which remained mainly esterified on the external positions (*sn-1/3*) of the glycerol backbone of TG. Lipid emulsification promoted the internalisation (*sn-2*) of linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3). EPA and DHA, as for them, remained exclusively esterified in *sn*-1/3 positions. By structuring PL in liposomes, the FA distribution in lymph TG was considerably modified, by decreasing the internal proportion of linoleic acid (18:2 n-6) and promoting the esterification of SFA, arachidonic acid (20:4 n-6), α-linolenic acid (18:3 n-3) and EPA (p<0.01) in this position. In particular, only the liposome group led to a switch from externalised positions (*sn-1/3*) of EPA to an equal molar distribution in the three positions. In lymph PL, irrespective of the groups, SFA and MUFA were mainly esterified in the external positions (*sn-1/3*) and PUFA in the internal one (*sn-2*). However, structuring lipids both in emulsion and in liposomes, favoured the externalisation (*sn-1/3*) of SFA and the internalisation (*sn-2*) of PUFA, notably arachidonic acid (20:4 n-6) and EPA (p<0.01), compared with

the bulk phase group. The proportion of α-linolenic acid (18:3 n-3) in the *sn-2* position of lymph PL decreased only in the

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Table 4: Effect of the supramolecular form of n-3 long-chain PUFA carried by marine phospholipids on the fatty acid distribution in triglycerides (TG) and phospholipids (PL) of lymph, 6 h after rat intubation

	Lymph TG			Lymph PL		
	Bulk phase	**Emulsion**	**Liposomes**	**Bulk phase**	**Emulsion**	**Liposomes**
	% esterified in the internal sn-2 position					
Σ SFA	28.8 ± 3.4[2ab]	25.6 ± 2.0[a]	32.5 ± 4.4[b]	13.7 ± 3.6[a]	9.2 ± 1.5[b]	9.6 ± 1.8[b]
16:0	29.5 ± 2.1[a]	26.5 ± 2.4[a]	35.0 ± 3.8[b]	14.7 ± 3.8[a]	10.3 ± 1.6[b]	9.7 ± 1.6[b]
18:0	23.2 ± 3.7[a]	21.3 ± 6.8[a]	32.7 ± 8.1[b]	10.9 ± 3.4[a]	7.1 ± 1.6[a]	10.2 ± 2.7[a]
Σ MUFA	33.5 ± 1.2[a]	35.2 ± 1.1[a]	33.8 ± 1.4[a]	35.7 ± 8.3[a]	35.6 ± 2.6[a]	33.9 ± 3.7[a]
18:1 n-9	34.1 ± 0.9[ab]	36.0 ± 1.4[a]	33.9 ± 1.8[b]	46.8 ± 8.4[a]	43.4 ± 2.3[a]	45.4 ± 2.9[a]
Σ PUFA	39.2 ± 1.5[a]	40.1 ± 2.3[b]	33.5 ± 2.9[b]	85.7 ± 5.7[a]	92.6 ± 1.1[b]	91.5 ± 3.7[ab]
Σ n-6 PUFA	43.1 ± 0.9[a]	45.9 ± 2.9[b]	38.0 ± 4.9[b]	86.7 ± 6.4[a]	93.3 ± 1.2[a]	92.5 ± 3.5[a]
18:2 n-6	44.9 ± 1.4[a]	47.5 ± 3.2[b]	38.2 ± 5.6[b]	87.4 ± 7.4[a]	92.4 ± 1.2[a]	89.4 ± 3.6[a]
20:4 n-6	32.1 ± 1.1[a]	35.5 ± 1.8[ab]	37.5 ± 3.7[b]	87.6 ± 6.4[a]	96.3 ± 1.2[b]	96.8 ± 4.0[b]
Σ n-3 PUFA	23.9 ± 2.4[a]	22.3 ± 2.6[a]	22.9 ± 2.9[a]	77.0 ± 13.7[a]	85.6 ± 1.3[a]	85.3 ± 6.4[a]
18:3 n-3	31.4 ± 1.1[a]	36.5 ± 3.1[b]	34.5 ± 0.6[b]	63.5 ± 7.2[a]	70.9 ± 11.0[a]	42.1 ± 19.5[b]
20:5 n-3	14.5 ± 3.6[a]	17.2 ± 3.0[a]	35.2 ± 7.4[b]	85.6 ± 11.7[a]	96.0 ± 1.8[ab]	99.8 ± 5.9[b]
22:6 n-3	17.3 ± 1.5[a]	16.7 ± 1.3[a]	18.7 ± 4.2[a]	78.4 ± 19.7[a]	88.0 ± 3.0[a]	86.3 ± 6.0[a]

[1]: Rats received a unique bolus of the lipid mixture under the three supramolecular forms: bulk phase, emulsion or liposomes.

[2]: Values are expressed as mean ± SD, n=6. Values with different superscript letters (a, b) in the same line are significantly different, p<0.05, ANOVA followed by Tukey post-hoc test.

Σ : sum ; SFA : saturated fatty acids; MUFA : monounsaturated fatty acids; PUFA : polyunsaturated fatty acids.

liposome group (p<0.02). The distribution of DHA remained mainly located in the *sn-2* position of lymph PL regardless the supramolecular form of marine PL.

4. Discussion

It is largely acknowledged that FA bioavailability is modulated by the molecular lipid species used as carrier[8,10,11,17,32–34] and the supramolecular form of dietary lipids, especially the emulsified state[18–24]. The increase in FA absorption when fish oil is emulsified[18] or dispersed in a food matrix[19,20,35] has been proved. However, to our knowledge, no study has used marine PL formulated in different colloidal systems in a single work. This was made possible by using simultaneously the different potentialities of marine PL, *i.e.* as n-3 LC-PUFA lipid carriers, associated with either their surfactant properties to stabilize emulsion or their self-assembly ability to form liposomes. Moreover, special attention was paid to ensure that the FA profile and amount in the different dietary groups were similar in order to obtain comparable results.

We clearly showed that the emulsification of a mixture of plant oils with marine PL, used to stabilize the O/W interface, increased lymph FA concentration without significant modification of the FA profile of total lymph lipids. This result is in agreement with the literature demonstrating a higher intestinal uptake of lipids emulsified with lecithins[21,22]. A recent study has even shown that PL were more effective for increasing lipid bioaccessibility than Tween 80 and casein[22]. Different phenomena could be associated to explain the potentiating role of emulsification on lipid absorption: (1) the preformed interface provided by the dietary emulsion should favour the anchoring of pancreatic lipase[23,36], and thus its lipolytic activity on TG, (2) an efficient dietary lipid lipolysis could promote a better micellization of the lipolysis products into mixed micelles[21,37–39], (3) as a result, a better intestinal absorption by passive diffusion of lipolysis products could occur due to a higher lipid concentration gradient in the intestinal lumen[21,22]. This latter point would also prevent the lipolysis products from undergoing a prolonged residence time in the gastrointestinal tract, thereby limiting their exposure to potentially oxidative conditions (*i.e.* acidic medium, prooxidant compounds and temperature)[40–42].

To our knowledge, only one study reported the use of marine liposomes for oral delivery of n-3 LC-PUFA[25]. In that instance, the increase in PUFA bioavailability observed with liposomes could not be entirely attributed to the supramolecular form. Indeed, the lipid reference taken in the

assays was fish oil in bulk phase. Moreover, rats were fed only PL that may have oriented the lipid synthesis pathways in the enterocytes. That is the reason why, in the present study, to ensure comparable results with the emulsion group, liposomes were completed with plant oils. Compared with the bulk phase group, structuring marine PL as liposomes did not increase FA amount in total lymph lipids. This suggested that the digestion process of oil TG was not influenced by the lipid structuration in liposomes. It is also in agreement with our previous study showing that the addition of PL in bulk oil did not influence FA absorption[17]. However, using liposomes as PL carriers specifically increased the proportions of the n-3 LC-PUFA esterified on the PL molecules, so that, after a 6 h-intubation time, the lymphatic concentrations of EPA and DHA were similar to those in the emulsion group. This demonstrated once again the importance of the preformed interface for the digestive enzymes to be more efficient in the lipolysis process: liposomes consisted of an adequate interface for PLA_2. However, in contrast with the organisation of PL in monolayers in the emulsion system, liposomes with their bilayer structure should provide a higher local concentration of substrates for PLA_2. Moreover, due to their smaller particle size, liposomes also provided a higher surface for PLA_2 adsorption than oil droplets for pancreatic lipase lipolysis.

After digestion in the gastrointestinal tract, the lipolysis products issued from TG and PL, *i.e.* free FA, 2-MG and lyso-PL, are absorbed by the enterocytes where they serve as precursors for the synthesis of different lipid species, mainly TG and PL, assembled in chylomicrons (CM), before their release into the lymph compartment. Interestingly, in the emulsion group, we showed that the enhanced intestinal absorption of dietary FA concerned both the TG and PL fractions. This suggested that the preformed interface of the dietary emulsion should favour both pancreatic lipase and PLA_2 activities. The simultaneous increase of the two major lipid species of CM could lead to a higher secretion of CM and/or a synthesis of larger CM than the bulk phase group, possibly more easily removed from the plasma stream and less atherogenic than smaller CM[43-44] . The fact that the FA profiles of TG and PL fractions were roughly the same in rats fed emulsion and lipids in bulk phase suggested that similar metabolic pathways were implied in the lipid synthesis although they seemed to be stimulated by a higher flux of precursors in the case of emulsion administration. In contrast, structuring marine PL as liposomes seemed to guide the increased input of EPA and DHA towards TG synthesis only. It may be assumed that the FA composition of PL was better regulated than that of TG.

As it appeared that a differential n-3 LC-PUFA absorption modulated the lymph lipid pathways, we investigated the distribution of FA on the glycerol backbone of lymph TG and PL fractions. We have previously shown that marine PL intake favoured the incorporation of n-3 LC-PUFA in the *sn-1/3* positions of lymph TG and in the *sn-2* position of lymph PL[17]. PL structuration in emulsion or in liposomes roughly maintained this distribution in these two lymph fractions. Interestingly, in the liposome group, the proportion of EPA esterified in the *sn-2* position of lymph TG switched from external positions to an equimolar distribution. Since EPA represented less than 1% of total dietary FA, this data needs to be confirmed by using marine PL with a higher level of EPA.

Conclusion

This study was undertaken to analyse the influence of the supramolecular form of dietary marine PL on the intestinal bioavailability of FA, notably EPA and DHA. We confirmed that the delivery of a preformed interface at the gastrointestinal site is a major parameter modulating the intestinal absorption of FA. We extended this result to emulsion stabilised by marine PL and to bilayer PL membranes. This suggested that it should be possible to specifically activate the lipolysis enzymes, i.e. pancreatic lipase and PLA_2 using emulsion and liposomes, respectively. As a result, while emulsion increased absorption of all dietary FA, liposomes increased specifically the intestinal absorption of FA esterified on the dietary PL (*i.e.* EPA and DHA), without increasing the lymph lipid load during the 6-h post-administration period. This difference in the digestion/absorption kinetics between the two supramolecular forms affected the metabolic pathways involved in lipid synthesis in the enterocytes, promoting n-3 LC-PUFA incorporation in lymph TG and/or lymph PL and, for some FA, leading to a new distribution on the glycerol moiety of TG and/or PL. The differences observed in the incorporation of n-3 LC-PUFA in lymph lipids may, in turn, influence the post-prandial lipemia and further lipid metabolism, especially in the liver. Concerning food science, marine PL rich products could appear as an alternative source to fish and seafood that provided n-3 LC-PUFA in TG carriers. Moreover, the structuration of marine PL in liposomes could increase n-3 LC-PUFA bioavailability without increasing that of the other FA present in the diet.

Conflicts of interest

All authors declare that they have no conflict of interest.

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