

Rapeseed Lecithin Increases Lymphatic Lipid Output and α -Linolenic Acid Bioavailability in Rats

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ABSTRACT

Background: Soybean lecithin, a plant-based emulsifier widely used in food, is capable of modulating postprandial lipid metabolism. With arising concerns of sustainability, alternative sources of vegetal lecithin are urgently needed, and their metabolic effects must be characterized.

Objectives: We evaluated the impact of increasing doses of rapeseed lecithin (RL), rich in essential α -linolenic acid (ALA), on postprandial lipid metabolism and ALA bioavailability in lymph-cannulated rats.

Methods: Male Wistar rats (8 weeks old) undergoing a mesenteric lymph duct cannulation were intragastrically administered 1 g of an oil mixture containing 4% ALA and 0, 1, 3, 10, or 30% RL (5 groups). Lymph fractions were collected for 6 h. Lymph lipids and chylomicrons (CMs) were characterized. The expression of genes implicated in intestinal lipid metabolism was determined in the duodenum at 6 h. Data was analyzed using either sigmoidal or linear mixed-effects models, or one-way ANOVA, where appropriate.

Results: RL dose-dependently increased the lymphatic recovery (AUC) of total lipids (1100 $\mu\text{g/mL}\cdot\text{h}$ per additional RL%; $P = 0.010$) and ALA (50 $\mu\text{g/mL}\cdot\text{h}$ per additional RL%; $P = 0.0076$). RL induced a faster appearance of ALA in lymph, as evidenced by the exponential decrease of the rate of appearance of ALA with RL ($R^2 = 0.26$; $P = 0.0064$). Although the number of CMs was unaffected by RL, CM diameter was increased in the 30%-RL group, compared to the control group (0% RL), by 86% at 3–4 h ($P = 0.065$) and by 81% at 4–6 h ($P = 0.0002$) following administration. This increase was positively correlated with the duodenal mRNA expression of microsomal triglyceride transfer protein (*Mttp*; $\rho = 0.63$; $P = 0.0052$). The expression of *Mttp* and secretion-associated, ras-related GTPase 1 gene homolog B (*Sar1b*, CM secretion), carnitine palmitoyltransferase 1A (*Cpt1a*) and acyl-coenzyme A oxidase 1 (*Acox1*, beta-oxidation), and fatty acid desaturase 2 (*Fads2*, bioconversion of ALA into long-chain n–3 PUFAs) were, respectively, 49%, 29%, 74%, 48%, and 55% higher in the 30%-RL group vs. the control group ($P < 0.05$).

Conclusions: In rats, RL enhanced lymphatic lipid output, as well as the rate of appearance of ALA, which may promote its subsequent bioavailability and metabolic fate. *J Nutr* 2020;00:1–12.

Keywords: nutrition, lecithin, emulsifier, lipid metabolism, α -linolenic acid, food

Introduction

Consumers are increasingly aware of the health and environmental impact of the foods they eat. Hence, there is a growing demand for sustainable food products with both environmentally friendly characteristics and positive health effects (1). Recent data concerning the suspected deleterious health effects of synthetic emulsifiers provide a further incentive to develop natural, eco-friendly alternatives (2, 3). Concomitantly, the desire to restrain from animal-derived

products has engendered a rise in the development of plant-based foods. The market of vegetal food-grade emulsifiers has consequently expanded in recent years, largely represented by vegetal lecithin. According to the official definition reported by the FAO and the European Food Safety Agency (EFSA), vegetal lecithin are a mixture of naturally occurring lipids containing more than 50% of phospholipids (PLs) (4), frequently used in foods for their emulsifying and stabilizing properties.

Both endogenous and food-derived PLs exert numerous important physiological effects (5, 6). The oral supplementation with vegetal PLs has been associated with beneficial effects on lipid and lipoprotein metabolism, notably in hyperlipidemic conditions (7, 8). This is of particular interest, as postprandial hyperlipemia is associated with metabolic syndrome and type 2 diabetes, and represents an independent risk factor for atherosclerosis and cardiovascular diseases (CVDs) (9, 10).

However, the impact of nutritional doses of lecithin remains inconclusive (11), and the differential effect of their consumption as food ingredients or as oral supplements must be explored.

Furthermore, the majority of the aforementioned studies have been carried out using soy lecithin. Soybean lecithin represents more than 90% of the global lecithin market (253,000 metric tons in 2015) (12). Yet, as highlighted in a recent report by the EFSA (13), in mice, the addition of soy lecithin to high-fat diets has been associated with higher markers of low-grade inflammation in the adipose tissue (14, 15). Furthermore, as concerns about sustainability and genetically modified organisms are rising, there is an expanding demand for the development of alternative sources of vegetable lecithin. In Europe and North America, the markets of locally produced sunflower and rapeseed lecithin (RL) are increasing.

RL is of particular nutritional interest, as it is rich in an essential n-3 PUFA, α -linolenic acid (ALA). ALA is implicated in numerous physiological processes and may play a preventive role in certain diseases, notably CVD and stroke (16). ALA is termed essential, as it may not be synthesized in humans and must consequently be obtained through the diet. Yet, the majority of the European population fails to meet the required daily intakes of ALA, i.e. 1% of total energy (17, 18). There is thus a need to develop strategies for food ingredients that would contribute to improving ALA status.

In this respect, there has been recently growing interest in PLs regarding their potential to increase the bioavailability of the fatty acids (FAs) they contain (19–21). Therefore, the potential of RL, rich in ALA-containing PLs, to improve ALA bioavailability deserves to be elucidated. This is all the

more important since RL represents an increasingly used food additive, which remains poorly studied (11).

The use of food additives is strictly regulated. In Europe, according to the EFSA, lecithin may be freely added to most food products according to the principle of *quantum satis* (no maximum level declared). Nonetheless, its use is regulated in certain foods, such as infant food formulas (maximum level allowed = 1%) or oils (3%). Furthermore, in practice, due to its thickening and emulsifying properties, lecithin may not be added to foods at high doses: the highest level reported is 10% w/w in diet-management foods (13).

To our knowledge, no study has compared the differential impacts of these various doses of lecithin on postprandial lipid metabolism.

On the basis of the above research gaps, the objective of the present study was to compare the impact of different doses of RL, ranging from low nutritional doses (<10%) to a higher level achievable only via supplementation (30%), on intestinal lipid absorption and ALA bioavailability. In order to do so, this study was performed using the mesenteric lymph-cannulated rat model, recognized as the gold-standard method to assess, in vivo, intestinal lipid absorption and native chylomicron (CM) secretion (22).

Methods

Preparation and characterization of the lipid formulations

RL was provided by Novastell. Commercial rapeseed, refined palm, and grapeseed oils were purchased by ITERG. The lipid formulations were prepared by mixing varying volumes of the aforementioned vegetable oils with increasing doses of RL under magnetic agitation, so as to obtain similar FA compositions for all formulations. Thereby, the lipid formulations differed in the proportion of ALA carried either by lecithin (predominantly PLs) or oil [triacylglycerols (TAGs)].

The FA compositions of RL and the lipid formulations (Tables 1 and 2) were determined using a GC equipped with a flame ionization detector (FID), as described below. The proportion and characterization of PLs in the lipid formulas was verified by high-performance thin layer chromatography. Migration was performed using chloroform, methanol, acetic acid, and distilled water (50:37.5:3.5:2, respectively). The spots corresponding to the TAG and PL fractions were then visualized under UV light after vaporization of 2,7-dichlorofluorescein in ethanol solution (0.2%, w/v). Their relative proportions were determined using the area of the spots.

Animals

In accordance with the European Community Council Directives (861,609/EEC), all experiments and procedures were approved by the French ministry. The protocol was registered under number 2,017,031,014,448,864 (Animal Care and Use Committee of Bordeaux, no. 9217). Experiments were performed using male Wistar rats (Elevage Janvier) that were 8 weeks old and weighed 300–350 g. During the 7 d prior to the experiment, the rats were housed 4 per cage and kept in a temperature- and humidity-controlled environment, with a 12-h light-dark cycle and free access to water and food.

Experimental procedure

Twenty-four hours prior to surgery, the rats were fed a fat-free diet (SAFE, France) with free access to water. The rats were then anesthetized via an intraperitoneal injection of a ketamine/xylazine mixture (100/10 mg/kg, respectively; Axience). A polyethylene catheter (0.95 mm \times 15 cm, Biotrol) was inserted into the main mesenteric lymph duct as described by Bollman et al. (23) and Couédelo et al. (24). Immediately after surgery, the rats were randomly assigned to 1 of the 5 experimental groups (6 rats/group). For each lipid

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Supplemental Table 1 and Supplemental Figures 1 and 2 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: *Acox1*, acyl-coenzyme A oxidase 1; ALA, α -linolenic acid; CM, chylomicron; *Cpt1a*, carnitine palmitoyltransferase 1A; CVD, cardiovascular disease; EFSA, European Food Safety Agency; *Elovl5*, elongation of very long chain fatty acids protein 5; FA, fatty acid; *Fads2*, fatty acid desaturase 2; FDR, false discovery rate; FID, flame ionization detector; *Mttp*, microsomal triglyceride transfer protein; OA, oleic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipid; *Pla2g2a*, a gene coding for group II phospholipase A₂; *Ppara*, peroxisome proliferator-activated receptor- α ; RL, rapeseed lecithin; *Sar1b*, secretion-associated, ras-related GTPase 1 gene homolog B; TAG, triacylglycerol; TEM, transmission electron microscopy.

TABLE 1 Phospholipid and fatty acid composition of rapeseed lecithin

Component	Amount
Phospholipid, g/100 g total ¹	
PC	34.0
PE	40.6
PI + PS	15.0
LysoPC	3.7
LysoPE	2.7
Others	4.0
FA composition ^{1,2}	
16:0	
g/100 g total FA	11.5
mol/100 mol total FA	12.5
18:1(n-9)	
g/100 g total FA	47.7
mol/100 mol total FA	47.0
18:2(n-6)	
g/100 g total FA	29.8
mol/100 mol total FA	29.6
18:3(n-3)	
g/100 g total FA	4.8
mol/100 mol total FA	4.8
20:5(n-3)	
g/100 g total FA	0.0
mol/100 mol total FA	0.0
22:6(n-3)	
g/100 g total FA	0.0
mol/100 mol total FA	0.0
Σ (SFA)	
g/100 g total FA	13.3
mol/100 mol total FA	14.0
Σ (MUFA)	
g/100 g total FA	51.3
mol/100 mol total FA	50.8
Σ (PUFA)	
g/100 g total FA	34.9
mol/100 mol total FA	34.6
Σ (n-6 PUFA)	
g/100 g total FA	29.9
mol/100 mol total FA	29.8
Σ (n-3 PUFA)	
g/100 g total FA	4.8
mol/100 mol total FA	4.8
Σ (TFA)	
g/100 g total FA	0.6
mol/100 mol total FA	0.5

Abbreviations: FA, fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TFA, trans-fatty acids.

¹Data were obtained by high-performance thin layer chromatography (phospholipids) and GC coupled to a flame ionization detector (fatty acid composition).

²Of note, RL was devoid of LC n-3 PUFAs.

formulation, a volume corresponding to 1 g of total FAs (1.0–1.1 mL) was administrated via intragastric gavage. Each rat was then placed in an individual restraining cage, in a warm environment with free access to water. Lymph was collected in 1-h time slots between 0–4 hours and for a 2-h time slot between 4–6 h, in Eppendorf tubes that were maintained in ice for 6 hours following intubation. The rats were then euthanized by an intraperitoneal injection of sodium pentobarbital (Axience) and lidocaine (Ceva). To prevent pain, rats received an intraperitoneal injection of buprenorphine (0.02 mg/kg; Axience) 1 h before and 2 h after surgery. The duodenum was immediately collected, and the lumen washed with PBS to remove the intestinal content.

Duodenal biopsies and lymph samples were stored at –80°C until analysis. The study design is presented in **Supplemental Figure 1**.

Lymph lipid analysis

Lymph samples were thawed to room temperature and submitted to direct (trans)methylation according to the 1-step methylation technique described by Lepage and Roy (25). Triheptadecanoin was added as an internal standard for FA quantification. Subsequent FA methyl esters were analyzed using GC-FID (TRACE GC, Thermo Fisher Scientific). A fused-silica capillary column (BPX 70, 60 m × 0.25 mm i.d., 0.25 μm film; SGE) was used with hydrogen as a carrier gas (inlet pressure: 120 kPa). The column temperature was increased from 160°C to 180°C (1.3°C/min) and maintained for 65 min before being increased to 230°C (25°C/min). Injector and detector temperatures were maintained at 250°C and 280°C, respectively. GC peaks were integrated using the Chromquest software (ThermoFinnigan). FA from Sigma France of known composition were used as standards for column calibration. The variation in peak area between injections was <2%.

Real-time quantitative RT-PCR analysis

Total RNA was extracted from the duodenal mucosa using the TRI Reagent (Ambion/Applied Biosystems). RNA quality and concentrations were measured using the Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific). Reverse transcription was performed using 1 μg of RNA and the PrimeScript RT (reverse transcription) reagent kit (Ozyme). Real-time PCR assays were then performed using a Rotor-Gene Q (Qiagen) and SYBR quantitative PCR Premix Ex Taq (Tli RNaseH Plus) reagents. The conditions of quantitative PCR conditions are available upon request (emmanuelle.meugnier@univ-lyon1.fr). The results were normalized using the expression of the TBP (TATA box-binding protein) gene as a reference.

Characterization of lymph CMs

The quantification of the number of CM particles was obtained by measuring lymph apoB48 using an apoB48 ELISA kit (Cusabio), as this apolipoprotein is specific to CM particles and 1 unit is present per CM. The hydrodynamic diameter of lymph CM was measured at 2, 4, and 6 h following intubation, via dynamic light scattering at 25°C using a ZetaSizer NanoS (Malvern Instruments) with 1.0658 cP as viscosity and 1.33 as refractive index of the aqueous phase [as previously described in Lecomte et al. (26)].

Chylomicron imaging by transmission electron microscopy

The lymph samples were collected at 2, 4, and 6 h following intubation and were diluted in PBS (1/10 dilution). The suspensions were adsorbed on 200 Mesh Nickel grids coated with formvar-C for 2 min at room temperature. The grids containing suspensions were then colored with 2% phosphotungstic acid for 2 min and observed through transmission electron microscopy (TEM; Jeol 1400 JEM) equipped with a Gatan camera (Orius 600) and Digital Micrograph Software.

Statistical analysis

Statistical analysis of the main kinetics outcomes.

The statistical analyses of the kinetics of lymph lipid composition were performed using R (version 3.5.1) and R studio (version 1.1.463). The impacts of RL on lymphatic output kinetics were investigated for the following 5 main responses: total FA concentration, ALA concentration (Figure 1), relative percentage of ALA, relative percentage of oleic acid (OA), and relative percentage of DHA (Figure 2). For each response, we fitted sigmoidal curves to the obtained data sets for individual rats and for each group, using the following equation:

$$y(t) = y_{\min} + \frac{\delta}{1 + e^{\frac{t_{\text{infl}} - t}{s}}} \quad (1)$$

Here, $y(t)$ is the fitted response, y_{\min} is the minimum of the sigmoid, δ is the difference between its maximum and its minimum, t_{infl} is the abscissa of its inflection point, and s is the steepness of the curve (Supplemental

TABLE 2 Main fatty acid and phospholipid profile and general composition of the lipid formulations

Fatty acid, g/100 g total FA ^{1,2}	Lipid mixtures				
	0% RL	1% RL	3% RL	10% RL	30% RL
12:0	0.070	0.070	0.070	0.069	0.060
14:0	0.25	0.26	0.26	0.26	0.24
15:0	0.032	0.028	0.032	0.034	0.046
16:0	13.7	13.8	14.0	14.0	13.8
18:0	2.9	2.9	2.9	2.8	2.6
18:1(n-9)	42.7	42.8	42.9	43.1	43.6
18:1(n-7)	1.9	1.9	1.9	1.9	1.9
18:2(n-6)	31.8	32.0	31.5	31.5	31.2
18:3(n-3)	4.5	4.5	4.4	4.4	4.3
20:0	0.41	0.41	0.41	0.39	0.35
20:5(n-3)	0.0	0.0	0.0	0.0	0.0
22:0	0.18	0.17	0.18	0.17	0.16
22:6(n-3)	0.0	0.0	0.0	0.0	0.0
24:0	0.082	0.081	0.085	0.086	0.084
Σ(SFA)	17.8	17.9	18.1	18.0	17.3
Σ(MUFA)	45.6	45.4	45.6	45.8	46.6
Σ(PUFA)	36.4	36.6	36.1	36.0	35.7
Σ(n-6 PUFA)	31.9	32.1	31.6	31.5	31.3
Σ(n-3 PUFA)	4.5	4.5	4.4	4.4	4.3
n-6/n-3	7.1	7.2	7.2	7.1	7.2
Σ(TFA)	0.2	0.1	0.2	0.2	0.4
Total phospholipids, g/100 g mixture ³	0.0	1.0	2.9	10	21
Rapeseed oil, g/100 g mixture	51	50	49	45	34
Palm oil, g/100 g mixture	22	22	22	21	17
Grapeseed oil, g/100 g mixture	27	27	26	24	19
Rapeseed lecithin, g/100 g mixture	0	1	3	10	30

Abbreviations: FA, fatty acid; RL, rapeseed lecithin; TFA, trans-fatty acids.

¹Fatty acid values were obtained by GC coupled to a flame ionization detector.

²Of note, the lipid formulations were devoid of LC n-3 PUFAs.

³The values were estimated by high-performance thin layer chromatography analysis.

Figure 2). We identified y_{\min} as the minimal observed value, while δ , t_{infl} , and s were adjusted using the NL2SOL algorithm, a non-linear least squares optimization method. For total lipid and ALA concentrations, we also computed the AUC for each individual fitted curve (Figure 1), using the “auc” function of the Miscellaneous Esoteric Statistical Scripts (MESS) package. The curves fitted to the group means were used for graphical illustration, while the curves fitted to individual kinetics were used for the statistical analysis. Specifically, we quantified the impact of lecithin dose by analyzing its effect on the individual values of δ , $\log(t_{\text{infl}})$, and s and on the AUC, using a linear model (t_{infl} did not respond linearly, hence the logarithmic transformation). The raw P values of these linear regressions are displayed on the figures. However, given that we performed a total of 17 linear regressions for this analysis, the raw P values obtained were corrected using the false discovery rate (FDR) method, as implemented in the “p.adjust” function. The FDR-corrected values are reported as \tilde{P} in the figures and text. For this analysis, \tilde{P} values lower than 0.05 were considered statistically significant, in order to ensure robust conclusions on the main outcomes.

Statistical analysis of mechanistic explorations.

The statistical analyses of the mechanistic explorations were performed using either the GraphPad Prism software version 7 (gene expression) or R version 3.5.1 (CM number and size). Means of gene expression (Figure 3) were compared across groups using a 1-way ANOVA, followed by Dunnett's post hoc test, using 0% RL as the control. As with the lipid analysis, the raw P values are presented on the figures, and the FDR-corrected P values are in the figure legends. The concentration of apoB48, as well as the size of lymph CM (Figure 4), were analyzed using linear mixed-effects models and Type I (sequential) sum of squares, where time, lecithin dose, and the interaction of both were the factors with fixed levels and rat number was the factor with random levels.

This linear mixed-effect model is equivalent to a 2-way ANOVA with repeated measures, but is more robust to missing data. When the analysis revealed a significant effect for the lecithin dose, Dunnett's post hoc test (using 0% RL as the control) was used to identify the doses contributing most to the effect. In addition, Spearman correlations were performed to evaluate possible relationships among the various outcomes (Figure 5). For these mechanistic explorations, raw P values lower than 0.05 were considered statistically significant, in order to avoid missing potential candidates for further exploration.

Results

RL and lipid formulation composition

RL was mainly composed of OA (48 g/100 g), linoleic acid (30 g/100 g), palmitic acid (11 g/100 g), and ALA (5 g/100 g; Table 1). As such, its FA composition resembled that of its oil-bearing seed, as reported in the literature (27). In terms of polar lipid composition, RL was mainly constituted of phosphatidylcholine (PC; 41 g/100 g) and phosphatidylethanolamine (PE; 34 g/100 g), alongside other minor PLs, such as phosphatidylinositol, phosphatidylserine, lysoPC, and lysoPE. Of note, RL did not contain any sphingomyelin, as this PL is characteristic of dairy polar lipids and is absent in plants.

The lipid formulations presented similar FA profiles, mainly composed of mono-unsaturated FA (mainly OA, ~44%) and PUFAs: linoleic acid (~32%), as well as similar ALA content (~4.5%, Table 2; ~16 mol in the 1 g of total administrated FAs, Supplemental Table 1). Consistent with our study design,

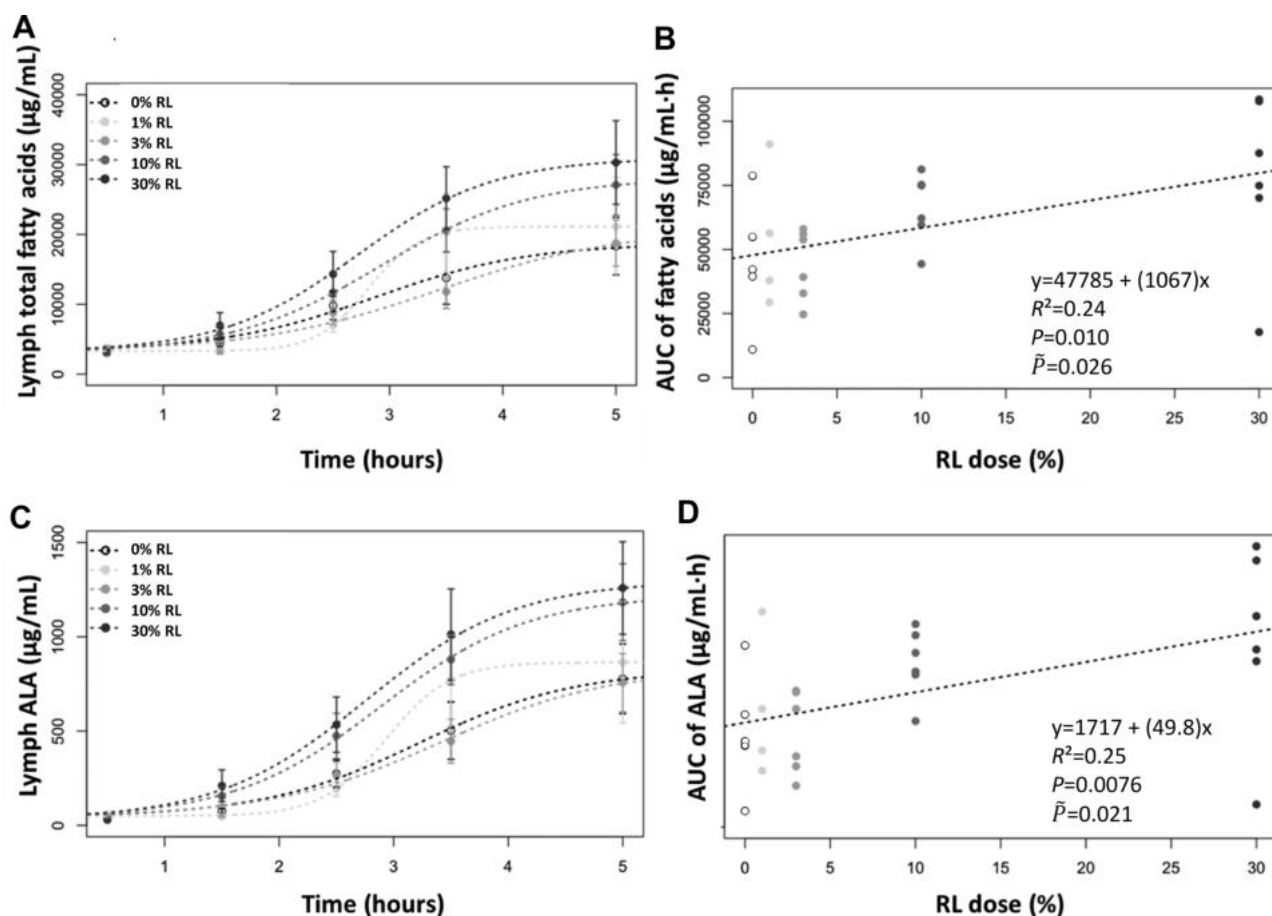


FIGURE 1 The concentrations of total FA (A) and ALA (C) in the lymph of rats following the intragastric administration of lipid mixtures containing 0 to 30% RL. The curves represent the fitted curves of the sigmoidal model for each group. Plot of the AUC of the fitted curve for total FA (B) and ALA (D) for each individual rat, according to RL dose. Values represent mean \pm SEM; $n = 4$ –6. Abbreviations: ALA, α -linolenic acid; FA, fatty acid; \tilde{P} , false discovery rate-corrected P value; RL, rapeseed lecithin.

while FA concentrations were alike amongst groups, their vectorization forms differed. In this way, the higher the lecithin dose present in the oil mixture, the higher the amount of ALA vectorized by polar lipids rather than TAG.

RL increased lipid recovery in lymph

For all groups, the concentration of lipids in lymph increased during the 6 h following the administration of the oil mixtures. The lymphatic recovery of total lipids, as well as individual lipids, such as ALA and OA, followed sigmoidal kinetics during the observed time period. The consumption of RL significantly and dose-dependently increased the AUC of total lipid concentration ($R^2 = 0.24$; $P = 0.010$; $\tilde{P} = 0.026$), so that the AUC of the 30%-RL group was 2.2 times greater than that of the control group (0% RL; Figure 1).

In addition, RL dose induced an increase in the AUC of the lymphatic ALA concentration with time ($R^2 = 0.25$; $P = 0.0076$; $\tilde{P} = 0.021$; Figure 1). In this way, the AUC of the ALA concentration was enhanced 1.8-fold in the 30%-RL group compared to the control group, despite the fact that all lipid mixtures contained the same ALA amount. The relative percentage of ALA in lymph lipids was not found to differ amongst groups during the first (0–1 h) and last (4–6 h) time intervals following intubation. However, 1 kinetic parameter was modified by RL: the inflection point of the curve

representing the percentage of ALA in lymph lipids with time significantly decreased with increasing RL doses ($R^2 = 0.26$; $P = 0.0064$; $\tilde{P} = 0.021$; Figure 2). This implies that the increasing proportion of RL within the lipid mixture induced a faster appearance of ALA in lymph.

OA, 1 of the main FAs found in RL, presented similar trends of lymph recovery (Figure 2). In effect, similarly to ALA, the inflection point of the curve representing the recovery of OA in lymph lipids with time was significantly reduced with increased RL amounts ($R^2 = 0.26$; $P = 0.0067$; $\tilde{P} = 0.021$), yet no difference was observed in its initial and final recoveries in lymph lipids. On the contrary, DHA, which was absent in the oil mixtures, presented inverse kinetics to ALA and OA: the percentage of DHA in lymph lipids decreased with time, and its inflection point decreased with increasing RL doses ($R^2 = 0.35$; $P = 0.0013$; $\tilde{P} = 0.012$; Figure 2).

RL increased CM size without altering CM number

As RL was found to modulate the output of lipids into lymph, we further investigated its impact on CM properties by measuring both their numbers and sizes (Figure 4). Indeed, absorbed dietary lipids are, for the most part, secreted into lymph via CM particles. No significant difference was observed between groups regarding the concentration of apoB48 in the lymph fractions collected between 1–2, 3–4, and 4–6

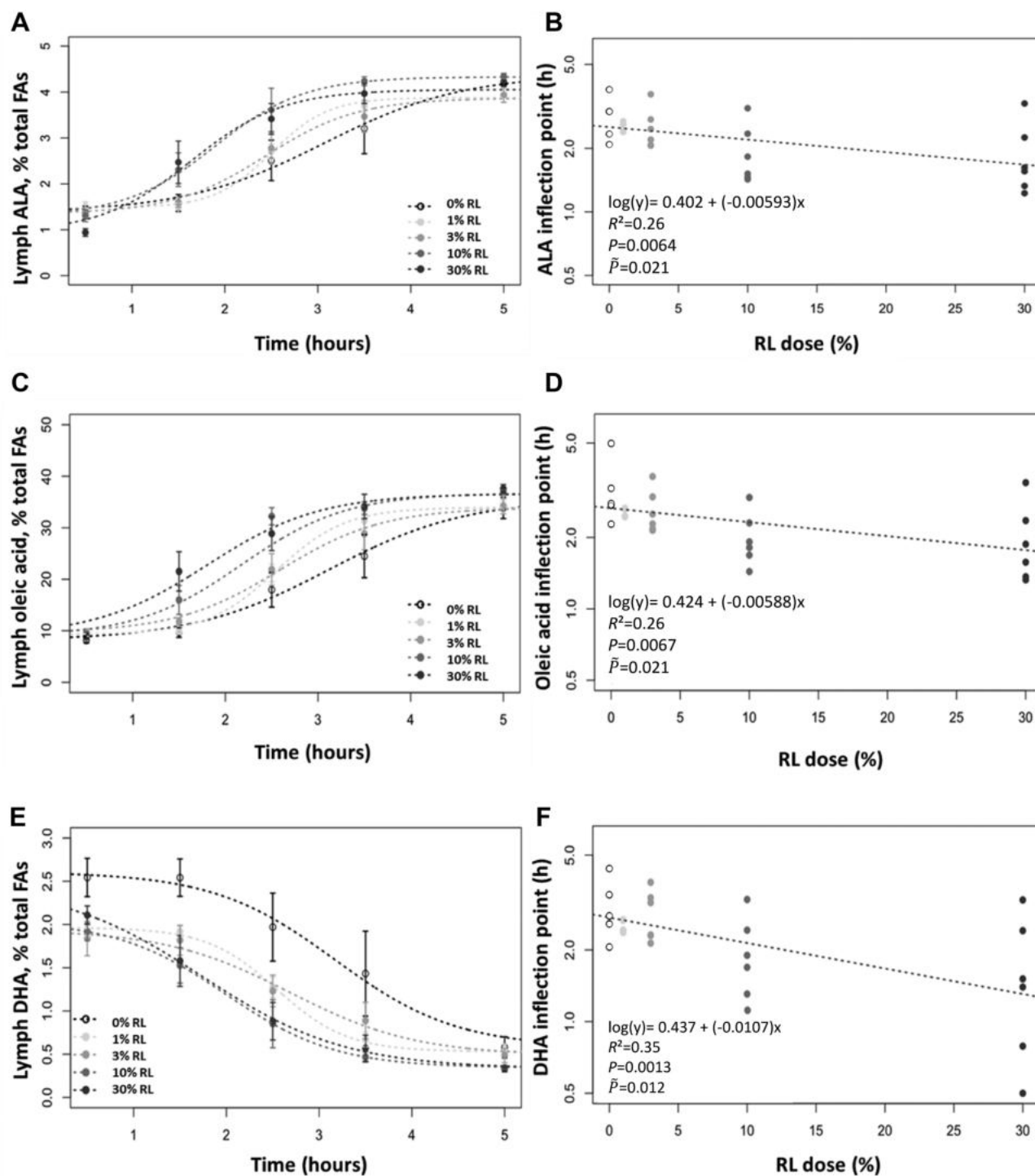


FIGURE 2 The relative percentages of ALA (A), oleic acid (B), and DHA (C) in total FAs in the lymph of rats following the intragastric administration of lipid mixtures containing 0 to 30% RL. Each curve represents the fitted line of the sigmoidal model. (D–F) The log-transformed inflection point of the corresponding fitted curve for each rat, according to RL dose. Values represent mean \pm SEM; $n = 4$ –6. Abbreviations: ALA, α -linolenic acid; FA, fatty acid; \bar{P} , false discovery rate–corrected P value; RL, rapeseed lecithin.

h following intubation, suggesting no impact of RL on the CM number. The lymph CM diameter, however, significantly rose with time for 6 h after intubation ($P < 0.0001$) and was found to be significantly greater in the lymph fractions collected over the 3–4 and 4–6 h intervals in the 30%-RL group, compared to the control group ($P = 0.0065$ and $P = 0.0002$, respectively). The images obtained via TEM corroborated these results (Figure 6).

Duodenal gene expression of lipid metabolism–related proteins

In order to explain the results above, we assessed, in the duodenal mucosa, the mRNA expression of key genes involved in intestinal lipid absorption and metabolism: notably, those implicated in CM secretion (Figure 3). In this way, the expression of secretion-associated, ras-related GTPase 1 gene homolog B (*Sar1b*), implicated in pre-CM formation and maturation, was

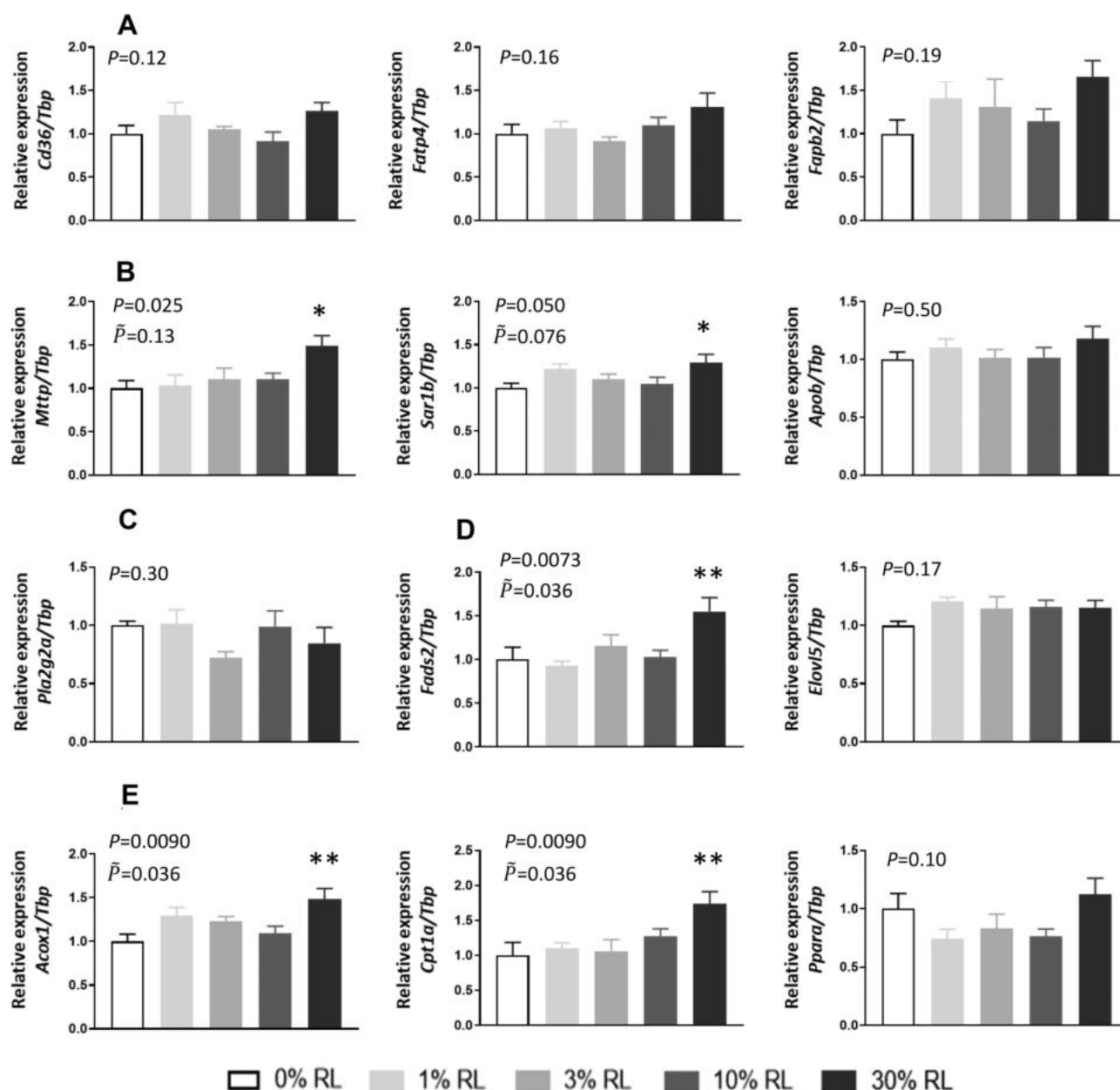


FIGURE 3 The mRNA expression of genes involved in (A) intestinal lipid absorption (*Cd36*, *Fatp4*, *Fabp2*), (B) chylomicron secretion and accretion (*Mttp*, *Sar1b*, *Apob*), (C) phospholipid metabolism (*Pla2g2a*), (D) bioconversion of n-3 PUFAs to long-chain PUFAs (*Fads2*, *Elovl5*), and (E) lipid beta-oxidation (*Acox1*, *Cpt1a*, *Ppara*) in the duodenal mucosa of rats following administration of oil mixtures containing 0 to 30% RL. Values are normalized to the levels of the mRNA of *Tbp* and expressed as relative amounts compared with control rats: that is, rats that consumed 0% RL. Gene expression was quantified by quantitative RT-PCR. Bars represent mean \pm SEM; $n = 5-6$. To test the impact of RL on gene expression, all groups were analyzed by one-way ANOVA, followed by Dunnett's post hoc test vs. 0% RL. ANOVA P values are shown. Post hoc P values vs. 0% RL: * $P < 0.05$; ** $P < 0.01$. When the latter are significant, \bar{P} are also shown on the graphs. Abbreviations: *Apob*, apolipoprotein B; *Acox1*, acyl-coenzyme A oxidase 1; *Cd36*, cluster of differentiation 36; *Cpt1a*, carnitine palmitoyltransferase 1A; *Elovl5*, elongation of very long chain fatty acids protein 5; *Fads2*, fatty acid desaturase 2; *Fabp2*, fatty acid binding protein 2; *Fatp4*, fatty acid transport protein 4; FDR, false discovery rate; *Mttp*, microsomal triglyceride transfer protein; \bar{P} , false discovery rate-corrected P value; *Pla2g2a*, a gene coding for group II phospholipase A₂; *Ppara*, peroxisome proliferator-activated receptor- α ; RL, rapeseed lecithin; *Sar1b*, secretion-associated, ras-related GTPase 1 gene homolog B; *Tbp*, TATA-box binding protein.

higher in the 30%-RL group compared to the control group ($P = 0.032$). That of microsomal triglyceride transfer protein (*Mttp*), involved in the accumulation of TAG in CM, was also higher in the 30%-RL group compared to the control group ($P = 0.013$). Furthermore, the CM diameter was positively and significantly correlated with the mRNA expression of *Mttp* ($\rho = 0.63$; $P = 0.0052$), but not *Sar1b* (Figure 5). The

addition of RL did not, however, alter the gene expression of *Apob*, nor were significant differences observed with regards to the gene expression of lipid transporters (cluster of differentiation 36 (*Cd36*), fatty acid binding protein (*Fatp2*), and fatty acid transport protein 4 (*Fabp4*). As lecithin is rich in PLs, we assessed the impact of RL on the expression of *Pla2g2a*, a gene coding for group II phospholipase A₂,

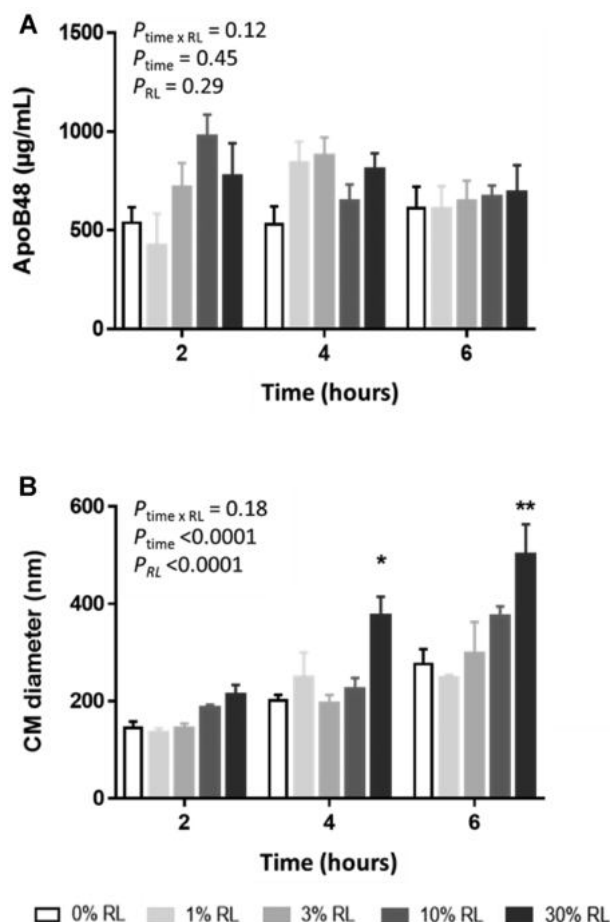


FIGURE 4 The apoB48 concentration (A) and the hydrodynamic diameter of CMs (B) present in the lymph of rats collected at 2, 4, and 6 h following administration of lipid formulations containing 0 to 30% RL. Bars represent mean \pm SEM; $n = 4-6$. To test the impact of RL doses on these parameters, all groups were analyzed using a linear mixed-effects model and sequential sum of squares, followed by Dunnett's post hoc test versus 0% RL. Means statistically different from 0% RL are presented as * $P < 0.01$; ** $P < 0.001$. Abbreviations: CM, chylomicron; RL, rapeseed lecithin.

a protein involved in the regulation of PL metabolism in biomembranes. We found that the consumption of RL did not significantly modify the expression of this gene in the duodenum.

In addition, we evaluated the impact of RL on the expression of genes implicated in the bioconversion of n-3 PUFAs to long chain LC PUFAs and lipid beta-oxidation, as they represent the major metabolic pathways for n-3 PUFAs, and hence of ALA, not only in the liver but also in the small intestine. The addition of 30% RL induced a significantly greater expression of peroxisomal acyl-coenzyme A oxidase 1 (*Acox1*; $P = 0.0031$) and carnitine palmitoyltransferase IA (*Cpt1a*; $P = 0.0046$), but not of peroxisome proliferator-activated receptor- α (*Ppara*), compared to the control group. Regarding the bioconversion of n-3 PUFA, whereas the expression of fatty acid desaturase 2 (*Fads2*) was higher in the duodenum of the 30%-RL group ($P = 0.0094$), that of the elongation of very long chain FAs protein 5 (*Elovl5*) gene was not modified by RL.

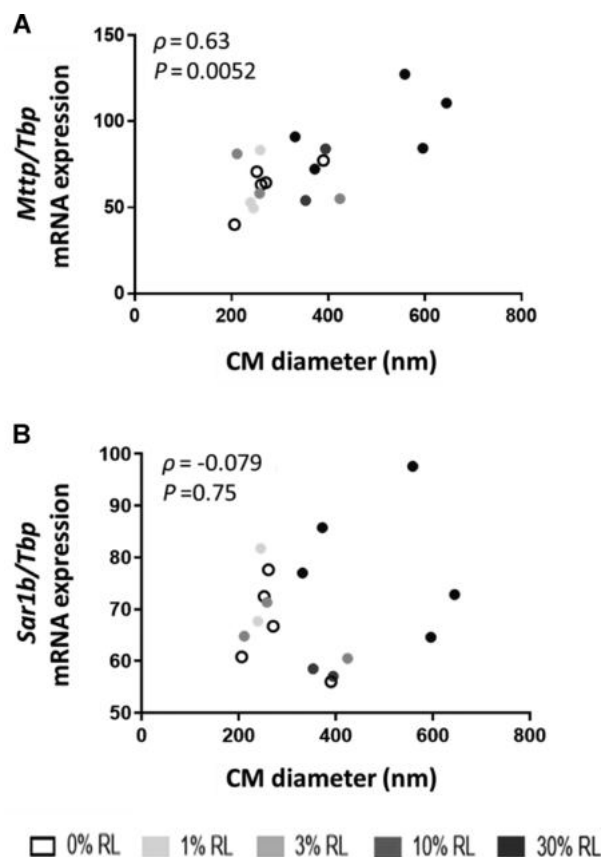


FIGURE 5 Spearman correlation between (A) duodenal *Mttp* mRNA expression or (B) duodenal *Sar1b* mRNA expression and the hydrodynamic diameter of CMs in the lymph of rats following the intragastric administration of oil mixtures containing 0 to 30% RL. Abbreviations: CM, chylomicron; *Mttp*, microsomal triglyceride transfer protein; RL, rapeseed lecithin; *Sar1b*, secretion-associated, ras-related GTPase 1 gene homolog B; *Tbp*, TATA-box binding protein.

Discussion

This is, to our knowledge, the first study to evaluate and compare different doses of RL, ranging from low nutritional doses ($\leq 10\%$) to a high level of 30% w/w achievable only via supplementation, on intestinal lipid absorption and ALA bioavailability (11). For optimal analysis, both quantitative and kinetic aspects of lymphatic output were assessed.

We hereby demonstrate that, in rats, the addition of RL in oils induces a dose-dependent increase in the lymphatic output of lipids. The maximum concentration of lipids in lymph was nearly doubled in the group which had consumed the highest dose of RL (30%), compared with the group that consumed oil only. This may not solely be attributed to an increase in intestinal lipid uptake, as RL did not alter the expression of genes involved in the uptake and intracellular transport of lipids in the duodenum. We show that this effect may be due, in part, to a stimulatory effect of RL on CM secretion and assembly, as *Mttp* and *Sar1b* were overexpressed in the presence of 30% RL, compared to oil. Likewise, lymphatic CM size increased with RL dose, and significantly differed from the control group to the 30%-RL group. As CM size, and not number, was increased with RL, it can be extrapolated that RL stimulates lipid output into lymph by facilitating the accretion of TAG in pre-CMs, thus generating large, TAG-rich CM. This complies with the positive

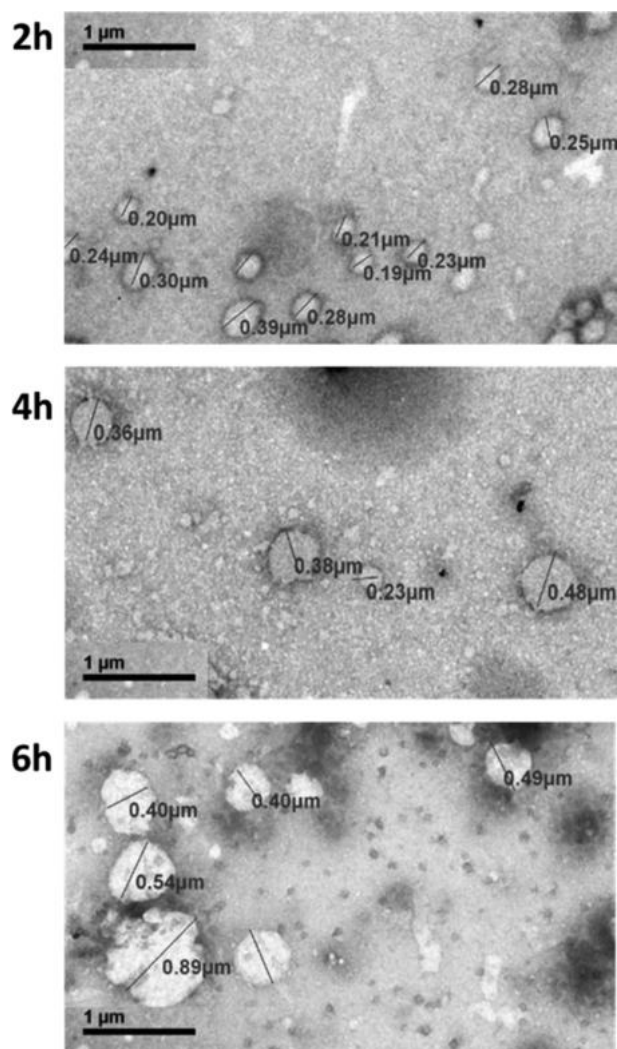


FIGURE 6 Images obtained via TEM of CMs present in lymph fractions collected at 2, 4, and 6 h following administration of an oil mixture containing 30% RL in a lymph-cannulated rat. Size indications are provided to guide the eye. Abbreviations: CM, chylomicron; RL, rapeseed lecithin; TEM, transmission electron microscopy.

correlation observed between CM diameter and the expression of *Mttp*.

This effect on lipoprotein size may have important repercussions on postprandial lipid metabolism (28). For a constant number of CM, large CM offer a greater binding surface for lipoprotein lipases than small CM particles. Therefore, they may be more effectively cleared from plasma, which can lead to beneficial impacts on metabolic and cardiovascular health (29). The observed effect of RL on CM size may be explained by the fact that RL, by providing amphiphilic PL, supplies the constituents of the surface of lipoproteins and increases their stability. The surfactant nature of the PL molecule depends, however, on its specific structural and interfacial properties. PC, which is cylindrical in shape, offers little curvature to the lipid droplet it stabilizes, whereas PE, via its conical shape, promotes a negative curvature and favors large droplets (30). Hence, this observed increase in CM size may be potentially explained by the increased intake of PE via RL. Nonetheless, it is important to note that this effect on CM was only significant when RL was added at levels attainable through supplementation, greater than those levels found in foods.

The dose-dependent effects of RL on intestinal lipid absorption described here corroborate previous findings in the literature using soy lecithin and other dietary PL sources. Nishimukai et al. (31) reported that supplementation with soybean PC at a high TAG:PL ratio of 3:1 induced an increase in lymphatic lipid output in lymph-cannulated rats that was not due to a rise in lipid absorption, but to an increase in CM secretion. In several studies carried out in lymph-cannulated rats, when dietary PLs were added at low levels, resembling those of levels in foods, no difference in intestinal lipid absorption was observed compared to the control group (32, 33). Similarly, Couëdelo et al. (34) denoted no difference in the duodenal expression of *Mttp*, *Apob*, and *Sar1b* in rats fed 250 mg flaxseed oil emulsified with 80 mg soy lecithin (i.e., 8% w/w of the emulsion), compared to rats fed flaxseed oil only. Hence, the data generated in this study explain the discrepancies reported in the literature. Most importantly, we highlight the importance of taking into consideration the dose of a food ingredient when evaluating its impact on metabolic and physiological processes.

The absorption of lipids is a complex process, which involves numerous steps (35). Thereby, the stimulatory effect of RL on lipid lymphatic output may not be solely ascribed to its impact on CM metabolism, but may also occur during the prior stages of lipid digestion: notably, their micellization in the lumen and lipolysis by digestive enzymes. This has not been investigated within this study, but such effects cannot be disregarded when considering postprandial lipid absorption. It would then be relevant to investigate in a future study the composition of residual lipids in the small intestinal lumen according to lecithin content and source. In effect, the gastrointestinal digestion of lipids can be considered as a limiting step of their bioavailability, as dietary lipids first have to be hydrolyzed by digestive enzymes in the gastrointestinal tract before being absorbed by the enterocyte. It is now recognized that PLs, and notably soy lecithin, are capable of increasing the rate and extent of the lipolysis of lipid micelles in the lumen (34, 36). PLs also have the ability to modulate the activity of certain digestive enzymes. In this way, Gargouri et al. (37) reported that vegetable lecithin, due to its rich phosphatidylcholine content, upregulates the activity of gastric lipase, and may therefore enhance lipolysis efficiency. The specific contribution of RL to digestive lipolysis deserves further investigation.

Moreover, PL have been recognized for their ability to increase the bioavailability of the FA they carry (11). As such, this study additionally aimed to determine the impact of RL on ALA bioavailability compared to oil. The lipid formulations were hence designed to provide a similar amount of ALA, originating either from the oil compartment, as TAG, or from the lecithin, as polar lipids, such as PL.

The study revealed the capacity of RL to modulate not only quantitative parameters, but also certain kinetic parameters of ALA absorption and recovery in lymph. The quantity of ALA, as described by its AUC, as well as the rate of appearance of ALA in lymph, were dose-dependently increased with RL. In order to confirm that these effects were a result of the vectorization of ALA as RL, we verified that OA, 1 of the main FAs found in RL, presented similar trends of lymph recovery. As reflected by its AUC and inflection point, the quantity and rate of appearance of OA also increased with RL dose. It may then be concluded that the vectorization form of these FAs in RL, as polar lipids, ameliorates their bioavailability, comparatively, to TAG.

It may then be interesting to evaluate, in future studies, whether the previously described seemingly proinflammatory

effects of soybean lecithin (14, 15), rich in n-6 PUFA-containing PLs, may be attributed to an increase in n-6 PUFA bioavailability.

The present results corroborate and complete preliminary results obtained by Couëdelo et al. (24), which showed that the lymphatic recovery of ALA was higher in rats fed linseed oil emulsified with soy lecithin vs. those receiving linseed oil only. Sugasini et al. (32) also observed no statistical difference in lymphatic TAG or PL concentrations in rats fed nonemulsified or PL-emulsified linseed oil, but reported higher ALA concentrations in the PL-emulsified group.

However, these studies focused on the bioavailability of ALA in its emulsified vs. non-emulsified form. It is now widely recognized that the pre-emulsification of an oil enhances its intestinal absorption (38–40). The size and composition of emulsion droplets vary immensely in foods, which in turn impact the rate and extent of their postprandial metabolism (41). In foods, the structural and compositional diversity of lecithin-stabilized emulsions is immense. Therefore, the novelty of the present study was therefore to determine the impact of the mere presence of lecithin in oil on the bioavailability of ALA, independently of its emulsifying effect. It is the first study, to our knowledge, to directly assess the capacity of RL to increase ALA bioavailability without pre-emulsification. Several studies concerning marine PL have suggested a higher bioavailability of marine LC n-3 PUFAs, such as EPA and DHA, when they were incorporated in PLs comparatively to TAGs (21, 42). Nonetheless, this is far from unequivocal. A study by Sehl et al. (43), also carried out in rats with mesenteric duct cannulation, demonstrated that the vectorization of DHA as PL did not alter its total concentration in lymph compared with TAG, but modified its distribution on the glyceride of TAGs, which may further impact its metabolic fate and tissue accretion. Another study in rats also denoted an increase in the incorporation of DHA in CM PL when DHA was provided as PL compared to TAG (44). This was not observed for EPA, however (43), which suggests a differential metabolic process depending on the FA species. The distribution of ALA in the lipid fractions of lymph CM vectorized as PL vs. TAG hence deserves to be assessed. Future research must be undertaken to determine the underlying mechanisms of such lipid remodeling. In effect, the distribution of FA in lymph lipoproteins may affect their subsequent metabolic fate and, consequently, their systemic bioavailability.

Moreover, in this study, the recovery of ALA in lymph was faster when incorporated in lecithin compared to TAG, rendering it more readily available for uptake by receiving tissues or subsequent metabolic processes. This is consistent with the observed increases of the expression of genes involved in the β -oxidation of FA (*Acox1* and *Cpt1a*) and the conversion to LC n-3 PUFAs (*Fads2*) with 30% RL, compared to the control. However, the mRNA expression of *Ppara*, a transcription factor implicated in the regulation of genes of FA oxidation, was not modulated by RL. Nevertheless, Morise et al. (45) demonstrated in mice that the regulation of *Ppara* expression by dietary factors, such as ALA, is gender-dependent and occurs mostly in females. Besides its preponderant oxidation, dietary ALA may be converted to LC n-3 PUFAs (16, 46). In fact, rat intestinal villi and crypt cells contain $\Delta 6$ and $\Delta 5$ desaturases that are capable of converting ALA to LC n-3 PUFAs (47). However, these desaturases only metabolize small amounts of dietary ALA, so that only 0.05% to 4% of ALA is enzymatically converted to DHA (48). In this study, the mRNA expression of *Fads2*, which codes for the $\Delta 6$ desaturase, but not that of the FA

elongase 5, *Elovl5*, was modulated by RL. In order to explore in more depth the effect of the vectorization of ALA by RL on its conversion to LC n-3 PUFAs, future studies must be undertaken to assess its metabolic fate not only in the small intestine, but also in hepatocytes, where bioconversion occurs primarily (32).

Of note, this increase of gene expression occurred only when RL was added at supplementation levels, which suggests that RL as an additive in foods may not significantly modulate postprandial lipid metabolism after acute consumption. Nevertheless, the long-term effects following repeated exposure remain unknown and must be assessed in future studies.

In addition, the limitations associated with the use of preclinical in vivo models must be taken into consideration in this study. While rats share many aspects of human metabolism, researchers must be aware of differences between the species (49), such as digestive enzymes (50) and the absence of a gallbladder, which subsequently allows the continuous flow of bile fluids in rats, as opposed to the non-continuous flow of bile fluids stored in the gallbladder in humans (51).

Nonetheless, the mesenteric lymph-cannulated rat model remains to this day the most appropriate and powerful tool to assess, in vivo, the concentration and the transit rate of dietary lipids that pass directly from the intestine to the lymphatic system (22). The obtained data are then a direct reflection of the absorption of lipids, prior to hepatic metabolism and dilution within the endogenous plasma pool, and hence of their bioavailability.

Altogether, we show that, in rats, the addition of RL in oil modulates postprandial lipid absorption both qualitatively and quantitatively. As such, RL not only increases lymphatic lipid output, but also acts as a more potent vector of ALA comparatively to TAG, as reflected by its ability to increase ALA bioavailability. Such an increase in bioavailability may then potentially affect its metabolic fate. These effects do not appear to be significant when RL is added at levels realistically achievable in foods ($\leq 10\%$), and only become significant at levels representative of supplementation (30%). Care must hence be taken when concluding on the effects of RL when provided as food-grade ingredients or as supplementation.

It is now widely acknowledged that postprandial lipemia, low-grade inflammation, and gut microbiota are codependent factors of metabolic disorders. Faced with the dramatic rise of obesity prevalence and the trending growth of the RL market, it is crucial that the long-term effects of RL be explored. This is all the more important since recent research has outlined the deleterious effects of certain synthetic emulsifiers (such as carboxymethyl cellulose and polysorbate 80) on these aspects (2, 3), which consequently also raises questions regarding more natural additives, such as lecithin. Clinical trials are currently being developed to assess the long-term impacts of soy lecithin on intestinal integrity, inflammation, and microbiota (52). Similar studies focusing on RL, which offers alternative, sustainable, and nutritional benefits, must also be undertaken.

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The authors' responsibilities were as follows—CR, LC, CV, and M-CM: designed the research; CR, LC, LF, CB, EE-C, EM, EL, CV, and M-CM: conducted the research; CR, CK, CB, EE-C, EM, and M-CM: analyzed the data; CR, LC, CK, EE-C, CV, and M-CM: wrote the paper; CR, CV, and M-CM: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

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