

Impact of Rapeseed and Soy Lecithin on Postprandial Lipid Metabolism, Bile Acid Profile, and Gut Bacteria in Mice

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Scope: Synthetic emulsifiers have recently been shown to promote metabolic syndrome and considerably alter gut microbiota. Yet, data are lacking regarding the effects of natural emulsifiers, such as plant lecithins rich in essential α -linolenic acid (ALA), on gut and metabolic health.

Methods and Results: For 5 days, male Swiss mice are fed diets containing similar amounts of ALA and 0, 1, 3, or 10% rapeseed lecithin (RL) or 10% soy lecithin (SL). Following an overnight fast, they are force-fed the same oil mixture and euthanized after 90 minutes. The consumption of lecithin significantly increased fecal levels of the *Clostridium leptum* group ($p = 0.0004$), regardless of origin or dose, without altering hepatic or intestinal expression of genes of lipid metabolism. 10%-RL increased ALA abundance in plasma triacylglycerols at 90 minutes, reduced cecal bile acid hydrophobicity, and increased their sulfatation, as demonstrated by the increased hepatic RNA expression of *Sult2a1* ($p = 0.037$) and cecal cholic acid-7 sulfate (CA-7S) concentration ($p = 0.05$) versus 0%-lecithin.

Conclusion: After only 5 days, nutritional doses of RL and SL modified gut bacteria in mice, by specifically increasing *C. leptum* group. RL also increased postprandial ALA abundance and induced beneficial modifications of the bile acid profile. ALA-rich lecithins, especially RL, may then appear as promising natural emulsifiers.

been shown that certain synthetic emulsifiers, such as polysorbate 80, may exert deleterious effects, including alterations of the gut microbiota, associated with low-grade inflammation and metabolic syndrome.^[4,5] Yet, data is lacking when it comes to natural emulsifiers.

Lecithins, complex lipids mixtures predominantly composed of phospholipids (PL),^[6] are the predominant natural emulsifiers used by the food industry. The vast majority of lecithin is obtained from plant sources, most particularly soy, which represents more than 90% of the global market.^[7] Recently, other alternative sustainable sources of lecithin have emerged, such as rapeseed and sunflower.^[8,9] Similarly to their respective oils, soy lecithin (SL) and rapeseed lecithin (RL) are rich in an essential n-3 polyunsaturated fatty acid (n-3 PUFA), α -linolenic acid (ALA).^[10] ALA is involved in numerous biological functions and may play a preventive role in cardiovascular diseases and in stroke.^[11,12] Furthermore, as an essential fatty acid (FA), it is not synthesized

endogenously and must be obtained through the diet. Yet, it is estimated that the majority of the European population fails to meet the recommended daily intakes of ALA, i.e., 1% of total energy.^[13] Since recent data suggests that PL are capable of increasing the bioavailability of the FA they contain compared to triacylglycerols (TAG),^[14–17] RL and SL may then appear as more potent vectors

1. Introduction

Over the last decade, the prevalence of chronic metabolic disorders and the concomitant increase in the use of food additives have led to a growing interest and need to understand the impact of such ingredients on metabolic health.^[1–3] In this regard, it has

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of ALA than their respective oils, and may thus help increase ALA intakes.

Moreover, in both preclinical and clinical studies, the supplementation with vegetal PL has been associated with both increased lipid absorption^[18,19] and beneficial effects on lipid and lipoprotein metabolism,^[20–22] which highlights the importance of PL in intestinal lipid absorption and in the regulation of postprandial lipid metabolism.^[23–25] Dietary PL not only impact lipid absorption and metabolism, but also strongly modulate the gut microbiota, which in turn regulates a plethora of metabolic and inflammatory processes, implicated in the development of chronic metabolic diseases.^[26–28] Indeed, the intestinal microbiota interacts with the host via a number of pathways, notably via the transformation of bile acids (BA).^[29] BA are increasingly recognized as important regulators of intestinal function and gut microbiota properties.^[30] In order to fully comprehend the impact of vegetable lecithins on host metabolism and gut health, it is therefore essential that their effects on the BA pool and the gut microbiota be characterized.

However, the metabolic impact of low nutritional doses of vegetable lecithins remains controversial.^[31,32] Indeed, in Europe, although lecithin may be added at *quantum satis* in most foods (no maximum authorized level declared), its use is restricted in certain food products, namely in infant formulas (maximum level authorized of 1% w/w) and in oils (3% w/w).^[33] Furthermore, for feasibility and palatable reasons, lecithin may not be added at high levels, so that its highest reported use is of 10% in diet management foods.

We have previously shown that incorporating lecithin within an ALA-rich oil can dose-dependently increase its postprandial intestinal absorption.^[34,35] However, these studies were all performed in rodents whose intestine had not previously been in contact with lecithins, while it has been reported that intestinal and metabolic adaptations may occur in mice after several days of lipid exposure.^[36,37] The present study hence aimed to assess, in mice, the potential modifications of host metabolism and gut microbiota that occur after several days of consumption of two ALA-rich lecithins, soy and rapeseed lecithin, at various nutritional doses and with a particular focus on ALA bioavailability, postprandial lipemia, the BA pool and gut microbiota composition.

2. Experimental Section

2.1. Preparation and Characterization of the Lipid Formulations and Diets

Soybean and rapeseed lecithin (SL and RL) were kindly provided by Novastell (Etrepagny, France). Grapeseed, rapeseed, and palm oils were provided by ITERG (Canéjan). The lipid formulations were prepared by mixing the aforementioned vegetable oils with varying doses of RL or SL under magnetic agitation, so as to obtain blends of similar FA profiles ($\approx 4\%$ ALA) with no prior emulsification. This hence allowed to focus on the sole presence of lecithins, with no additional effect of emulsified lipid structure. The lipid blends were then either stored under N_2 for future administration or incorporated into delipidated powdered diets (no. U8958 version 0244, SAFE, Augy). In order to avoid any confounding effect related to other nutrients, all of the diets contained the same amount of lipid-free diet base (95 wt%) and

of lipid blends (5 wt%), and were hence nutritionally balanced with equal macronutrient repartition and caloric input. Detailed composition of the diets is presented in Table S1, Supporting information.

The FA compositions of the two lecithins and lipid formulations were determined using a gas chromatograph (GC) equipped with a flame ionization detector (FID), as described in Section 2.5 (Tables 1 and 2). The quantification of the lipid fractions of the two lecithins was evaluated by high-performance thin layer chromatography (HPTLC) (Supplementary Experimental Section).

2.2. Animals and Experimental Procedure

All experiments and procedures were performed in accordance with the EU Council Directive for the Care and Use of Laboratory Animals (no. 2010/63/EU). The protocol was approved by the Animal Ethics Committee of National Institute of Applied Sciences of Lyon and registered under the number CETIL 01 2014. Experiments were performed using male Swiss mice (Elevage Janvier), obtained at 4 weeks old and weighing 30–40 g. The mice were acclimatized for 7 days prior to the experiment, during which they were housed six per cage and kept in a temperature-controlled environment (temperature $24 \pm 1^\circ\text{C}$; 12h light – 12h dark cycle) with free access to water and food.

After acclimatization, the mice were randomly assigned to one of the five experimental groups ($n = 12$ mice/group) and, during 5 days, were fed diets in which the oil blend contained no lecithin, 1%, 3%, or 10% RL, or 10% SL. In order to avoid a potential lipid carryover effect on postprandial lipids originating from previously deposited lipids within enterocytes,^[38] animals were transiently placed back on a standard chow diet and then food-deprived for 12 h before the intervention, upon which they received, via oral intubation, 150 μL of the same respective oil mixture they had consumed during the 5-day diet. The mice were euthanized 90 minutes after intubation by inhalation of isoflurane. Death was induced by subsequent cardiac puncture. Blood was collected directly in heparin-containing tubes and placed immediately in ice. Plasma was obtained by centrifugation (4600 rpm at 4°C for 5 min). The small intestine was segmented into three parts with length ratios of 1:3:2 corresponding to the duodenum:jejunum:ileum. The plasma, the liver, the intestinal segments, and caecum were weighed, frozen in liquid nitrogen, and stored at -80°C until analysis.

2.3. Dosage Information/Dosage Regimen

On average, the mice weighed 37 g and consumed 8.8 g of diet per day. According to the FDA's Human Equivalent Dose calculation, the daily doses of lecithin consumed by the mice are equivalent to 10 $\text{mg}\cdot\text{kg}^{-1}$ body weight in humans for the 1% lecithin group, 29 $\text{mg}\cdot\text{kg}^{-1}$ for the 3% lecithin group and 97 $\text{mg}\cdot\text{kg}^{-1}$ for both 10% lecithin groups.^[39] Concerning the gavage, the mice received a 150 μL bolus of oil mixtures containing 0, 1, 3, or 10% lecithin. In humans, these doses are equivalent to 0, 3, 10, or 33 $\text{mg}\cdot\text{kg}^{-1}$ body weight, respectively. According to the EFSA Panel on Food Additives, the estimated average exposure to lecithins as food additives ranges from 32 to 177 $\text{mg}\cdot\text{kg}^{-1}$ body weight per day in

Table 1. Main fatty acid profile and general composition of the lipid formulations.

	Control	1% RL	3% RL	10% RL	10% SL
Fatty acid composition (g 100 g ⁻¹ of total fatty acids) ^{a)}					
12:0	0.06	0.06	0.07	0.06	0.05
14:0	0.2	0.2	0.3	0.3	0.2
15:0	0.03	0.03	0.03	0.04	0.03
16:0	14.2	14.3	14.3	14.5	14.0
18:0	2.8	2.8	2.8	2.7	2.8
18:1(n-9)	45.4	45.1	45.1	45.2	47.2
18:1(n-7)	1.9	1.9	1.9	1.9	1.9
18:2(n-6)	28.8	28.9	28.8	28.7	27.2
18:3(n-3)	4.3	4.3	4.3	4.3	4.4
20:0	0.4	0.4	0.4	0.4	0.4
20:5(n-3) ^{b)}	–	–	–	–	–
22:0	0.2	0.2	0.2	0.2	0.2
22:6(n-3) ^{b)}	–	–	–	–	–
24:0	0.08	0.08	0.08	0.08	0.1
Σ(SFA) ^{c)}	18.1	18.1	18.2	18.2	17.9
Σ(MUFA) ^{c)}	48.5	48.3	48.2	48.3	50.2
Σ(PUFA) ^{c)}	33.1	33.2	33.1	33.0	31.5
Σ(n-6 PUFA) ^{c)}	28.8	28.9	28.8	28.8	27.2
Σ(n-3 PUFA) ^{c)}	4.3	4.3	4.3	4.3	4.4
Ratio n-6/n-3 PUFA ^{c)}	6.7	6.7	6.7	6.7	6.2
Σ(TFA) ^{c)}	0.3	0.4	0.4	0.4	0.3
Ingredient (g 100 g ⁻¹ of final mixture)					
Rapeseed oil	51	50	49	45	46
Palm oil	22	22	22	21	19
Grapeseed oil	27	27	26	24	20
Rapeseed lecithin	–	1.0	3.0	10	–
Soybean lecithin	–	–	–	–	10

^{a)} Fatty acid values were obtained by gas chromatography coupled to a flame ionization detector (GC-FID); ^{b)} Of note, the lipid formulations were devoid of LC n-3 PUFAs; ^{c)} MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; RL, rapeseed lecithin; SFA, saturated fatty acids; SL, soybean lecithin; TFA, trans-fatty acids.

adolescents, and from 70 to 118 mg.kg⁻¹ body weight per day in adults.^[33] The doses used in this study are hence representative of the daily intakes of the population.

2.4. Plasma and Hepatic Lipid Analysis

Plasma and liver samples were thawed to room temperature. Free FA were extracted according to the technique described by Bligh et Dyer.^[40] PL and TAG were separated by thin layer chromatography (TLC), derived into FAME by transmethylation,^[41,42] which were analyzed by GC-FID (Supplementary Experimental Section).

2.5. Real-time Quantitative RT-PCR Analysis

Total RNA was extracted from the jejunum, ileum and liver using the TRI Reagent (Ambion/Applied Biosystems). RNA quality and concentration were measured using the Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). As described in,^[43] reverse transcription was performed using 1 µg of RNA and the PrimeScript RT reagent kit

(Ozyme, Saint Quentin-en-Yvelines, France). Real-time PCR assays were then performed using a Rotor-Gene Q (Qiagen, Hilden, Germany) and SYBR qPCR Premix Ex Taq (Tli RNaseH Plus) reagents. PCR primers are listed in Table S2, Supporting information. The results were normalized using the expression of the *Tbp* (TATA-box-binding protein) gene as a reference.

2.6. Fecal Microbiota Analysis

Feces were collected after the 5-day diet. The analysis of fecal microbiota populations was performed, as previously described.^[44,45] Briefly, feces were subjected to a lysozyme treatment (100 mg of feces in 500 µL of a TE solution containing 30 mg mL⁻¹ of lysozyme) with an incubation of 30 min at 37°C. DNA was then extracted using 200 µL of the obtained solution and the NucleoSpin Soil Genomic DNA isolation kit (Macherey-Nagel, France). Several primer pairs were selected to evaluate specific bacterial populations of interest: total *Bacteroidetes*, total *Firmicutes*, *Bifidobacteria*, *Escherichia coli*, *Akkermansia muciniphila*, *Clostridium coccoides*, *Clostridium*

Table 2. Fatty acid and phospholipid composition of both rapeseed and soybean lecithin.

	Rapeseed lecithin	Soybean lecithin
Fatty acid composition (g 100 g ⁻¹ of total FA) ^{a)}		
16:0	11.5	22.0
18:0	0.8	3.0
18:1 (n-9)	47.7	15.4
18:2 (n-6)	29.8	51.9
18:3 (n-3)	4.8	4.1
20:5 (n-3) ^{b)}	—	—
22:6 (n-3) ^{b)}	—	—
Σ (SFA) ^{c)}	13.3	26.1
Σ (MUFA) ^{c)}	51.3	17.6
Σ (PUFA) ^{c)}	34.9	56.1
Σ (n-6 PUFA) ^{c)}	29.9	52.0
Σ (n-3 PUFA) ^{c)}	4.8	4.1
Σ (TFA) ^{c)}	0.6	0.2
Phospholipid composition (g 100 g ⁻¹ of total PL) ^{a)}		
PC	34.0	34.8
PE	40.6	38.6
PI + PS	15.0	14.7
Lyso-PC	3.7	1.9
Lyso-PE	2.7	5.5
Others	4.0	4.5

^{a)} Data was obtained by gas chromatography coupled to a flame ionization detector (GC-FID) and high-performance thin layer chromatography (HPTLC), respectively; ^{b)} Of note, both lecithins are devoid of LC n-3 PUFAs; ^{c)} MUFA, mono-unsaturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, poly-unsaturated fatty acids; SFA, saturated fatty acids; TFA, trans-fatty acids.

leptum group, *Lactic acid bacteria*, and *Faecalibacteria prausnitzii*. The quantification of these populations was performed using primers synthesized by Biomers (France) and listed in Table S3, Supporting information. PCR reactions were carried out on a CFX96 System (Bio-Rad) using iTaq SYBR Green Universal Supermix (BioRad, France). The resulting data were collected and analyzed in CFX Maestro (BioRad), using standard curves obtained from pure cultures.

2.7. Cecal Bile Acid Analysis

Cecal BA molecular species concentrations were measured by HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) using a 5500Q-trap (Sciex), as previously described.^[46] The hydrophobicity index reflects BA hydrophobicity, taking into account the concentration and the retention time of different BA on a C18 column with a gradient of methanol; lithocholic acid has the highest retention time, and tauroursodeoxycholic acid-3S the lowest.

2.8. Statistical Analysis

All statistical analyses were performed using the GraphPad Prism software (version 7). Data normality and homogeneity of

variance were verified using a Shapiro-Wilk test and Bartlett test, respectively. Means of lipid and BA concentration and relative abundance, mRNA gene expression, and bacterial count were compared across groups using one-way ANOVA. For non-normal data, a Kruskal-Wallis test was performed instead. When the analysis revealed a significant effect, a Dunnett's post hoc test (using 0% lecithin as control) was used to identify the dose of lecithin contributing most to the effect. To evaluate possible relationships among the various outcomes, Spearman correlations were performed. Conventional values of $p < 0.05$ were considered statistically significant. Data in tables and figures is presented as mean \pm SEM.

3. Results

3.1. Lecithin and Diet Composition

As revealed by GC-FID analysis, the two plant-derived lecithins differed in their FA composition (Table 2). Although both were predominantly composed of oleic acid (OA), linolenic acid (LA), and palmitic acid (PA), RL contained higher amounts of OA, while SL was characterized by its high LA content. Both lecithins contained similar amounts of ALA (≈ 4 g 100 g⁻¹). As such, the FA composition of the lecithin resembled that of its oil-bearing seed, as reported in the literature.^[6] The polar lipid compositions of SL and RL were equally similar, both mainly constituted of phosphatidylcholine (PC) and phosphatidylethanolamine (PE). SL contained twice the amount of lyso-PE and half that of lyso-PE found in RL.

In accordance to the study design, all formulations presented similar FA profiles, mainly composed of OA (≈ 45 g 100 g⁻¹), LA (≈ 28 g 100 g⁻¹), PA (≈ 14 g 100 g⁻¹), and ALA (≈ 4.3 g 100 g⁻¹), which differed only in their vectorization form (Table 1). In this way, the higher the lecithin dose present in the oil mixture, the higher the amount of ALA vectorized by polar lipids.

Of note, both the lecithins and the lipid mixtures were obtained from plant sources and were consequently devoid of the long-chain (LC) n-3 PUFAs, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).

3.2. The Impact of Lecithin on Postprandial Lipemia

In order to assess the impact of ALA-rich lecithin on postprandial lipemia and ALA bioavailability, the FA composition of both the TAG and the PL fractions in plasma were evaluated at 90 minutes after gavage (**Figure 1**). Whereas the concentration of total PL in plasma was not found to differ between groups, that of total TAG was increased 1.6-fold in the 10%-RL group compared to control, i.e., those who had not consumed any lecithin, although this effect was not found to be statistically significant.

Regarding ALA specifically, the consumption of 10% RL induced a significant increase in the relative percentage of ALA in plasma TAG, compared to control ($p = 0.011$). Interestingly, this increase was not observed in the 10%-SL group, suggesting a specific effect of RL. The relative percentage of ALA in plasma PL was not found to differ.

Interestingly, the percentage of EPA and DHA in plasma PL was significantly decreased in all groups of mice, which had

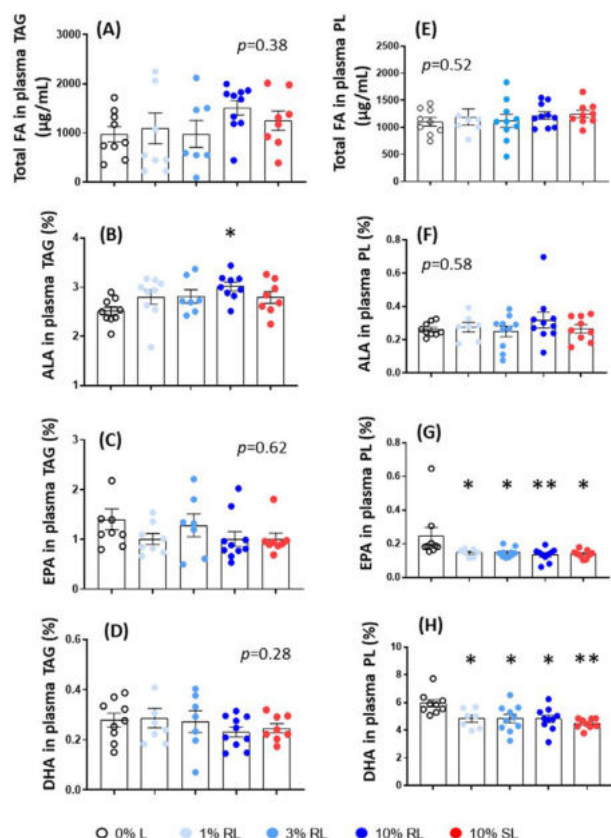


Figure 1. The concentration of total fatty acids in (A) the TAG fraction of plasma, and their relative percentage of (B) ALA, (C) EPA, and (D) DHA, as well as concentration of total fatty acids in the (E) PL fraction of plasma, and the relative percentage of (F) ALA, (G) EPA, and (H) DHA within the PL fraction, in mice following the oral administration of lipid mixtures containing 0% to 10% RL or SL. Values represent mean \pm SEM, $n = 7-11$. To test the impact of lecithin on these plasmatic lipids, all groups were analyzed by one-way ANOVA, followed by Dunnett's post-hoc test versus 0% lecithin (control). Means statistically different from control are presented as * $p < 0.05$; ** $p < 0.01$. ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PL, phospholipid; RL, rapeseed lecithin; SL, soybean lecithin; TAG, triacylglycerol.

consumed lecithin versus control ($p = 0.024$ and $p = 0.0032$, respectively), regardless of lecithin dose or origin.

In order to evaluate whether the observed decrease in the relative abundance of EPA and DHA in plasma at 90 min was the result of their dilution amongst the FA pool, the lipid composition of both the TAG and PL fractions in the liver of these mice was also determined (Figure 2). No difference in FA composition, be it ALA, EPA, or DHA, was observed in either lipid fractions in the liver.

As RL increased the abundance of ALA in plasma TAG at 90 min, we evaluated the impact of RL on 1) the expression of genes implicated in lipid absorption in the jejunum (Figure S4, Supporting Information) and on 2) the intestinal and hepatic expression of genes involved in n-3 PUFA metabolic pathways, such as beta-oxidation and bioconversion to n-3 LC-PUFAs (Figure S5, Supporting Information). No difference was observed.

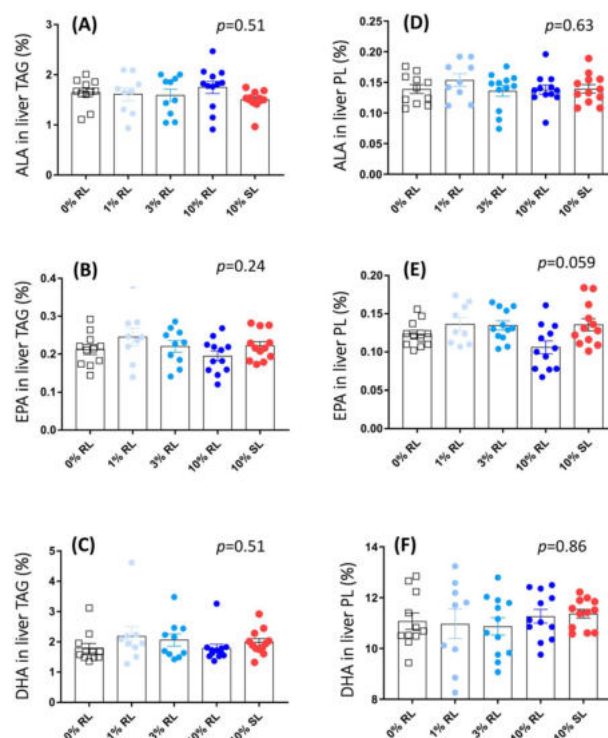


Figure 2. The relative percentage of (A) ALA, (B) EPA, and (C) DHA within the TAG fraction of liver lipids, as well as the relative percentage of (D) ALA, (E) EPA, and (F) DHA within the hepatic PL fraction, in mice following the oral administration of lipid mixtures containing 0% to 10% RL or SL. Values represent mean \pm SEM, $n = 9-12$. To test the impact of lecithin on these hepatic lipids, all groups were analyzed by one-way ANOVA. ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PL, phospholipid; RL, rapeseed lecithin; SL, soybean lecithin; TAG, triacylglycerol.

3.3. Impact on Gut Bacterial Groups

As both dietary lipids and synthetic emulsifiers have been shown to alter the gut microbiota, we also assessed the impact of both lecithins on gut microbiota composition. Total bacterial count was evaluated, as well as the prevalence of specific bacterial groups families and species of known interesting metabolic effects (described in the Methods section) (Figure S6, Supporting Information). The total bacterial count, as well as the *Firmicutes/Bacteroidetes* ratio did not differ between groups (Figure 3). Of all bacterial populations tested, only one group was found to be significantly modified by lecithin consumption. The count corresponding to the *Clostridium leptum* group was increased in all groups which had consumed lecithin, regardless of dose or origin ($p = 0.0004$, Figure 3).

3.4. Rapeseed Lecithin Modified Bile Acid Composition in the Caecum

As the gut microbiota and BA are known to be strongly intertwined, we further investigated the impact of SL and RL on the BA pool in the caecum (Figure 4).

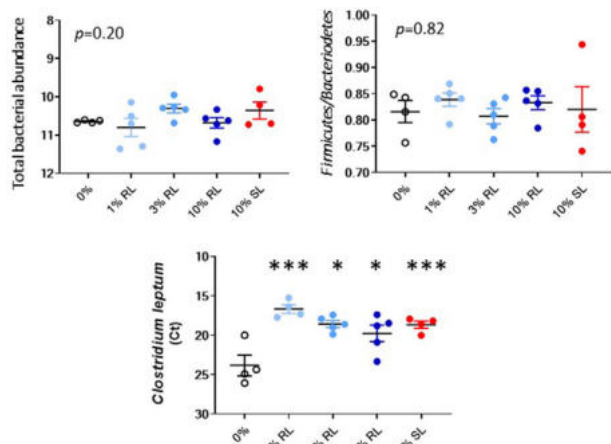


Figure 3. Total bacteria abundance, ratio of *Firmicutes/Bacteroidetes* bacteria and DNA expression of *Clostridium leptum* in the feces of Swiss mice following 5 days of consumption of diets containing 0–10% RL or SL. Bars represent mean ± SEM, $n = 4$ –5. To test the impact of lecithin on these parameters, all groups were analyzed by one-way ANOVA, followed by Dunnett's post hoc test versus 0% lecithin. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Ct, number of cycles; RL, rapeseed lecithin. SL, soybean lecithin.

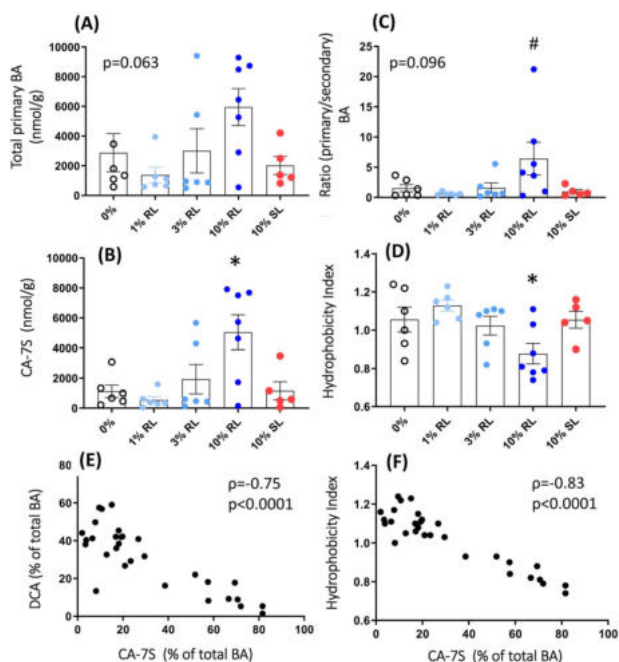


Figure 4. The concentration of (A) total primary bile acids and (B) CA-7S, (C) the ratio of primary to secondary bile acids, and (D) the hydrophobicity index of the bile acid pool in the caecum of mice, following 5 days of consumption of diets containing 0–10% RL or SL. E) The Spearman correlation between the relative percentage of CA-7S and DCA; F) The Spearman correlation between the relative percentage of CA-7S and the bile acid pool hydrophobicity index. Bars represent mean ± SEM, $n = 5$ –7. To test the impact of lecithin on the bile acid pool, all groups were analyzed by one-way ANOVA, followed by Dunnett's post-hoc test versus control. Means statistically different from control are presented as #0.1 < $p < 0.05$; * $p < 0.05$. BA, bile acid; CA-7S, cholic acid–7sulfate; DCA, deoxycholic acid; RL, rapeseed lecithin; SL, soybean lecithin.

Herein, no statistically significant differential effect between groups was observed regarding the weight of the caecum or the amount of total cecal BA (cecal BA profiles are presented in Figure S7, Supporting Information). However, the amount of total primary BA was doubled in the 10%-RL group compared to control, leading to a higher ratio of primary to secondary BA ($p = 0.096$) in the 10%-RL group versus control. This may be primarily attributed to a significant increase in the concentration of sulfated cholic acid (CA-7S) in the 10%-RL group ($p = 0.05$) versus control, as this sulfated BA represented more than 80% on average in the 10%-RL group. The increase in sulfated BA was associated with a decrease in the hydrophobic index of the cecal BA pool ($p = 0.05$) in the 10%-RL group versus control, as evidenced by the inverse correlation between the two parameters ($\rho = -0.83$, $p < 0.0001$). The increase in CA-7S was also inversely correlated with the relative abundance of the secondary BA, deoxycholic acid (DCA) ($\rho = -0.75$, $p < 0.0001$). Again, these changes were not observed in the 10%-SL group, suggesting a specific effect of RL.

3.5. Gene Expression of Entero-hepatic Bile Acid Metabolism and Inflammation

In order to explore potential mechanisms for the sulfation of BA, we assessed, in both the liver and the ileum (Figures 5 and 6), the mRNA expression of genes involved in enterohepatic BA metabolism. In the liver, *Sult2a1* was overexpressed in the 10%-RL compared to control ($p = 0.037$). In the ileum, the mRNA expression of none of the main genes involved in the main metabolic pathways of BA was modified.

4. Discussion

The present study aimed to evaluate the impact of the short-term consumption of nutritional doses of two ALA-rich vegetable lecithins on postprandial lipemia and ALA bioavailability, as well as other regulators of lipid homeostasis and intestinal health, such as BA and gut microbiota.

Dietary PL have been previously reported to increase postprandial lipid absorption.^[18,31,47] Nonetheless, their stimulatory effect seems to be dose-dependent and the effects of low nutritional doses of lecithin remain controversial.^[32] In this way, we have recently demonstrated that the gastric administration of RL in lymph-cannulated rats dose-dependently increased the output of lipids in lymph, but that this stimulatory effect only became significant at high supplementation doses (30%).^[34] This has been confirmed in other studies in which the administration of dietary PL at low PL/TAG ratios (1/16 – 1/7) did not generate an increase in lipid lymphatic output.^[48,49]

The results obtained in this study complete these previous findings, as we show that, after 5 days of intestine exposure, nutritional doses ($\leq 10\%$) of RL and SL do not significantly increase the postprandial absorption of lipids. This is reflected by the absence of a significant effect of either lecithin on both the amount of plasmatic lipids and the expression of lipid absorption-related genes in the small intestine. Altogether, these results highlight the differential metabolic impacts of vegetable lecithins consumed either as food ingredients or as supplements. In the current context of obesity and related hyperlipidemia, the fact that nutritional

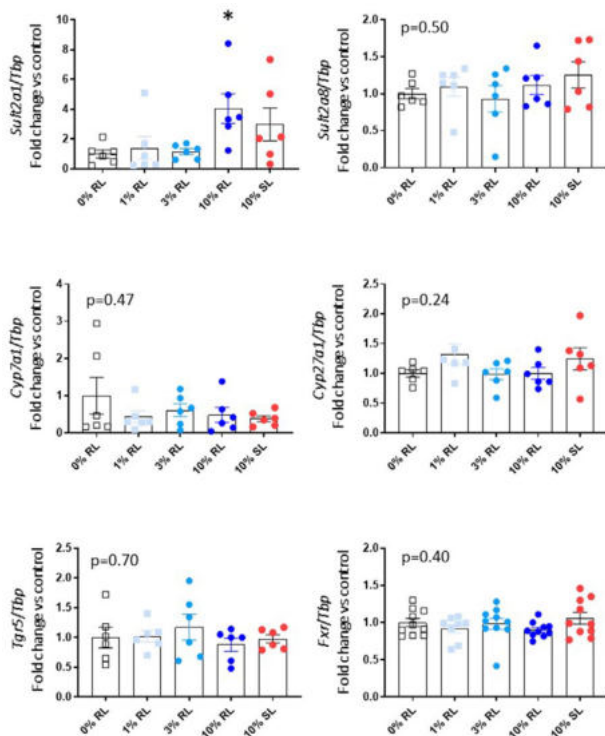


Figure 5. The mRNA expression of genes involved in the enterohepatic bile acid metabolism in the liver of mice. Values are normalized to the levels of the mRNA expression of *Tbp* and expressed as relative amount compared with control (0% lecithin). Bars represent mean \pm SEM, $n = 8-9$. To test the impact of lecithin on the expression of these genes, all groups were analyzed by one-way ANOVA, followed by Dunnett's post-hoc test versus control. Means statistically different from control are presented as $*p < 0.05$. *Cyp27a1*, sterol 27-hydroxylase; *Cyp7a1*, cholesterol 7- α -hydroxylase; *Fxr*, farnesoid X receptor; RL, rapeseed lecithin; *Sult2a1*, sulfotransferase 2A1; *Sult2a8*, sulfotransferase 2A8; SL, soybean lecithin; *Tbp*, TATA box binding protein; *Tgr5*, Takeda G Protein-coupled Receptor 5.

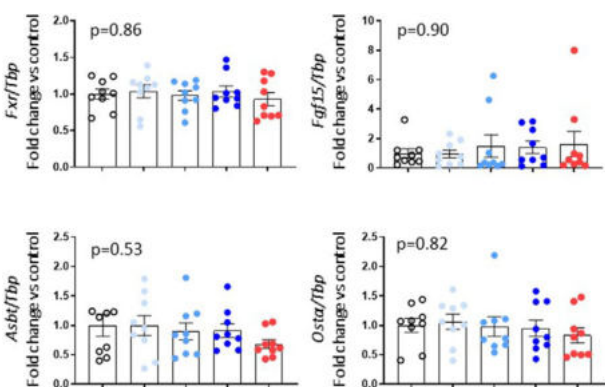


Figure 6. The mRNA expression of genes involved in the enterohepatic bile acid metabolism in the ileum. Values are normalized to the levels of the mRNA of *Tbp* and expressed as relative amount compared with control (0% lecithin). Bars represent mean \pm SEM, $n = 8-9$. To test the impact of lecithin on the expression of these genes, all groups were analyzed by one-way ANOVA. *Asbt*, apical sodium–bile acid transporter; *Fgf15*, fibroblast growth factor 15; *Fxr*, farnesoid X receptor; *Osta*, organic solute transporter alpha; R, rapeseed lecithin; SL, soybean lecithin; *Tbp*, TATA box binding protein.

doses of vegetable lecithins do not increase lipemia conveys a rather reassuring message regarding their use as emulsifiers, which deserves to be confirmed in humans.

Moreover, the present study is the first, to our knowledge, to compare two plant sources of a similar type of natural emulsifier. We demonstrate that the addition of 10% RL, but not SL, induced an increase in the abundance of ALA in plasma TAG at 90 min. As both lecithins have a similar PL content, this stimulatory effect of RL on ALA plasmatic abundance may not be solely attributed to its vectorization as PL. Indeed, although recent data has described PL as potential preferential vectors of FA compared to TAG, notably concerning marine n-3 LC-PUFAs,^[14–17] their capacity to increase systemic FA bioavailability remains controversial.^[42,50] In this way, using a similar study design to the one employed here, but in piglets, Amate et al. concluded that the bioavailability of n-3 LC PUFAs in lymph was unchanged, but that their distribution within lipoproteins was modulated, when provided as egg PL comparatively to marine TAG.^[51]

Nevertheless, the observed difference in plasma ALA abundance between RL and SL may result from differences in lipolysis and intestinal absorption rates. Indeed, the emulsification of dietary lipids and their interaction with bile salts and digestive enzymes in both the stomach and the small intestine are strongly impacted by the nature of the emulsifier.^[35,52] As such, in the present study, the different FA composition of rapeseed and soy lecithins may have led to differences in lipolytic rate. Indeed, the FA composition of vegetable oils, as well the distribution of the FA within TAG molecules, has been shown to modulate their digestion rates and extent.^[53,54] The observed difference between the two lecithins on plasma ALA abundance may also arise from a synergistic effect of ALA with other FA. In a combination of in vitro and in vivo studies, the digestion and absorption of ALA was greater when it was incorporated in rapeseed oil, rich in oleic acid and ALA, than that in sunflower oil, rich in n-6 PUFA.^[55] It may then be of interest to compare, in future studies, the lipolysis rates, in vitro digestion and enterocyte absorption of RL and SL.

Contrarily to the plasma fraction, which is subject to constant fluctuations in FA composition, the liver offers a more stable reflection of lipid metabolic status. Hence, the identical abundance of the different n-3 PUFAs (ALA, DHA, EPA) in the TAG and PL compartments of the liver in all mice suggests that the observed differences in plasma PL are the result of differential postprandial kinetics, rather than nutritional status. It may then be suggested that nutritional doses of vegetable lecithins are not sufficient to significantly modify ALA bioavailability and body lipid status in the short term. This concurs with the fact that the hepatic and intestinal expression of genes involved in the metabolism of ALA and n-3 PUFAs, such as beta-oxidation or bioconversion to n-3 LC-PUFAs, did not differ among groups.

Besides their role on lipid metabolism, dietary PL represent potential modulators of gut microbiota. As recent reports have highlighted detrimental effects of synthetic emulsifiers on gut microbiota,^[4,5,56] we additionally aimed to assess the short-term impact of RL and SL on the abundance of specific bacterial groups, families, and species known for their metabolic effects. Indeed, it has recently been shown in humans randomized to either plant-based or animal-based diets that the gut microbiome is capable of responding quickly (2–4 days) to changes in nutrient

microenvironment.^[57] We show here that the fecal bacterial count of *Clostridium leptum* group was increased in mice after only 5 days of consumption of vegetable lecithins, regardless of their source or dose. *C. leptum* group, also commonly referred to as Clostridial cluster IV, represents one of the dominant groups of fecal bacteria in humans (16–25%).^[58] The *C. leptum* subgroup is largely composed of fibrolytic, butyrate-producing bacteria, generally associated with beneficial anti-inflammatory effects.^[59] Patients suffering from chronic inflammatory diseases, such as Crohn's disease and ulcerative colitis, have been shown to have reduced fecal levels of *C. leptum*.^[60] The short-term consumption of SL and RL hence seems to induce beneficial shifts in gut microbiota populations. Nonetheless, the impact of RL and SL on the gut microbiota was assessed on a limited number of fecal microbial species. A more global metagenomic analysis is now required to grasp a more in-depth understanding of the impact of these natural emulsifiers on bacterial diversity and function.

Bile acids are increasingly recognized as important regulators of lipid metabolism and gut microbiota properties.^[30,61] In fact, changes in the BA pool precede and causally impact shifts in intestinal microbial populations.^[62] We hereby demonstrate that the short-term consumption of 10% RL, but not SL, induces an increase in the abundance of sulfated BA, primarily of CA-7S. This observed sulfation appears to take place predominantly in the liver, as demonstrated by the increase in the hepatic mRNA expression of *Sult2a1* in the 10% RL group versus control.

In hepatocytes, the sulfation of BA is catalyzed by sulfotransferases, notably at the 3, 7, and 12 positions.^[63] This renders them more hydrophilic, thereby promoting their elimination in urine or feces and reducing their toxicity.^[64] In addition, BA-sulfates are poor substrates for the apical sodium-dependent BA transporter (ASBT),^[64] thus the sulfation of BA markedly inhibits their passive re-absorption. Furthermore, the increase in sulfation of CA at the 7 position in the liver inhibits its microbial conversion to DCA,^[65] which corroborates the observed decrease in the relative abundance of DCA in the 10%-RL group. As DCA is considered toxic, this increase in BA sulfation by RL may then result in beneficial effects on intestinal health.

Nevertheless, these observations in mice may not be directly transposed to humans, as differences in BA metabolism between the two species are important^[30]; future studies must be undertaken to specifically explore the effects of vegetable lecithins on BA metabolism in humans.

The presence of emulsifiers within foods is evermore prevalent, which leads us to question their health impacts. This study hence aimed to assess the short-term impact of two natural emulsifiers on markers of gut and metabolic health. Our results demonstrate that nutritional doses (<10%) of RL and SL, despite a potential effect on postprandial plasmatic lipid kinetics, do not affect plasmatic or hepatic lipid concentrations and metabolism. However, after only 5 days of consumption, both vegetable lecithins were capable of modifying certain gut bacterial groups, by increasing *C. leptum*, a health-promoting, anti-inflammatory bacterial group. In the current context of obesity and associated low-grade inflammation, the use of these natural ingredients therefore seems preferential over that of synthetic emulsifiers, whose detrimental effects on the microbiota and host gut health have been recently reported.

Interestingly, our study also highlights some differential metabolic impacts of the two plant-derived lecithins. RL, but not SL, induced an increase in the postprandial abundance of ALA in plasma and in the sulfation of primary BA, suggesting a specific beneficial impact on metabolic and intestinal health. As most studies using vegetable lecithins have focused on soy, which displays important societal concerns regarding sustainability and the use of genetically modified organisms, rapeseed lecithin appears as a novel food ingredient with promising beneficial nutritional benefits.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

C.R., L.C., H.A., and C.V. are employees of ITERG. M.-C. M. is an external academic expert member of the Scientific Committee of ITERG. C.R. acknowledges a CIFRE PhD grant from ANRT. M.-C.M. received other research funding on other topics from Sodial-Candia R&D, the Centre National Interprofessionnel de l'Economie Laitière (CNIEL, French Dairy Interbranch Organization) and Nutricia Research and had consultancy activities for food & dairy companies. These activities had no link with the present article. All authors are members of UMT (Mixed Technological Unit) ACTIA BALI (BioAvailability of Lipids and Intestine). Other authors have no conflict of interest to declare.

Authors Contribution

C.R., C.B., C.V., and M.-C.M. designed the research; C.R., C.B., F.L., H.B., L.H., D.R., E.M., E.L., F.C., J.V.W., M.U., C.V., and M.-C.M. conducted the research; C.R., C.B., and M.-C.M. analyzed the data and performed statistical analyses; C.R., E.M., E.L., D.R., L.H., C.V., and M.-C.M. provided useful scientific insight; C.B. and F.L. participated in the in vivo experiments; E.M. and E.L. performed qPCR analysis; J.V.W. and M.U. performed fecal microbiota analysis; L.H. and D.R. performed bile acid analysis; C.R. and M.-C.M. wrote the paper; C.R., C.V., and M.-C.M. had primary responsibility for final content. All authors read and approved the final manuscript.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Keywords

absorption, food additive, intestine, nutrition, phospholipid

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