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Long-term intake of *Lacticaseibacillus helveticus* enhances bioavailability of omega-3 fatty acids in the mouse retina

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Abstract

Omega-3 (n-3) polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA), are required for the structure and function of the retina. They could also help to prevent or delay the development of retinopathies. Given the accumulating evidence showing the role of gut microbiota in regulating retinal physiology and host lipid metabolism, we evaluated the potential of long-term dietary supplementation with the Gram-positive bacterium *Lacticaseibacillus helveticus* strain VEL12193 to modulate the retinal n-3 PUFA content. A set of complementary approaches was used to study the impact of such a supplementation on the

gut microbiota and host lipid/fatty acid (FA) metabolism. *L. helveticus*-supplementation was associated with a decrease in retinal saturated FAs (SFAs) and monounsaturated FAs (MUFAs) as well as an increase in retinal n-3 and omega-6 (n-6) PUFAs. Interestingly, supplementation with *L. helveticus* enriched the retina in C22:5n-3 (docosapentaenoic acid, DPA), C22:6n-3 (DHA), C18:2n-6 (linoleic acid, LA) and C20:3n-6 (dihomo gamma-linolenic acid, DGLA). Long-term consumption of *L. helveticus* also modulated gut microbiota composition and some changes in OTUs abundance correlated with the retinal FA content. This study provides a proof of concept that targeting the gut microbiota could be an effective strategy to modulate the retinal FA content, including that of protective n-3 PUFAs, thus opening paths for the design of novel preventive and/or therapeutical strategies for retinopathies.

Introduction

The retina is the tissue that lines the back of the eyes and converts light into electrical signals for the brain. It consists of the neuroretina that contains the light-sensitive cells, laying on the retinal pigmentary epithelium (RPE), a single layer of post-mitotic cells that nourishes and protects the neuroretina. The retina is the third tissue with the highest content in lipids in the human body after the adipose tissue and the brain. Retinal lipids are mostly phospholipids, representing 87.3% of total lipids in the neuroretina and 58.3% in the RPE [1,2]. This high content in phospholipids makes the retina very rich in fatty acids (FAs), particularly in docosahexaenoic acid (DHA) that is a polyunsaturated fatty acid (PUFA) belonging to the omega-3 (n-3) series [2]. DHA and its derivatives are crucial for visual function as well as for protecting retinal cell against inflammation, oxidative stress, apoptosis or neovascularization [3-7]. The essential role of n-3 PUFAs in the retina physiology is also supported by a number of observational studies indicating that a high dietary intake of fish rich in n-3 long chain (LC)-PUFAs is associated with a reduced risk of developing retinopathies such as age-related macular degeneration (AMD) [8].

The retinal physiology, including its FA composition, is very sensitive to diet [9-12]. In addition to impacting host lipids through the nature of the lipids it provides, diet can also indirectly influence host lipid metabolism by acting on the gut microbiota [13]. Indeed, the gut microbiota is involved in the regulation of different aspects of the host lipid metabolism (e.g., intestinal absorption, tissue storage, systemic transport and endogenous biosynthesis) [14-18].

A growing body of evidence suggests the existence of a gut microbiota-retina axis. Alterations of the gut microbiota have been described in patients with retinal diseases, including AMD [19-21]. Moreover, several studies suggest that the gut microbiota could influence pathophysiological mechanisms in the retina such as neurodegeneration, pathological vascularization and inflammation [19,22-25]. The gut microbiota could also affect the retina lipid

content. Comparison of the lipidome of retinas from germ-free mice and conventionally raised mice revealed that the presence of gut microbiota is associated with change in the glycerophospholipids profile of the retina [26,27]. Moreover, we recently reported that modulating gut microbiota composition through a prebiotic-based approach leads to alterations in liver FA content, which is known to address FA-rich lipoproteins to the retina [14,28].

In light of the evidence supporting the existence of a gut microbiota-retina axis and the role of the gut microbiota in regulating host lipid metabolism, manipulating the gut microbiota to modulate retinal lipid content seems an attractive approach. In this line, the use of probiotics, defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" could be an interesting strategy [29]. The lactic acid bacteria (LAB) are a group of microorganisms commonly used as probiotics. Interestingly, several experimental studies suggest that retinal physiology could be influenced by oral administration of probiotics [30-32]. In the present study, we investigated the impact of long-term dietary intake of a LAB strain, *Lacticaseibacillus helveticus* (*L. helveticus*) VEL12193, on the bioavailability of FAs to the retina. For that purpose, mice were fed either a control diet or a diet enriched in *L. helveticus* for 6 months. Gut microbiota composition was analyzed, and host lipid metabolism was studied in different organs/tissues of interest (liver, plasma and retina).

Results

Body weight, food intake and fat deposition

Weight gain was evaluated in mice after a 6-month exposure period to a diet supplemented or not with *L. helveticus* VEL12193. Administration of *L. helveticus* was well-tolerated by the mice, with no noticeable side effects, including on the consistency of feces. We observed that supplementating mice with *L. helveticus* significatively limited weight gain compared to control mice (**Fig. 1a**). This phenotype was not the consequence of a change in eating behavior since

92 the amount of food consumed daily was identical for the two groups (**Fig. 1b**). It was also not
93 associated with modification in visceral fat deposition, as evidenced by measurement of
94 epididymal fat weight (**Fig. 1c**).

95

96 Lipid metabolism in the liver

97 We investigated whether long-term consumption of *L. helveticus* VEL12193 impacts lipids in
98 the liver, a central organ of lipid metabolism. Analysis of the distribution of the lipids in the
99 different classes showed that dietary supplementation with *L. helveticus* only significantly
100 decreased the abundance of cholesteryl esters (CE; control group: 1.2% ± 0.1% and *L.*
101 *helveticus* group: 0.9% ± 0.1% of total lipids; **Fig. 2**).

102 As changes in the composition of the gut microbiota can affect the metabolism of hepatic
103 FAs [14,17], the FA profile and the expression level of a set of genes involved in FA biosynthesis
104 were analyzed in the liver of mice fed *L. helveticus*-supplemented diet (**Fig. 3** and
105 **Supplementary Table 1**). Dietary supplementation with *L. helveticus* had little effect on liver
106 FA content. A significant decrease in the hepatic abundance of total SFAs, which probably
107 ensues from the significant reduction of the main SFA species (C16:0, palmitic acid), was
108 observed in *L. helveticus*-treated mice compared to control mice (**Fig. 3a** and **Supplementary**
109 **Table 1**). The decrease in C16:0 was not associated with a modulation of the expression of
110 *Fasn*, which encodes FAS, an enzyme involved in *de novo* lipogenesis from simple precursors
111 and whose primary product is C16:0 (**Fig. 3b**). Besides, no effect of *L. helveticus* was observed
112 neither on MUFAs nor on PUFAs levels (**Fig. 3a** and **Supplementary Table 1**). Moreover, no
113 modification of the expression level of genes coding for desaturases (*Fads1*, *Fads2* and *Scd1*),
114 elongases (*Elovl1*, *Elovl2*, *Elovl3*, *Elovl5* and *Elovl6*) and plasmalogen biosynthesis (*Far1*,
115 *Agps* and *Gnpat*) was observed (**Fig. 3b** and **3c**).

Taken together, these results suggest that long-term exposure to *L. helveticus* only affect SFAs in the liver and that this phenotype was not related to a modulation of the hepatic expression level of enzymes involved in their biosynthesis.

Circulating lipids

As plasma is the fluid that supplies FAs to the organs through lipoproteins, we analyzed the impact of long-term consumption of *L. helveticus* on the relative abundance of plasma lipid classes and FAs (**Fig. 4**, **Fig. 5** and **Supplementary Table 2**). Plasma levels of cholesterol, cholesteryl esters, phospholipids, triglycerides and free FAs were similar in mice fed a diet supplemented with *L. helveticus* when compared to those measured in mice fed a control diet (**Fig. 4**). However, some changes were observed in the abundance of plasma FAs in *L. helveticus*-supplemented mice (**Fig. 5**). They were characterized by a significant decrease in the amounts of total SFAs, C14:0, C16:0 and C16:1n-9, and by a significant increase in the amounts of C20:0, C22:0, C22:1n-9, total PUFAs, total PUFAs n-3, total PUFAs n-6 and C20:2n-6.

In addition to fatty methyl esters (FAMEs), GC-FID enables the detection of dimethyl acetals (DMAs) that result from the acid-catalyzed transmethylation of the aldehyde aliphatic groups from the sn-1 position of plasmalogens, a specific class of glycerophospholipids [33]. Modifications in the distribution of DMAs species were observed in the plasma of *L. helveticus*-supplemented mice compared to control mice. Indeed, the amount of DMA C16:0 was decreased and that of DMA C18:0 was increased (**Fig. 5** and **Supplementary Table 2**).

These results indicate that long-term exposure to *L. helveticus* is associated with remarkable changes in the plasma FA content.

Lipid profile and metabolism in the retina

Analysis of the retinal FA content revealed profound changes in mice fed a *L. helveticus*-supplemented diet (**Fig. 6** and **Supplementary Table 3**). As observed in liver and plasma, *L. helveticus* consumption was associated with a significant decrease in the amount of total SFAs in the retina that may result from the decrease in C16:0 (**Fig. 6a** and **Supplementary Table 3**). The retina of the *L. helveticus* group of mice also exhibited a reduced amount of total MUFAs compared to control mice. This might be related to a decrease in the amounts of several individual MUFA species belonging both to the n-7 (C16:1n-7) and n-9 series (C16:1n-9 and C20:1n-9) (**Fig. 6a** and **Supplementary Table 3**). These changes in SFAs and MUFAs levels were balanced by an enrichment of the retina in PUFAs from both the n-6 and n-3 series. Particularly, *L. helveticus* promoted a significant enrichment of the retina in C22:5n-3 (n-3 docosapentaenoic acid, n-3 DPA), C22:6n-3 (docosahexaenoic acid, DHA), C18:2n-6 (linoleic acid, LA) and C20:3n-6 (dihomo gamma-linolenic acid, DGLA) (**Fig. 6a** and **Supplementary Table 3**). We investigated whether changes in the retinal FA content could be associated with modulation of the expression of enzymes involved in their biosynthesis (**Fig. 6b**). Unexpectedly, retinal expression of the gene encoding the elongase ELOVL5, involved in the elongation of PUFAs to LC-PUFAs, was significantly decreased in mice fed a *L. helveticus*-supplemented diet (**Fig. 6b**).

In the retina, FAs are almost exclusively esterified on phospholipids. In order to find out accurately which phospholipid species were affected by the FA changes occurring in the retina, an HPLC-MS analysis was performed (**Supplementary Table 4**). We identified 128 phospholipid species, including 26 phosphatidylethanolamine (PE) species, 18 plasmenylethanolamine (PlE) species, 35 phosphatidylcholine (PC) species, 3 plasmenylcholine (PlC) species, 12 phosphatidylserine (PS) species, 1 plasmenylserine (PlS) species, 19 phosphatidylinositol (PI) species and 14 sphingomelin (SM) species

(**Supplementary Table 4**). No change in the abundance of SMs was observed in the retina of mice fed a *L. helveticus*-supplemented diet when compared to control mice. However, the abundance of 5 PEs, 4 PCs and 4 PIs was significantly increased in the mouse retina as a consequence of long-term consumption of *L. helveticus* (**Supplementary Table 4**). Interestingly, among these species, the probiotic increased the relative abundance of two PE species esterified with DHA at the sn-2 position (namely PE(16:0/22:6), which is the second most abundant PE species in the retina, and PE(16:1/22:6)). In addition, a significant decrease in the abundance of the main PI species, PI(18:0/20:4), was observed in the retina of the *L. helveticus* group (**Supplementary Table 4**). The relative abundance of 5 PlEs was increased in *L. helveticus*-supplemented mice (**Supplementary Table 4**), but these changes were not associated with a modification in the expression level of genes encoding enzymes involved in Pls biosynthesis (**Fig. 6b**).

Altogether, these results showed that long-term consumption of *L. helveticus* modulates the retinal FA content and, particularly, enriches this tissue in PUFAs having beneficial properties for retinal health.

Impact of long-term consumption of *L. helveticus* on gut microbiota communities

Since *L. helveticus* might indirectly affects host lipids by modulating the resident gut microbiota, we evaluated whether long-term consumption of *L. helveticus* has impacted its composition. Comparison of the fecal microbiota between mice fed a *L. helveticus*-supplemented diet and control mice showed no significant difference regarding the Hill's diversity indices, indicating that this long-term bacterial supplementation did not affect the gut microbiota alpha-diversity (**Fig. 7**). The relative abundance of the major phyla and genera were

also similar in the fecal microbiota of control mice and *L. helveticus*-supplemented mice (**Supplementary Fig. 1a** and **Supplementary Fig. 2a**). Non-metric multidimensional scaling (NMDS) ordination of communities at the phylum level or at the genus level did not reveal any different microbial clustering between control mice and *L. helveticus*-supplemented mice (**Supplementary Fig. 1b** and **Supplementary Fig. 2b**). In addition, PERMANOVA analysis were not significantly different between control mice and *L. helveticus*-supplemented mice showing that the overall distributions and abundances of phyla and genera were similar in the two groups (**Supplementary Fig. 1b** and **Supplementary Fig. 2b**). Altogether, these results indicated that long-term consumption of *L. helveticus* did not markedly altered the initial composition of the resident gut microbiota in mice.

To go further in the analysis of the microbial communities and find a pattern of bacterial species able to describe the changes in microbiota composition of *L. helveticus*-supplemented mice, we conducted a DESeq2 analysis at the OTU level. The DESeq2 differential abundance multiple-testing results were displayed on the volcano plot presented in **Fig. 8**. The abundance of 21 OTUs was significantly decreased (blue dots) and that of 4 OTUs (red dots) was significantly increased in the fecal microbiota of *L. helveticus*-supplemented mice compared to that of control mice (**Fig. 8** and **Supplementary Fig. 3**). For each OTU identity, the seed sequence was selected and compared to the 16S ribosomal database of NCBI with BLASTN and default parameters (**Table 1**).

Among the 25 OTUs whose abundance were modified by the *L. helveticus* supplementation, 21 belonged to the *Firmicutes* phylum, 2 belonged to the *Actinobacteria* phylum, 1 belonged to the *Verrucomicrobia* phylum and 1 was not classified (**Table 1**). Twenty-three of the OTU sequences presented percentage identities of less than 98.5% when they were compared with the sequences of the NCBI database. However, among the *Firmicutes*-related OTUs, the sequence of OTU00000130 displayed 100% identity with the species

Acutalibacter muris. In addition, the sequence of the *Verrucomicrobia*-related OTU (OTU00000403) matched with 100% identity to the species *Akkermansia muciniphila* (**Table 1**). No modification in the *Firmicutes*/*Bacteroidetes* ratio was observed (**Supplementary Fig. 4**).

Correlation between retinal fatty acids and changes in the gut microbiota associated with long-term consumption of *L. helveticus*

To investigate any potential links between gut microbiota changes and the modifications of the FA content in the retina observed in *L. helveticus*-supplemented mice, we correlated the abundance of FAs or the expression level of enzymes involved in FA biosynthesis that were significantly modified in *L. helveticus*-supplemented mice with the abundance of individual OTU identified by the DESeq2 analysis (**Fig. 8** and **Fig. 9**). No correlation was found between the abundance of 3 OTUs (OTU00000012, OTU00000036 and OTU00005085) and the retinal level of FAs or expression level of genes encoding FA-related enzymes. Regarding the other 22 OTUs, we observed that on the one hand the OTUs whose abundance was increased in the gut microbiota of *L. helveticus*-supplemented mice (OTU00000107 and OTU00000507) and on the other hand the OTUs whose abundance was decreased in the gut microbiota of *L. helveticus*-supplemented mice segregated (**Fig. 9** and **Supplementary Fig. 5** to **Fig. 9**). Indeed, when a positive correlation was found between the abundance of OTU00000107 and/or OTU00000507 and the retinal level of FAs or expression level of genes encoding FA-related enzymes, a negative correlation was found for the other OTUs, and conversely (**Fig. 9** and **Supplementary Fig. 5** to **Fig. 9**). Of note, we did not identify any positive and negative correlations between the OTUs significantly modified by the *L. helveticus* supplementation and the retinal amounts of total n-3 PUFAs, DHA (C22:6n-3) and C16:1n-9 (**Fig. 9**).

The abundances of two OTUs, OTU00000107 and OTU00000403, were oppositely correlated to 12 of the 18 changes observed in the retina at the FA or gene expression level. The abundance of OTU00000107 (*Firmicutes*, 88.45 % of identity with *Clostridium cellulovorans* 743B, **Table 1**) was positively correlated with the retinal amount of C15:0, C20:1n-9, total PUFAs, n-6 PUFAs, C18:2n-6, C20:3n-6, DMA C18:1n-7 and *Fads2* expression in the retina, and negatively correlated with the retinal amount of total SFAs, C16:0, C20:0, MUFAs n-7, C16:1n-7, DMA C16:0 and *Elovl6* expression in the retina (**Fig. 9** and **Supplementary Fig. 5** to **Fig. 9**). The abundance of OTU00000403 (*Verrucomicrobia*, 100% identity with *A. muciniphila*, **Table 1**) was positively correlated with the retinal amount of total SFAs, C16:0, C20:0, n-7 MUFAs, C16:1n-7 and DMA C16:0 and *Elovl6* expression in the retina, and negatively correlated with the retinal amount of total PUFAs, C22:5n-3, PUFAs n-6, C18:2n-6, C20:3n-6 and DMA C18:1n-7 (**Fig. 9** and **Supplementary Fig. 5 to Fig. 9**). In addition, we observed that a consortium of 9 OTUs was correlated (positive correlation: OTU00000107; negative correlation: OTU00000069, OTU00000130, OTU00000403, OTU00000409, OTU00000442, OTU00000630, OTU00000642 and OTU00003016) to the retinal amount of n-6 PUFAs (**Fig. 9** and **Supplementary Fig. 5** to **Fig. 9**). Finally, a negative correlation of the retinal amount of C22:5n-3 (docosapentaenoic acid, DPA) and the abundance of a consortium of 3 OTUs (OTU00000403, OTU00000630 and OTU00005682) was observed.

Discussion

PUFAs of the n-3 series and their derivatives are crucial for the retinal physiology and play a pivotal protective role in AMD [8]. In light of recent literature supporting the existence of a gut microbiota-retina axis and studies showing the influence of the gut microbiota on host FA metabolism, targeting the gut microbiota to modulate the FA content of the retina seems an attractive strategy to prevent retinopathies such as AMD. The aim of this study was to

investigate in mice the effect of long-term consumption of *L. helveticus* strain VEL12193 on the composition of the gut microbiota, the host lipid metabolism and the FA content of the retina.

Long-term consumption of *L. helveticus* strain VEL12193 limited weight gain in mice. However, other studies have suggested that lactobacilli could exert different effects on body weight depending on several factors that include the bacterial species or strains studied, the study model, the mouse diet, and the mode of administration of the bacteria [34-36]. In our conditions, the limitation in weight gain of *L. helveticus*-supplemented mice was neither associated with a reduction in visceral fat nor a lower food intake. Several hypotheses could be explored to characterize the origin of this *L. helveticus* VEL12193-related phenotype. The first-one would be a loss of muscle and/or bone mass, but such an hypothesis is unlikely since lactobacilli have been shown to instead have the opposite effect [37-40]. A second possibility would be a modulation by *L. helveticus* VEL12193 of the gut microbiota capacities on nutrient absorption, energy expenditure and/or fat oxidation [41,42]. Although results in humans are inconsistent, an increased *Firmicutes*/*Bacteroidetes* ratio has been reported to modify the metabolic function of the gut microbiota, including the production of short chain fatty acids (SCFAs; e.g., acetate, propionate, and butyrate) that could be involved in body weight control [43-46]. No modification of the *Firmicutes*/*Bacteroidetes* ratio was observed in *L. helveticus*-supplemented mice. However, the sequences of the 4 OTUs whose abundances were increased in the gut microbiota by *L. helveticus* supplementation were assigned to 4 bacterial genera encompassing some species that are known as butyrate-producers (namely *Roseburia faecis*, *Clostridium cellulovorans*, *Faecalibaculum rodentium* and *Butyribacter intestini*) [47-50]. Whether *L. helveticus* VEL12193-supplementation impacts SCFAs production by the gut microbiota remains to be determined.

Evidence has accumulated showing the influence of the gut microbiota on the host lipid metabolism through the modulation of key metabolic pathways in the liver, including those involved in cholesterol metabolism [51]. A cholesterol-lowering effect has been described for several species of lactobacilli [52-55]. In this study we showed that long-term consumption of *L. helveticus* VEL12193 did not modify the cholesterol level in the liver and the plasma. It should be noted that few studies have investigated the effect of *L. helveticus* species on cholesterol metabolism and their results are not consensual [56,57].

The liver is a key organ in lipid metabolism. The analysis of its FA content revealed that long-term supplementation with *L. helveticus* affected the amount of total SFAs. This phenotype could be directly related to the hepatic decrease observed in the major FA of this class, namely palmitic acid (C16:0). It is unlikely that the alteration of the C16:0 level was due to a difference in the diet composition since the incorporation of the bacteria into the dietary preparation did not modify the amounts of FA. In addition, no modulation of the hepatic expression level of genes encoding enzymes involved in FA biosynthesis was observed including for *Fasn* that encodes FAS, an enzyme involved in *de novo* lipogenesis and whose primary reaction product is C16:0. Another hypothesis that could explain the decrease in the hepatic C16:0 amount is an alteration in its intestinal absorption and/or in its esterification/transfer into lipoproteins. To test this hypothesis, the intestinal expression level of genes encoding acylglycerol transferase (MOGAT, DGAT) and proteins involved in FA uptake (e.g., CD36) and lipoprotein assembly (e.g., MTTP) could be analyzed in *L. helveticus*-supplemented mice. Finally, it has been reported that *Lacticaseibacillus rhamnosus* strain GG has the ability to consume FAs, including C16:0, a property that reduces intestinal FA absorption [58].

Plasma FA content was more importantly affected by the *L. helveticus* supplementation than liver since changes were observed among SFAs (decrease in C14:0, C16:0 and total SFA

311 amounts; increase in C20:0 and C22:0 amounts), MUFAs (decrease in C16:1n-9 amount;
312 increase in C22:1n-9 amount) and also PUFAs (increase in total PUFAs, total n-6 and n-3
313 PUFAs, and C20:2n-6). The DMA C16:0 amount was also altered. The *L. helveticus*-associated
314 plasma alterations in C16:0 and total SFAs may directly reflect the FA liver status of these FAs.
315 It is likely that this is also the case for the decrease in DMA C16:0, since no difference in the
316 expression of genes encoding plasmalogen synthesis enzymes (*Far1*, *Agps*, and *Gnpat*) was
317 observed in *L. helveticus*-supplemented mice. We can also assume that the decrease in C16:1n-
318 9 may result from reduced hepatic C16:0 abundance rather than a desaturation defect since the
319 hepatic expression of the gene encoding SCD-1, which catalyzes insertion of the double bond
320 at the delta-9 position of C16:0, was unchanged in *L. helveticus* supplemented mice. As a
321 consequence of the plasma decreases in SFAs and MUFAs, the relative abundance of PUFAs
322 was increased. However, neither the liver PUFA content nor the hepatic expression of genes
323 encoding elongase and desaturase were modified in *L. helveticus*-supplemented mice.

324 As expected, some of the changes observed in the plasma of *L. helveticus*-supplemented
325 mice were also found in the retina, particularly those affecting C16:0 and its MUFA derivatives.
326 These changes were balanced by an increase in the abundance of PUFAs. At the species level,
327 retinal alterations in PUFAs were different from those observed in the plasma where only an
328 increase in the level of C20:2n-6 and an upward trend for that of C22:6n-3 were measured.
329 Among the n-6 PUFAs, we noticed an increased retinal incorporation of C18:2n-6 (linoleic
330 acid, LA), which is the precursor for n-6 PUFAs that is only provided by the diet. This
331 phenotype could result from an enhanced expression of FA transporters at the retinal barrier
332 such as FATPs (fatty acid transport proteins), FABPs (fatty acid binding proteins) or FA
333 translocase [59]. In an *in vitro* model of human brain microvascular endothelial cells, it has been
334 shown that FATP4-knockdown reduced the transport of LA, thus indicating that this protein is
335 involved in LA transport [60]. Interestingly, some gut microbes could influence the expression

336 level of these proteins in organs/tissues. Indeed, treatment of mice with *A. muciniphila* activated
337 hepatic expression of *Fatp4* and *Cd36* [61]. However, in our study supplementing mice with *L.*
338 *helveticus* VEL12193 was associated with a reduction of the OTU403, assigned to *A.*
339 *muciniphila,* in the fecal microbiota.

340 In addition to LA, the abundance of C20:3n-6 (dihomo-gamma-linolenic acid, DGLA)
341 was increased in the retina of *L. helveticus*-supplemented mice. DGLA is the elongation product
342 of C18:3n-6 (gamma-linolenic acid, GLA) that it is itself the desaturation product of LA. Once
343 produced, DGLA can integrate two different pathways that can lead to the production of
344 bioactive molecules (eicosanoids) with different inflammatory properties. On the one hand
345 DGLA can serve as a precursor for the biosynthesis of prostaglandins and thromboxane of the
346 series-1 that are generally viewed as having mainly anti-inflammatory properties. But, on the
347 other hand, desaturation of DGLA by FADS2 will lead to the production of C20:4n-6
348 (arachidonic acid, AA), a precursor of prostaglandins and thromboxane of the series-2 and
349 leukotrienes of the series-4, having mainly pro-inflammatory properties. No modification in the
350 amount of C20:4n-6 was observed in the retina of *L. helveticus* supplemented mice suggesting
351 that DGLA is probably not desaturated. However, further experiments that could give
352 information on the retina inflammatory status are needed to conclude on the beneficial *versus*
353 harmful effect of increased DGLA level observed in the retina of *L. helveticus* supplemented-
354 mice.

355 Several studies support that a diet enriched in n-3 LC-PUFAs as well as high
356 concentration of plasma n-3 PUFAs are protective against AMD [8,62-64]. Conversely, low dietary
357 intake of n-3 LC-PUFAs has been correlated with a higher risk of developing the disease [8,63].
358 Interestingly, we showed that dietary supplementation with *L. helveticus* VEL12193 was
359 associated with an increase in retinal n-3 PUFAs. More specifically, the amounts of two n-3
360 PUFA were increased in the retina of *L. helveticus*-supplemented mice compared to controls:

C22:5n-3 (n-3 docosapentaenoic acid, n-3 DPA) and DHA. DPA is an intermediate product between C20:5n-3 (eicosapentanoic acid, EPA) and DHA. *In vitro* and *in vivo* studies have shown that DPA can be retro-converted into EPA [65]. Retinal DPA conversion to DHA has also been reported in miniature poodle dogs [66]. Many beneficial biological effects have been described for EPA and DHA in the retina, including protection against oxidative stress, neovascularization and inflammation, which are mechanisms involved in AMD [67]. In addition, although its biological effects have been until now under-explored, DPA may also possess beneficial properties for retinal health [65].

Predominant lipids in the retina are phospholipids, with phosphatidylcholine (PCs) and phosphatidylethanolamine (PEs) accounting for the majority (≈ 90%) of the retinal phospholipids [1,2]. In accordance with previous studies, we observed that the two predominant retinal PCs species contain disaturated FAs (PC(16:0/16:0), 17.5% of total PCs) and saturated/monounsaturated FAs (PC(16:0/18:1), 17.9% of total PCs), whereas the two major PEs species contain saturated/polyunsaturated (DHA) FAs (PE(18:0/22:6), 26.1% of total PEs; PE(16:0/22:6), 14.8% of total PEs). Interestingly, long-term dietary supplementation with *L. helveticus* enriched the retinal content of two PE species esterified with DHA, including PE(16:0/22:6).

Correlative analyses between OTUs whose abundance was altered by *L. helveticus* supplementation and retinal FAs abundance have enabled identifying a consortium of 9 OTUs associated with retinal n-6 PUFA changes and a consortium of 3 OTUs associated with retinal n-3 PUFA changes. Two OTUs were common to these 2 consortia: OTU630, belonging to Actinobacteria-p and whose sequence has 84.91% of identity with *Faecalimonas umbilicata* and OTU403, belonging to *Verrucomicrobia* and whose sequence has 100% identity with *A. muciniphila* [68]. Such an observation raises the possibility that changes in the FA content of the

386 retina associated with *L. helveticus* supplementation resulted from a reshaping of the gut
387 microbiota composition rather than the action of a unique bacteria strain. Indeed, reshaping of
388 the gut microbiota can affect its metabolic functions and thus modify its communication with
389 the host at the level of the gut mucosa but also at the level of other distant organs. Some studies
390 suggest that some products derived from the metabolic activities of the gut microbiota such as
391 SCFAs (e.g., propionate, butyrate) and secondary bile acids (e.g., ursodeoxycholic acid,
392 UDCA; tauroursodeoxycholic acid, TUDCA) could take part in the dialogue between the gut
393 microbiota and the eye [69-77]. Interestingly, in addition to SCFAs, studies suggest that UDCA
394 and TUDCA could also be involved in the regulation of the host FA metabolism [17,78,79]. To
395 further understand the molecular mechanisms linking the reshaping of the gut microbiota
396 induced by *L. helveticus* and its consequences on the bioavailability of FAs for the retina, an
397 analysis of the gut microbiota-derived metabolites at the gut and systemic levels is required.

398 In conclusion, we showed that long-term dietary supplementation with *L. helveticus*
399 enriched the retina in DGLA and DHA that are two PUFAs having beneficial health properties
400 that could help to protect the retina against deleterious age-related mechanisms/stresses. These
401 *L. helveticus*-induced retinal lipid modifications were associated with a reshaping of the gut
402 microbiota composition. Further investigations are now required to (i) determine whether the
403 PUFA-enrichment induced by long-term consumption of *L. helveticus* would be effective in
404 protecting the retina from the harmful effects of aging and (ii) identify the molecular actors
405 linking the changes induced by *L. helveticus* in the gut microbiota and their effect on the retinal
406 physiology.

407

408 Methods

409 Mice

The use of animals was in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. French legal and institutional ethics committee review board approvals were obtained (2018072513005644).

Eight-week-old male C57BL/6JRj SPF mice were purchased from Janvier Labs, France. They were maintained at INRAE, Dijon, France until euthanasia (C21 231 010 EA) with *ad libitum* access to food and water and exposed to 12h:12h light:dark cycles. After one week of acclimation, mice were randomly divided into two groups: one group received standard diet (control group; n=10) and the other group received the same diet as controls but supplemented with *L. helveticus* (*L. helveticus* group; n=10). Mice were maintained on these diets for 6 months. Fecal samples were collected for microbiota analyses one day before the end of the experiment. Prior to euthanasia, mice were fasted for 15 h. They were euthanized by cervical dislocation. Liver, retina and blood were collected. Hemolysis-free serum was generated by centrifugation (1800× g, 10 min, 4 °C).

Diet

L. helveticus strain VEL12193 [80] was grown overnight under anaerobic conditions at 37°C without shaking in Man-Rogosa-Sharpe medium (Condalab), pH 5.8. The bacterial culture was centrifuged at 5000 g for 10 min at room temperature. The bacterial pellet was washed twice in PBS and resuspended in sterile water at a concentration of 2.10^9 CFUs/mL. This bacterial suspension was then mixed with complete maintenance diet powder for adult mice (SAFE® A04) to obtain a final bacterial concentration in the diet of 1.10^9 CFUs/g. Food portions (approximatively 20g) were molded into Petri dishes, dried for 24 h at 4°C and then stored anaerobically at 4°C. Fresh diet was prepared weekly. The food portion were renewed in the cages every 2 days from the stock stored in anaerobic conditions at 4°C. The viability of *L.*

helveticus in food portions stored under these conditions was checked (**Supplementary Fig. 10**). The FA content of the diets is provided in **Supplementary Table 5**.

Microbiota analysis

An optimized and standardized DNA extraction protocol dedicated to bacterial DNA extraction from stool samples has been used (GenoScreen, Lille, France).

Genomic DNA extraction from stools samples was done with the QIAamp Fast DNA stool mini kit (Qiagen, Germany) with an optimized protocol for lysis step. After DNA extraction, the concentration was determined with the SybrGreen assay Kit (Life Technologies, USA).

A 16S rRNA gene fragment comprising V3 and V4 hypervariable regions was amplified using an optimized and standardized 16S-amplicon-library preparation protocol (Metabiote®, GenoScreen, Lille, France). Briefly, 16S rRNA gene PCR was carried out using 5ng of genomic DNA according to Metabiote® protocol (or maximal of DNA volume) instructions using 192 bar-coded primers (Metabiote® MiSeq Primers, GenoScreen, Lille, France) at final concentrations of 0.2 µM and an annealing temperature of 50°C for 30 cycles. PCR products were cleaned up with Agencourt AMPure XP-PCR Purification system (Beckman Coulter, Brea, USA), quantified according to the manufacturer's protocol, and multiplexed at equal concentration. Sequencing was performed using a 250-bp paired-end sequencing protocol on the Illumina MiSeq platform (Illumina, San Diego, USA) at GenoScreen, Lille, France.

Bioinformatic analyses were performed using the BIOCOM-PIPE pipeline, with default parameters, except when parameters were clearly described [81]. First, the 16S raw reads were sorted according to each sample using multiplex identifiers, and low-quality reads were deleted based on their length (less than 350-bp for 16S reads), their number of ambiguities and their primer(s) sequence(s). Then a PERL program was applied for rigorous dereplication (i.e.

458 clustering of strictly identical sequences). The dereplicated reads were globally aligned using
459 the Infernal tool [82], and clustered into operational taxonomic units (OTUs) using a similarity
460 threshold of 97%. A filtering step was carried out to remove chimeras based on the quality of
461 their taxonomic assignments. Finally, the retained reads were homogenized by random
462 selection (28,663 reads for 16S rRNA gene sequences) to compare the datasets efficiently and
463 avoid biased community comparisons. The retained high-quality reads were used to determine
464 alpha-diversity metrics after clustering refining with ReClustOR to improve OTUs definition
465 [83], and taxonomy-based analysis was performed using USEARCH against the SILVA 16S
466 rRNA reference database (r132).

467 The raw datasets are available in the EBI database system under project accession
468 number PRJEB56822.

469

470 ## Lipid class distributions

471 Total lipids were extracted from plasma, livers and retinas following the Folch's procedure [84].
472 The distribution of lipids into different classes [phospholipids (PL), triglycerides (TG),
473 diglycerides (DG), free fatty acids (FFA), free cholesterol (Chol), and/or cholesteryl esters
474 (CE)] was determined using a combination of thin-layer chromatography on silica gel-coated
475 quartz rods and flame ionization detection (Iatroscan® system, Iatron, Tokyo, Japan), according
476 to Ackman's technique [85]. The values obtained for each compound were corrected according to
477 their response factor using specific calibration curves. Data were reported as a percentage
478 relative to total lipids in the sample (considered as 100%).

479

480 ## FAME and DMA profiles

Total lipids were extracted as described above [84]. Boron trifluoride in methanol was used for transmethylation [86]. Hexane was used to extract fatty methyl esters (FAMEs) and dimethyl acetals (DMAs). Analyses were performed on a GC Trace 1310 (Thermo Scientific) gas chromatograph (GC) using a CPSIL-88 column (100 ×0.25 mm i.d., film thickness 0.20 µm; Varian). This device was coupled to a flame ionization detector (FID). The configuration was: inlet pressure of hydrogen 210 kPa, oven temperature 60°C for 5 min + 165°C at 15°C per min and upholding for 1 min, + 225°C at 2°C per min and upholding at 225°C for 17 min. The injector and the detector were maintained at 250°C. Comparisons with commercial and synthetic standards enabled the identification of FAMEs and DMAs. The ChromQuest software (Thermo Scientific) was used to process the data.

Analysis of phospholipid molecular species by liquid chromatography coupled to high-resolution mass spectrometer

Phosphorus content of the total lipid extract was determined according to the method developed by Bartlett and Lewis [87]. The total phospholipids were dried under a stream of nitrogen and diluted to the appropriate concentration of 500 µg/µL of phospholipids in CHCl3/CH3OH (1:1, v/v). Ten microliters of internal standard mixture containing PC(14:0/14:0) 320 µg/mL, PE(14:0/14:0) 160 µg/mL, PS(14:0/14:0) 80 µg/mL, PI(8:0/8:0) 100 µg/mL, and SM(d18:1/12:0) 80 µg/mL were added into 200 µL of this phospholipid solution.

Phospholipid classes were separated under hydrophilic interaction liquid chromatograph (HILIC) conditions using a Kinetex HILIC 100 x 2.1-mm, 1.7-µm column (Phenomenex, Sydney, NSW, Australia) as described previously [88]. Ultra-high-performance liquid chomatography (UHPLC) separation was achieved using an ULTIMATE 3000 LC pump and an ULTIMATE 3000 Autosampler (Thermo Scientific, San Jose, CA, USA). The mobile phase

consisted of (A) CH3CN/H2O (96/4, v/v) containing 10mM ammonium acetate and (B) CH3CN/H2O (50/50, v/v) containing 10mM ammonium acetate. The chosen solvent-gradient system of the analytical pump was as follows : 0 min 100% A, 12 min 80% A, 18 min 50% A, 18.1–30 min 100% A. The flow rate was 500 µL/min, the injection volume was 10 µL and the column was maintained at 50°C. The liquid chromatography system was controlled by Standard Instrument Integration (SII) software based on Dionex Chromeleon TN 7.

The process of identification and quantification of phospholipid species was performed on an orbitrap FusionTM Tribrid Mass Spectrometer equipped with an EASY-MAX NG Ion Source (Heated Electrospray Ionization H-ESI) (Thermo Scientific, San Jose, CA, USA). Phospholipid species were detected by high-resolution mass spectrometry (HRMS) analysis. H-ESI source parameters were optimized and set as follows: ion transfer tube temperature of 285°C, vaporizer temperature of 370°C, sheath gas flow rate of 35 au, sweep gas of 1 au, auxiliary gas flow rate of 25 au. Positive and negative ions were monitored alternatively by switching the polarity approach with a static spray voltage at 3500V and 2800V in positive and negative mode respectively. Mass spectra in full scan mode were obtained using the Orbitrap mass analyzer with the normal mass range and a target resolution of 240,000 (FWHM at m/z 200), in a mass-to-charge ratio m/z ranging from 200 to 1600 using a Quadrupole isolation in a normal mass range. All MS data were recorded using a maximum injection time of 100 ms, automatic gain control (AGC) target (%) at 112.5, RF lens (%) at 50, and one microscan. An intensity threshold filter of 1.103 counts was applied.

For tandem mass spectrometry (MS/MS) analyses, the data-dependent mode was used for the characterization of phospholipid species. Precursor isolation was performed in the Quadrupole analyzer with an isolation width of m/z 1.6. Higher-energy collisional dissociation was employed for the fragmentation of phospholipid species with an optimized stepped collision energy of 27%. The linear ion trap was used to acquire spectra for fragment ions in

data-dependent mode. The AGC target was set to 2.104 with a maximum injection time of 50 ms. All MS and MS/MS data were acquired in the profile mode. The Orbitrap Fusion was controlled by XcaliburTM 4.1 software (Thermo Scientific, San Jose, CA, USA). The identification of all PL species was performed using the high-accuracy data and the information collected from fragmentation spectra with the help of LIPIDSEARCH software version 4.1.16 (Thermo Scientific, San Jose, CA, USA) and the LIPID MAPS® database (https://www.lipidmaps.org/).

Gene expression

Total RNA was extracted using TRIzol reagent (Life Technologies). Reverse transcription was performed using PrimeScript RT reagent Kit with gDNA Eraser (Takara Bio). Gene expression was determined by real-time PCR using SYBR Green (Biorad) and a CFX96 Real-Time PCR system (Biorad). Hprt was used as the internal control for normalization. Primer sequences are given in **Supplementary Table 6**.

Statistical analyses

The data are presented as mean ± standard deviation of the mean (SEM), except those including bacterial communities. Statistical analyses were performed using the GraphPad Prism software for all analyses except those including bacterial communities, which were performed with R (version 4.1.2). The non-parametric Mann and Whitney or Krus-kal-Wallis tests were used to compare data from the two groups (after Bonferroni correction). The p-values of less than 0.05 were considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$).

OTUs differences between the two groups (mice receiving *L. helveticus* against those of the control group) were assessed by pairwise comparison of normalized sequence counts using Negative Binomial Wald Tests from the DESeq2 package [89]. More precisely, the DESeq2 package performed three steps: (1) estimation of size factors, which are used to normalize library sizes in a model-based fashion; (2) estimation of dispersions from the negative binomial likelihood for each feature, and subsequent shrinkage of each dispersion estimate towards the local trendline by empirical Bayes; (3) fitting each feature to the specified class groupings with negative binomial generalized linear models and performing hypothesis testing, for which we chose the default Wald test. Then, DeSeq2 helped to decrease the false discovery rate of OTUs, using the Benjamini and Hochberg method by default. The adjusted *p*-values < 0.1 were considered as significant for the DeSeq2 analysis.

To compare the OTUs modified by the probiotic supplementation with either fatty acid amounts or gene expression levels, Spearman correlation analyses (and related *p*-values) were performed with the 'cor.test' function from R, considering a *p*-value less than 0.05 as significant.

Data availability

For microbiota, the raw datasets are available in the EBI database system under project accession number PRJEB56822. All other data supporting the findings reported herein are available on reasonable request from the corresponding author.

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Author contributions

P.L. and M.-A.B. conceived the study and supervised the experimental work. M.-A.B drafted the manuscript. S.T. performed the analyses and interpretation of the Illumina data. P.L., M.-A.B, L.P., L.M., B.B., S.G. and S.C. performed the experiments. M.-A.B., N.A. and O.B. performed the analyses and interpretation of the lipid data. N.A., A.R., L.B.-H., P.-H.G. and C.C.-G. revised the manuscript.

Competing interests statement

Authors declare no competing interests.

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Figure Legends

Figure 1. Effect of *L. helveticus* on weight gain, epididymal fat deposition and food intake. (**a**) Weight gain. Results are expressed as the percentage of weight gained after a 6 months period of exposure to a control diet or *L. helveticus*-supplemented diet. (**b**) Food intake. Results are expressed as the weight (g) of food consumed per day and per mouse. Nine independent measurements were performed per cage. (**c**) Epididymal fat deposition. The epididymal adipose tissue from the left fat pad was weighted. Results are expressed in grams (g). Mann-Whitney test (* $p<0.05$).

Figure 2. Effect of long-term consumption of *L. helveticus* on lipid classes in the liver. (**a**) Cholesterol (Chol). (**b**) Cholesteryl esters (CE). (**c**) Phospholipids (PL). (**d**) Triglycerides (TG). (**e**) Diglycerides (DG). (**f**) Free fatty acids (FFA). Results are expressed as abundance (%) relative to total lipids defined as 100%. Mann-Whitney test (* $p<0.05$).

Figure 3. Fatty acid content in the liver of mice exposed lengthily to *L. helveticus*. (**a**) Heat map showing the hepatic abundance of each fatty acid methyl esters (FAME) or dimethylacetal (DMA) relative to total FAMEs + DMAs (defined as 100%) in mice fed a control diet or fed a diet supplemented with *L. helveticus*. The ratio of total n-6 PUFAs/total n-3 PUFAs was calculated (n-6/n-3 ratio). (**b**) Hepatic expression of genes encoding enzymes involved in the biosynthesis of fatty acids: acyl-CoA (8-3)-desaturase (*Fads1*), acyl-CoA 6-desaturase (*Fads2*), acyl-CoA desaturase 1 (*Scd1*), elongation of very long chain fatty acids proteins 1, 2, 3, 5 and 6 (*Elovl1*, *Elovl2*, *Elovl3*, *Elovl5* and *Elovl6*), and fatty acid synthase (Fasn). (**c**) Hepatic expression of genes encoding enzymes involved in the biosynthesis of plasmalogens: fatty acyl-CoA reductase 1 (*Far1*), alkylglycerone-phosphate synthase (*Agps*) and dihydroxyacetone phosphate acyltransferase (*Gnpat*). The levels of mRNA were normalized to *Hprt* mRNA level for calculation of the relative levels of transcripts. mRNA levels are illustrated as fold change. Mann-Whitney test (** $p<0.001$).

Figure 4. Effect of *L. helveticus* on lipid class distribution in the plasma. (**a**) Cholesterol (Chol). (**b**) Cholesteryl esters (CE). (**c**) Phospholipids (PL). (**d**) Triglycerides (TG). (**e**) Free fatty acids (FFA). Results are expressed as abundance (%) relative to total lipids defined as 100%.

Figure 5. Fatty acid content in the plasma of mice lengthily exposed to *L. helveticus*. Heat map showing the plasma abundance of each FAME or DMA relative to total FAMEs + DMAs (defined as 100%) in mice fed a control diet or fed a diet supplemented with *L. helveticus*. The ratio of total n-6 PUFAs/total n-3 PUFAs was calculated (n-6/n-3 ratio). Mann-Whitney test (* $p<0.05$ and ** $p<0.01$).

Figure 6. Fatty acid content in the retina of mice lengthily exposed to *L. helveticus*. (**a**) Heat map showing the retinal abundance of each FAME or DMA relative to total FAMEs + DMAs (defined as 100%) in mice fed a control diet or fed a diet supplemented with L. helveticus. The ratio of total n-6 PUFAs/total n-3 PUFAs was calculated (n-6/n-3 ratio). (**b**) Retinal expression of genes involved encoding enzymes involved in the biosynthesis of fatty acids: acyl-CoA (8-3)-desaturase (*Fads1*) and acyl-CoA 6-desaturase (*Fads2*), acyl-CoA desaturase 1 (*Scd1*), and elongation of very long chain fatty acids proteins 1, 2, 4, 5 and 6 (*Elovl1*, *Elovl2*, *Elovl4*, *Elovl5* and *Elovl6*). (**c**) Hepatic expression of genes encoding enzymes involved in the biosynthesis of plasmalogens: fatty acyl-CoA reductase 1 (*Far1*), alkylglycerone-phosphate synthase (*Agps*) and dihydroxyacetone phosphate acyltransferase (*Gnpat*). The levels of mRNA were normalized to *Hprt* mRNA level for calculation of the relative levels of transcripts. mRNA levels are illustrated as fold change. Mann-Whitney test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$).

Figure 7. Hill's diversity of the gut microbiota in *L. helveticus*-supplemented mice based on OTUs determination after ReClustOR refining. (**a**) q=0 (species richness). (b) q=1 (exponential of Shannon entropy). (**c**) q=2 (reciprocal of Simpson index).

Figure 8. Volcano plot highlighting OTU fold changes in the gut microbiota of *L. helveticus*-supplemented mice. Each point represents an operational taxonomic unit (OTU). The x-axis represents the log2 of the fold change whilst the y-axis is the negative log10 of DESeq2 p values adjusted for multiple testing using the false discovery rate method. The vertical lines are fold-change cutoff that correspond to a log 2-fold change of 0.5 and 0.5. Blue points to the left of the plot with negative log2FoldChange values represent OTUs with increased abundance in control mice relative to mice fed *L. helveticus*-supplemented diet. Red points to the right of the plot with positive log2FoldChange values represent OTUs with increased abundance in mice fed *L. helveticus*-supplemented diet relative to control mice.

Figure 9. Schematic representation of the correlations between abundance of OTUs in the gut microbiota and the amount of fatty acids or expression of genes encoding enzymes involved in fatty acid biosynthesis in the retina. The abundance of OTUs identified to be significantly different between the two group of mice (control mice and mice fed a diet supplemented with *L. helveticus*) by DESeq2 were tested for correlation with the retinal amounts of fatty acids or the retinal expression levels of genes involved in fatty acid biosynthesis found to be significantly modified (or with a high tendency (Elovl2 p=0.0524)) between the two groups of mice by using Spearman linear correlations. Significant ($p<0.05$) positive and negative correlation are sum-up on this figure. Each OTU is represented by a colored square.

Tables

Table 1. Taxonomic identification of OTUs.

DBOTU	Phylum	Identification (BLASTN best match)	Identity (%)	Accession number	Log2FoldChange	Adjusted *p*-value [a]
00000012	*Firmicutes*	*Roseburia faecis*	95.56	NR_042832.1	2.54	4.2E-02
00000036	*Firmicutes*	*[Ruminococcus] gnavus* ATCC 29149	93.32	NR_118690.1	-2.26	8.9E-02
00000069	*Firmicutes*	*[Clostridium] scindens*	98.27	NR_028785.1	-3.74	5.6E-03
00000107	*Firmicutes*	*Clostridium cellulovorans* 743B	88.45	NR_102875.1	5.66	3.3E-04
00000130	*Firmicutes*	*Acutalibacter muris*	100.00	NR_144605.1	-1.66	8.2E-02
00000145	*Firmicutes*	*Lacrimispora aerotolerans*	93.81	NR_119068.1	-3.34	8.8E-03
00000194	*Firmicutes*	*Ruminiclostridium cellulolyticum* H10	88.40	NG_041947.1	-2.43	6.5E-02
00000287	*Firmicutes*	*Murimonas intestini*	95.05	NR_134772.1	-3.09	4.8E-02
00000303	*Firmicutes*	*Falcatimonas natans*	92.57	NR_152688.1	-3.01	8.5E-02
00000347	*Firmicutes*	*Lacrimispora aerotolerans*	89.34	NR_119068.1	-3.53	1.2E-02
00000403	*Verrucomicrobia*	*Akkermansia muciniphila*	100.00	NR_074436.1	-3.70	2.1E-02
00000409	*Unknown*	*[Eubacterium] rectale* ATCC 33656	85.78	NR_074634.1	-4.33	2.1E-02
00000442	*Firmicutes*	*Roseburia faecis*	95.31	NR_042832.1	-4.09	1.2E-02
00000462	*Firmicutes*	*Turicibacter sanguinis*	88.55	NR_028816.1	-3.69	8.5E-02
00000491	*Firmicutes*	*Bifidobacterium animalis*	86.08	NR_043438.1	-3.87	5.2E-02
00000507	*Firmicutes*	*Faecalibaculum rodentium*	96.96	NR_146011.1	2.41	5.2E-02
00000591	*Actinobacteria-p*	*Bifidobacterium animalis*	83.92	NR_043438.1	-3.38	8.5E-02
00000630	*Actinobacteria-p*	*Faecalimonas umbilicata*	84.91	NR_156907.1	-4.63	1.0E-02
00000642	*Firmicutes*	*Bacillus anthracis*	95.77	NR_118536.1	-2.77	6.5E-02
00000733	*Firmicutes*	*Kineothrix alysoides*	95.05	NR_156081.1	-3.90	8.9E-02
00001567	*Firmicutes*	*Roseburia faecis*	95.06	NR_042832.1	-5.33	3.3E-04
00001846	*Firmicutes*	*[Clostridium] populeti*	95.56	NR_026103.1	-3.71	5.2E-02
00003016	*Firmicutes*	*Oscillibacter ruminantium GH1*	91.13	NR_118156.1	-3.30	8.4E-02
00005085	*Firmicutes*	*Butyribacter intestini*	95.80	NR_173596.1	3.60	5.2E-02
00005682	*Firmicutes*	*Turicibacter sanguinis*	93.47	NR_028816.1	-3.89	6.2E-02

[a] Adjusted *p*-value computed during the DeSeq2 analysis with the Benjamini and Hochberg method.

Supplementary information

Supplementary Figures

Supplementary Figures are provided in a separate file.

Legends of supplementary Figures :

Supplementary Figure 1. Relative abundances of the major phyla in the gut microbiota of *L. helveticus*-supplemented mice. (**a**) bar graph presenting the relative abundance of the major phyla in the gut microbiota of control mice and in that of mice fed a diet supplemented with L. helveticus. (**b**) Non-metric multidimensional scaling (NMDS) ordination of communities at the phylum level.

Supplementary Figure 2. Relative abundances of the major genera in the gut microbiota of *L. helveticus*-supplemented mice. (**a**) bar graph presenting the relative abundance of the major genera in the gut microbiota of control mice and in that of mice fed a diet supplemented

with *L. helveticus*. (**b**) Non-metric multidimensional scaling (NMDS) ordination of communities at the genus level.

Supplementary Figure 3. Relative abundances of OTUs identified by DESeq2 differential abundance multiple-testing. Data are presented as as corrected values for each OTU after DESeq2 normalization, with adjusted p-values (padj).

Supplementary Figure 4. *Firmicutes*/*Bacteroidetes* ratio.

Supplementary Figure 5. Spearman linear correlations on corrected values for OTUs after DESeq2 normalization (X axis) and the retinal amount of SFAs (Y axis), showing significant correlations among both datasets.

Supplementary Figure 6. Spearman linear correlations on corrected values for OTUs after DESeq2 normalization (X axis) and the retinal amount of MUFAs (Y axis), showing significant correlations among both datasets.

Supplementary Figure 7. Spearman linear correlations on corrected values for OTUs after DESeq2 normalization (X axis) and the retinal amount of PUFAs (Y axis), showing significant correlations among both datasets.

Supplementary Figure 8. Spearman linear correlations on corrected values for OTUs after DESeq2 normalization (X axis) and the retinal amount of DMAs (Y axis), showing significant correlations among both datasets.

Supplementary Figure 9. Spearman linear correlations on corrected values for OTUs after DESeq2 normalization (X axis) and the retinal expression level of elongases and desaturases (Y axis), showing significant correlations among both datasets.

Supplementary Figure 10. Viability of *L. helveticus* following its incorporation into the diet and its storage at 4°C. The probiotic was incorporated into the diet at the concentration of 1.10^9 CFUs of *L. helveticus*/g of food. Portions were molded using Petri dishes (20g/dish) and stored at 4°C under anaerobic conditions. The number of viable CFUs of *L. helveticus*/portion was determined by resuspending the diet, plating dilutions of the suspension on MRS agar plates and enumerating the number of CFUs. Results are expressed as CFUs/g of diet at day 0 (the day the food containing *L. helveticus* was prepared), and into portions stored during 4 days or 8 days at 4°C under anaerobic conditions.

Supplementary Tables

Supplementary Table 1. Fatty acid composition in the liver of mice fed a control diet or a diet supplemented with *L. helveticus*.

	Control	+ *L. helveticus*
C14:0	0.472 ± 0.014	0.438 ± 0.015
C15:0	0.110 ± 0.005	0.117 ± 0.004
C16:0**	24.019 ± 0.238	22.893 ± 0.205
C17:0	0.122 ± 0.004	0.122 ± 0.005
C18:0	4.023 ± 0.250	3.786 ± 0.145
C20:0	0.121 ± 0.009	0.128 ± 0.015
Total SFAs**	**28.868 ± 0.292**	**27.484 ± 0.307**
C16:1n-7	6.153 ± 0.210	6.555 ± 0.357
C18:1n-7	5.348 ± 0.456	4.586 ± 0.275
Total MUFAs n-7	**11.501 ± 0.496**	**11.141 ± 0.461**
C16:1n-9	1.027 ± 0.093	0.962 ± 0.051
C18:1n-9	32.696 ± 1.580	31.571 ± 0.917
C20:1n-9	0.559 ± 0.055	0.499 ± 0.034
C22:1n-9	0.060 ± 0.002	0.067 ± 0.007
Total MUFAs n-9	**34.341 ± 1.726**	**33.099 ± 0.997**
C18:1t	0.070 ± 0.006	0.076 ± 0.011
Total MUFAs	**45.912 ± 2.142**	**44.316 ± 1.061**
C18:3n-3	0.281 ± 0.041	0.255 ± 0.026
C20:5n-3	0.323 ± 0.032	0.400 ± 0.017
C22:5n-3	0.308 ± 0.030	0.348 ± 0.013
C22:6n-3	4.058 ± 0.383	4.344 ± 0.154
Total PUFAs n-3	**4.970 ± 0.458**	**5.347 ± 0.194**
C18:2n-6	14.339 ± 1.161	16.748 ± 0.714
C18:3n-6	0.332 ± 0.058	0.413 ± 0.065
C20:2n-6	0.131 ± 0.004	0.141 ± 0.004
C20:3n-6	0.172 ± 0.009	0.157 ± 0.011
C20:4n-6	4.918 ± 0.425	4.934 ± 0.164
C22:4n-6	0.167 ± 0.013	0.166 ± 0.009
C22:5n-6	0.101 ± 0.012	0.095 ± 0.008
Total PUFAs n-6	**20.160 ± 1.619**	**22.654 ± 0.894**
Total PUFAs	**25.130 ± 2.065**	**28.001 ± 1.078**
n-6/n-3 ratio	**4.102 ± 0.099**	**4.236 ± 0.057**
DMA C18:1n-9	0.201 ± 0.009	0.199 ± 0.005

Results are expressed as percentages of total fatty acid methyl esters (FAMEs) + dimethylacetals (DMAs). SFAs: saturated fatty acids. MUFAs: monounsaturated fatty acids. PUFAs: polyunsaturated fatty acids.**p<0.01.

Supplementary Table 2. Fatty acid composition in the plasma of mice fed a control diet or a diet supplemented with *L. helveticus*.

	Control	*+ L. helveticus*
C14:0*	0.048 ± 0.008	0.029 ± 0.006
C15:0	0.057 ± 0.005	0.044 ± 0.005
C16:0*	15.784 ± 0.726	13.447 ± 0.429
C17:0	0.186 ± 0.004	0.185 ± 0.008
C18:0	8.142 ± 0.156	7.938 ± 0.176
C20:0*	0.193 ± 0.008	0.232 ± 0.012
C22:0*	0.065 ± 0.004	0.090 ± 0.009
C24:0	0.084 ± 0.006	0.102 ± 0.006
Total SFAs**	**24.559 ± 0.668**	**22.067 ± 0.402**
C16:1n-7	2.509 ± 0.207	2.161 ± 0.167
C18:1n-7	3.249 ± 0.133	3.133 ± 0.146
C20:1n-7	0.179 ± 0.009	0.206 ± 0.011
Total MUFAs n-7	**5.938 ± 0.286**	**5.500 ± 0.301**
C16:1n-9*	0.279 ± 0.027	0.199 ± 0.015
C18:1n-9 (p=0.506)	16.401 ± 0.262	15.615 ± 0.260
C20:1n-9	0.422 ± 0.017	0.476 ± 0.030
C22:1n-9**	0.584 ± 0.047	0.856 ± 0.092
C24:1n-9	0.148 ± 0.009	0.174 ± 0.010
Total MUFAs n-9	**17.835 ± 0.260**	**17.321 ± 0.271**
C18:1t**	0.157 ± 0.008	0.201 ± 0.014
Total MUFAs	**23.930 ± 0.521**	**23.022 ± 0.446**
C18:3n-3	0.273 ± 0.011	0.280 ± 0.008
C20:5n-3	0.628 ± 0.034	0.690 ± 0.019
C22:5n-3	0.344 ± 0.018	0.385 ± 0.016
C22:6n-3 (p=0.0653)	7.797 ± 0.404	8.605 ± 0.246
Total PUFAs n-3*	**9.042 ± 0.430**	**9.960 ± 0.256**
C18:2n-6	19.081 ± 0.757	20.751 ± 0.545
C18:3n-6	0.337 ± 0.038	0.380 ± 0.039
C20:2n-6*	0.135 ± 0.006	0.149 ± 0.004
C20:3n-6	1.662 ± 0.105	1.613 ± 0.067
C20:4n-6	20.010 ± 0.661	20.776 ± 0.575
C22:4n-6	0.119 ± 0.004	0.131 ± 0.007
C22:5n-6	0.076 ± 0.011	0.082 ± 0.004
Total PUFAs n-6*	**41.420 ± 0.735**	**43.882 ± 0.541**
n-6/n-3 ratio	**4.647 ± 0.159**	**4.422 ± 0.088**
C20:3n-9	0.575 ± 0.060	0.593 ± 0.035
Total PUFAs*	**51.036 ± 1.087**	**54.435 ± 0.709**
DMA C16:0*	0.142 ± 0.013	0.112 ± 0.008
DMA C18:0*	0.168 ± 0.009	0.204 ± 0.012
DMA C18:1n-7	0.040 ± 0.002	0.042 ± 0.001
DMA C18:1n-9	0.125 ± 0.004	0.118 ± 0.004
Total DMAs	**0.475 ± 0.021**	**0.476 ± 0.012**

978 Results are expressed as percentages of total fatty acid methyl esters (FAMEs) +
979 dimethylacetals (DMAs). SFAs: saturated fatty acids. MUFAs: monounsaturated fatty acids.
980 PUFAs: polyunsaturated fatty acids. *p<0.05 and **p<0.01.

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Supplementary Table 3. Fatty acid composition in the retina of mice fed a control diet or a diet supplemented with *L. helveticus*.

	Control	+ *L. helveticus*
C14:0	0.081 ± 0.017	0.068 ± 0.008
C15:0**	0.061 ± 0.007	0.110 ± 0.012
C16:0***	16.954 ± 0.793	13.470 ± 0.478
C17:0	0.155 ± 0.007	0.142 ± 0.007
C18:0	24.449 ± 0.546	24.962 ± 0.273
C20:0*	0.223 ± 0.009	0.270 ± 0.015
Total SFAs**	**41.923 ± 0.756**	**39.022 ± 0.528**
C16:1n-7**	0.421 ± 0.036	0.266 ± 0.023
C18:1n-7	3.323 ± 0.037	3.272 ± 0.060
Total MUFAs n-7*	**3.744 ± 0.042**	**3.538 ± 0.078**
C16:1n-9*	0.186 ± 0.017	0.145 ± 0.008
C18:1n-9	11.024 ± 0.166	10.624 ± 0.171
C20:1n-9**	0.427 ± 0.060	0.425 ± 0.009
C22:1n-9	0.376 ± 0.032	0.368 ± 0.043
Total MUFAs n-9	**12.013 ± 0.196**	**11.562 ± 0.196**
C18:1t	0.119 ± 0.007	0.118 ± 0.008
Total MUFAs (p=0.0630)	**15.876 ± 0.225**	**15.218 ± 0.238**
C18:3n-3	0.191 ± 0.009	0.184 ± 0.016
C20:5n-3	0.359 ± 0.012	0.349 ± 0.015
C22:5n-3**	0.762 ± 0.021	0.851 ± 0.027
C22:6n-3*	25.795 ± 0.813	28.135 ± 0.672
Total PUFAs n-3*	**27.107 ± 0.829**	**29.519 ± 0.690**
C18:2n-6****	1.819 ± 0.056	2.847 ± 0.171
C18:3n-6	0.069 ± 0.006	0.141 ± 0.035
C20:2n-6	0.370 ± 0.040	0.379 ± 0.014
C20:3n-6***	0.905 ± 0.017	0.998 ± 0.009
C20:4n-6	7.299 ± 0.141	7.343 ± 0.058
C22:4n-6	0.814 ± 0.024	0.870 ± 0.016
C22:5n-6	0.106 ± 0.013	0.109 ± 0.018
Total PUFAs n-6**	**11.382 ± 0.127**	**12.687 ± 0.180**
n-6/n-3 ratio	**0.423 ± 0.011**	**0.432 ± 0.012**
Total PUFAs**	**38.489 ± 0.916**	**42.206 ± 0.725**
DMA C16:0**	1.361 ± 0.041	1.135 ± 0.042
DMA C18:0	1.896 ± 0.026	1.942 ± 0.062
DMA C18:1n-7**	0.207 ± 0.004	0.240 ± 0.008
DMA C18:1n-9	0.249 ± 0.006	0.246 ± 0.006
Total DMAs*	**3.713 ± 0.023**	**3.563 ± 0.065**

Results are expressed as percentages of total fatty acid methyl esters (FAMEs) + dimethylacetals (DMAs). SFAs: saturated fatty acids. MUFAs: monounsaturated fatty acids. PUFAs: polyunsaturated fatty acids. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

Supplementary Table 4. Relative amounts of phospholipid species in the retina.

	Control	+ *L. helveticus*
Ethanolanine glycerophospholipids		
Phosphatidylethanolamine (PE)		
PE(16:0/16:0)	0.252 ± 0.012	0.241 ± 0.006
PE(16:0/18:0)	0.370 ± 0.016	0.351 ± 0.010
PE(16:0/18:1)	2.122 ± 0.094	2.197 ± 0.053
PE(16:0/20:4)	1.274 ± 0.052	1.230 ± 0.022
PE(16:0/20:5)	0.364 ± 0.007	0.359 ± 0.008
PE(16:0/22:6)*	14.769 ± 0.113	15.334 ± 0.204
PE(16:1/22:6)*	0.208 ± 0.004	0.225 ± 0.005
PE(18:0/18:1)	2.465 ± 0.106	2.586 ± 0.059
PE(18:0/22:4)	0.646 ± 0.064	0.760 ± 0.009
PE(18:0/22:6)	26.065 ± 0.427	25.647 ± 0.500
PE(18:1/18:1)**	0.602 ± 0.053	0.730 ± 0.015
PE(18:1/18:2)**	0.401 ± 0.017	0.481 ± 0.015
PE(18:1/22:6)	3.614 ± 0.068	3.637 ± 0.101
PE(18:2/22:6)	0.275 ± 0.008	0.307 ± 0.017
PE(20:0/22:6)	0.183 ± 0.003	0.184 ± 0.004
PE(20:1/22:6)	0.350 ± 0.006	0.356 ± 0.010
PE(20:2/22:6)	0.255 ± 0.009	0.237 ± 0.012
PE(20:3/22:6)	0.485 ± 0.021	0.419 ± 0.043
PE(20:4/22:6)	0.285 ± 0.015	0.253 ± 0.010
PE(22:4/22:6)	0.330 ± 0.015	0.334 ± 0.010
PE(22:5/22:6)	1.258 ± 0.064	1.205 ± 0.051
PE(22:6/22:6)	6.643 ± 0.380	6.139 ± 0.278
PE(24:5/22:6)	0.205 ± 0.013	0.203 ± 0.009
PE(24:6/22:6)	0.554 ± 0.039	0.560 ± 0.031
PE(18:0/20:4); PE(18:1/20:3)	5.769 ± 0.293	5.396 ± 0.100
PE(18:0/20:5); PE(18:1/20:4)*	2.362 ± 0.037	2.472 ± 0.045
Plasmenylethanolamine (PlE)		
PE(P-16:0/16:0)	0.187 ± 0.009	0.194 ± 0.008
PE(P-16:0/18:1)*	1.047 ± 0.035	1.137 ± 0.042
PE(P-16:0/18:2)**	0.244 ± 0.011	0.306 ± 0.018
PE(P-16:0/20:3)*	0.552 ± 0.009	0.588 ± 0.017
PE(P-16:0/20:4) (p=0.0654)	3.521 ± 0.080	3.269 ± 0.068
PE(P-16:0/20:5)	0.252 ± 0.018	0.257 ± 0.014
PE(P-18:0/16:0)	0.675 ± 0.029	0.682 ± 0.023
PE(P-18:0/18:1)*	0.265 ± 0.009	0.280 ± 0.007
PE(P-18:0/18:2)*	0.196 ± 0.006	0.224 ± 0.007
PE(P-18:0/20:3)	0.450 ± 0.008	0.445 ± 0.008
PE(P-18:0/20:4)	3.774 ± 0.079	3.608 ± 0.074
PE(P-18:1/22:6)	0.801 ± 0.034	0.811 ± 0.023
PE(P-20:0/20:3)	0.173 ± 0.004	0.171 ± 0.004
PE(P-16:0/22:6); PE(P-18:1/20:5)	4.972 ± 0.132	5.174 ± 0.129
PE(O-16:0/22:6)§; PE(P-18:1/20:4)	2.600 ± 0.041	2.676 ± 0.075
PE(P-18:0/22:4); PE (P-20:0/20:4)	0.680 ± 0.081	0.701 ± 0.011
PE(P-18:0/22:6); PE(P-18:1/22:5)	6.357 ± 0.210	6.232 ± 0.150

PE(O-18:0/22:6)§; PE(P-20:0/20:5)	1.144 ± 0.164	1.401 ± 0.042
Choline glycerophospholipids		
Phosphatidylcholine (PC)		
PC(14:0/16:0)*	0.881 ± 0.046	0.981 ± 0.029
PC(16:0/16:0)	17.509 ± 0.506	18.176 ± 0.398
PC(16:0/16:1)*	2.194 ± 0.062	2.413 ± 0.062
PC(16:0/18:0)	4.028 ± 0.158	4.147 ± 0.093
PC(16:0/18:1)	17.869 ± 0.428	17.914 ± 0.363
PC(16:0/18:2); PC(16:1/18:1)*	1.223 ± 0.026	1.344 ± 0.042
PC(16:0/18:3)	0.565 ± 0.044	0.621 ± 0.046
PC(16:0/20:3); PC(18:1/18:2)**	0.813 ± 0.012	0.859 ± 0.014
PC(16:0/20:4)	2.445 ± 0.079	2.393 ± 0.048
PC(16:0/20:5)	0.159 ± 0.004	0.161 ± 0.003
PC(16:0/22:6)	10.252 ± 0.301	10.089 ± 0.261
PC(16:1/22:6)	0.226 ± 0.012	0.235 ± 0.013
PC(18:0/18:0)	0.747 ± 0.029	0.747 ± 0.031
PC(18:0/18:1)	6.765 ± 0.199	6.804 ± 0.211
PC(18:0/20:2)	0.163 ± 0.006	0.175 ± 0.006
PC(18:0/20:4); PC(16:0/22:4)	3.284 ± 0.081	3.214 ± 0.051
PC(18:0/22:6)	13.748 ± 0.435	13.518 ± 0.338
PC(18:1/18:1)	1.931 ± 0.049	2.007 ± 0.053
PC(18:1/20:4)	1.423 ± 0.041	1.385 ± 0.036
PC(18:1:22:6)	1.437 ± 0.054	1.137 ± 0.171
PC(20:0/22:6)	0.159 ± 0.004	0.156 ± 0.005
PC(20:1/22:6)	0.142 ± 0.005	0.144 ± 0.006
PC(20:3/22:6)	0.627 ± 0.062	0.646 ± 0.061
PC(20:4/22:6); PC(20:5/22:5)	0.210 ± 0.025	0.217 ± 0.012
PC(22:6/22:6)	3.111 ± 0.204	2.674 ± 0.188
PC(24:6/22:6)	0.229 ± 0.014	0.203 ± 0.014
PC(32:5/22:6)	0.384 ± 0.021	0.340 ± 0.022
PC(32:6/22:6)	1.244 ± 0.069	1.108 ± 0.078
PC(34:5/22:6)	0.640 ± 0.038	0.622 ± 0.043
PC(34:6/22:6)	1.621 ± 0.101	1.589 ± 0.119
PC(36:6/22:6)	0.196 ± 0.017	0.224 ± 0.024
PC(37:6)	0.202 ± 0.011	0.214 ± 0.018
PC(38:3)	0.529 ± 0.007	0.543 ± 0.007
PC(40:5)	2.000 ± 0.064	1.967 ± 0.050
PC(40:9)	0.315 ± 0.036	0.317 ± 0.034
Plasmenylcholine (PlC)		
PC(P-16:0/16:0)	0.172 ± 0.011	0.176 ± 0.007
PC(P-18:0/16:0)	0.182 ± 0.009	0.172 ± 0.018
PC(P-18:1/16:0); PC(P-16:0/18:1)	0.373 ± 0.014	0.369 ± 0.019
Serine glycerophospholipids		
Phosphatidylserine (PS)		
PS(16:0/20:4)	1.543 ± 0.164	1.747 ± 0.103
PS(18:0/20:4)	3.685 ± 0.402	3.244 ± 0.279
PS(18:0/22:6)	11.832 ± 1.764	9.329 ± 0.883
PS (22:6/22:6)	7.100 ± 1.708	4.180 ± 0.545

PS(37:3)	2.417 ± 0.260	2.726 ± 0.140
PS(37:4) (p=0.0524)	1.697 ± 0.196	2.186 ± 0.123
PS(37:5)	4.039 ± 0.363	4.748 ± 0.325
PS(39:5)	5.941 ± 0.526	6.353 ± 0.446
PS(39:6)	2.697 ± 0.277	3.066 ± 0.209
PS(40:3)	7.338 ± 0.684	8.218 ± 0.336
PS(42:3)	11.845 ± 0.510	12.244 ± 0.381
PS(42:5)	38.492 ± 1.571	40.979 ± 1.098
Plasmenylserine (PlS)		
PS(P-16:0/20:4)	1.373 ± 0.124	0.980 ± 0.218
Inositol glycerophospholipids		
Phosphatidylinositol (PI)		
PI(16:0/16:0)	0.061 ± 0.009	0.070 ± 0.004
PI(16:0/18:0)	0.111 ± 0.007	0.124 ± 0.007
PI(16:0/18:1)	0.684 ± 0.030	0.742 ± 0.049
PI(16:0/18:2)*	0.259 ± 0.010	0.310 ± 0.018
PI(16:0/20:3)	2.833 ± 0.063	2.889 ± 0.045
PI(16:0/20:4)	18.659 ± 0.234	18.911 ± 0.179
PI(16:0/20:5)*	1.314 ± 0.036	1.441 ± 0.064
PI(16:0/22:6)	3.110 ± 0.062	3.104 ± 0.122
PI(17:0/20:4)	0.187 ± 0.003	0.199 ± 0.013
PI(18:0/20:3)	8.800 ± 0.072	8.658 ± 0.050
PI(18:0/20:4)*	53.580 ± 0.466	52.303 ± 0.318
PI(18:0/22:6)	1.442 ± 0.036	1.375 ± 0.044
PI(18:1/22:6)	0.519 ± 0.015	0.506 ± 0.021
PI(18:1/20:4); PI(18:0/20:5)**	7.126 ± 0.622	8.090 ± 0.071
PI(20:4/22:6)	0.355 ± 0.015	0.319 ± 0.014
PI(22:6/22:6)	0.059 ± 0.007	0.063 ± 0.005
PI(36:1)	0.251 ± 0.011	0.257 ± 0.011
PI(36:2)	0.275 ± 0.013	0.309 ± 0.018
PI(40:5)	0.374 ± 0.007	0.330 ± 0.032
Sphingomyelins (SM)		
SM(d18:0/16:0)	3.251 ± 0.038	3.302 ± 0.034
SM(d18:0/18:0)	2.970 ± 0.050	3.014 ± 0.036
SM(d18:1/16:0)	33.164 ± 0.438	33.836 ± 0.352
SM(d18:1/18:0)	27.862 ± 0.454	28.319 ± 0.408
SM(d18:1/18:1)	2.686 ± 0.070	2.565 ± 0.143
SM(d18:1/20:0)	6.634 ± 0.181	6.366 ± 0.150
SM(d18:1/20:1)	1.564 ± 0.105	1.705 ± 0.257
SM(d18:1/22:0)	2.935 ± 0.187	2.621 ± 0.156
SM(d18:1/22:1)	1.092 ± 0.074	0.892 ± 0.119
SM(d18:1/24:0)	1.252 ± 0.089	0.881 ± 0.213
SM(d18:1/24:1)	2.664 ± 0.100	2.629 ± 0.102
SM(d18:2/18:1)	6.769 ± 0.335	6.809 ± 0.313
SM(d18:2/20:1)	5.915 ± 0.285	5.954 ± 0.229
SM(d18:2/22:1)	1.241 ± 0.121	1.108 ± 0.134

Lipid species were analyzed by LC-MS². For each phospholipid class, results are expressed as abundance (in percentage) of each species relative to that of total species, defined as 100%. It should be noted that for some isobaric phospholipid species, the different possible combinations of fatty acids moiety position are presented. § Plasmanylethanolamine species. *p<0.05 and **p<0.01.

Supplementary Table 5. Fatty acid profiles of diets.

	Not supplemented	*+ L. helveticus*
C14:0	0.477 ± 0.006	0.460 ± 0.010
C15:0	0.183 ± 0.006	0.180 ± 0.000
C16:0	20.510 ± 0.026	20.150 ± 0.170
C17:0	0.133 ± 0.006	0.130 ± 0.000
C18:0	2.270 ± 0.017	2.240 ± 0.000
C20:0	0.290 ± 0.010	0.293 ± 0.006
C22:0	0.193 ± 0.012	0.183 ± 0.006
C24:0	0.203 ± 0.006	0.200 ± 0.010
Total SFAs	**24.260 ± 0.026**	**23.837 ± 0.172**
C16:1n-7	0.510 ± 0.000	0.503 ± 0.012
C18:1n-7	1.230 ± 0.026	1.250 ± 0.010
Total MUFAs n-7	**1.740 ± 0.026**	**1.753 ± 0.006**
C16:1n-9	0.100 ± 0.000	0.097 ± 0.006
C18:1n-9	20.380 ± 0.056	19.917 ± 0.060
C20:1n-9	0.767 ± 0.006	0.753 ± 0.015
C22:1n-9	0.090 ± 0.000	0.090 ± 0.010
Total MUFAs n-9	**21.337 ± 0.055**	**20.857 ± 0.074**
Total MUFAs	**23.077 ± 0.032**	**22.610 ± 0.072**
C18:3n-3	3.307 ± 0.012	3.397 ± 0.025
C20:5n-3	0.300 ± 0.000	0.297 ± 0.006
C22:5n-3	0.160 ± 0.010	0.157 ± 0.006
C22:6n-3	0.497 ± 0.012	0.503 ± 0.012
Total PUFAs n-3	**4.263 ± 0.021**	**4.353 ± 0.023**
C18:2n-6	48.173 ± 0.042	48.987 ± 0.093
C20:2n-6	0.130 ± 0.017	0.120 ± 0.000
C20:4n-6	0.097 ± 0.006	0.093 ± 0.006
Total PUFAs n-6	**48.400 ± 0.046**	**49.200 ± 0.095**
Ratio n-6/n-3	**11.353 ± 0.065**	**11.302 ± 0.054**
Total PUFAs	**52.663 ± 0.029**	**53.553 ± 0.108**

Results are expressed as percentages of total fatty acid methyl esters (FAMEs). SFAs: saturated fatty acids. MUFAs: monounsaturated fatty acids. PUFAs: polyunsaturated fatty acids.

Supplementary Table 6. Primers used in this study.

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)
Fads1	CGCCAAACGCGCTACTTTAC	CCACAAAAGGATCCGTGGCA
Fads2	CGTGGGCAAGTTCTTGAAGC	TCTGAGAGCTTTTGCCACGG

Scd1	CAGGAGGGCAGGTTTCCAAG	CGTTCATTTCCGGAGGGAGG
Elovl1	CCTGAAGCACTTCGGATGGT	TCACTTGCCCGTCCTTCTTC
Elovl2	GTGATGTCCGGGTAGCCAAG	GGACGCGTGGTGATAGACAT
Elovl3	TACTTCTTTGGCTCTCGCCC	AGCTTACCCAGTACTCCTCCA
Elovl4	TGAAGTCAGGATAGCTGGCG	AGTGAACATGGTGCAGTGGT
Elovl5	TGATGAACTGGGTTCCCTGC	CAGCTGCCCTTGAGTGATGT
Elovl6	AGAACACGTAGCGACTCCGA	TCAGATGCCGACCACCAAAG
Fasn	GACTCGGCTACTGACACGAC	CGAGTTGAGCTGGGTTAGGG
Far1	GCTCGGAAGCATCTCAACAAG	GTGCTGGATGCTCGGAAGTAT
Gnpat	TCACCGCAGCTACATTGACT	GCAGCTCACTGACCACTCTC
Agps	GTGCAGGGTGACACAGACTT	CCATGGTGATGTGACAGGCT
Hprt	CAGTCCCAGCGTCGTGATTA	TGGCCTCCCATCTCCTTCAT

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Figures

Figure 1

Figure 7

Figure 2

Figure 1

Figure 3

Figure 9

Figure 4

Figure 8

Figure 5

Figure 5

Figure 6

Figure 3

Figure 7

Figure 2

Figure 8

Figure 6

Figure 9

Figure 4

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