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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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# 1 Long-term intake of *Lacticaseibacillus helveticus* 2 enhances bioavailability of omega-3 fatty acids in the 3 mouse retina

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20

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22 Fatty acids, Phospholipids, Docosahexaenoic acid.

23

## 24 **Abstract**

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

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

## 42 **Introduction**

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

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

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

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

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

84

## 85 **Results**

### 86 **Body weight, food intake and fat deposition**

87 Weight gain was evaluated in mice after a 6-month exposure period to a diet supplemented or  
88 not with *L. helveticus* VEL12193. Administration of *L. helveticus* was well-tolerated by the  
89 mice, with no noticeable side effects, including on the consistency of feces. We observed that  
90 supplementating mice with *L. helveticus* significantly limited weight gain compared to control  
91 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:  $1.2\% \pm 0.1\%$  and *L.*  
101 *helveticus* group:  $0.9\% \pm 0.1\%$  of total lipids; **Fig. 2**).

102 As changes in the composition of the gut microbiota can affect the metabolism of hepatic  
103 FAs<sup>14,17</sup>, 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**).

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

119

## 120 **Circulating lipids**

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

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

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

138

## 139 **Lipid profile and metabolism in the retina**

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

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

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

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

179

## 180 **Impact of long-term consumption of *L. helveticus* on gut microbiota** 181 **communities**

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

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

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

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

213 *Acutalibacter muris*. In addition, the sequence of the *Verrucomicrobia*-related OTU  
214 (OTU00000403) matched with 100% identity to the species *Akkermansia muciniphila* (**Table**  
215 **1**). No modification in the *Firmicutes/Bacteroidetes* ratio was observed (**Supplementary Fig.**  
216 **4**).

217

### 218 **Correlation between retinal fatty acids and changes in the gut microbiota** 219 **associated with long-term consumption of *L. helveticus***

220 To investigate any potential links between gut microbiota changes and the modifications of the  
221 FA content in the retina observed in *L. helveticus*-supplemented mice, we correlated the  
222 abundance of FAs or the expression level of enzymes involved in FA biosynthesis that were  
223 significantly modified in *L. helveticus*-supplemented mice with the abundance of individual  
224 OTU identified by the DESeq2 analysis (**Fig. 8** and **Fig. 9**). No correlation was found between  
225 the abundance of 3 OTUs (OTU00000012, OTU00000036 and OTU00005085) and the retinal  
226 level of FAs or expression level of genes encoding FA-related enzymes. Regarding the other  
227 22 OTUs, we observed that on the one hand the OTUs whose abundance was increased in the  
228 gut microbiota of *L. helveticus*-supplemented mice (OTU00000107 and OTU00000507) and on  
229 the other hand the OTUs whose abundance was decreased in the gut microbiota of *L. helveticus*-  
230 supplemented mice segregated (**Fig. 9** and **Supplementary Fig. 5 to Fig. 9**). Indeed, when a  
231 positive correlation was found between the abundance of OTU00000107 and/or OTU00000507  
232 and the retinal level of FAs or expression level of genes encoding FA-related enzymes, a  
233 negative correlation was found for the other OTUs, and conversely (**Fig. 9** and **Supplementary**  
234 **Fig. 5 to Fig. 9**). Of note, we did not identify any positive and negative correlations between  
235 the OTUs significantly modified by the *L. helveticus* supplementation and the retinal amounts  
236 of total n-3 PUFAs, DHA (C22:6n-3) and C16:1n-9 (**Fig. 9**).

237 The abundances of two OTUs, OTU00000107 and OTU00000403, were oppositely  
238 correlated to 12 of the 18 changes observed in the retina at the FA or gene expression level. The  
239 abundance of OTU00000107 (*Firmicutes*, 88.45 % of identity with *Clostridium cellulovorans*  
240 743B, **Table 1**) was positively correlated with the retinal amount of C15:0, C20:1n-9, total  
241 PUFAs, n-6 PUFAs, C18:2n-6, C20:3n-6, DMA C18:1n-7 and *Fads2* expression in the retina,  
242 and negatively correlated with the retinal amount of total SFAs, C16:0, C20:0, MUFAs n-7,  
243 C16:1n-7, DMA C16:0 and *Elovl6* expression in the retina (**Fig. 9** and **Supplementary Fig. 5**  
244 to **Fig. 9**). The abundance of OTU00000403 (*Verrucomicrobia*, 100% identity with *A.*  
245 *muciniphila*, **Table 1**) was positively correlated with the retinal amount of total SFAs, C16:0,  
246 C20:0, n-7 MUFAs, C16:1n-7 and DMA C16:0 and *Elovl6* expression in the retina, and  
247 negatively correlated with the retinal amount of total PUFAs, C22:5n-3, PUFAs n-6, C18:2n-  
248 6, C20:3n-6 and DMA C18:1n-7 (**Fig. 9** and **Supplementary Fig. 5 to Fig. 9**). In addition, we  
249 observed that a consortium of 9 OTUs was correlated (positive correlation: OTU00000107;  
250 negative correlation: OTU00000069, OTU00000130, OTU00000403, OTU00000409,  
251 OTU00000442, OTU00000630, OTU00000642 and OTU00003016) to the retinal amount of  
252 n-6 PUFAs (**Fig. 9** and **Supplementary Fig. 5 to Fig. 9**). Finally, a negative correlation of the  
253 retinal amount of C22:5n-3 (docosapentaenoic acid, DPA) and the abundance of a consortium  
254 of 3 OTUs (OTU00000403, OTU00000630 and OTU00005682) was observed.

255

## 256 **Discussion**

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

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

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

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

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

309 Plasma FA content was more importantly affected by the *L. helveticus* supplementation  
310 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<sup>59</sup>. 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<sup>60</sup>. 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*<sup>61</sup>. 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<sup>8,62-64</sup>. Conversely, low dietary  
357 intake of n-3 LC-PUFAs has been correlated with a higher risk of developing the disease<sup>8,63</sup>.  
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:

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

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

378

379         Correlative analyses between OTUs whose abundance was altered by *L. helveticus*  
380 supplementation and retinal FAs abundance have enabled identifying a consortium of 9 OTUs  
381 associated with retinal n-6 PUFA changes and a consortium of 3 OTUs associated with retinal  
382 n-3 PUFA changes. Two OTUs were common to these 2 consortia: OTU630, belonging to  
383 Actinobacteria-p and whose sequence has 84.91% of identity with *Faecalimonas umbilicata*  
384 and OTU403, belonging to *Verrucomicrobia* and whose sequence has 100% identity with *A.*  
385 *muciniphila*<sup>68</sup>. 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<sup>69-77</sup>. Interestingly, in addition to SCFAs, studies suggest that UDCA  
394 and TUDCA could also be involved in the regulation of the host FA metabolism<sup>17,78,79</sup>. 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**

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

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

423

## 424 **Diet**

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

434 *helveticus* in food portions stored under these conditions was checked (**Supplementary Fig.**  
435 **10**). The FA content of the diets is provided in **Supplementary Table 5**.

436

### 437 **Microbiota analysis**

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

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

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

453 Bioinformatic analyses were performed using the BIOCOM-PIPE pipeline, with default  
454 parameters, except when parameters were clearly described <sup>81</sup>. First, the 16S raw reads were  
455 sorted according to each sample using multiplex identifiers, and low-quality reads were deleted  
456 based on their length (less than 350-bp for 16S reads), their number of ambiguities and their  
457 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 <sup>82</sup>, 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 <sup>83</sup>, 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 <sup>84</sup>.  
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 <sup>85</sup>. 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**

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

491

#### 492 **Analysis of phospholipid molecular species by liquid chromatography coupled** 493 **to high-resolution mass spectrometer**

494 Phosphorus content of the total lipid extract was determined according to the method developed  
495 by Bartlett and Lewis<sup>87</sup>. The total phospholipids were dried under a stream of nitrogen and  
496 diluted to the appropriate concentration of 500 μg/μL of phospholipids in CHCl<sub>3</sub>/CH<sub>3</sub>OH (1:1,  
497 v/v). Ten microliters of internal standard mixture containing PC(14:0/14:0) 320 μg/mL,  
498 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  
499 SM(d18:1/12:0) 80 μg/mL were added into 200 μL of this phospholipid solution.

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

505 consisted of (A) CH<sub>3</sub>CN/H<sub>2</sub>O (96/4, v/v) containing 10mM ammonium acetate and (B)  
506 CH<sub>3</sub>CN/H<sub>2</sub>O (50/50, v/v) containing 10mM ammonium acetate. The chosen solvent-gradient  
507 system of the analytical pump was as follows : 0 min 100% A, 12 min 80% A, 18 min 50% A,  
508 18.1–30 min 100% A. The flow rate was 500  $\mu$ L/min, the injection volume was 10  $\mu$ L and the  
509 column was maintained at 50°C. The liquid chromatography system was controlled by Standard  
510 Instrument Integration (SII) software based on Dionex Chromeleon TN 7.

511 The process of identification and quantification of phospholipid species was performed  
512 on an orbitrap Fusion<sup>TM</sup> Tribrid Mass Spectrometer equipped with an EASY-MAX NG Ion  
513 Source (Heated Electrospray Ionization H-ESI) (Thermo Scientific, San Jose, CA, USA).  
514 Phospholipid species were detected by high-resolution mass spectrometry (HRMS) analysis.  
515 H-ESI source parameters were optimized and set as follows: ion transfer tube temperature of  
516 285°C, vaporizer temperature of 370°C, sheath gas flow rate of 35 au, sweep gas of 1 au,  
517 auxiliary gas flow rate of 25 au. Positive and negative ions were monitored alternatively by  
518 switching the polarity approach with a static spray voltage at 3500V and 2800V in positive and  
519 negative mode respectively. Mass spectra in full scan mode were obtained using the Orbitrap  
520 mass analyzer with the normal mass range and a target resolution of 240,000 (FWHM at  $m/z$   
521 200), in a mass-to-charge ratio  $m/z$  ranging from 200 to 1600 using a Quadrupole isolation in a  
522 normal mass range. All MS data were recorded using a maximum injection time of 100 ms,  
523 automatic gain control (AGC) target (%) at 112.5, RF lens (%) at 50, and one microscan. An  
524 intensity threshold filter of 1.103 counts was applied.

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

530 data-dependent mode. The AGC target was set to 2.10<sup>4</sup> with a maximum injection time of 50  
531 ms. All MS and MS/MS data were acquired in the profile mode. The Orbitrap Fusion was  
532 controlled by Xcalibur™ 4.1 software (Thermo Scientific, San Jose, CA, USA). The  
533 identification of all PL species was performed using the high-accuracy data and the information  
534 collected from fragmentation spectra with the help of LIPIDSEARCH software version 4.1.16  
535 (Thermo Scientific, San Jose, CA, USA) and the LIPID MAPS® database  
536 (<https://www.lipidmaps.org/>).

537

### 538 **Gene expression**

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

544

### 545 **Statistical analyses**

546 The data are presented as mean ± standard deviation of the mean (SEM), except those including  
547 bacterial communities. Statistical analyses were performed using the GraphPad Prism software  
548 for all analyses except those including bacterial communities, which were performed with R  
549 (version 4.1.2). The non-parametric Mann and Whitney or Krus-kal-Wallis tests were used to  
550 compare data from the two groups (after Bonferroni correction). The p-values of less than 0.05  
551 were considered statistically significant (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  and \*\*\*\*  $p <$   
552  $0.0001$ ).

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

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

568

## 569 **Data availability**

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

573

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581

## 582 **Author contributions**

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

588

## 589 **Competing interests statement**

590 Authors declare no competing interests.

591

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834

## 835 **Figure Legends**

836 **Figure 1. Effect of *L. helveticus* on weight gain, epididymal fat deposition and food intake.**

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

843

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

848

849 **Figure 3. Fatty acid content in the liver of mice exposed lengthily to *L. helveticus*. (a)** Heat  
850 map showing the hepatic abundance of each fatty acid methyl esters (FAME) or dimethylacetal  
851 (DMA) relative to total FAMES + DMAs (defined as 100%) in mice fed a control diet or fed a  
852 diet supplemented with *L. helveticus*. The ratio of total n-6 PUFAs/total n-3 PUFAs was  
853 calculated (n-6/n-3 ratio). **(b)** Hepatic expression of genes encoding enzymes involved in the  
854 biosynthesis of fatty acids: acyl-CoA (8-3)-desaturase (*Fads1*), acyl-CoA 6-desaturase (*Fads2*),  
855 acyl-CoA desaturase 1 (*Scd1*), elongation of very long chain fatty acids proteins 1, 2, 3, 5 and  
856 6 (*Elovl1*, *Elovl2*, *Elovl3*, *Elovl5* and *Elovl6*), and fatty acid synthase (*Fasn*). **(c)** Hepatic  
857 expression of genes encoding enzymes involved in the biosynthesis of plasmalogens: fatty acyl-  
858 CoA reductase 1 (*Far1*), alkylglycerone-phosphate synthase (*Agps*) and dihydroxyacetone  
859 phosphate acyltransferase (*Gnpat*). The levels of mRNA were normalized to *Hprt* mRNA level  
860 for calculation of the relative levels of transcripts. mRNA levels are illustrated as fold change.  
861 Mann-Whitney test (\*\*  $p < 0.001$ ).

862

863 **Figure 4. Effect of *L. helveticus* on lipid class distribution in the plasma. (a)** Cholesterol  
864 (Chol). **(b)** Cholesteryl esters (CE). **(c)** Phospholipids (PL). **(d)** Triglycerides (TG). **(e)** Free  
865 fatty acids (FFA). Results are expressed as abundance (%) relative to total lipids defined as  
866 100%.

867

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

873

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

887

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

891

892 **Figure 8. Volcano plot highlighting OTU fold changes in the gut microbiota of *L. helvet-***  
893 ***icus*-supplemented mice.** Each point represents an operational taxonomic unit (OTU). The x-  
894 axis represents the log<sub>2</sub> of the fold change whilst the y-axis is the negative log<sub>10</sub> of DESeq2 p  
895 values adjusted for multiple testing using the false discovery rate method. The vertical lines are  
896 fold-change cutoff that correspond to a log 2-fold change of 0.5 and 0.5. Blue points to the left  
897 of the plot with negative log<sub>2</sub>FoldChange values represent OTUs with increased abundance in  
898 control mice relative to mice fed *L. helveticus*-supplemented diet. Red points to the right of the  
899 plot with positive log<sub>2</sub>FoldChange values represent OTUs with increased abundance in mice  
900 fed *L. helveticus*-supplemented diet relative to control mice.

901

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

912

## 913 **Tables**

914 **Table 1. Taxonomic identification of OTUs.**

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

915 <sup>a</sup> Adjusted *p*-value computed during the DeSeq2 analysis with the Benjamini and Hochberg  
916 method.

917

## 918 **Supplementary information**

### 919 ***Supplementary Figures***

920 **Supplementary Figures are provided in a separate file.**

921 **Legends of supplementary Figures :**

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

927

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

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

933

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

937

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

939

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

943

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

947

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

951

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

955

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

959

960 **Supplementary Figure 10.** Viability of *L. helveticus* following its incorporation into the diet  
961 and its storage at 4°C. The probiotic was incorporated into the diet at the concentration of  $1.10^9$   
962 CFUs of *L. helveticus*/g of food. Portions were molded using Petri dishes (20g/dish) and stored  
963 at 4°C under anaerobic conditions. The number of viable CFUs of *L. helveticus*/portion was  
964 determined by resuspending the diet, plating dilutions of the suspension on MRS agar plates  
965 and enumerating the number of CFUs. Results are expressed as CFUs/g of diet at day 0 (the  
966 day the food containing *L. helveticus* was prepared), and into portions stored during 4 days or  
967 8 days at 4°C under anaerobic conditions.

969 *Supplementary Tables*970 **Supplementary Table 1. Fatty acid composition in the liver of mice fed a control diet or a**  
971 **diet supplemented with *L. helveticus*.**

	Control	+ <i>L. helveticus</i>
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
<b>Total SFAs**</b>	<b>28.868 ± 0.292</b>	<b>27.484 ± 0.307</b>
C16:1n-7	6.153 ± 0.210	6.555 ± 0.357
C18:1n-7	5.348 ± 0.456	4.586 ± 0.275
<b>Total MUFAs n-7</b>	<b>11.501 ± 0.496</b>	<b>11.141 ± 0.461</b>
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
<b>Total MUFAs n-9</b>	<b>34.341 ± 1.726</b>	<b>33.099 ± 0.997</b>
C18:1t	0.070 ± 0.006	0.076 ± 0.011
<b>Total MUFAs</b>	<b>45.912 ± 2.142</b>	<b>44.316 ± 1.061</b>
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
<b>Total PUFAs n-3</b>	<b>4.970 ± 0.458</b>	<b>5.347 ± 0.194</b>
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
<b>Total PUFAs n-6</b>	<b>20.160 ± 1.619</b>	<b>22.654 ± 0.894</b>
<b>Total PUFAs</b>	<b>25.130 ± 2.065</b>	<b>28.001 ± 1.078</b>
<b>n-6/n-3 ratio</b>	<b>4.102 ± 0.099</b>	<b>4.236 ± 0.057</b>
DMA C18:1n-9	0.201 ± 0.009	0.199 ± 0.005

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

975

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

	Control	+ <i>L. helveticus</i>
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
<b>Total SFAs**</b>	<b>24.559 ± 0.668</b>	<b>22.067 ± 0.402</b>
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
<b>Total MUFAs n-7</b>	<b>5.938 ± 0.286</b>	<b>5.500 ± 0.301</b>
C16:1n-9*	0.279 ± 0.027	0.199 ± 0.015
C18:1n-9 ( <i>p</i> =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
<b>Total MUFAs n-9</b>	<b>17.835 ± 0.260</b>	<b>17.321 ± 0.271</b>
C18:1t**	0.157 ± 0.008	0.201 ± 0.014
<b>Total MUFAs</b>	<b>23.930 ± 0.521</b>	<b>23.022 ± 0.446</b>
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 ( <i>p</i> =0.0653)	7.797 ± 0.404	8.605 ± 0.246
<b>Total PUFAs n-3*</b>	<b>9.042 ± 0.430</b>	<b>9.960 ± 0.256</b>
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
<b>Total PUFAs n-6*</b>	<b>41.420 ± 0.735</b>	<b>43.882 ± 0.541</b>
<b>n-6/n-3 ratio</b>	<b>4.647 ± 0.159</b>	<b>4.422 ± 0.088</b>
C20:3n-9	0.575 ± 0.060	0.593 ± 0.035
<b>Total PUFAs*</b>	<b>51.036 ± 1.087</b>	<b>54.435 ± 0.709</b>
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
<b>Total DMAs</b>	<b>0.475 ± 0.021</b>	<b>0.476 ± 0.012</b>

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.

981

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

	Control	+ <i>L. helveticus</i>
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
<b>Total SFAs**</b>	<b>41.923 ± 0.756</b>	<b>39.022 ± 0.528</b>
C16:1n-7**	0.421 ± 0.036	0.266 ± 0.023
C18:1n-7	3.323 ± 0.037	3.272 ± 0.060
<b>Total MUFAs n-7*</b>	<b>3.744 ± 0.042</b>	<b>3.538 ± 0.078</b>
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
<b>Total MUFAs n-9</b>	<b>12.013 ± 0.196</b>	<b>11.562 ± 0.196</b>
C18:1t	0.119 ± 0.007	0.118 ± 0.008
<b>Total MUFAs (p=0.0630)</b>	<b>15.876 ± 0.225</b>	<b>15.218 ± 0.238</b>
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
<b>Total PUFAs n-3*</b>	<b>27.107 ± 0.829</b>	<b>29.519 ± 0.690</b>
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
<b>Total PUFAs n-6****</b>	<b>11.382 ± 0.127</b>	<b>12.687 ± 0.180</b>
<b>n-6/n-3 ratio</b>	<b>0.423 ± 0.011</b>	<b>0.432 ± 0.012</b>
<b>Total PUFAs**</b>	<b>38.489 ± 0.916</b>	<b>42.206 ± 0.725</b>
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
<b>Total DMAs*</b>	<b>3.713 ± 0.023</b>	<b>3.563 ± 0.065</b>

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

987

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

	Control	+ <i>L. helveticus</i>
<b>Ethanolamine glycerophospholipids</b>		
<b>Phosphatidylethanolamine (PE)</b>		
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
<b>Plasmenylethanolamine (PIE)</b>		
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) ( <i>p</i> =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
<b>Choline glycerophospholipids</b>		
<i>Phosphatidylcholine (PC)</i>		
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
<i>Plasmenylcholine (PIC)</i>		
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
<b>Serine glycerophospholipids</b>		
<i>Phosphatidylserine (PS)</i>		
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) ( <i>p</i> =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
<b>Plasmalogen (PLS)</b>		
PS(P-16:0/20:4)	1.373 ± 0.124	0.980 ± 0.218
<b>Inositol glycerophospholipids</b>		
<b>Phosphatidylinositol (PI)</b>		
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
<b>Sphingomyelins (SM)</b>		
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

989 Lipid species were analyzed by LC-MS<sup>2</sup>. For each phospholipid class, results are expressed as  
 990 abundance (in percentage) of each species relative to that of total species, defined as 100%. It  
 991 should be noted that for some isobaric phospholipid species, the different possible combinations  
 992 of fatty acids moiety position are presented. § Plasmanylethanolamine species. \* $p < 0.05$  and  
 993 \*\* $p < 0.01$ .

994

995 **Supplementary Table 5. Fatty acid profiles of diets.**

	<i>Not supplemented</i>	<i>+ L. helveticus</i>
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
<b>Total SFAs</b>	<b>24.260 ± 0.026</b>	<b>23.837 ± 0.172</b>
C16:1n-7	0.510 ± 0.000	0.503 ± 0.012
C18:1n-7	1.230 ± 0.026	1.250 ± 0.010
<b>Total MUFAs n-7</b>	<b>1.740 ± 0.026</b>	<b>1.753 ± 0.006</b>
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
<b>Total MUFAs n-9</b>	<b>21.337 ± 0.055</b>	<b>20.857 ± 0.074</b>
<b>Total MUFAs</b>	<b>23.077 ± 0.032</b>	<b>22.610 ± 0.072</b>
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
<b>Total PUFAs n-3</b>	<b>4.263 ± 0.021</b>	<b>4.353 ± 0.023</b>
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
<b>Total PUFAs n-6</b>	<b>48.400 ± 0.046</b>	<b>49.200 ± 0.095</b>
<b>Ratio n-6/n-3</b>	<b>11.353 ± 0.065</b>	<b>11.302 ± 0.054</b>
<b>Total PUFAs</b>	<b>52.663 ± 0.029</b>	<b>53.553 ± 0.108</b>

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

998

999 **Supplementary Table 6. Primers used in this study.**

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
<i>Fads1</i>	CGCCAAACGCGCTACTTTAC	CCACAAAAGGATCCCGTGGA
<i>Fads2</i>	CGTGGGCAAGTTCTTGAAGC	TCTGAGAGCTTTTGCCACGG

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

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1000

# 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

## **Supplementary Files**

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