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The effect of gut microbiota on the intestinal lipidome of mice

Gerhard Liebisch [a,*], Johannes Plagge [b], Marcus Höring [a], Claudine Seeliger [b], Josef Ecker [b,**]

[a] Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany
[b] ZIEL - Institute for Food & Health, Research Group Lipid Metabolism, Technical University of Munich, Freising, Germany

A R T I C L E I N F O

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A B S T R A C T

Gut microbiota significantly influence the plasma and liver lipidome. An interconnecting metabolite is acetate generated after degradation and fermentation of dietary fiber by the gut microbiota, which is metabolized in the liver into longer chain fatty acids and complex lipids reaching the circulation. Whether these systemic changes are accompanied by alternations of the intestinal lipidome is unclear. Therefore, we quantified glycerophospholipids, sphingolipids and sterols in ileum and colon, the two segments containing the highest densities of microbes in the gastrointestinal tract, of germfree and specific pathogen free mice using mass spectrometry-based lipidomics. We found that the presence of gut microbes lowers the free cholesterol content in colon while elevating phosphatidylcholine levels. Further, PUFA-containing phosphatidylcholine and -ethanolamine fractions are increased in ileum and colon of germfree compared to SPF mice. A total fatty acid analysis by GC–MS revealed higher levels of arachidonic and docosahexaenoic acid in the ileum of germfree mice indicating that the gut microbiota inhibits PUFA metabolism in the small intestine.

1. Introduction

Interactions between gut microbiota and host lipid metabolic processes are highly relevant for host physiology and pathophysiology. The gut microbiota processes food components and makes non-digestible food components available for further metabolism in the host. This includes the de-polymerization and fermentation of complex carbohydrates from dietary fiber to the short chain fatty acids (SCFA) acetate (FA 2:0), propionate (FA 3:0) and butyrate (FA 4:0) in the gut lumen. Acetate is the predominant SCFA with levels of ~0.5 mmol/g in human feces and ~0.05 mmol/g in mouse colon content (Liebisch et al., 2019). Changes in gut microbiota composition (e.g. by antibiotic treatment) or variation of dietary fiber intake significantly affect SCFA levels in mice and humans. After uptake by the intestinal epithelium, acetate reaches the liver via the portal vein with concentrations up to 1.0 mmolar in mice, rats and humans (Cummings et al., 1987; Jakobsdottir et al., 2013; Kindt et al., 2018). We have previously identified that acetate originating from gut microbial degradation of dietary fiber is a major precursor for synthesis of long chain fatty acids (FA) in the liver (Kindt et al., 2018). It is metabolized to C16 and C18 fatty acids and integrated into phospholipids that are released into the circulation.

The diversity of the mammalian lipidome is enormous with various lipid classes (e.g. phosphatidylcholine (PC)) consisting of numerous lipid species (e.g. PC 34:1, PC 34:2, PC 36:1, etc.). Cellular lipidomes are cell type and tissue-specific, and adapted to cellular functions (Hermansson et al., 2011; Schweizer et al., 2019). Particularly membrane lipid composition is precisely controlled during cell growth and differentiation (Ecker et al., 2010a), since it is crucial for its biophysical properties including lipid packing density and fluidity. Cholesterol for example reduces fluidity by intercalating between phospholipid chains due to its planar and rigid structure (Ernst et al., 2016). Glycerophospholipids containing saturated acyl chains pack with higher density forming less-fluid bilayers, whereas poly-unsaturated fatty acyls such as docosahexaenoic acid (DHA) are very flexible and can switch

Abbreviations: CE, cholesteryl ester; Cer, ceramide; DB, double bond; DHA, docosahexaenoic acid; ELOVL, fatty acid elongase; FADS, fatty acid desaturase; FA, fatty acid; FC, free cholesterol; GF, germfree; GPL, glycerophospholipid; LPC, lysophosphatidylcholine; MUFA, monounsaturated fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PE P, PE-based plasmalogens; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SAFA, saturated fatty acid; SL, sphingolipid; SM, sphingomyelin; SPF, specific pathogen-free.

* Corresponding author at: Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany.

** Corresponding author at: Research Group Lipid Metabolism, ZIEL – Institute for Food & Health, Technical University of Munich, Gregor-Mendel-Str. 2, 85354 Freising, Germany.

E-mail addresses: gerhard.liebisch@ukr.de (G. Liebisch), josef.ecker@tum.de (J. Ecker).

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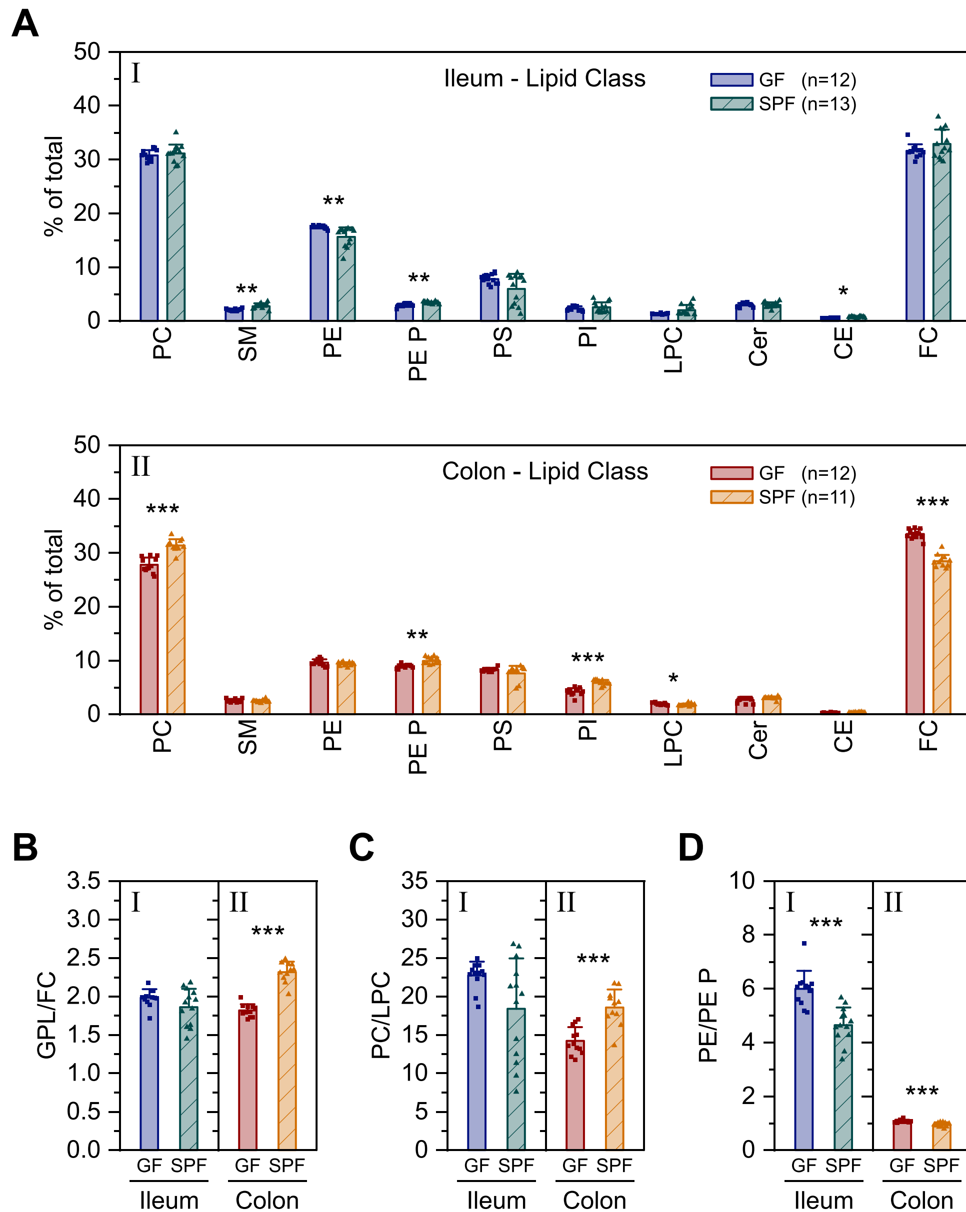

Fig. 1. Lipid class composition of ileum and colon from GF and SPF mice.
(A) Composition of all analyzed lipid classes, (B) GPL/FC, (C) PC/LPC, (D) PE/ PE P, in (I) ileum and (II) colon. Shown are means ± SD of n = 11–13 mice. Significant difference was tested as described in the methods section (*q<0.05, **q<0.01, ***q<0.001).

easily between different conformations, increasing membrane fluidity (Pinot et al., 2014). External perturbations of membrane biophysical properties including dietary lipid inputs are compensated by rapid acyl chain remodeling in phospholipids or adjustment of the cholesterol content (Levental et al., 2020).

Since we recently found that the gut microbiota significantly alters hepatic and plasma fatty acid and glycerophospholipid composition of mice (Kindt et al., 2018), the aim of the present study was to investigate its effects on the intestinal lipidome. Therefore, we quantified glycerophospholipids, sphingolipids and sterols in the small and large intestine of germfree and SPF mice using mass spectrometry-based lipidomics. We analyzed ileum and colon, because these two segments contain the highest amounts microbes in the gastrointestinal tract as the microbial density increases along the path from the stomach to the colon (Sekirov et al., 2010). Our results indicate that gut microbiota alter total PC and cholesterol contents in colon, as well as polyunsaturated fatty acid (PUFA) metabolism in the small intestine.

2. Methods

2.1. Mouse housing

SPF and GF C57BL/6 N mice were housed at 22 ± 1 °C and 50–60 % relative humidity with a 12 h light–dark cycle, fed a chow diet (autoclaved, V1534, Ssniff) *ad libitum* and sacrificed at 10 weeks of age. SPF mice were kept in individually ventilated cages, and GF mice were housed in open cages within flexible film isolators ventilated via HEPA-filtered air at the ZIEL Institute for Food & Health.

Sterility of GF mice was routinely confirmed by culturing and microscopic observation of feces after Gram-staining and a 16S rRNA gene-targeted PCR of GF cecal content was performed at the end of the study. The breeding and experimental use of mice in the facilities at the Technische Universität München (School of Life Sciences Weihenstephan) was approved by the local institution in charge (Regierung von Oberbayern; approval number 55.2-1-54-2531-99-13 and 55.2-1-54-2532-17-2015; 55.2-1-54-2532-192-2016).

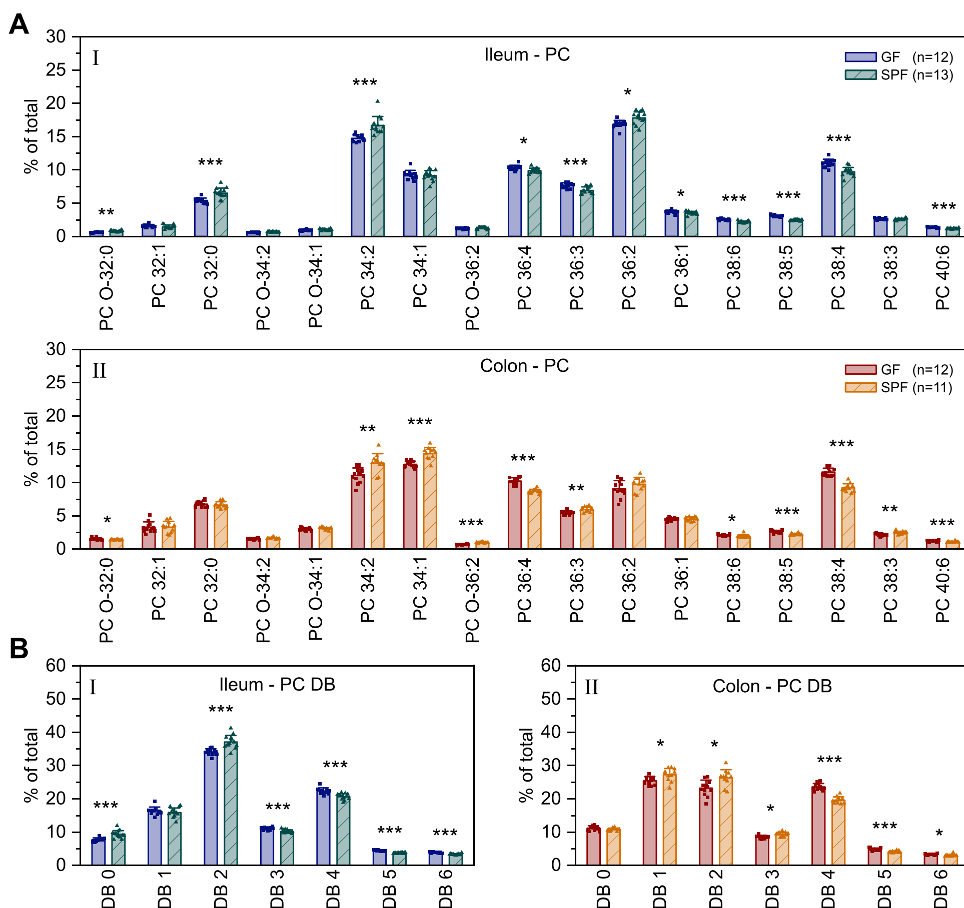

Fig. 2. Lipid species profiles of PC from ileum and colon of SPF mice.
(A) PC Species composition, (B) saturation, as number of double bonds in the sum of FA moieties, in (I) ileum and (II) colon. Shown are means ± SD (n = 11–13) of PC and PC O species with fractions >1 %. PC O were annotated based on the assumption that only even acyl chains are present. Significant difference was tested as described in the methods section (*q<0.05, **q<0.01, ***q<0.001).

2.2. Ileum and colon sampling

Sampling of intestines was performed on ice. For ileum samples, the distal 8 cm the small intestine was used, colon was sampled in whole. Both, ileum and colon were rinsed with 0.9 % NaCl solution to remove intestinal contents and subsequently snap-frozen in liquid nitrogen.

2.3. Total fatty acid analysis

FA analysis was performed as described previously (Ecker et al., 2012). Briefly, fatty acid methyl esters (FAMEs) were generated by acetyl chloride and methanol treatment and extracted with hexane. Total FA analysis was carried out using a Shimadzu 2010 GC–MS system. FAMEs were separated on a BPX70 column (10 m length, 0.10 mm diameter, 0.20 μm film thickness) from SGE using helium as the carrier gas. The initial oven temperature was 50 °C and was programed to increase at 40 °C/min to 155 °C, 6 °C/min to 210 °C, and finally 15 °C/min to 250 °C. The FA species and their positional and *cis/trans* isomers were characterized in scan mode and quantified by single ion monitoring, to detect specific fragments of saturated and unsaturated FAs (saturated, *m/z* 74; mono-unsaturated, *m/z* 55; di-unsaturated, *m/z* 67; poly-unsaturated, *m/z* 79). The internal standard was non-naturally-occurring C21:0 iso.

2.4. Glycerophospholipid, sphingolipid and sterol analyses

Lipids were quantified by electrospray ionization tandem mass spectrometry (ESI-MS/MS) in positive ion mode as described previously (Ecker et al., 2010b). In brief, samples were analyzed by direct flow injection using a HTS PAL autosampler, an Agilent 1100 binary pump, and triple quadrupole mass spectrometer (Quattro Ultima, Micromass). A precursor ion scan of *m/z* 184 specific for phosphocholine-containing lipids was used for PC (Liebisch et al., 2004). Neutral loss scans of 141 and 185 were used for PE and PS, respectively. Sphingosine (d18:1) based ceramides were analyzed using *m/z* 264 (Liebisch et al., 1999). PE-based plasmalogens (PE P) were analyzed according to the principles described by Zemski-Berry (Zemski Berry and Murphy, 2004). Free cholesterol (FC) and cholesteryl ester (CE) were quantified using a fragment ion of *m/z* 369 after selective derivatization of FC using acetyl chloride (Liebisch et al., 2006). Correction of isotopic overlap of lipid species and data analysis by self-programmed Excel macros were performed for all lipid classes. For all lipid classes, non-naturally occurring lipid species were used as internal standards. Quantification was performed by standard addition calibration to tissue homogenates using a number of naturally occurring lipid species for each lipid class.

2.5. RNA isolation and quantitative RT-PCR analysis

Total RNA was extracted from total tissue using the RNEasy Mini Kit (Qiagen). The purity and integrity of the RNA were assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies). For real-time PCR, 2 μg RNA was transcribed into cDNA using the Reverse Transcription System from Promega. Real-time quantitative RT-PCR analysis was performed using the Light Cycler LC 480 (Roche). Relative quantification was carried out using the LightCycler 480 SW 1.5.1 (Roche). GAPDH was used as a reference gene, relative mRNA expression values were calculated using delta delta threshold cycle (Ct) analysis (Langmann et al., 2003).

2.6. Statistical analyses

Lipid class and species profiles were calculated from molar

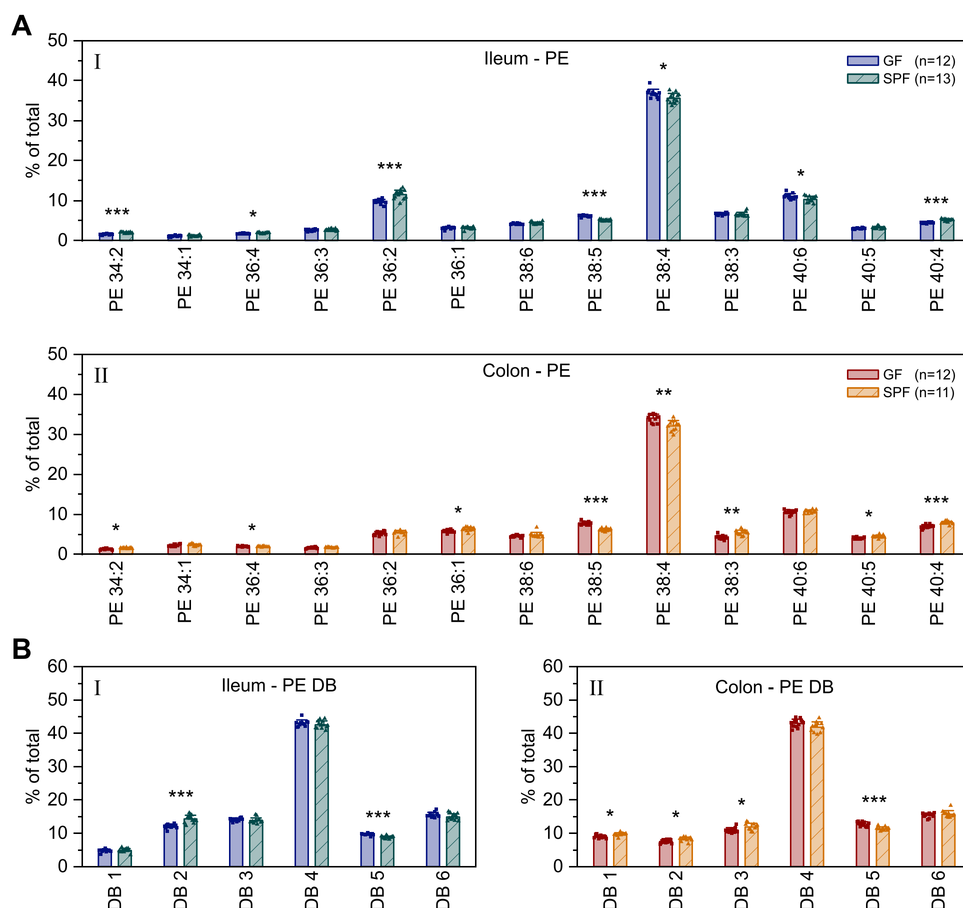

Fig. 3. Lipid species profiles of PE from ileum and colon of SPF mice.
(A) PE Species composition, (B) saturation, as number of double bonds in the sum of FA moieties, in (I) ileum and (II) colon. Shown are means ± SD (n = 11–13) of PE species with fractions >1 %. Significant difference was tested as described in the methods section (*q<0.05, **q<0.01, ***q<0.001).

concentrations. Significant difference between GF and SPF condition was tested using a two-tailed two-sample *t*-test. Shown are Benjamini–Hochberg-corrected p-values (* q<0.05, ** q<0.01, *** q<0.001).

3. Results

3.1. The gut microbiota alters total phosphatidylcholine and free cholesterol content of the large intestine

To test whether gut microbiota affect the intestinal lipidome, we quantified cellular glycerophospho- (GPL), sphingolipids (SL) and sterols using direct infusion electrospray ionization coupled to tandem mass spectrometry (ESI-MS/MS) in ileum and colon samples from germfree and SPF mice. Microbiota composition of SPF mice was described previously after high-throughput sequencing of V3-V4 amplicons of 16S rRNA genes in cecal contents (Kindt et al., 2018).

Our lipidomic analyses comprised:

(I) GPL including phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), PE-based plasmalogens (PE P), phosphatidylinositol (PI), phosphatidylserine (PS)
(II) SL including sphingomyelin (SM) and ceramide (Cer)
(III) Sterols, free cholesterol (FC) and cholesteryl ester (CE)

All analyzed lipids are predominantly part of membranes, expect for the storage lipid CE. PC (28–31 %) and FC (29–34 %) were the major lipid classes in ileum and colon (Fig. 1A). Sphingolipids represented only a minor fraction of intestinal lipids (2–3 % each). The lipid class composition of small and large intestine was comparable, except for their PE and PE P contents. Ileum contained ~4-fold more PE than PE P, while in colon both fractions were similar (Fig. 1D).

Gut microbiota significantly affected the large intestinal lipidome. GF mice contained less PC, but higher proportions of FC leading to a 30 % elevated GPL/FC ratio in mice harboring gut microbiota (Fig. 1A-B). Further, SPF mice contained elevated levels of LPC (colon) and ether-containing PE fraction leading to significantly altered PC/LPC and PE/PE P ratios (Fig. 1C-D).

3.2. Germfree mice contain elevated proportions of PUFA-containing PC and PE species in colon and ileum

Next, we asked whether the species profiles of GPL, SL and sterols depend on gut microbiota. For both intestinal segments, the major PC species were mono-unsaturated and di-unsaturated PC 34–36 as well as tetra-unsaturated PC 36–38 (Fig. 2A-B, Figure SI 1A-B). However, colon contained elevated fractions of PC 34:1 compared to ileum.

Importantly, we found a shift from shorter mono- and di- to longer tetra-unsaturated PC species in ileum and colon comparing SPF to GF mice (Fig. 2B). Proportions of PC 34:1 and PC 34:2 were higher in SPF mice, while GF mice contained elevated fractions of PC 36:4 and 38:4 (Fig. 2A). Surprisingly, the LPC species profile was not altered (data not shown).

PE acyl chains were generally longer and more unsaturated than PC (Fig. 3A-B, Figure SI 2A-B). The mono-unsaturated PE fraction (PE 34:1 and 36:1) was higher in colon than in ileum. Similar to PC, we could detect slightly higher levels of PUFA-containing species in GF mice compared to SPF animals, i.e. PE 38:4 and 38:5, in the small and large intestine. We did neither observe significant differences for species

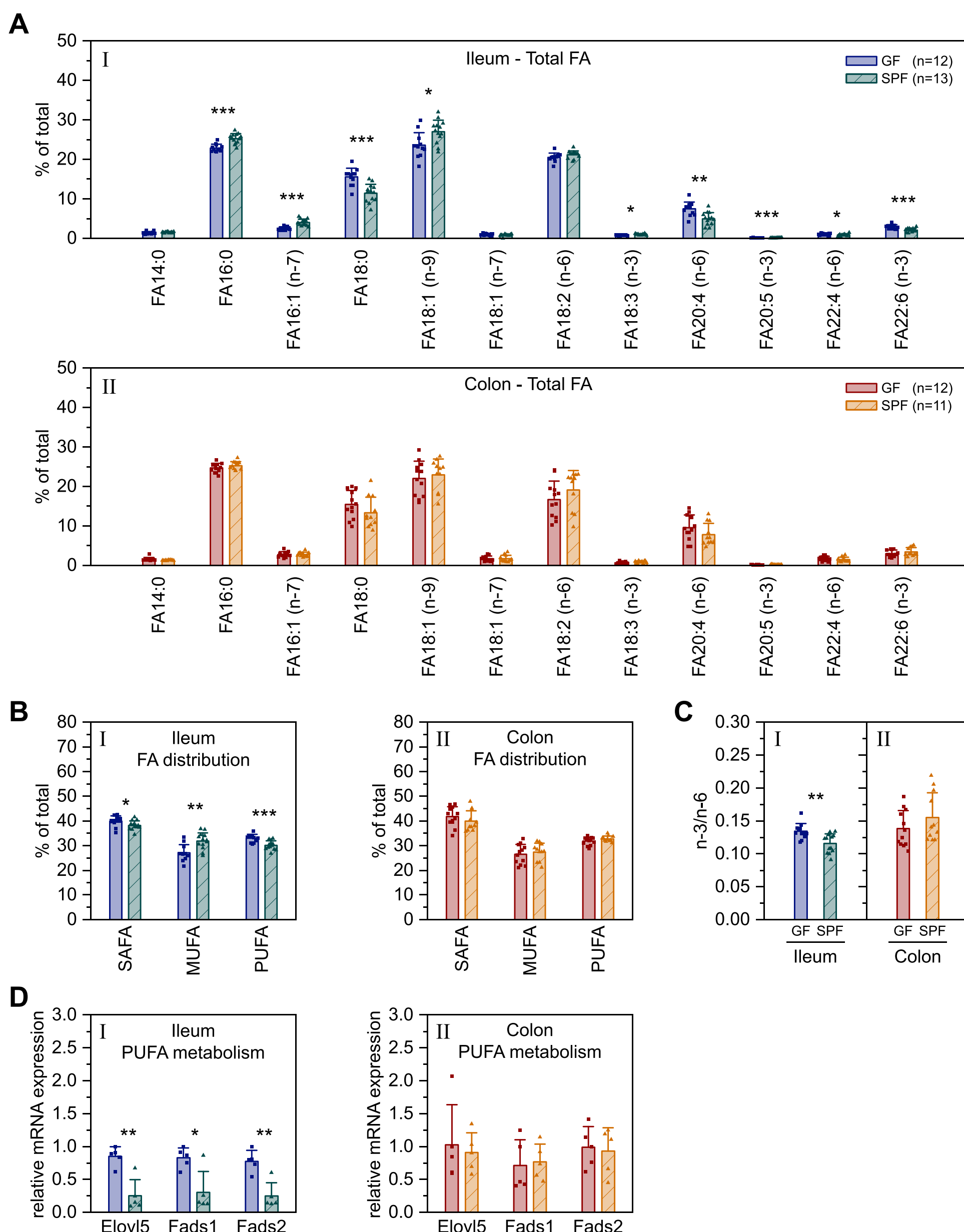

Fig. 4. FA metabolism of ileum and colon of GF and SPF mice.
(A) Total FA composition, (B) saturation, as sum of SAFA, MUFA and PUFA, (C) n-3/n-6 FA ratio, in (I) ileum and (II) colon. Shown are means ± SD of n = 11–13. (D) mRNA expression of PUFA elongase and desaturases. Shown are means ± SD of n = 5 per group, in (I) ileum and (II) colon. Significant difference was tested as described in the methods section (*q<0.05, **q<0.01, ***q<0.001).

profiles of the other analyzed GPL (LPC, PS, PG and PI), nor for the SL Cer and SM comparing GF with SPF mice (data not shown). Quantification of CE revealed ~1.4-fold elevated mono- and di-unsaturated species in ileum compared to colon, and ~1.3-fold higher levels of CE 20:4 in ileum of GF vs. SPF mice (data not shown).

Together, these data show that the gut microbiota affects lipid species of GPL in the small and large intestine. Mice harboring gut microbiota contain less PUFA-containing PC, PE and CE species in their ileum and colon.

3.3. *Gut microbiota inhibit PUFA metabolism in the small intestine*

To determine whether gut microbiota affects intestinal PUFA metabolism, we finally quantified total FA profiles using GC–MS. Our analysis includes 44 FA species (Ecker et al., 2012), of which 12 were detected in our samples. While FA profiles were similar between ileum and colon, the microbiome had a significant effect on the degree of FA unsaturation in the small intestine (Fig. 4A and B). GF mice had elevated fractions of arachidonic (FA 20:4 n-6) and docosahexaenoic acid (FA 22:6 n-3), but lower levels of palmitic acid (FA 16:0), palmitoleic (FA 16:1) and oleic acid (FA 18:1 n-9) in ileum. The fraction of n-3 FA was elevated in GF mice ileum samples (Fig. 4C). These results suggest a higher PUFA metabolism in the absence of gut microbiota.

This hypothesis is supported by the finding that GF mice show a higher mRNA expression of genes relevant for PUFA elongation and desaturation (Fig. 4D). The transcription of Fads1 and 2 and Elovl 5 are 2.5–3.9- fold higher in the ileum of GF mice, but unchanged in colon. Elovl 2 expression was not detected in ileum and colon.

Together, these data indicate that gut microbiota inhibit PUFA metabolism in the small intestine.

4. Discussion

The aim of the present study was to provide quantitative lipidomic data of small and large intestinal tissue from GF and SPF mice to investigate potential effects of gut microbiota on intestinal lipid metabolism. Although the lipidome of plasma/serum, liver and adipose tissue has been reported for GF mice, to the best of our knowledge, the small

and large intestinal lipidome is not yet available (Kindt et al., 2018; Velagapudi et al., 2010).

Our analyses revealed less PC and elevated FC fractions in the colon of germfree mice. The GPL/FC cholesterol ratio was higher in mice harboring gut microbiota potentially increasing membrane fluidity in large intestinal enterocytes. In contrast, in ileum and colon, SPF mice showed less PUFA-containing GPL and higher amounts of PE P permitting tighter membrane lipid packing (Koivuniemi, 2017). Thus, gut microbiota might have differential effects on biophysical membrane parameters in the small and large intestine. The presence of microbes might increase membrane fluidity in colon through modification of the PC and FC content, but decrease it in ileum by changing PUFA levels.

We found that the absence of microbes increased FA 20:4 n-6 and FA22:6 n-3 in ileum. While arachidonic acid is a precursor of inflammatory eicosanoids, DHA is metabolized to very potent pro-resolving mediators including the resolvins (Buckley et al., 2014; Schmitz and Ecker, 2008). n-3 PUFA have been exploited in therapy of inflammatory bowel disease. Although their effects on clinical endpoints are still under debate, a negative association between fish consumption and the risk of Crohn's disease and an inverse correlation between n-3 PUFA and ulcerative colitis was identified in a recent meta-analysis (Mozaffari et al., 2020). Krill oil, rich in n-3 PUFA including DHA, attenuates intestinal damage in porcine and mouse colitis models and restores gut microbial dysbiosis (Liu et al., 2020).

PUFAs are either taken up directly from the nutrition or metabolized from dietary fatty acids including linoleic acid (FA18:2 n-6) or alpha-linolenic acid (FA18:3 n-3) by fatty acid desaturases and elongases (Schmitz and Ecker, 2008). In our experiments, GF mice had lower expression of FADS1/2 and ELOVL5. Just recently, it was reported that changes in gut microbiota composition can be associated hepatic FADS1/2 and ELOVL 5 expression (Albouery et al., 2019). Large-scale, genome-wide association studies identified strong associations of SNPs in the region of the FADS gene cluster (FADS 1-2-3) with liver function, inflammation, cardiovascular disease and type 2 diabetes (Chambers et al., 2011; Dupuis et al., 2010; Lettre et al., 2011; Martinelli et al., 2008).

Because we recently showed that the gut microbiota promotes hepatic *de novo* FA synthesis, we hypothesize that GF mice might compensate their lower synthesis capacity by a higher lipid uptake from the nutrition leading to higher PUFA levels in the ileum (Kindt et al., 2018). Dietary long-chain FA (> 12 carbons) are absorbed in the upper parts of the small intestine, also by jejunal and ileal enterocytes (Glatz et al., 2010). In rats, it was shown that the ileum has the same capacity as the jejunum for uptake of PUFA (Saunders and Sillery, 1976). In humans, the jejunum seems to be the major site of fat absorption in case of moderate food intake. The ileum becomes more important in case of a higher intake of dietary fat (Booth et al., 1961). In Zebrafish as well as in mice that were fed a high fat diet, it was shown that gut microbiota rather stimulate than inhibit FA uptake (Martinez-Guryn et al., 2018; Semova et al., 2012). Another factor that may influence crosstalk between gut microbiota and host metabolism is diet. It has been shown that in absence of microbiota mice were resistant to diet induced obesity when a cholesterol-rich lard-based high-fat diet was fed, whereas on a cholesterol-free palm oil-based high-fat diet GF mice gain similar weight as SPF (Kubeck et al., 2016). Intestinal lipid transport could be also affected by microbiota derived-lipids which can represent about 25 % of all fecal lipids (Yasuda et al., 2020). However, a detailed study investigating the influence of gut microbiota on absorption of dietary lipids under physiological conditions in mammalians is not yet available.

In summary, our study revealed significant differences of colon cholesterol and PC and altered PUFA metabolism comparing GF to SPF mice. Thus, gut microbiota might influence biophysical membrane properties and generation of inflammatory mediators from PUFA in enterocytes relevant for the progression of gastrointestinal diseases.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijmm.2021.151488.

References

Albouery, M., Buteau, B., Gregoire, S., Cherbuy, C., Pais de Barros, J.P., Martine, L., Chain, F., Cabaret, S., Berdeaux, O., Bron, A.M., Acar, N., Langella, P., Bringer, M.A., 2019. Age-related changes in the gut microbiota modify brain lipid composition. Front. Cell. Infect. Microbiol. 9, 444.

Booth, C.C., Alldis, D., Read, A.E., 1961. Studies on the site of fat absorption: 2 Fat balances after resection of varying amounts of the small intestine in man. Gut 2, 168–174.

Buckley, C.D., Gilroy, D.W., Serhan, C.N., 2014. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. Immunity 40, 315–327.

Chambers, J.C., Zhang, W., Sehmi, J., Li, X., Wass, M.N., Van der Harst, P., Holm, H., Sanna, S., Kavousi, M., Baumeister, S.E., Coin, L.J., Deng, G., Gieger, C., Heard-Costa, N.L., Hottenga, J.J., Kuhnel, B., Kumar, V., Lagou, V., Liang, L., Luan, J., Vidal, P.M., Mateo Leach, I., O'Reilly, P.F., Peden, J.F., Rahmioglu, N., Soininen, P., Speliotes, E.K., Yuan, X., Thorleifsson, G., Alizadeh, B.Z., Atwood, L.D., Borecki, I.B., Brown, M.J., Charoen, P., Cucca, F., Das, D., de Geus, E.J., Dixon, A.L., Doring, A., Ehret, G., Eyjolfsson, G.I., Farrall, M., Forouhi, N.G., Friedrich, N., Goessling, W., Gudbjartsson, D.F., Harris, T.B., Hartikainen, A.L., Heath, S., Hirschfield, G.M., Hofman, A., Homuth, G., Hypponen, E., Janssen, H.L., Johnson, T., Kangas, A.J., Kema, I.P., Kuhn, J.P., Lai, S., Lathrop, M., Lerch, M.M., Li, Y., Liang, T.J., Lin, J.P., Loos, R.J., Martin, N.G., Moffatt, M.F., Montgomery, G.W., Munroe, P.B., Musunuru, K., Nakamura, Y., O'Donnell, C.J., Olafsson, I., Penninx, B.W., Pouta, A., Prins, B.P., Prokopenko, I., Puls, R., Ruokonen, A., Savolainen, M.J., Schlessinger, D., Schouten, J.N., Seedorf, U., Sen-Chowdhry, S., Siminovitch, K.A., Smit, J.H., Spector, T.D., Tan, W., Teslovich, T.M., Tukiainen, T., Uitterlinden, A.G., Van der Klauw, M.M., Vasan, R.S., Wallace, C., Wallaschofski, H., Wichmann, H.E., Willemsen, G., Wurtz, P., Xu, C., Yerges-Armstrong, L.M., et al., 2011. Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. Nat. Genet. 43, 1131–1138.

Cummings, J.H., Pomare, E.W., Branch, W.J., Naylor, C.P., Macfarlane, G.T., 1987. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 28, 1221–1227.

Dupuis, J., Langenberg, C., Prokopenko, I., Saxena, R., Soranzo, N., Jackson, A.U., Wheeler, E., Glazer, N.L., Bouatia-Naji, N., Gloyn, A.L., Lindgren, C.M., Magi, R., Morris, A.P., Randall, J., Johnson, T., Elliott, P., Rybin, D., Thorleifsson, G., Steinthorsdottir, V., Henneman, P., Grallert, H., Dehghan, A., Hottenga, J.J., Franklin, C.S., Navarro, P., Song, K., Goel, A., Perry, J.R., Egan, J.M., Lajunen, T., Grarup, N., Sparso, T., Doney, A., Voight, B.F., Stringham, H.M., Li, M., Kanoni, S., Shrader, P., Cavalcanti-Proenca, C., Kumari, M., Qi, L., Timpson, N.J., Gieger, C., Zabena, C., Rocheleau, G., Ingelsson, E., An, P., O'Connell, J., Luan, J., Elliott, A., McCarroll, S.A., Payne, F., Roccasecca, R.M., Pattou, F., Sethupathy, P., Ardlie, K., Ariyurek, Y., Balkau, B., Barter, P., Beilby, J.P., Ben-Shlomo, Y., Benediktsson, R., Bennett, A.J., Bergmann, S., Bochud, M., Boerwinkle, E., Bonnefond, A., Bonnycastle, L.L., Borch-Johnsen, K., Bottcher, Y., Brunner, E., Bumpstead, S.J., Charpentier, G., Chen, Y.D., Chines, P., Clarke, R., Coin, L.J., Cooper, M.N., Cornelis, M., Crawford, G., Crisponi, L., Day, I.N., de Geus, E.J., Delplanque, J., Dina, C., Erdos, M.R., Fedson, A.C., Fischer-Rosinsky, A., Forouhi, N.G., Fox, C.S., Frants, R., Franzosi, M.G., Galan, P., Goodarzi, M.O., Graessler, J., Groves, C.J., Grundy, S., Gwilliam, R., Gyllensten, U., Hadjadj, S., et al., 2010. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat. Genet. 42, 105–116.

Ecker, J., Liebisch, G., Englmaier, M., Grandl, M., Robenek, H., Schmitz, G., 2010a. Induction of fatty acid synthesis is a key requirement for phagocytic differentiation of human monocytes. Proc Natl Acad Sci U S A 107, 7817–7822.

Ecker, J., Liebisch, G., Scherer, M., Schmitz, G., 2010b. Differential effects of conjugated linoleic acid isomers on macrophage glycerophospholipid metabolism. J. Lipid Res. 51, 2686–2694.

Ecker, J., Scherer, M., Schmitz, G., Liebisch, G., 2012. A rapid GC-MS method for quantification of positional and geometric isomers of fatty acid methyl esters. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 897, 98–104.

Ernst, R., Ejsing, C.S., Antonny, B., 2016. Homeoviscous adaptation and the regulation of membrane lipids. J. Mol. Biol. 428, 4776–4791.

Glatz, J.F., Luiken, J.J., Bonen, A., 2010. Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. Physiol. Rev. 90, 367–417.

Hermansson, M., Hokynar, K., Somerharju, P., 2011. Mechanisms of glycerophospholipid homeostasis in mammalian cells. Prog. Lipid Res. 50, 240–257.

Jakobsdottir, G., Jadert, C., Holm, L., Nyman, M.E., 2013. Propionic and butyric acids, formed in the caecum of rats fed highly fermentable dietary fibre, are reflected in portal and aortic serum. Br. J. Nutr. 110, 1565–1572.
Kindt, A., Liebisch, G., Clavel, T., Haller, D., Hormannsperger, G., Yoon, H., Kolmeder, D., Sigruener, A., Krautbauer, S., Seeliger, C., Ganzha, A., Schweizer, S., Morisset, R., Strowig, T., Daniel, H., Helm, D., Kuster, B., Krumsiek, J., Ecker, J., 2018. The gut microbiota promotes hepatic fatty acid desaturation and elongation in mice. Nat. Commun. 9, 3760.
Koivuniemi, A., 2017. The biophysical properties of plasmalogens originating from their unique molecular architecture. FEBS Lett. 591, 2700–2713.
Kubeck, R., Bonet-Ripoll, C., Hoffmann, C., Walker, A., Muller, V.M., Schuppel, V.L., Lagkouvardos, I., Scholz, B., Engel, K.H., Daniel, H., Schmitt-Kopplin, P., Haller, D., Clavel, T., Klingenspor, M., 2016. Dietary fat and gut microbiota interactions determine diet-induced obesity in mice. Mol. Metab. 5, 1162–1174.
Langmann, T., Mauerer, R., Zahn, A., Moehle, C., Probst, M., Stremmel, W., Schmitz, G., 2003. Real-time reverse transcription-PCR expression profiling of the complete human ATP-binding cassette transporter superfamily in various tissues. Clin. Chem. 49, 230–238.
Lettre, G., Palmer, C.D., Young, T., Ejebe, K.G., Allayee, H., Benjamin, E.J., Bennett, F., Bowden, D.W., Chakravarti, A., Dreisbach, A., Farlow, D.N., Folsom, A.R., Fornage, M., Forrester, T., Fox, E., Haiman, C.A., Hartiala, J., Harris, T.B., Hazen, S. L., Heckbert, S.R., Henderson, B.E., Hirschhorn, J.N., Keating, B.J., Kritchevsky, S.B., Larkin, E., Li, M., Rudock, M.E., McKenzie, C.A., Meigs, J.B., Meng, Y.A., Mosley, T. H., Newman, A.B., Newton-Cheh, C.H., Paltoo, D.N., Papanicolaou, G.J., Patterson, N., Post, W.S., Psaty, B.M., Qasim, A.N., Qu, L., Rader, D.J., Redline, S., Reilly, M.P., Reiner, A.P., Rich, S.S., Rotter, J.I., Liu, Y., Shrader, P., Siscovick, D.S., Tang, W.H., Taylor, H.A., Tracy, R.P., Vasan, R.S., Waters, K.M., Wilks, R., Wilson, J. G., Fabsitz, R.R., Gabriel, S.B., Kathiresan, S., Boerwinkle, E., 2011. Genome-wide association study of coronary heart disease and its risk factors in 8,090 African Americans: the NHLBI CARe Project. PLoS Genet. 7, e1001300.
Levental, K.R., Malmberg, E., Symons, J.L., Fan, Y.Y., Chapkin, R.S., Ernst, R., Levental, I., 2020. Lipidomic and biophysical homeostasis of mammalian membranes counteracts dietary lipid perturbations to maintain cellular fitness. Nat. Commun. 11, 1339.
Liebisch, G., Drobnik, W., Reil, M., Trumbach, B., Arnecke, R., Olgemoller, B., Roscher, A., Schmitz, G., 1999. Quantitative measurement of different ceramide species from crude cellular extracts by electrospray ionization tandem mass spectrometry (ESI-MS/MS). J. Lipid Res. 40, 1539–1546.
Liebisch, G., Lieser, B., Rathenberg, J., Drobnik, W., Schmitz, G., 2004. High-throughput quantification of phosphatidylcholine and sphingomyelin by electrospray ionization tandem mass spectrometry coupled with isotope correction algorithm. Biochim. Biophys. Acta 1686, 108–117.
Liebisch, G., Binder, M., Schifferer, R., Langmann, T., Schulz, B., Schmitz, G., 2006. High throughput quantification of cholesterol and cholesteryl ester by electrospray ionization tandem mass spectrometry (ESI-MS/MS). Biochim. Biophys. Acta 1761, 121–128.
Liebisch, G., Ecker, J., Roth, S., Schweizer, S., Ottl, V., Schott, H.F., Yoon, H., Haller, D., Holler, E., Burkhardt, R., Matysik, S., 2019. Quantification of fecal short chain fatty acids by liquid chromatography tandem mass spectrometry-investigation of pre-analytic stability. Biomolecules 9.
Liu, F., Smith, A.D., Solano-Aguilar, G., Wang, T.T.Y., Pham, Q., Beshah, E., Tang, Q., Urban Jr, J.F., Xue, C., Li, R.W., 2020. Mechanistic insights into the attenuation of intestinal inflammation and modulation of the gut microbiome by krill oil using in vitro and in vivo models. Microbiome 8, 83.
Martinelli, N., Girelli, D., Malerba, G., Guarini, P., Illig, T., Trabetti, E., Sandri, M., Friso, S., Pizzolo, F., Schaeffer, L., Heinrich, J., Pignatti, P.F., Corrocher, R., Olivieri, O., 2008. FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease. Am. J. Clin. Nutr. 88, 941–949.
Martinez-Guryn, K., Hubert, N., Frazier, K., Urlass, S., Musch, M.W., Ojeda, P., Pierre, J. F., Miyoshi, J., Sontag, T.J., Cham, C.M., Reardon, C.A., Leone, V., Chang, E.B., 2018. Small intestine microbiota regulate host digestive and absorptive adaptive responses to dietary lipids. Cell Host Microbe 23, 458-469 e455.
Mozaffari, H., Daneshzad, E., Larijani, B., Bellissimo, N., Azadbakht, L., 2020. Dietary intake of fish, n-3 polyunsaturated fatty acids, and risk of inflammatory bowel disease: a systematic review and meta-analysis of observational studies. Eur. J. Nutr. 59, 1–17.
Pinot, M., Vanni, S., Pagnotta, S., Lacas-Gervais, S., Payet, L.A., Ferreira, T., Gautier, R., Goud, B., Antonny, B., Barelli, H., 2014. Lipid cell biology. Polyunsaturated phospholipids facilitate membrane deformation and fission by endocytic proteins. Science (80-) 345, 693–697.
Saunders, D.R., Sillery, J., 1976. Lecithin inhibits fatty acid and bile salt absorption from rat small intestine in vivo. Lipids 11, 830–832.
Schmitz, G., Ecker, J., 2008. The opposing effects of n-3 and n-6 fatty acids. Prog. Lipid Res. 47, 147–155.
Schweizer, S., Liebisch, G., Oeckl, J., Hoering, M., Seeliger, C., Schiebel, C., Klingenspor, M., Ecker, J., 2019. The lipidome of primary murine white, brite, and brown adipocytes-Impact of beta-adrenergic stimulation. PLoS Biol. 17, e3000412.
Sekirov, I., Russell, S.L., Antunes, L.C., Finlay, B.B., 2010. Gut microbiota in health and disease. Physiol. Rev. 90, 859–904.
Semova, I., Carten, J.D., Stombaugh, J., Mackey, L.C., Knight, R., Farber, S.A., Rawls, J. F., 2012. Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. Cell Host Microbe 12, 277–288.
Velagapudi, V.R., Hezaveh, R., Reigstad, C.S., Gopalacharyulu, P., Yetukuri, L., Islam, S., Felin, J., Perkins, R., Boren, J., Oresic, M., Backhed, F., 2010. The gut microbiota modulates host energy and lipid metabolism in mice. J. Lipid Res. 51, 1101–1112.
Yasuda, S., Okahashi, N., Tsugawa, H., Ogata, Y., Ikeda, K., Suda, W., Arai, H., Hattori, M., Arita, M., 2020. Elucidation of gut microbiota-associated lipids using LC-MS/MS and 16S rRNA sequence analyses. iScience 23, 101841.
Zemski Berry, K.A., Murphy, R.C., 2004. Electrospray ionization tandem mass spectrometry of glycerophosphoethanolamine plasmalogen phospholipids. J. Am. Soc. Mass Spectrom. 15, 1499–1508.