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Formation & diplômes

- 2014** **Baccalauréat Scientifique**, option Physique-Chimie.
Lycée Jean Moulin, Saint-Amand Montrond (18).
- 2014-2017** **Licence en Sciences de la vie, « Biologie Cellulaire et Physiologie »**
Université Clermont Auvergne, Clermont-Ferrand (63).
- 2017-2019** **Master Biologie-Santé, parcours « Génétique, Physiologie et Pathologies », Mention Assez bien**
Université Clermont Auvergne, Clermont-Ferrand (63).
- 2019-2023** **Doctorat d'université, spécialité « biochimie, biologie cellulaire & moléculaire & physiologie »**
Ecole doctorale « Environnements – Santé », Université de Bourgogne Franche-Comté (21).

Parcours professionnel de recherche

- 2018** **Stage de Master 1 Recherche (2 mois)**
Oxford Expression Technologies, Oxford Brookes University, Oxford, Royaume-Uni.
Sujet : Mise au point d'un test diagnostic rapide de la fièvre hémorragique de Crimée-Congo.
Encadrant : Robert Possee
- 2019** **Stage de Master 2 Recherche (6 mois)**
Equipe « Proteostasis », Unité de Nutrition Humaine, Centre INRAE de Theix.
Sujet : Impact des polyphénols sur la voie de signalisation GCN2/eif2 α /ATF4.
Encadrant : Dr. Julien Averous
- 2023** **Doctorat d'Université « biochimie, biologie cellulaire & physiologie » (3 ans et 10 mois)**
Equipe « Œil, nutrition et signalisation cellulaire », Centre INRAE de Dijon.
Sujet : Impact de facteurs alimentaires sur la physiologie des tissus nerveux : focus sur les lipides cérébraux et rétiens et le mécanisme d'autophagie
Encadrant : Dr. Marie-Agnes Bringer
- 2024** **Chercheur postdoctoral en biologie cellulaire, moléculaire & virologie (CDD 36 mois)**
Équipe « Interactions hôte-virus » Institut Cochin, Paris
Sujet : Étude de la contribution des protéines relatives à l'autophagie et des lipides sur les particules virales du VIH-1.
Encadrant : Dr. Clarisse Berlioz-Torrent

Productions & communications scientifiques

Productions scientifiques :

- 1- **Bizeau JB**, Albouery M, Grégoire S, Buteau B, Martine L, Crépin M, Bron AM, Berdeaux O, Acar N, Chassaing B, Bringer MA. Dietary Inulin Supplementation Affects Specific Plasmalogen Species in the Brain. *Nutrients*. 2022. PMID : 35956273.

- 2- Plasmalogens Regulate Retinal Connexin 43 Expression and Müller Glial Cells Gap Junction Intercellular Communication and Migration. Karadayi R, Mazzocco J, Leclerc L, Buteau B, Gregoire S, Belloir C, Koudsi M, Bessard P, **Bizeau JB**, Dubus E, Fenech C, Briand L, Bretillon L, Bron AM, Fioramonti X, Acar N. Front Cell Dev Biol. 2022.PMID : 35433704.
- 3- Reciprocal interactions between gut microbiota and autophagy. Lapaquette P, **Bizeau JB**, Acar N, Bringer MA. World J Gastroenterol. 2021 27(48):8283-8301. PMID : 35068870.
- 4- Modulation of the brain lipid content after long-term exposure to diets varying in their ratio and content in omega-6 and omega-3 polyunsaturated fatty acids in mice. **Bizeau JB**, Martine L, Peltier C, Grégoire S, Buteau B, Bron AM, Acar N and Marie-Agnès Bringer (*en préparation*)
- 5- Impact of dietary omega-6 and omega-3 polyunsaturated fatty acids on retinal autophagy during aging in mice. **Bizeau JB**, Buteau B, Martine L, Dubus E, Leclère L, Lapaquette P, Peltier C, Bron AM, Acar N and Bringer MA (*en préparation*)

Prix de thèse :

Mai 2022 Lauréat du **prix coup de cœur 2022** décerné par le jury de la Fondation Roquette pour la Santé.
Prévention en santé : quels enjeux et quelles approches autour de l'alimentation et de la nutrition ?

Communications orales :

- ❖ Impact of dietary fatty acids on autophagy in the retina during aging. **Bizeau JB**, Buteau B, Martine L, Grégoire S, Leclère L, Dubus E, Bron AM, Acar N and Bringer MA.
- Forum des jeunes chercheurs. Ecole doctorale « Environnements-Santé », Dijon, 23-24 juin 2021.
- Journée des doctorants. UMR CSGA. Dijon, 25 juin 2021 et 24 juin 2022.
- ❖ Impact of dietary fatty acids on autophagy in the retina. **Bizeau JB**, Buteau B, Martine L, Grégoire S, Leclère L, Dubus E, Bron AM, Acar N and Bringer MA.
- 10^{ème} Congrès du Club Francophone de l'Autophagie (CFATG10). Besançon – 31 au 02 juin 2022.
- ❖ Impact of different omega-6/omega-3 ratio precursors on autophagy in the retina. **Bizeau JB**, Buteau B, Martine L, Grégoire S, Leclère L, Dubus E, Bron AM, Acar N and Bringer MA.
- Semaine du cerveau. Dijon, 15 mars 2023
- Journée de Labex LIpSTIC. Dijon, 1 et 2 juin 2023

Communication affichée :

- ❖ Impact of dietary fatty acids on autophagy in the retina. **Bizeau JB**, Buteau B, Martine L, Grégoire S, Leclère L, Dubus E, Bron AM, Acar N and Bringer MA.
- 10^{ème} Congrès du Club Francophone de l'Autophagie (CFATG10). Besançon – 31 au 02 juin 2022.
- Journées Chevreul 2023 (80 ans de la SFEL). Paris – 18 au 20 janvier 2023

Encadrement

2021 Paul Dumas, DUT « Génie biologique », option « Analyses biologiques et biochimiques ». Université de Bourgogne-Franche-Comté, Dijon. Stage de 2^{ème} année (9 semaines).
Sujet : Impact des lipides sur l'autophagie rétinienne.

- 2022** Katia Ihadadene, Master 1 parcours « Biologie cellulaire et physiologie animale ». Université de Bourgogne-Franche-Comté, Dijon (8 semaines).
Sujet : **Impact des acides gras alimentaires sur l'autophagie des tissus nerveux.**
- Youssra Laïka Comtois, DUT « Génie biologique », option « Analyses biologiques et biochimiques ». Université de Bourgogne-Franche-Comté, Dijon. Stage de 2^{ème} année (9 semaines).
Sujet : **Impact des acides gras alimentaires sur l'autophagie des tissus nerveux.**
- 2023** Samuel Cegarra, BUT « Génie biologique », option « Analyses biologiques et biochimiques ». Université de Bourgogne-Franche-Comté, Dijon. Stage de 2^{ème} année (8 semaines).
Sujet : **Impact d'un régime riche en graisse sur l'autophagie rétinienne**

Enseignement

- 2019-2020** Vacataire à l'Université de Bourgogne :
 - UE « Biologie Cellulaire et physiologie membranaire » : 19h TP (Licence 1 Biologie)
 - UE « Biologie animale » : 24h TP (Licence 1 Biologie)
 - UE « Physiologie nerveuse animale » : 12h TP (Licence 2 Biologie)
- 2020-2021** Activités complémentaires d'enseignements à l'Université de Bourgogne :
 - UE « Physiologie nerveuse animale » : 64h TP (Licence 2 Biologie)

Autres compétences & activités

Scientifique :

- Membre du « CFATG » (<http://cfatg.org/>).
- Membre du comité d'organisation du 10^{ème} congrès du CFATG (Besançon, 31 mai-02 juin 2022).
- Représentant des doctorants à l'évaluation HCERES du CSGA en janvier 2023.
- ❖ Obtention du diplôme d'expérimentation animale (2018) sur les rongeurs et les lagomorphes de niveau 1 durant la 1^{ère} année de Master 1 - Université Clermont Auvergne, Clermont-Ferrand (63).
- ❖ Analyses moléculaires (RT-qPCR), biochimiques (Western blot, ELISA), lipidiques (Extraction et analyse en chromatographie phase gazeuse, couche mince, LC-MS), cellulaire (expérimentation *ex-vivo*, cytométrie en flux)
- ❖ Physiologie animale (entretien de lignées transgénique, génotypage)
- ❖ Culture cellulaire (entretien de lignée, transfection, transduction).
- ❖ Virologie (production virale, infection)
- ❖ Immuno marquage et microscopie confocale.
- ❖ Informatique (logiciels GraphPad, Las X, ImageLab et ImageJ).

Langues étrangères :

- ❖ Anglais : B2/C1
- ❖ Espagnol : A2

Article

Dietary Inulin Supplementation Affects Specific Plasmalogen Species in the Brain

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Abstract: Plasmalogens (PIs) are glycerophospholipids that play critical roles in the brain. Evidence supports the role of diet and that of the gut microbiota in regulating brain lipids. We investigated the impact of dietary intake of inulin—a soluble fiber used as prebiotic—on the PI content of the cortex in mice. No global modification in the PI amounts was observed when evaluated by gas chromatographic analysis of dimethyl acetals (DMAs). However, the analysis of individual molecular species of PIs by liquid chromatography revealed a reduced abundance of major species of ethanolamine PIs (PIsEtn)—PE(P-18:0/22:6) and PE(P-34:1)—in the cortex of mice fed a diet supplemented with inulin. DMA and expression levels of genes (*Far-1*, *Gnpat*, *Agps*, *Pla2g6* and *Tmem86b*) encoding key enzymes of PI biosynthesis or degradation were not altered in the liver and in the cortex of mice exposed to inulin. In addition, the fatty acid profile and the amount of lyso forms derived from PIsEtn were not modified in the cortex by inulin consumption. To conclude, inulin affects the brain levels of major PIsEtn and further investigation is needed to determine the exact molecular mechanisms involved.

Keywords: dietary fibers; inulin; lipid; glycerophospholipid; plasmalogen; fatty acid; docosahexaenoic acid; brain; cortex; liver



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1. Introduction

The brain is the second-richest organ in terms of lipid content after adipose tissue. Lipids account for about half of the dry weight of the brain and are essential components in the structure and function of this organ [1–3]. The crucial role of lipids in maintaining the health status of the brain is well illustrated by the existence of neurological disorders (e.g., mood disorder, bipolar disorders and schizophrenia) and neurodegenerative diseases (e.g., Alzheimer's disease (AD) and Parkinson's disease (PD)) that are associated with alterations in lipid homeostasis in the brain [1,2]. In addition to displaying a high lipid content, the brain is also characterized by a high lipid diversity, which relies mainly on fatty acids [4,5]. In the brain, phospholipids are the reservoirs of fatty acids, and particularly of arachidonic acid (ARA, C20:4*n*-6) and docosahexaenoic acid (DHA, C22:6*n*-3), which are polyunsaturated fatty acids (PUFAs) involved in the regulation of the structure and functions of brain cells [6,7]. Indeed, in addition to serving as an energy source, fatty acids

also act as structural components of the cell membrane, and their derivatives are involved in cell signaling processes [6].

One of the specific features of brain phospholipid composition is its enrichment in a unique subclass of glycerophospholipids termed “plasmalogens” (Pls) [8]. Pls are characterized by a vinyl ether bond linking a long-chain fatty alcohol to the glycerol backbone in the *sn*-1 position of glycerol instead of an ester bond as found in other glycerophospholipids. The fatty alcohols in Pls are C16:0, C18:0 and C18:1 (*n*-7 or *n*-9). The fatty acid esterified at the *sn*-2 position of glycerol is predominantly ARA or DHA. In the brain, the polar head at the *sn*-3 position of the glycerol molecule is mainly ethanolamine. The brain has the highest content of ethanolamine Pls (or plasmenyl-ethanolamine, PlsEtn), which account for more than half of the total ethanolamine phospholipids in this tissue [8]. Pls can be derived from dietary intake and/or can be endogenously synthesized in tissues [9–11]. Moreover, some studies suggest that the liver might provide Pls for other tissues, but this concept remains controversial. Indeed, the amount of Pls and the level of activity of enzymes involved in their biosynthesis were found to be very low in the liver [8,12]. Pls are carried into the blood via chaperone proteins, low-density lipoprotein (LDL) being the major carrier. Pls are thereafter delivered to tissues through the LDL receptor pathway [13]. Studies suggest that Pls and their precursors might cross the blood–brain barrier, but incorporation of dietary Pls or their precursors in the brain does not seem to be as efficient when compared to peripheral organs [14–16]. The endogenous synthesis of Pls in the brain is thought to be the main source of brain Pls but this is still under debate [12,17]. The biosynthesis of Pls starts in peroxisomes and ends in the endoplasmic reticulum (Figure 1). Critical steps in Pl biosynthesis include the reduction of fatty acid to fatty alcohol by fatty acyl-CoA reductase 1 (encoded by *Far1*), which is an enzyme located in the outer surface of the peroxisomal membrane, and by the two peroxisomal enzymes DHAP-AT/DAP-AT (dihydroxyacetone phosphate acyltransferase, encoded by *Gnpat*) and alkyl-DHAP synthase (alkylglycerone-phosphate synthase, encoded by *Agps*) [12].

Several functions and properties have been attributed to Pls. The high susceptibility of the vinyl ether bond to oxidative damage has been described as a property of Pls that may protect other lipids in cell membranes and lipoproteins against oxidative stress. However, this hypothesis is controversial [18]. Pls also influence the physical and chemical properties of biomembranes (e.g., fluidity, thickness and lateral pressure) and thereby cellular and subcellular processes such as vesicle formation and membrane fusion events [17]. The enrichment of “lipid rafts” with Pls may also affect the initiation of signal transduction in membranes [19]. Pls are involved in the composition of glycosyl-phosphatidyl-inositol anchors, a post-translational modification of membrane proteins [20]. In addition, they constitute reservoirs of biologically active lipid mediators that are produced subsequently to the release of ARA and DHA by phospholipase A2 hydrolysis [21,22]. Lysoplasmalogens that are generated following the fatty acyl cleavage from Pls may also have biological functions, both as precursors of Pls and as metabolites [21]. Pls have been identified as a major structural component of the brain, and particularly of myelin and synaptic membranes. They also modulate processes that are important for maintaining brain homeostasis and functions such as neurotransmission, oxidative stress and neuroinflammation. The important role of Pls in brain physiology is highlighted by the association of neurological diseases/disorders with an abnormal composition or with abnormal levels of Pls or of enzymes involved in their biosynthesis [16]. In addition, beneficial effects of supplementation with Pls/Pl precursors on brain functions have been reported, particularly in the context of AD [11,23].

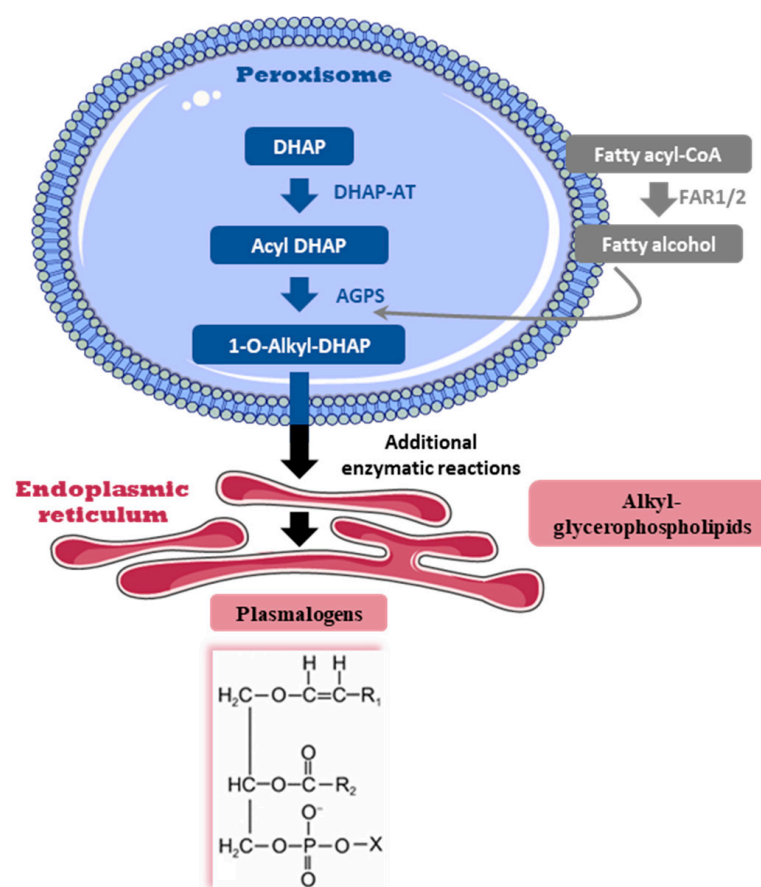


Figure 1. Schematic representation of plasmalogen biosynthesis. The biosynthesis of plasmalogens (Pls) is initiated in the peroxisome with three critical steps catalyzed by the enzymes FAR1 (fatty acyl-CoA reductase 1), DHAP-AT (dihydroxyacetone phosphate acyltransferase) and alkyl-DHAP synthase (alkylglycerone-phosphate synthase, AGPS). The biosynthesis of Pls is then continued in the endoplasmic reticulum by additional enzymatic reactions leading to the synthesis of alkyl-glycerophospholipid intermediates. The chemical structure of Pls is presented here. R1 denotes the carbon chain at the *sn*-1 position, and R2 at the *sn*-2 position. The polar head group, denoted by X, is most commonly choline or ethanolamine. acyl-CoA, acyl coenzyme A.

It is well documented that the gut microbiota influences the physiology of organs at distance from the gut mucosa, including the nervous tissues [24,25]. In particular, there is evidence that the gut microbiota modulates the lipid composition of both the brain and the retina—the neurosensorial tissue that lines the back of the eye and that is known to be an extension of the central nervous system. Indeed, analysis of the retinal lipidome of germ-free mice and conventionally raised mice showed that the gut microbiota influences the PlsEtn content of the retina [26]. In addition, comparison of the lipid profile of germ-free mice colonized with the gut microbiota of young or old donor mice revealed that the composition of the gut microbiota affects the cholesterol and phospholipid content of the cortex, including phosphatidylcholine (PChol), phosphatidylethanolamine (PEtn) and PlsEtn species [27].

Diet and the gut microbiota are intrinsically linked [28]. Among dietary factors shaping the gut microbiota and influencing its functions is the consumption of dietary fibers [29]. Dietary fibers can be categorized according to their water solubility. Whereas insoluble fibers (e.g., cellulose or hemicellulose) are poorly digested in the colon by the gut microbiota, soluble fibers (e.g., inulin-type fructans) can be fermented by gut bacteria. The fermentation of soluble fibers by bacteria generates metabolites (e.g., short-chain fatty acids (SCFAs)) that can have biological effects on the host, including effects on lipid metabolism [30]. A lack of fibers has been shown to alter the composition, diversity and richness of the gut

microbiota [31–33]. Soluble dietary fibers may influence the gut microbial ecosystem in several ways. The consumption of soluble dietary fibers favors not only the expansion of gut bacteria that are enzymatically equipped to degrade these substrates, but also that of gut bacteria that will take advantage of the physicochemical changes associated with the presence of fibers (e.g., acid environment) and/or benefit from the intermediate products or metabolites arising from the fiber degradation. The influence of inulin on the gut microbiota is particularly well documented. Data obtained from mouse models as well as from studies of humans showed that inulin consumption is associated with the expansion of bacteria that are described as conferring health benefits and with a reduction in pathobionts [34–37]. Modulation of the host lipid metabolism is also associated with inulin consumption. Indeed, effects of inulin on triglyceride and cholesterol blood levels have been reported, but these findings are still controversial [38–40]. In addition, we recently showed that supplementation of a low- or high-fat diet with inulin affects the fatty acid content of mouse liver [34]. Although no direct causal relationship has been established, some inulin-induced changes in the gut microbiota were correlated with modification of the expression of genes encoding enzymes involved in fatty acid biosynthesis [34]. The aim of this study was to investigate whether dietary intake of inulin affects the PI content of the brain. To this end, mice were exposed to a diet supplemented with either cellulose or inulin. The abundance and the diversity of PIs were explored in the liver and the cortex of mice through gas and liquid chromatographic techniques. The expression levels of the key enzymes involved in PI biosynthesis and cleavage/degradation were also determined.

2. Materials and Methods

2.1. Mice and Diets

For this study, 5-week-old male C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed at Georgia State University, Atlanta, GA, USA until euthanasia under institutionally approved protocols (Institutional Animal Care and Use Committee IACUC #A18006). Mice were maintained on 12 h light:dark cycles with ad libitum access to food and water. After 1 week of acclimation, mice were randomly divided into two groups: a control group (CTRL; $n = 12$) received a purified diet supplemented with 50 g cellulose/kg (Research Diet; #D12450J) and an inulin group (INU; $n = 11$) received a purified diet supplemented with 200 g inulin/kg (Research Diet; #D13081108) [34]. Cellulose as a source of fiber is generally poorly fermented by the gut. The diet containing cellulose served as a control. The source of inulin was chicory (average degree of polymerization ≥ 23 ; Orafiti[®] HP; BENE0-Orafiti, Tienen, Belgium). Mice were maintained on these respective diets for 11 weeks. Blood was collected by retrobulbar venous plexus puncture in heparinized tubes and plasma was isolated after centrifugation ($1800 \times g$, 10 min, 4 °C). They were then euthanized by cervical dislocation and the cortex and liver were collected.

2.2. Lipid Extraction and Determination of Fatty Methyl Ester and Dimethyl Acetal Profiles

Total lipids from cortex, plasma and livers were extracted using Folch's procedure [41]. Boron trifluoride in methanol was used for transmethylation [42]. Hexane was used to extract fatty acid methyl esters (FAMES) and dimethyl acetals (DMAs). Analyses were performed on a GC Trace 1310 (Thermo Scientific, Illkirch, France) gas chromatograph (GC) using a CPSIL-88 column (100 m \times 0.25 mm inside diameter, film thickness 0.20 μ m; Agilent, CA, USA). This device was coupled to a flame ionization detector (FID). The configuration was: inlet pressure of hydrogen 210 kPa, oven temperature 60 °C for 5 min + 165 °C at 15 °C per min and upholding for 1 min, +225 °C at 2 °C per min and upholding at 225 °C for 17 min. The injector and the detector were maintained at 250 °C. Comparisons with commercial and synthetic standards enabled the identification of FAMES and DMAs. The ChromQuest 5.0 version 3.2.1 software (Thermo Scientific, Illkirch, France) was used to process the data.

2.3. Analysis of Phospholipid Molecular Species

The phosphorus content of the total lipid extract was determined according to the method developed by Bartlett and Lewis [43]. The total phospholipids were dried under a stream of nitrogen and diluted to the appropriate concentration of 500 µg/µL of phospholipids in chloroform/methanol (CHCl₃/CH₃OH) (1:1, *v/v*). Ten microliters of internal standard mixture containing PC(14:0/14:0) 320 µg/mL, PE(14:0/14:0) 160 µg/mL, PS(14:0/14:0) 80 µg/mL, PI(8:0/8:0) 100 µg/mL and SM(d18:1/12:0) 80 µg/mL were added into 200 µL of this phospholipid solution.

The process of identification and quantification of phospholipid species was performed on a Thermo UltiMate™ 3000 coupled to an Orbitrap Fusion™ Tribrid Mass Spectrometer equipped with an EASY-MAX NGTM Ion Source (H-ESI) (Thermo Scientific, Waltham, MA, USA).

Separation of phospholipid classes was achieved under hydrophilic interaction liquid chromatography (HILIC) conditions using a Kinetex HILIC 100 m × 2.1 mm, 1.7 µm column (Phenomenex, Sydney, Australia), with a flow of 0.5 mL/min. The mobile phase consisted of (A) acetonitrile/water (CH₃CN/H₂O) (96:4, *v/v*) containing 10 mM ammonium acetate and (B) CH₃CN/H₂O (50:50, *v/v*) containing 10 mM ammonium acetate. The chosen solvent-gradient system of the analytical pump was as follows: 0 min 100% A, 12 min 80% A, 18 min 50% A, 18.1–30 min 100% A. The injection volume was 10 µL and the column was maintained at 50 °C.

Phospholipid species were detected by high-resolution mass spectrometry (HRMS) analysis. H-ESI source parameters were optimized and set as follows: ion transfer tube temperature of 285 °C, vaporizer temperature of 370 °C, sheath gas flow rate of 35 au, sweep gas of 1 au, auxiliary gas flow rate of 25 au. Positive and negative ions were monitored alternatively by switching the polarity approach with a static spray voltage at 3500 V and 2800 V in positive and negative mode, respectively. Mass spectra in full scan mode were obtained using the Orbitrap mass analyzer with the normal mass range and a target resolution of 240,000 (full width at half maximum (FWHM) at *m/z* 200), in a mass-to-charge ratio *m/z* ranging from 200 to 1600 using a Quadrupole isolation in a normal mass range. All mass spectrometry (MS) data were recorded using a maximum injection time of 100 ms, automatic gain control (AGC) target (%) at 112.5, radio frequency lens (%) at 50 and one microscan. An intensity threshold filter of 1.103 counts was applied.

For tandem mass spectrometry (MS/MS) analyses, the data-dependent mode was used for the characterization of phospholipid species. Precursor isolation was performed in the Quadrupole analyzer with an isolation width of *m/z* 1.6. Higher-energy collisional dissociation was employed for the fragmentation of phospholipid species with an optimized stepped collision energy of 27%. The linear ion trap was used to acquire spectra for fragment ions in data-dependent mode. The AGC target was set to 2.104 with a maximum injection time of 50 ms. All MS and MS/MS data were acquired in the profile mode.

The Orbitrap Fusion was controlled by Xcalibur™ 4.1 software (Thermo Scientific, Waltham, MA, USA). Data of high accuracy and the information collected from fragmentation spectra, with the help of the LipidSearch™ 2.0 software (Thermo Scientific, Waltham, MA, USA) and the LIPID MAPS® database [44], were used for phospholipid species identification.

2.4. Gene Expression

Total RNA was extracted using TRIzol reagent (Fisher Scientific, Illkirch, France). Reverse transcription was performed with the PrimeScript RT reagent kit containing gDNA Eraser (Takara Bio Europe, Saint Germain-En-Laye, France) and using 500 ng of total RNA. Gene expression was determined by real-time polymerase chain reaction (PCR) using SYBR Green (Bio-Rad, Marnes-La-Coquette, France) and a CFX96 Real-Time PCR system (Bio-Rad, Marnes-La-Coquette, France). *Hprt* was used as the internal control for normalization. Fold induction was calculated with the delta-delta Ct (ddCt) method. Primer sequences are given in Table 1.

Table 1. Primer sequences.

Genes (ID)	Sense (5'-3')	Antisense (5'-3')
<i>Hprt</i> (15452)	CAGTCCCAGCGTCGTGATTA	TGGCCTCCCATCTCCTTCAT
<i>Far1</i> (67420)	GCTCGGAAGCATCTCAACAAG	GTGCTGGATGCTCGGAAGTAT
<i>Gnpat</i> (14712)	TCACCGCAGCTACATTGACT	GCAGCTCACTGACCACTCTC
<i>Agps</i> (228061)	GTGCAGGGTGACACAGACTT	CCATGGTGATGTGACAGGCT
<i>Pla2g6</i> (53357)	AAAGTCCCCTCAAGTGCCTG	ACAGTCCACGACCATCTTGC
<i>Tmem86b</i> (68255)	TGGGGTGCTGTGCTCTTTAC	CACTAGGCGGGCAAAAGGTA
<i>Cat</i> (12359)	CAACAGCTTCAGCGCACCAG	GGCCGGCAATGTTCTCACAC
<i>Gpx1</i> (14775)	GGAATGCCTTGCCAAACACCC	GTCGATGGTACGAAAGCGGC
<i>Nos2</i> (18126)	AGAGCCACAGTCCTCTTTGC	ACCACCAGCAGTAGTTGCTC
<i>Sod1</i> (20655)	GATGAAAGCGGTGTGCGTGC	TGGACGTGGAACCCATGCTG
<i>Cox-2</i> (19225)	TTGCATTCTTTGCCAGCAC	TTAAGTCCACTCCATGGCCC
<i>Sqstm1</i> (18412)	TAAAAGCTGGGCTCTCGGCG	CGTGAACGACGCCATAACCG

2.5. Statistical Analysis

Statistical analyses were performed using Prism 6 software (GraphPad Software Inc., San Diego, CA, USA). The non-parametric Mann–Whitney test was used to compare data from the two groups. All *p* values of less than 0.05 were considered statistically significant (* *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001, and **** *p* < 0.0001).

3. Results

3.1. Effect of Inulin on the Level of Total Pls in the Liver, in the Plasma and in the Cortex

The liver has been proposed as the primary organ of PI biosynthesis. However, in contrast to the brain whose PI content is very high, the hepatic level of Pls is very low due to a low storage rate and a high rate of export to other organs [8,12]. The amount of Pls in the liver and in the cortex was measured by GC-FID. *Acid-catalyzed* transmethylation of the aldehyde aliphatic groups from the *sn*-1 position of Pls resulted in the production of DMAs (DMA 16:0, DMA 18:0, DMA 18:1*n*-7 and DMA 18:1*n*-9) whose amounts could be determined concomitantly with FAMES by GC-FID. As expected, we observed that the amount of DMAs in the liver of control mice represented only 0.06% ± 0.005% of the total FAMES and DMAs (Figure 2a). Only one class of DMAs was detected: DMA 16:0 (Figure 2a). Supplementation of the diet with inulin did not modify the hepatic level of DMA 16:0 (Figure 2a).

The level of total Pls was also measured in the plasma. As for the liver, the mean level of DMAs in the plasma was low (0.80% ± 0.10% of total DMAs and FAMES in CTRL mice; Figure 2b). Inulin did not modify the total amount of DMAs in this transport fluid (Figure 2b). However, the analysis at the species level revealed that the relative abundance of the two DMA species detected in the plasma (DMA 16:0 and DMA 18:0) was modified by inulin consumption: the plasma level of DMA 16:0 was significantly decreased and that of DMA 18:0 significantly increased in the plasma of INU mice compared to CTRL mice (Figure 2c,d).

In the cortex, the amount of total DMAs represented 9.24% ± 0.11% of the total FAMES and DMAs (Figure 2e). Four DMA classes were detected (Figure 2f–i). Among DMAs, DMA 18:0 was the most widely represented (44.08% ± 0.51% of total DMAs in CTRL mice, Figure 2g), followed by DMA 16:0 (23.34% ± 0.26%, Figure 2f), DMA 18:1*n*-7 (17.02% ± 0.31%, Figure 2h), and DMA 18:1*n*-9 (15.55% ± 0.18%, Figure 2i). No effect of inulin was observed neither on the amount of total DMAs in the cortex nor on the amounts of individual subclasses of DMAs (Figure 2e–i).

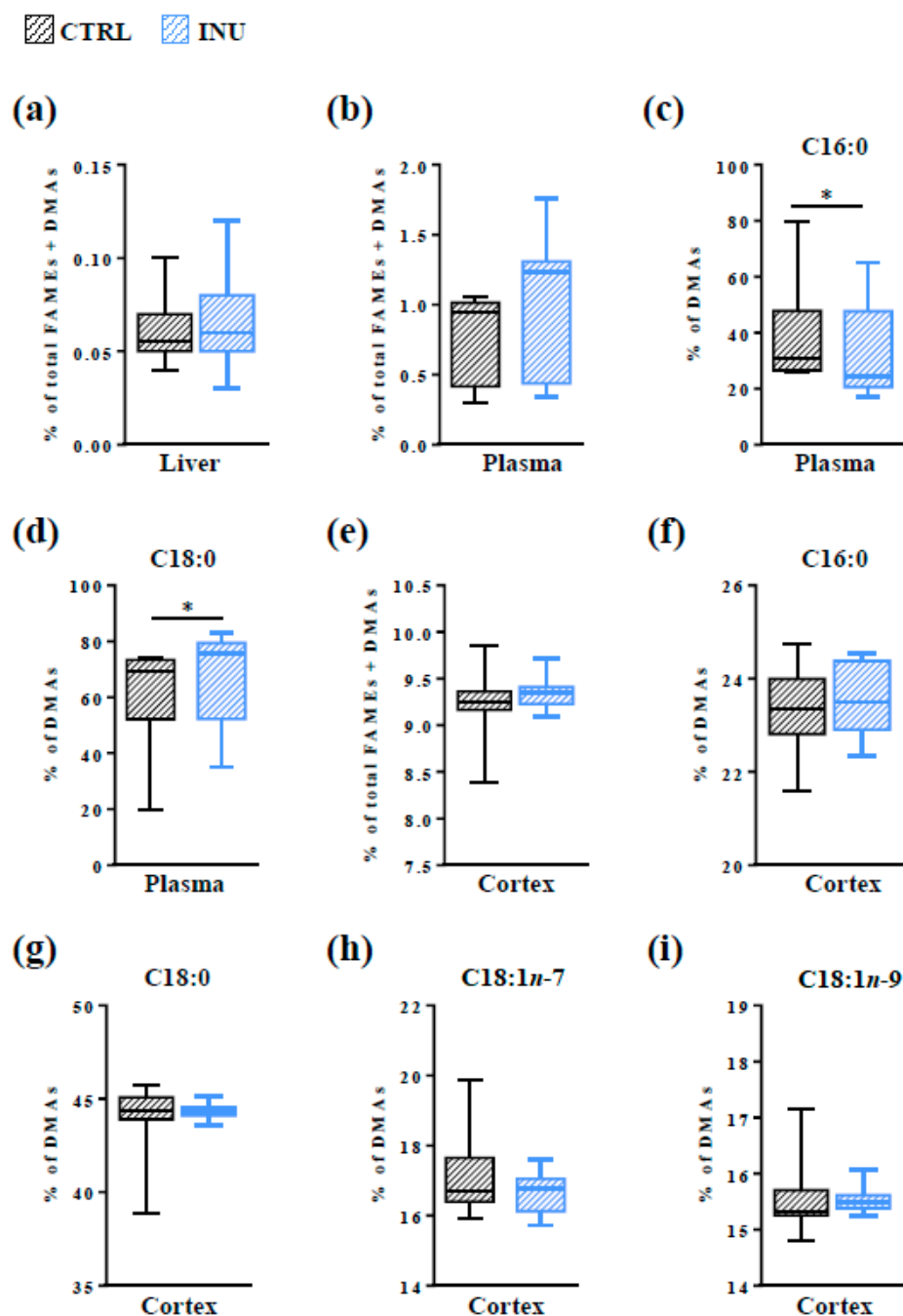


Figure 2. Evaluation of plasmalogen (PI) content in the liver, plasma and cortex by GC-FID. The results represent the quantification of dimethylacetals (DMAs, derivatives of aldehyde aliphatic groups from the *sn*-1 position of PIs) by GC-FID. (a,b,e) Results are expressed as percentages of total DMAs relative to total fatty acid methyl esters (FAMES) + total DMAs, defined as 100%, in the liver (a), in the plasma (b) and in the cortex (e). (c,d,f–i) percentages of (c,f) DMA 16:0, (d,g) DMA 18:0, (h) DMA 18:1n-7, and (i) DMA 18:1n-9 relative to total DMAs (defined as 100%), in the plasma (c,d) and in the cortex (f–i). CTRL: mice fed a control diet. INU: mice fed a diet supplemented with inulin. Data are presented in box and whisker plot format (median; min. to max.). Mann–Whitney test for comparison of lipid abundance between CTRL and INU mice, * $p < 0.05$. GC-FID, gas chromatography with flame-ionization detection.

These data indicate that, despite its effects on PI classes in the plasma, inulin had no impact on the total amount of PIs in the cortex.

3.2. Impact of Dietary Supplementation with Inulin on the Plasmalogen Content of the Cortex

3.2.1. Overview of Plasmalogen Species

In the cortex, PlsEtn are the most abundant Pls [8,45]. A total of 102 glycerophospholipid species were identified in the cortex of control mice by liquid chromatography-tandem mass spectrometry method (HPLC-MS²) analyses. Among them, five were alkyl-glycerophospholipids (AKGs), which are intermediate molecules in the biosynthesis of Pls, and 16 were alkenyl-glycerophospholipids, namely, Pls (Figure 1 and Table 2). As expected, the large majority (76.2%) of AKGs and Pls belonged to the ethanolamine subclass (Table 2). PlsEtn represented $46.155 \pm 1.303\%$ of the overall ethanolamine glycerophospholipid species. The three most abundant PlsEtn were PE(P-18:0/22:6), PE(P-16:0/22:6), and PE(P-18:0/20:4), which represented $10.857 \pm 0.530\%$, $5.189 \pm 0.299\%$, and $5.001 \pm 0.230\%$ of total ethanolamine glycerophospholipids in CTRL mice, respectively (Table 2).

Table 2. Relative amounts of alkyl-glycerophospholipid and plasmalogen species in the different classes of glycerophospholipids measured in mouse cerebral cortex.

Glycerophospholipids	Relative Abundance (%)
Ethanolamine glycerophospholipids	
<i>Alkyl-glycerophospholipids</i>	
PE(O-18:0/20:4)	0.619 ± 0.011
<i>Plasmalogens</i>	
PE(P-16:0/16:0)	0.178 ± 0.011
PE(P-16:0/18:2)	0.233 ± 0.010
PE(P-16:0/20:3)	0.365 ± 0.014
PE(P-16:0/20:4)	1.546 ± 0.071
PE(P-16:0/22:6)	5.189 ± 0.299
PE(P-18:0/16:0)	0.715 ± 0.021
PE(P-18:0/20:4)	5.001 ± 0.230
PE(P-18:0/22:4)	2.084 ± 0.126
PE(P-18:0/22:6)	10.857 ± 0.530
PE(P-18:1/18:1)	4.206 ± 0.172
PE(P-18:1/20:4)	3.240 ± 0.109
PE(P-18:1/22:4)	2.250 ± 0.117
PE(P-18:1/22:6)	2.757 ± 0.187
PE(P-16:0/18:1); PE(P-18:1/16:0) *	3.855 ± 0.147
PE(P-16:0/20:1); PE(P-18:0/18:1) *	3.681 ± 0.164
Total PlsEtn	46.155 ± 1.303
Choline glycerophospholipids	
<i>Alkyl-glycerophospholipids</i>	
PC(O-32:0)	0.134 ± 0.011
PC(O-34:1)	0.361 ± 0.013
PC(O-16:0/20:4)	0.108 ± 0.004
<i>Plasmalogens</i>	
PC(P-32:0)	0.125 ± 0.010
Inositol glycerophospholipids	
<i>Alkyl-glycerophospholipids</i>	
PI(O-16:0/20:4)	0.109 ± 0.004

For each glycerophospholipid class, results are expressed as abundance (in percentage) of each species relative to that of total species, defined as 100%. Data are expressed as mean \pm SEM. * based on ion precursor fragmentation information given for both molecular ions according to their fatty acids moiety position. SEM, standard error of the mean.

Among the other glycerophospholipids, we identified three AKG species in the choline (AKGChol) and one in the inositol subclasses (Table 2). Only one PI species, PC(P-32:0), was detected in the class of choline glycerophospholipids. This represented only $0.125 \pm 0.010\%$ of total choline glycerophospholipids (Table 2).

3.2.2. Impact of Dietary Inulin Supplementation on the Abundance of Plasmalogen Species in the Liver and the Cortex

For each species and each glycerophospholipid class, we compared the abundance of the individual species of AKGs and Pls in the cortex of INU mice relative to that of CTRL mice (Figure 3).

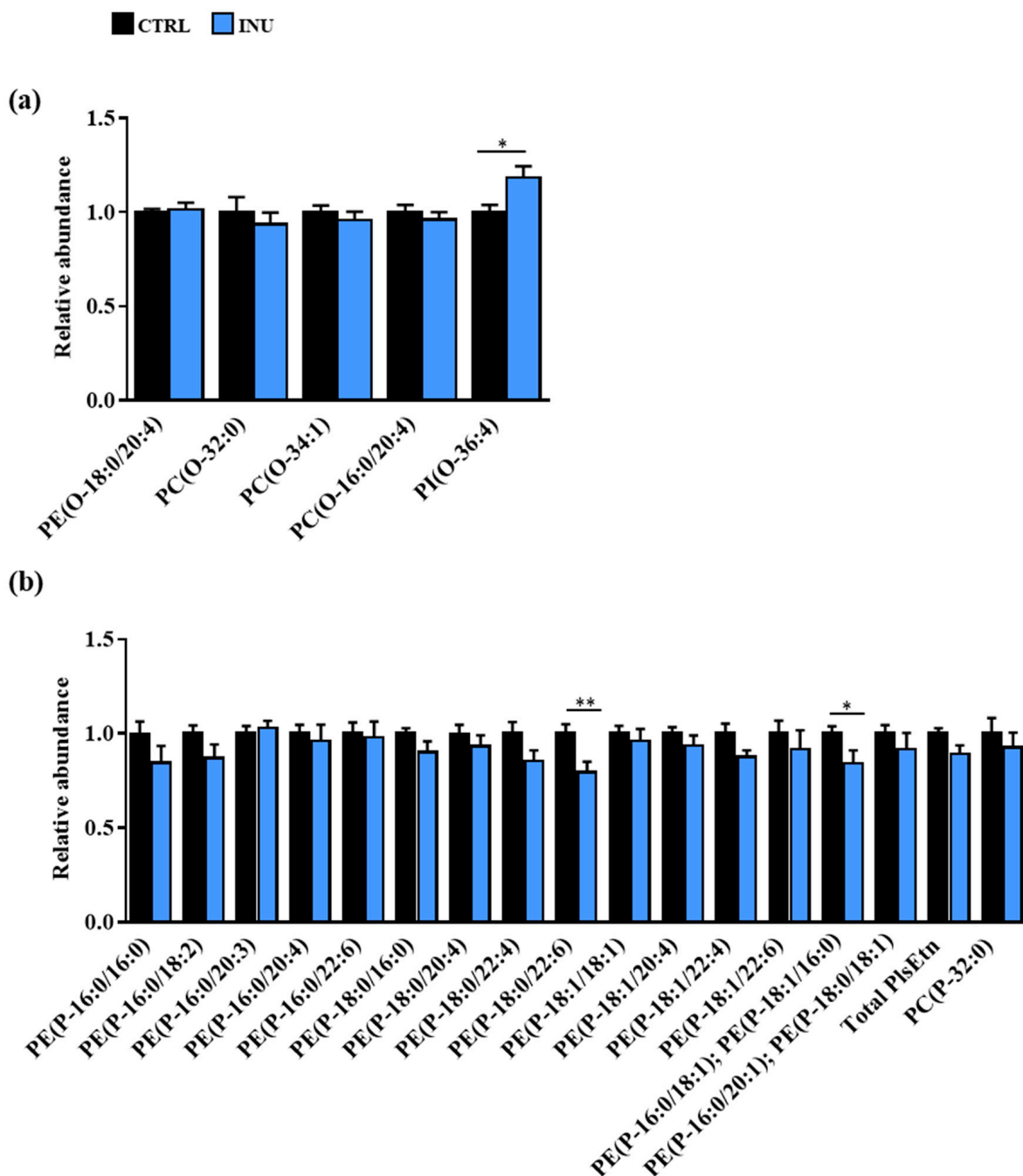


Figure 3. Changes in the abundance of AKG and PI species in the cortex of mice fed a diet supplemented with inulin. (a) AKGs and (b) Pls. Results are expressed as fold change in the cortex of INU mice relative to the mean level observed in the cortex of CTRL mice, defined as 1.0. All data are presented as mean \pm SEM. Mann–Whitney test for comparison of each AKG or PI species abundance between CTRL and INU mice, * $p < 0.05$, ** $p < 0.01$. AKG, alkyl-glycerophospholipid; PI, Plasmalogen; SEM, standard error of the mean.

Whereas the abundance of the AKGEtn (AKG species in the ethanolamine subclass) and AKGChol was unchanged, an $18.5\% \pm 6.0\%$ increase in the abundance of PI(O-16:0/20:4) was observed in the cortex of INU mice compared with CTRL mice (Figure 3a). In addition, among the 15 PlsEtn species identified, two were significantly decreased in the cortex of INU mice compared with CTRL mice (Figure 3b). Indeed, inulin supplementation was associated with a $20.5\% \pm 5.5\%$ decrease in PE(P-18:0/22:6), which is the most abundant PlsEtn species in the cortex, and with a $15.7\% \pm 6.8\%$ decrease in PE(P-34:1) [PE(P-16:0/18:1); PE(P-18:1/16:0)] (Figure 3b). The abundance of PlsChol was not modified by inulin (Figure 3b).

Altogether, these results show that supplementation of the diet with inulin modifies the abundance of specific AKG and individual PI species in the cortex.

3.3. Effect of Inulin on the Expression of Genes Encoding Enzymes Involved in Plasmalogen Biosynthesis

Fatty acyl-CoA reductase 1 (encoded by *Far1*), alkyl-DHAP synthase (encoded by *Agps*), and DHAP-AT/DAP-AT (encoded by *Gnpat*) are key enzymes involved in PI biosynthesis (Figure 1). As their level of expression could be a factor modulating the amount of PI, we compared the mRNA levels encoding these enzymes in the liver (Figure 4a) and in the cortex (Figure 4b) of INU and CTRL mice. As estimated by the comparison of the DeltaCt (ΔCt), the expression levels of *Far1*, *Agps* and *Gnpat* in CTRL mice were significantly lower in the liver than in the cortex (Appendix A Figure A1). Diet supplementation with inulin did not modulate gene expression in either organ (Appendix A Figures 4 and A1).

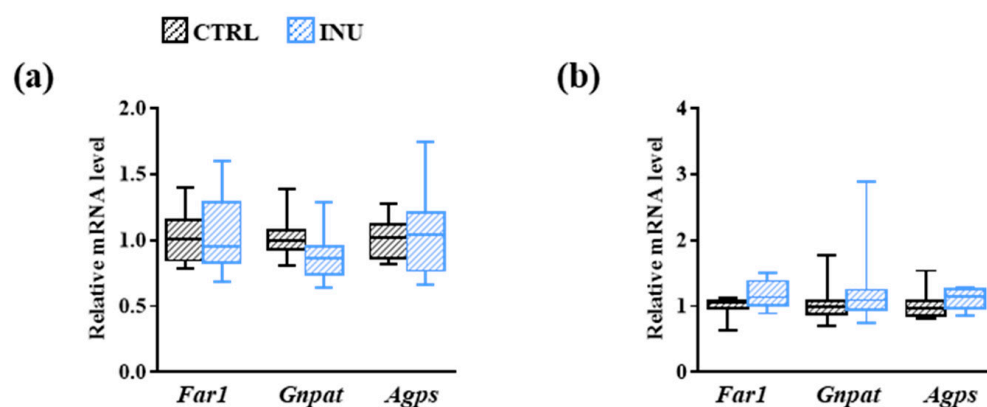


Figure 4. Effect of inulin on the expression of genes encoding enzymes involved in the biosynthesis of plasmalogens. Liver (a) and cortex (b) expression of genes encoding fatty acyl-CoA reductase 1 (*Far1*), DHAP-AT/DAP-AT (*Gnpat*), and alkyl-DHAP synthase (*Agps*) in mice fed a control diet or a diet supplemented with inulin. The levels of mRNA were normalized to *Hprt* mRNA level for calculation of the relative levels of transcripts. mRNA levels are illustrated as fold change. Data are presented in box and whisker plot format (median, min. to max.). Mann–Whitney test for comparison of the level of each mRNA between CTRL and INU mice.

3.4. Modulation of the Fatty Acid Content of the Cortex by the Dietary Intake of Inulin

Another limiting factor that could have affected the amounts of PlsEtn PE(P-18:0/22:6) and PE(P-34:1) [PE(P-16:0/18:1); PE(P-18:1/16:0)] in the cortex is the bioavailability of fatty acids entering the biosynthesis of these lipid species. Therefore, we analyzed the fatty acid composition of the cortex by GC-FID in INU mice compared with CTRL mice (Table 3). We observed that inulin supplementation has a weak effect on the saturated fatty acid (SFA) content of the cortex, since only the abundance of two minor SFAs (C15:0 and C17:0) was significantly modified (Table 3). Among monounsaturated fatty acids (MUFAs), a trend toward a decrease in the amount of total MUFAs of the *n*-7 series ($p = 0.0572$) and a significant decrease in the abundance of C16:1 n -7 were observed in the cortex of mice exposed to inulin compared to those fed a control diet (Table 3). However, the dietary intake of inulin modulated the abundance of several PUFAs in the cortex. The abundance

of C22:5 n -3, C18:2 n -6 and C20:3 n -6 was decreased whereas that of C22:5 n -6 and C20:3 n -9 was increased in the cortex of INU mice compared to CTRL mice (Table 3).

Table 3. Relative abundance of ester-linked fatty acids in the cortex.

Fatty Acids	CTRL	INU
Saturated fatty acids (SFAs)		
C14:0	0.143 ± 0.004	0.136 ± 0.004
C15:0 ***	0.044 ± 0.001	0.059 ± 0.003
C16:0	22.033 ± 0.153	22.014 ± 0.167
C17:0 ****	0.146 ± 0.002	0.181 ± 0.006
C18:0	21.454 ± 0.081	21.471 ± 0.059
C20:0	0.273 ± 0.009	0.274 ± 0.006
C22:0	0.183 ± 0.006	0.181 ± 0.009
C24:0	0.207 ± 0.008	0.219 ± 0.017
Total	44.482 ± 0.170	44.535 ± 0.159
Monounsaturated fatty acids (MUFAs)		
C16:1 n -7 **	0.735 ± 0.016	0.671 ± 0.022
C18:1 n -7	4.102 ± 0.042	4.038 ± 0.033
C20:1 n -7	0.384 ± 0.013	0.364 ± 0.011
C16:1 n -9	0.173 ± 0.002	0.167 ± 0.002
C18:1 n -9	17.532 ± 0.170	17.534 ± 0.119
C20:1 n -9	1.548 ± 0.057	1.574 ± 0.049
C22:1 n -9	0.152 ± 0.005	0.148 ± 0.005
C24:1 n -9	0.450 ± 0.019	0.454 ± 0.030
Total n -7 MUFAs ($p = 0.0572$)	5.221 ± 0.066	5.073 ± 0.056
Total n -9 MUFAs	19.854 ± 0.239	19.878 ± 0.188
Total MUFAs	25.076 ± 0.271	24.950 ± 0.216
Polyunsaturated fatty acids (PUFAs)		
C20:5 n -3	0.062 ± 0.002	0.061 ± 0.002
C22:5 n -3 *	0.168 ± 0.002	0.157 ± 0.003
C22:6 n -3	15.360 ± 0.172	15.328 ± 0.111
C18:2 n -6 **	0.660 ± 0.022	0.570 ± 0.014
C20:2 n -6	0.094 ± 0.005	0.088 ± 0.005
C20:3 n -6 ****	0.463 ± 0.005	0.411 ± 0.010
C20:4 n -6	10.598 ± 0.072	10.768 ± 0.074
C22:4 n -6	2.551 ± 0.024	2.590 ± 0.020
C22:5 n -6 *	0.303 ± 0.006	0.351 ± 0.023
C20:3 n -9 **	0.127 ± 0.003	0.141 ± 0.004
Total n -3 PUFAs	15.590 ± 0.171	15.546 ± 0.111
Total n -6 PUFAs	14.668 ± 0.083	14.778 ± 0.091
Total PUFAs	30.386 ± 0.249	30.466 ± 0.163
n -6 PUFAs/ n -3 PUFAs	0.942 ± 0.006	0.951 ± 0.008

The percentage of each fatty acid methyl ester (FAME) relative to that of total FAMES (100%) was determined. Data are expressed as mean ± SEM. Mann-Whitney test for comparison of the abundance of each fatty acid between control group (CTRL) and inulin group (INU) mice, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. SEM, standard error of the mean.

These results indicate that feeding mice an inulin-supplemented diet influences the fatty acid content of the cortex. However, alterations induced by inulin did not concern the fatty acids involved in the composition of PlsEtn PE(P-18:0/22:6) and PE(P-34:1) [PE(P-16:0/18:1); PE(P-18:1/16:0)], namely, C16:0, C18:0, C18:1 and C22:6 n -3.

3.5. Influence of Inulin on the Production of Lyso-Glycerophospholipids in the Cortex

A decrease in the amounts of glycerophospholipids can result from an enhanced production of metabolic intermediates termed “lyso-glycerophospholipids” that are generated by the release of the fatty acid esterified at the sn -2 position of the glycerol molecule following the action of the enzyme phospholipase A(2) encoded by the *Pla2g6* gene [46].

The vinyl-ether bond of lysoplasmalogens can then be cleaved by the enzyme lysoplasmalogenase encoded by the *Tmem86b* gene. As estimated by the analysis of the ΔC_t levels, the expression levels of these genes was significantly higher in the liver than in the cortex (Appendix A Figure A1). We observed no modification of the expression levels of *Pla2g6* and *Tmem86b* in liver and cortex of INU mice compared to CTRL mice (Figure 5).

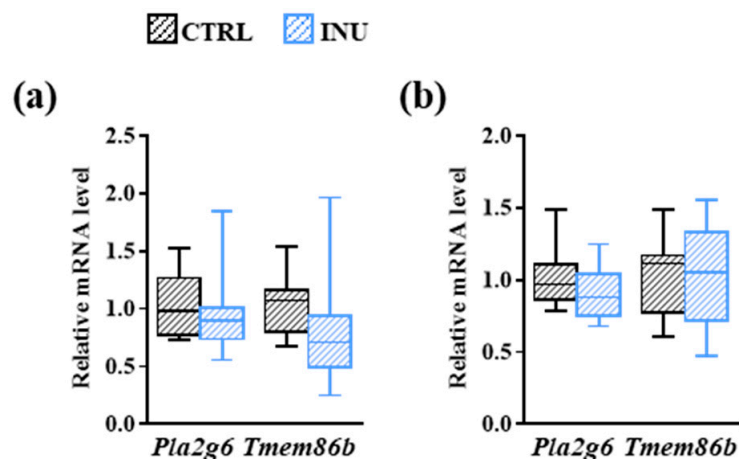


Figure 5. Effect of inulin on the expression of gene-encoding enzymes involved in the degradation of plasmalogens. Liver (a) and cortex (b) expression of genes encoding phospholipase A(2) (*Pla2g6*) and lysoplasmalogenase (*Tmem86b*) in mice fed a control diet or a diet supplemented with inulin. The levels of mRNA were normalized to *Hprt* mRNA level for calculation of the relative levels of transcripts. mRNA levels are illustrated as fold change. Data are presented in box and whisker plot format (median, min. to max.). Mann-Whitney test for comparison of the level of each mRNA between CTRL and INU mice.

Using HPLC-MS², we analyzed and compared the amounts of lyso-ethanolamine glycerophospholipids in the cortex of mice fed control or inulin-supplemented diet (Table 4). In total, 21 species of lyso-phosphatidylethanolamine (LPEs) species were identified but no lyso form of PlsEtn was detected (Table 4). No significant modification of the ratio of total LPEs/total ethanolamine glycerophospholipids was observed in the cortex of CTRL mice compared to that of INU mice (Table 4). In addition, we observed at the individual species level that the INU diet affected the abundance of LPE 14:0 (Table 4).

Altogether, these results suggest that dietary intake of inulin is not associated with an increase in LPEs in the cortex.

3.6. Influence of Inulin on Oxidative Stress-Related Mechanisms in the Cortex

As oxidative-stress-related molecules could cause PI degradation by attacking their vinyl-ether bond [47,48], we compared the expression level of a set of genes involved in oxidative stress-related mechanisms: *Cat* encoding catalase, *Gpx1* encoding for glutathione peroxidase 1, *Nos2* encoding for inducible NO synthase, *Sod1* encoding for superoxide dismutase (Cu-Zn), *Cox-2* encoding for cyclooxygenase-2 and *Sqstm1* encoding for sequestosome-1 (ubiquitin-binding protein p62). As presented in Figure 6, we did not observe any modification in the expression levels of these genes in the cortex of mice from the INU group compared to CTRL mice, suggesting that oxidative stress-related mechanisms were not modulated by inulin.

Table 4. Relative abundance of lyso-phosphatidylethanolamine species in the cortex.

	CTRL	INU
^a LPE 14:0 *	0.274 ± 0.053	0.259 ± 0.131
LPE 16:0 (<i>p</i> = 0.0576)	5.485 ± 0.553	4.438 ± 0.462
LPE 18:0	7.184 ± 1.712	5.552 ± 1.782
LPE 20:0	0.653 ± 0.044	0.522 ± 0.068
LPE 22:0	0.034 ± 0.004	0.032 ± 0.005
LPE 14:1	0.016 ± 0.004	0.026 ± 0.010
LPE 16:1	0.712 ± 0.051	0.652 ± 0.043
LPE 18:1	21.223 ± 0.722	21.005 ± 0.914
LPE 19:1	0.113 ± 0.009	0.121 ± 0.012
LPE 20:1	8.779 ± 0.396	8.129 ± 0.732
LPE 22:1	0.760 ± 0.046	0.695 ± 0.077
LPE 18:2	2.624 ± 0.502	2.463 ± 0.440
LPE 20:2	0.500 ± 0.049	0.513 ± 0.037
LPE 22:2	0.134 ± 0.011	0.127 ± 0.014
LPE 18:3	0.116 ± 0.013	0.136 ± 0.025
LPE 20:3	0.907 ± 0.078	0.938 ± 0.052
LPE 20:4	13.377 ± 0.520	14.498 ± 0.589
LPE 22:4	7.068 ± 0.830	8.058 ± 0.715
LPE 20:5	0.060 ± 0.008	0.059 ± 0.007
LPE 22:5	0.626 ± 0.045	0.750 ± 0.063
LPE 22:6	29.357 ± 1.145	31.032 ± 1.146
^b LPEs/PEs	0.807 ± 0.273	1.096 ± 0.487

^a The percentage of each lyso-phosphatidylethanolamine (LPE) species relative to that of total LPEs (100%) was determined. ^b Ratio of total LPEs/total ethanolamine glycerophospholipids (PEs, non-plasmalogens). Total LPEs and total PEs were calculated as the sum of the area under the peak of each species corrected with that of the internal control (PE(28:0)). Data are expressed as mean ± SEM. * *p* < 0.05. Mann–Whitney test for comparison of the abundance of LPE species or LPEs/PEs between CTRL and INU mice. SEM, standard error of the mean.

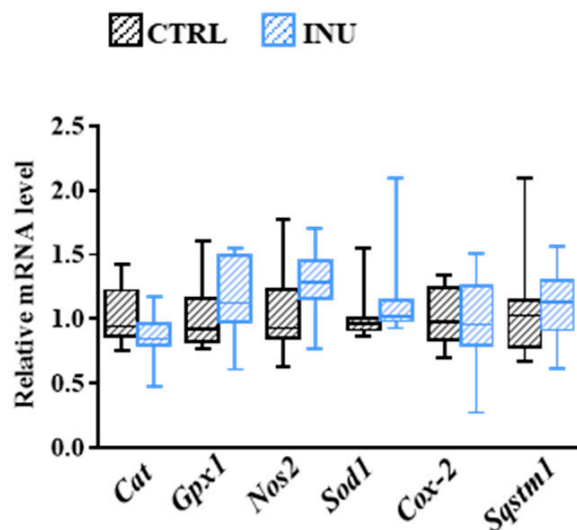


Figure 6. Effect of inulin on the cortex expression of gene-encoding proteins involved in oxidative stress-related mechanisms. *Cat* encodes catalase, *Gpx1* encodes glutathione peroxidase 1, *Nos2* encodes inducible nitric oxide (NO) synthase, *Sod1* encodes superoxide dismutase (Cu-Zn), *Cox-2* encodes cyclooxygenase-2, and *Sqstm1* encodes sequestosome-1 (ubiquitin-binding protein p62). The levels of mRNA were normalized to *Hprt* mRNA level for calculation of the relative levels of transcripts. mRNA levels are illustrated as fold change. Data are presented in box and whisker plot format (median; min. to max.). The Mann–Whitney test was used for comparison of the level of each mRNA between CTRL and INU mice.

4. Discussion

The brain is highly enriched in PIs, where they are essential in maintaining structure (e.g., myelination) and homeostasis (e.g., anti-oxidative properties, regulation of inflammation) as well as the functioning of specific processes (e.g., neurotransmission) [16]. It is now well recognized that the lipid composition of the brain is modulated by the lipid composition of the diet [49–53]. Thanks to the efforts made over the past decades to understand the link between diet, gut microbiota, and host metabolism, it has become evident that diet modulates not only host lipids by bringing lipids and their precursors to the host but also by acting through the gut microbiota [54]. A body of evidence indicates that the gut microbiota influences different aspects of host lipid metabolism as well as the lipid composition of organs, including that of the brain [27,55–58]. In this study, we investigated the effect of inulin, a soluble dietary fiber with prebiotic properties, on the content and composition of PIs in the brain.

Determination of the DMA profile in the cortex of mice fed a control diet revealed that PIs represent approximately 9.3% of total fatty acids in this brain structure and that they are distributed into four classes (DMA 16:0, DMA 18:0, DMA 18:1 n -7 and DMA 18:1 n -9), with DMA 18:0 and DMA 16:0 being the most abundant. These results are in agreement with previous studies [59]. We found that the dietary supplementation with inulin did not alter the DMA content or the distribution of DMAs into the different classes, suggesting that dietary intake of this prebiotic does not affect the whole PI content of the cortex, or the distribution of PIs according to their *sn*-1 position.

In addition to the fatty alcohol moiety linked by a vinyl-ether bond at the *sn*-1 position (whose trans-methylation yields the DMA derivatives), the diversity of PI species is also ensured by the fatty acid esterified at the *sn*-2 position as well as by the polar head group at the *sn*-3 position of glycerol. Analysis by liquid chromatography coupled to MS/MS of the diversity of glycerophospholipids in the cortex of mice enabled the identification of five AKG species that are intermediate metabolites of PI synthesis, as well as 16 species of PIs. As reported in other studies, we observed that most of them (76.2%) were PlsEtn [8]. It has been shown in the context of PlsEtn deficiency that the level of PEtn is adjusted to keep the level of PlsEtn + PEtn constant [60]. In our study, no modification in the total amount of PlsEtn or PEtn was observed in the cortex of mice fed an inulin-supplemented diet compared to those fed a control diet. However, two PI species were affected by supplementation of the diet with inulin, namely, PE(P-34:1) [PE(P-16:0/18:1); PE(P-18:1/16:0)] and PE(P-18:0/22:6), the latter being the most abundant PI species of the cortex (10.9% of the PlsEtn). Their abundance was decreased in the cortex of mice fed a diet supplemented with inulin compared to those fed a control diet.

The inulin-dependent effect on PlsEtn could have deleterious effects on the brain tissue since PE(P-18:0/22:6) constitutes a major reservoir of C22:6 n -3 (DHA). Indeed, DHA and its derivatives are essential for the development and maintenance of brain structure and function [61]. Epidemiological studies also support a link between dietary intake of DHA and the development of brain diseases and disorders such as AD [61,62]. In addition, decreased amounts of PE(P-18:0/22:6) and PE(P-16:0/18:1) have been reported in the cerebrum of patients with AD [63]. Potential harmful effects of inulin have already been described. Dietary intake of inulin has been shown to aggravate colitis, exacerbate atherosclerosis, enhance hepatic inflammation and fibrosis, disturb hepatic and bile acid metabolism, and cause hepatocellular carcinoma in specific genetic contexts associated with dysbiosis [39,64–67]. In addition, we have recently shown that although inulin prevents some of the alterations in the hepatic fatty acid metabolism caused by chronic consumption of a high-fat diet (HFD), it also exacerbates others [34]. Indeed, inulin consumption prevented the HFD-induced increase in C16:1 n -9 and C20:3 n -6 as well as the HFD-induced modulation of expression and/or activity of enzymes involved in fatty acid biosynthesis (*Elovl2*, *Elovl5* and *FADS2*) in mouse liver. However, this dietary fiber also exacerbated the HFD-induced increase in the hepatic amount of C17:0.

To expand our understanding of the mechanisms underlying the inulin-dependent decrease in some PI species in the cortex, we explored several hypotheses. Since the liver has been proposed as the primary site of PI biosynthesis, we investigated whether alterations of PIs in the cortex could have a hepatic origin. To this end, we evaluated the DMA content of the liver as well as the expression level of genes encoding key enzymes involved in the initial three steps of PI biosynthesis (*Far1*, *Gnpat* and *Agps*) and compared them between mice fed a control diet and those fed an inulin-supplemented diet. Only DMA 16:0 was detected in the liver of mice at very low levels, which is consistent with previous studies [8]. No effect of inulin was observed, neither on the DMA content nor on the expression levels of *Far1*, *Gnpat* and *Agps* in the liver. This is in line with previous results showing no modification in the expression levels and activities of enzymes involved in the biosynthesis of fatty acids following inulin supplementation [34]. To go further in the exploration of a hepatic origin for the changes we observed in the PI content of the cortex, we analyzed the PIs in the plasma. Indeed, we previously showed that the inulin supplementation, as we provided in the diet of this study, induced changes in the composition of the gut microbiota [34], and changes in the composition of the gut microbiota following inulin consumption have been associated with serum PI levels [68]. No modification of the total DMA content was observed in the plasma of mice fed a diet supplemented with inulin. However, intra-class modifications were observed: the relative abundance of DMA 16:0 was decreased and counterbalanced by an increase in DMA 18:0. Whereas this result might account for the decrease in PE(P-34:1) [PE(P-16:0/18:1); PE(P-18:1/16:0)] in the cortex, it does not explain that in PE(P-18:0/22:6). Altogether, these data suggest that it is unlikely that the PI changes observed in the cortex have an extra-brain or hepatic origin.

Another hypothesis that could explain the decreased abundance of PlsEtn PE(P-18:0/22:6) and PE(P-34:1) [(PE(P-16:0/18:1); PE(P-18:1/16:0))] in the cortex of inulin-fed mice is a modulation of the endogenous biosynthesis of PIs. However, no modification in the expression levels of *Far-1*, *Gnpat* and *Agps* was observed in the cortex of mice fed an inulin-supplemented diet.

The bioavailability of the fatty acids in the cortex required for their biosynthesis was also analyzed. We observed no modification in the abundance of C16:0, C18:0, C18:1*n*-7, C18:1*n*-9 or C22:6*n*-3 in the cortex of mice fed an inulin-supplemented diet. However, the level of docosapentaenoic acid (DPA) from the *n*-3 series (C22:5*n*-3), which is an intermediate between eicosapentaenoic acid (EPA, C20:5*n*-3) and DHA (C22:6*n*-3), was decreased. Finally, as the decrease in the abundance of some PlsEtn could also be the consequence of their hydrolysis, the expression levels of enzymes involved in PI cleavage/degradation and the level of lyso species were evaluated. No modification of the expression level of *Pla2g6* and *Tmem86b* genes, encoding phospholipase A(2) and lysoplasmalogenase, respectively, was observed in mice fed an inulin-supplemented diet. Another cause of PI degradation could be an attack on the vinyl-ether bond by oxidative stress-related molecules [47,48]. To test this hypothesis, the level of oxidative stress as well as the amount of oxidized derivatives of PIs should be evaluated. However, our results showed that dietary intake of inulin did not modify the expression level of a set of genes involved in oxidative stress-related mechanisms (*Cox-2*, *Cat*, *Gpx1*, *Sod1*, *Nos2* and *Sqstm1*) in the cortex. In addition, no lyso form of PlsEtn was detected in the cortex of mice fed a control diet or an inulin-supplemented diet and no modification of the ratio of LPEs/PEs was observed in the cortex of mice exposed to inulin. Taken together, these data suggest that the dietary intake of inulin does not enhance glycerophospholipid hydrolysis. However, as intermediate products arising from PI degradation may only have a short-lived existence, further experiments such as assessment of phospholipase A(2) and lysoplasmalogenase activities are needed to rule out the existence of an impact of inulin consumption on PlsEtn degradation.

Finally, despite the use of compositionally controlled diets, we cannot exclude that the amount of fiber consumed by mice fed an inulin-supplemented diet was different to that of the control mice that received a cellulose-containing diet. Indeed, we have previously reported that inulin supplementation can slightly decrease food consumption, very likely

linked to the energy provided by fermentable fiber compared to non-fermentable fiber [32]. More importantly, the dose of inulin used in the current study is relatively high and cannot be transposed to human nutrition. Hence, future studies appear warranted to investigate the effect of lower doses of inulin on cortex PIs.

5. Conclusions

In this study, we showed that dietary supplementation with inulin do not modify the global amount of PIs in the cortex of mice but affects its content at the species level. In particular, dietary intake of this prebiotic induces a decrease in the abundance of the most widely represented PIsEtn species, PE(P-18:0/22:6), which represents a major reservoir of DHA, a fatty acid essential for brain development and function. This study joins others that suggest inulin may have deleterious effects. The consequences of these alterations on the physiology and the functioning of the brain, as well as the molecular mechanisms that link inulin/gut microbiota and PI levels in the brain, remain to be elucidated.

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Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Georgia State University (protocol number A18006 approved on 25 September 2017).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Appendix A

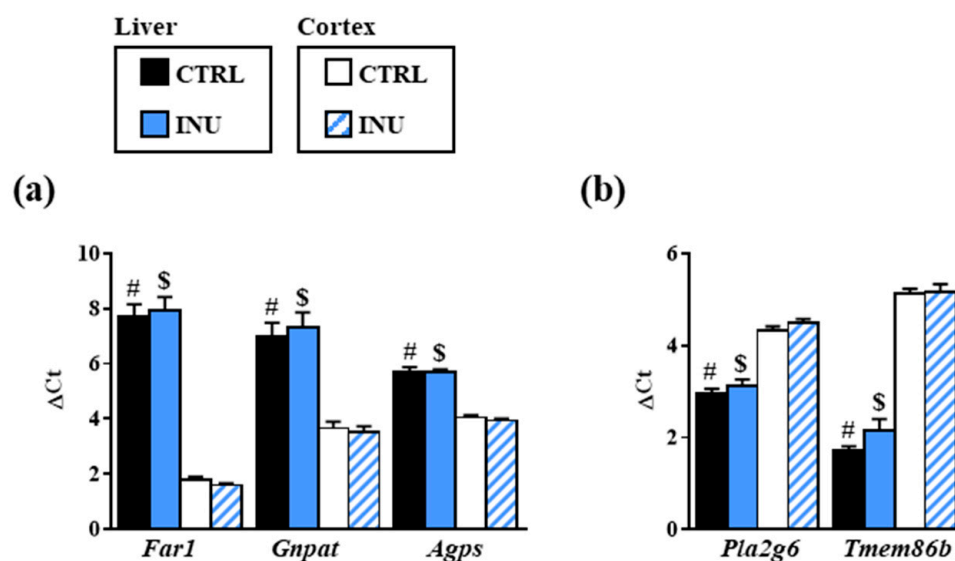


Figure A1. Expression levels of genes encoding enzymes involved in the biosynthesis (a) or in the degradation (b) of plasmalogens in liver and cortex of mice fed a control diet or a diet supplemented with inulin. (a) Expression levels of genes encoding fatty acyl-CoA reductase 1 (*Far1*), DHAP-AT/DAP-AT (*Gnpat*), and alkyl-DHAP synthase (*Agps*). (b) Expression levels of genes encoding phospholipase A(2) (*Pla2g6*) and lysoplasmalogenase (*Tmem86b*). DeltaCt (Δ Ct) are presented as mean \pm SEM. #, $p < 0.0001$, Mann–Whitney test for comparison between Δ Ct in liver and Δ Ct in cortex of control group (CTRL) mice. \$, $p < 0.0001$, Mann–Whitney test for comparison between Δ Ct in liver and Δ Ct in cortex of inulin group (INU) mice. acyl-CoA, acyl coenzyme A; SEM, standard error of the mean.

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Reciprocal interactions between gut microbiota and autophagy

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Abstract

A symbiotic relationship has set up between the gut microbiota and its host in the course of evolution, forming an interkingdom consortium. The gut offers a favorable ecological niche for microbial communities, with the whole body and external factors (e.g., diet or medications) contributing to modulating this microenvironment. Reciprocally, the gut microbiota is important for maintaining health by acting not only on the gut mucosa but also on other organs. However, failure in one or another of these two partners can lead to the breakdown in their symbiotic equilibrium and contribute to disease onset and/or progression. Several microbial and host processes are devoted to facing up the stress that could alter the symbiosis, ensuring the resilience of the ecosystem. Among these processes, autophagy is a host catabolic process integrating a wide range of stress in order to maintain cell survival and homeostasis. This cytoprotective mechanism, which is ubiquitous and operates at basal level in all tissues, can be rapidly down- or up-regulated at the transcriptional, post-transcriptional, or post-translational levels, to respond to various stress conditions. Because of its sensitivity to all, metabolic-, immune-, and microbial-derived stimuli, autophagy is at the crossroad of the dialogue between changes occurring in the gut microbiota and the host responses. In this review, we first delineate the modulation of host autophagy by the gut microbiota locally in the gut and in peripheral organs. Then, we describe the autophagy-related mechanisms affecting the gut microbiota. We conclude this review with the current challenges and an outlook toward the future interventions aiming at modulating host autophagy by targeting the gut microbiota.

Key Words: Gut microbiota; Autophagy; Probiotic; Brain; Liver; Muscle

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Core Tip: We are now aware that maintaining a fine equilibrium between the host and its gut microbiota is a prerequisite to maintain host homeostasis and promote long-term health. Several host and microbial processes interact dynamically to respond to external stresses. Among these processes, host autophagy acts as a cytoprotective mechanism responsive to a wide range of stress conditions, including metabolic, immune, and microbial stimuli. Autophagy was initially described as a degradative process active upon nutrient starvation. However, this process fulfils a wide range of other functions that are essential to host homeostasis. We discuss herein reciprocal interactions of autophagy with the gut microbiota in health and disease conditions.

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INTRODUCTION

The commensal microbiota living in the human gut is a unique ecosystem that has co-evolved with human to establish a symbiotic relationship. This microbial community is estimated to encompass about 10^{14} resident microorganisms, dominated by bacteria, but containing also populations of archaea, fungi, protozoa, and viruses[1]. The host provides nutrients and a favorable environment (*i.e.*, ecological niches) for its microbial inhabitants. In return, the gut microbiota plays multiple roles that contribute to the host whole-body homeostasis, in particular by metabolizing dietary nutrients, by preventing colonization by enteric pathogens, and by regulating the host immune system and metabolism. The gut microbiota is, for instance, essential for the synthesis of vitamins (*e.g.*, K and B-group vitamins) and the fermentation of dietary fibers and carbohydrates, which generate short-chain fatty acids (SCFAs). These fermentation products are used as energy source by organs and are also involved in the regulation of various cellular processes (*e.g.*, intestinal barrier integrity, mucus production, and inflammation)[2,3].

Through their interactions with the host, gut microbes and their derived products are involved not only in the physiological regulation of the gut mucosa but also in that of organs located at distance from the gut mucosa, as illustrated by the studies detailing molecular features of the gut-microbiota-brain axis[4-6]. Keeping the mutualistic relationship between the gut microbiota and the host throughout host's life is thus essential to maintain the health status of the host[7]. Deleterious shifts in the composition of the gut microbiota, called dysbiosis, can unbalance its functions, leading to the disruption of host homeostasis. This is particularly well illustrated by the ability of fecal microbiota transplantation (FMT) to transmit detrimental metabolic and/or pro-inflammatory traits from a sick donor to healthy recipient mice[8-10]. In addition to environmental stresses, the symbiotic equilibrium of the gut microbiota and the host can also be broken by dysfunctions/alterations in the host metabolism and immune system, which are conditions that can contribute to dysbiosis[8,11,12]. In this context, the roles of autophagy in strengthening the intestinal barrier and in maintaining host metabolic and inflammatory balance position it as the cornerstone of the symbiotic relationship between the gut microbiota and the host[4,13].

Macroautophagy/autophagy is an intracellular and multistep process starting with the formation of a membranous cup-shaped structure, called phagophore, which engulfs portions of the cytoplasm. The phagophore elongates and finally closes to form a sealed double-membraned vacuole, called autophagosome, whose maturation ends by its fusion with lysosomes[14-16]. Autophagy was initially described as a lysosomal catabolic process occurring under starvation that degrades and recycles cytoplasmic macromolecules (*e.g.*, proteins, lipids, and carbohydrates) for the biosynthesis of essential cellular components and to restore energy balance[17]. Nowadays, autophagy process and autophagy-related proteins are recognized as key cellular components whose roles are not restricted to the regulation of energy balance[18,19]. These roles include, but are not limited to, the regulation of the inflammatory response, the cytoprotection by preventing the accumulation of intracellular waste (*e.g.*, damaged organelles and misfolded or aggregated proteins), the protection against

intracellular pathogens (*e.g.*, bacteria, fungi, or viruses), the membrane dynamic (*e.g.*, transport or secretion), and the regulation of cell differentiation and survival. Autophagy also regulates specific functions related to the features of organs. For example, at the gut mucosa - the first tissue at the interface between the gut microbiota and the host - autophagy is involved in the regulation of the functions of the secretory cells and of the intestinal stem cell[4]. In the central nervous system, autophagy plays roles in neuronal development and survival and other various functions[20]. The central role of autophagy in maintaining homeostasis, and thus the health status, is supported by the observed embryonic or neonatal lethality of mice deficient for most autophagy-related (*Atg*) core genes (*Becn1*, *Vps34*, *Atg9a*, *Ulk1/2*, *Atg3*, *Atg5*, *Atg7*, and *Atg16l1*) as well as association of numerous diseases and disorders with autophagy defects[19,21].

Of note, a growing number of recent studies highlight that most of the proteins of the autophagy machinery also mediate autophagy-independent functions, including phagocytosis, exocytosis, cytokinesis, DNA repair, or innate and adaptive immune signaling[22]. To exert their numerous functions, the machineries involving autophagy proteins are intricately with molecular sensors specialized in the detection of various stimuli such as microbial sensors [*e.g.*, Toll-like receptors (TLR) and Nod-like receptors (NLR)], stress sensors (*e.g.*, HMGB1, Sestrins, ER-stress sensor proteins, P2XR, and cGAS-STING pathway), or energy status sensors (*e.g.*, AMPK and mTOR pathways) [23-29].

In this review, we summarize the current knowledge on how the gut microbiota influences host autophagy locally in the gut mucosa or remotely in peripheral organs (brain, heart, liver, or muscles), and how autophagy or autophagy-related proteins can reciprocally shape the gut microbiota composition and modify its functions (Figure 1). We finally discuss the potential of targeting the gut microbiota as a strategy to modulate autophagy or restore its functionality in pathological context.

INFLUENCE OF THE MICROBIOTA ON GUT AUTOPHAGY

A first clue that points out a direct implication of the gut microbiota in the regulation of host autophagy has been provided by analyzing autophagy in germ-free mice (*i.e.*, mice lacking microorganisms and bred in isolators without any microbial exposure). Basal autophagy was decreased in the colonic epithelium of germ-free mice compared to conventionally raised mice, suggesting that the gut microbiota influences intestinal autophagy in physiological condition[30]. The increase in basal activity of autophagy in germ-free mice was attributed to an energy-deprived status of colonocytes. Treatment of these cells with butyrate, a SCFA generated by some gut bacteria and serving as main energy source for colonocytes, was sufficient to reverse the phenotype. *In vivo*, colonization of germ-free mice with the butyrate-producing bacterial strain *Butyrivibrio fibrisolvens* was sufficient to restore autophagy steady state. In addition to butyrate, other bacteria-derived metabolites may have the ability to reduce basal autophagy in the colon. They include indole-3-lactate, which is a tryptophan metabolite produced notably by the bacteria belonging to the *Lactocaseibacillus*, *Lactobacillus*, *Bifidobacterium*, *Megamonas*, *Roseburia*, or *Ruminococcus* genus[31,32].

Pathogen-associated molecular patterns (PAMPs), which are conserved microbial molecules, are also able to modulate autophagy usually by stimulating the process [23]. These effects have been particularly well described for pathogens. PAMPs mainly act by interacting with specific host cell receptors that belong to the TLR and NLR families. This has been illustrated by the ability of the lipopolysaccharide (LPS) from Gram negative bacteria to stimulate autophagy through its binding to TLR4[33], or the peptidoglycan (PGN) from Gram positive bacteria through NOD1-, NOD2-, and TLR2-associated signaling[34,35]. Besides those of bacteria, fungal PAMPs can also mobilize components of the autophagy machinery. This is true for β -glucans that are found in fungal cell walls and stimulate autophagy-related processes through their binding to the host receptor Dectin-1[36,37]. Trehalose, a non-reducing disaccharide produced by bacteria and fungi, is also a potent autophagy inducer, for which the ability to stimulate colonic autophagy during colitis in mice has been described[38,39]. In addition, in-depth studies of the infectious cycle of some pathogenic bacteria have shed the light on the existence of secreted bacterial effectors able to activate (*e.g.*, Ats-1 protein from *Anaplasma phagocytophilum*) or inhibit (*e.g.*, RavZ protein from *Legionella pneumophila*) autophagy at various stages of the process[40,41]. It is not excluded that some commensal microorganisms in the gut express such proteins that influence host autophagy.

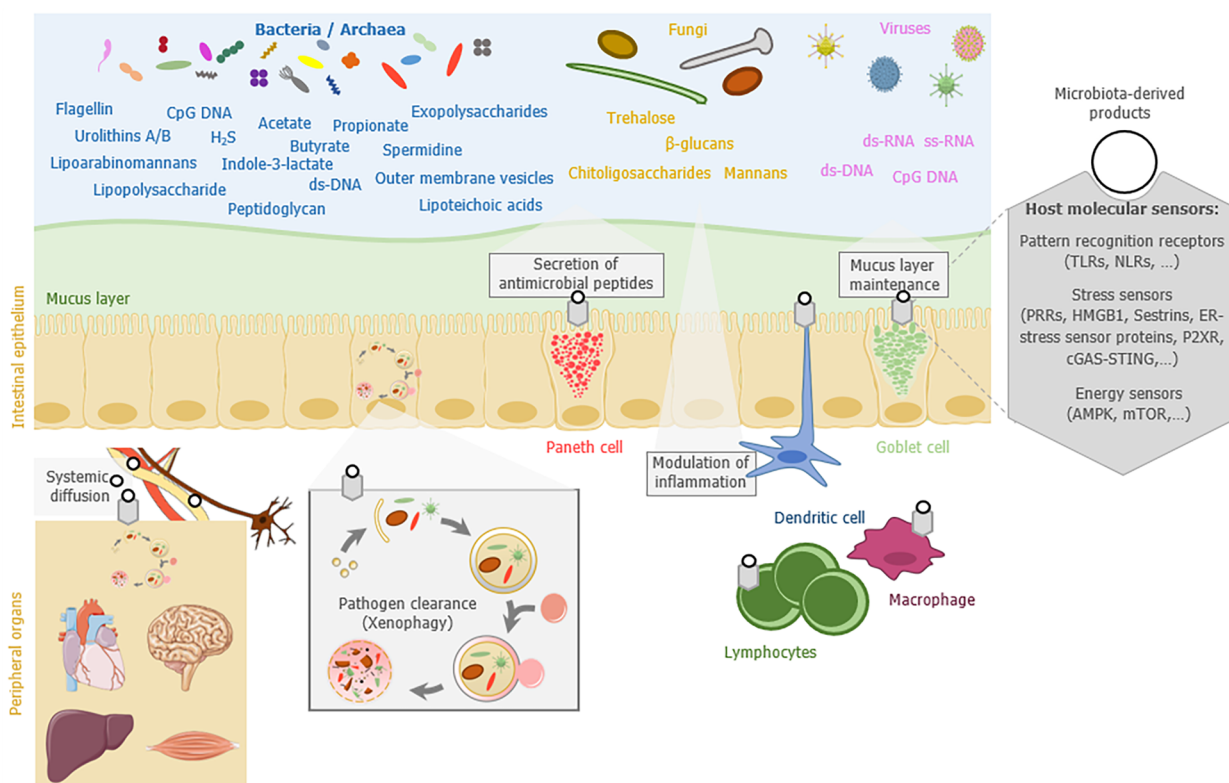


Figure 1 Complex interplay between gut microbiota and autophagy. The interactions between the gut microbiota and autophagy are bidirectional. Autophagy is involved in the regulation of several mechanisms (grey boxes) that shape the gut microbiota. Reciprocally, some bacterial- (blue), fungal- (orange), or viral-derived (pink) compounds are able to modulate autophagy in the gut mucosa as well as in distant organs through systemic pathways (circulatory system, nervous system ...). Modulation of autophagy by the gut microbiota involves microbiota-derived products such as microbial compounds (lipopolysaccharide, peptidoglycan ...), microbial derived-compounds (short chain fatty acids, secondary biliary acids ...), or signaling molecules (cytokines, hormones ...). They stimulate a wide range of host molecular sensors (pattern recognition receptors, stress sensors, and energy sensors; grey hexagons) located in the gut or peripheral organs. PRR: Pattern recognition receptor; TLR: Toll-like receptor; NLR: Nod-like receptor.

Given the influence of gut microbiota-related factors on autophagy, one could expect that alterations in the composition of the gut microbiota would affect autophagy in the gut mucosa. Indeed, an increase in the expression of some autophagy-related proteins (FoxO1, FoxO3, GABARAP, and ATG7) and LC3-II/LC3-I ratio and a decrease in AKT activation have been reported in newborn piglets receiving FMT[42]. In addition, alteration of the gut microbiota resulting from the administration of a cocktail of broad-spectrum antibiotics increased the basal activity of autophagy as well as the expression of some autophagy-related proteins (ATG16L1, ATG5, and IRGM1) in the ileal mucosa of mice[43,44]. Interestingly, oral administration of a single bacterial species (*e.g.*, *Desulfovibrio* spp., *Fusobacterium nucleatum*, or *Escherichia coli*) in conventional mice can also be sufficient to modulate gut autophagy [42,44,45]. Altogether, these studies suggest that autophagy regulatory network is sensitive to changes in the gut microbiota.

SYSTEMIC EFFECTS OF THE GUT MICROBIOTA ON HOST AUTOPHAGY

Microbial-derived metabolites (*e.g.*, PAMPs), compounds that are issued from the gut microbiota metabolism (*e.g.*, neuroactive compounds and SCFAs) and host bioactive molecules that are produced in response to its interaction with the gut microbiota (*e.g.*, cytokines), can have large systemic effects and modulate the physiology of organs that are distant from the gut. Influence of the gut microbiota on the brain is a well-documented example of such effects[6]. Several communication routes (immune system, autonomic nervous system, neuroendocrine system, hypothalamic – pituitary – adrenal axis, and other metabolic pathways) between the microbiota and the brain have been identified[6]. It is very likely that similar pathways and microbiota-derived players, or at least some of them, modulate as well the physiology of other organs in the body. Evidence is accumulating on the modulation of autophagy by the gut

microbiota in distant organs and several of these are presented below (Table 1).

Modulation of autophagy in nervous tissues

Although few studies are available on this emerging topic, they suggest that the gut microbiota could influence autophagy in the brain throughout life in both physiological and pathological conditions.

Diet is a key environmental factor that drives the composition and metabolic functions of the gut microbiota[46,47]. In particular, maternal diet can influence post-natal gut microbiota and neurological development of the offspring[48]. In a recent study, Wang and colleagues reported that feeding mothers with a high sugar and high fat (HSHF) diet, a condition that modifies the gut microbiota of the offspring, modulates also the expression of neuronal and autophagy markers in the brain during early life stage[49]. Particularly, they observed that the LC3A and LC3B levels were modified in the brain of the offspring in the HSHF group compared to controls before 28 d of age, and then decreased, meaning that autophagy may be differentially regulated in HSHF offspring[49].

Aging is associated with a decline of host autophagy including in the brain[50]. Influence of the gut microbiota on brain autophagy in aging has been evidenced in *in vivo* models. Alteration of autophagy has been reported in the brain of D-gal-treated mice, a model of accelerated aging[51,52]. These alterations were characterized by decreases in the LC3-II/LC3-I ratio and in the expression of ATG7 and SIRT1, as well as by increased phosphorylation of the master negative regulator of autophagy mTOR (S2448) and expression of p62 in the hippocampus tissue of D-gal-induced aging mice [52]. Interestingly, the administration of urolithin A (UA), a bioactive metabolite generated by the gut microbiota, was efficient in rescuing these autophagy-related defects. To note, UA administration also allowed to reverse increases in the LC3-II/LC3-I ratio, the expression of p62, and the phosphorylation of mTOR (S2448), as well as the decreased expression of Sirt-1 and ATG7 observed in the hippocampus of 12-mo-old mice[52].

Autophagy defect is thought to play a role in neurodegenerative processes associated with numerous diseases, including Alzheimer's disease (AD)[53]. Interestingly, although a causal relationship remains to be demonstrated, a few studies suggest that dysbiosis associated with AD could influence brain autophagy[54]. Decreased Beclin-1 expression and increased expression of p62 have been observed in the brain of old 3xTg-AD mice (a transgenic mouse model of AD) compared to young control mice, indicating alterations in autophagy[55]. Interestingly, in addition to modifying the composition and predicted function of the gut microbiota, oral supplementation of old 3xTg-AD mice with a combination of nine probiotic strains (*Streptococcus thermophilus*, *Bifidobacterium longum*, *B. breve*, *B. infantis*, *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum*, *Lactocaseibacillus paracasei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Levilactobacillus brevis*; SLAB51 formulation) also partially restored defects in autophagy[55]. Moreover, SLAB51 was also effective in restoring the impaired expression level and activity of SIRT1, a positive regulator of autophagy, in the brain of 3xTg-AD mice[56,57].

In another context, changes in the composition of the fecal microbiota have been reported in patients with acute ischemic stroke (AIS), a common cerebrovascular disease caused by sudden loss of blood circulation in a specific brain area[58,59]. Interestingly, anal administration of the fecal supernatant obtained from an AIS patient to antibiotics-treated mice resulted in increased expression of genes encoding Beclin-1, ATG12, and LC3 as well as increased expression of Beclin-1 at the protein level and an increased level of LC3-II in brain tissue compared to antibiotics-treated mice that received the fecal supernatant of healthy controls[59].

The retina, which is the light sensitive neural tissue that lines the back of the eyes, displays numerous similarities with the brain either anatomically or functionally[60]. Neurodegenerative conditions that affect the brain seem to compromise the retina, and *vice versa*[60-62]. Similarly to the brain, the retina is also highly sensitive to nutritional variations[63]. Retina autophagy[64,65] as well as modifications in the gut microbiota [66-69] is suspected to contribute to retinal diseases such as diabetic retinopathy, age-related macular degeneration, and glaucoma. Although no causal relationship has been yet established, one can assume that, as in the brain, the gut microbiota might influence retinal autophagy and that changes in its composition might alter retinal autophagy and contribute to the development of retinopathies.

Modulation of liver autophagy

Evidence of the influence of the gut microbiota on liver autophagy came from studies in gut microbiota-deprived mouse models. Comparison of germ-free mice and altered

Table 1 Data supporting the existence of a systemic regulation of autophagy by the gut microbiota

Ref.		Impact on autophagy		
		Brain	Liver	Muscles
[49,74-76]	Diet-induced changes in the gut microbiota	Feeding of mother mice with an HSHF diet: Changes in the expression levels of LC3A-I/LC3A-II/ LC3B-I/LC3B-II in the offspring.	Feeding mice or rats with an HF diet: Changes in the expression levels of LC3, p62, mTOR, and p-AKT and modulation of the LC3-II amount.	
[55,56,59,70]	Mice with specific gut microbiota	AD mice ¹ : Modulation of the lysosomal activity (Cathepsin L) and SIRT1 activity and changes in the expression levels of Beclin-1, p62, and SIRT1. FMT from patients with AIS to mice: Changes in the expression levels of <i>Becn1</i> , <i>ATG12</i> , and <i>LC3</i> expression and in the amount of LC3-II.	ASF colonized mice: Changes in the expression of a set of genes related to autophagy/membrane trafficking (<i>Uvr9g</i> , <i>Atg14</i> , <i>Becn1</i> , <i>Bcl2l1</i> , and <i>Pik3c3</i>) and lysosomal functions (<i>Chmp4c</i> and <i>Chmp2a</i>) compared to germ-free mice.	
[71,79]	Germ free or antibiotic-treated animals		Antibiotic treatment of mice fed a normal diet: Alteration of the basal expression of LC3 compared to controls.	Germ free piglets: Changes in the expression levels of LC3A, LC3B, and <i>Becn1</i> and of mTOR, p-mTOR, AKT, and p-AKT levels compared to normal and/or FMT piglets.
[55,56,75,76,78]	Probiotics	SLAB51 ² : Modulation of SIRT1 activity and changes in the expression levels of Beclin-1, p62, and SIRT-1 as well as in the LC3-II amount in AD mice ¹ .	<i>Limosilactobacillus reuteri</i> : Modulation of the expression levels of mTOR and p-AKT in HFD-fed rats.	<i>Lactocaseibacillus rhamnosus</i> , <i>Pediococcus acidilactici</i> , <i>Bifidobacterium adolescentis</i> : Changes in the expression levels of LC3 and ATG7 in rats fed a high-calorie diet.
[52,71,74,77,80]	Gut microbiota-derived products	UA: Modulation of LC3-II/LC3-I and p-mTOR/mTOR ratio and changes in the expression levels of ATG7 and p62 in mouse models of aging ³ .	SCFAs: Activation of the PPAR γ -UCP2-AMPK pathway, and induction of autophagy flux and lysosomal activity in mouse hepatocyte AML-12 cells. FXR and TGR5 ⁴ : Involved in autophagy modulation.	UA: Induction of mitophagy in <i>Caenorhabditis elegans</i> and in rodents. UB: Modulation of LC3-II/LC3-I, p-mTOR/mTOR and p-ULK1/ULK1 ratio and change in the expression level of p62 in a rat model of ischemia/reperfusion injury.

¹AD mice: Mouse model of Alzheimer's disease (3xTg-AD mice).²SLAB51: A combination of nine probiotic strains (*Streptococcus thermophilus*, *Bifidobacterium longum*, *B. breve*, *B. infantis*, *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum*, *Lactocaseibacillus paracasei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Levilactobacillus brevis*).³D-gal-treated mice and 12-mo-old mice.⁴FXR and TGR5: Bile acid receptors.

HSHF diet: High sugar and high fat diet; HF diet: High fat diet; FMT: Fecal microbiota transplantation; SCFAs: Short chain fatty acids (propionate and butyrate); AIS: Acute ischemic stroke; ASF: Altered Schaedler's flora; UA: Urolithin A; UB: urolithin B.

Schaedler's flora (a community of eight bacterial species) colonized mice revealed that absence of the gut microbiota altered hepatic expression of genes involved in autophagy and lysosomal functions[70]. In another study, a decrease in the expression of LC3 at the protein level has been reported in the liver of mice deprived from gut microbiota as a consequence of chronic treatment with antibiotics (ampicillin and neomycin) compared to control mice[71]. In addition, those authors showed that microbial-derived SCFAs (propionate and butyrate) activated autophagy, induced lysosomal activity, and increased autophagy flux *in vitro* in mouse hepatocyte AML-12

cells[71]. The mechanism involves the activation of the PPAR γ -UCP2-AMPK pathway [71].

Primary bile acids are synthesized from cholesterol in the liver and are converted into secondary bile acids by the gut microbiota[72]. Bile acids are signaling molecules that can activate nuclear hormone receptors including FXR and TGR5 (also known as GPBAR1), which is a cell-surface receptor of the G protein-coupled receptor family [73]. These two bile acid receptors have been described to modulate autophagy in the liver and adipose tissue in fed and fasted states[74].

Several alterations of autophagy, including a decreased amount of LC3 mRNA and LC3-II and an increased amount of p62, have been observed in the liver of mice fed a high-fat diet (HFD), a potent inducer of dysbiosis[74]. Chronic exposure of rats to an HFD can lead to NASH (non-alcoholic fatty steatohepatitis). Development of this liver disease has been associated with dysbiosis and alterations in autophagy, particularly increased expression of hepatic mTOR and p-AKT[75,76]. Interestingly, supplementation of an HFD with a probiotic strain (*Limosilactobacillus reuteri*) and/or treatment of NASH mice with antibiotics (metronidazole) tended to normalize the hepatic content of these two autophagy-related proteins, as well as SCFAs and *Firmicutes* and *Bacteroidetes* fecal contents, thus suggesting a role of the gut microbiota in the modulation of hepatic autophagy[75,76]. To note, some data suggest a role for TGR5 in the regulation of autophagy in response to HFD[74].

Modulation of autophagy in muscle tissues

An induction of autophagy, characterized by decreased phosphorylation of mTOR (S2448) and ULK1 (S757), an increased amount of LC3-II, and decreased expression of p62, has been reported in a rat model of ischemia/reperfusion injury[77]. Interestingly, intraperitoneal injection of urolithin B (UB), a gut microbiota-derived metabolite, was able to reverse this phenotype[77]. The inhibitory effect of UB on autophagy is thought to activate the Nrf2-related antioxidant response by increasing p62 accumulation and favoring p62-Keap1 interaction[77]. Another argument that suggests the influence of the gut microbiota on heart autophagy has been provided by changes in the expression levels of LC3 and ATG7 observed in heart tissue of rats fed a high-calorie diet supplemented with probiotics (*Lactocaseibacillus rhamnosus*, *Pediococcus acidilactici*, and *Bifidobacterium adolescentis*)[78].

In addition to the heart, autophagy might be regulated by the gut microbiota in other muscles. Recently, high-throughput RNA-seq analysis revealed that the expression levels of autophagy-related genes (*LC3A*, *LC3B*, and *Beclin-1*) were modulated in the skeletal muscles of germ-free piglets compared to control piglets [79]. Moreover, germ-free piglets harbored decreased expression of mTOR and AKT and their phosphorylated forms, phospho-mTOR (S2448) and phospho-AKT (S473), respectively, compared to control piglets[79]. FMT of germ-free piglets with stools collected on healthy donors pigs was effective in restoring the amounts of phospho-AKT and mTOR to a level similar to that of controls[79]. Some microbial-derived metabolites able to influence the muscle autophagy have been identified. For example, a role of UA as a mitophagy (selective degradation of mitochondria by autophagy) inducer in the muscle tissue has been described in the model organism *Caenorhabditis elegans* and in rodents[80].

SHAPING OF THE GUT MICROBIOTA BY AUTOPHAGY

As developed in the first part of this review, the gut microbiota is able to influence host autophagy by several pathways and through complex regulatory networks governing the autophagy machinery. Reciprocally, autophagy and autophagy-related proteins can shape the gut microbiota (Figure 1). This is particularly well illustrated by changes in the gut microbiota composition observed in mice conditionally deficient for autophagy (*Atg5*^{-/-}, *Atg7*^{-/-}, and *ATG16L1* T300A knock-in) in the gut[81-83]. Interestingly, alterations of autophagy in peripheral organs such as the liver have been shown to influence the composition of the gut microbiota[84].

A first overall reason that would explain why autophagy activity in the gut mucosa can modulate the abundance of gut microorganisms is that this process is essential to maintain homeostasis of their ecological niche. Indeed, basal autophagy is crucial to maintain the integrity of Lgr5-positive intestinal stem cells that give rise to all differentiated lineages of the intestinal epithelium throughout life[85]. In addition, autophagy contributes to the maintaining of intestinal barrier integrity, particularly by regulating proteins involved in tight junctions (*e.g.*, Claudin-2 and Occludin) on the apical side of

intestinal epithelial cells and by promoting cell survival upon various stress (*e.g.*, bacterial or viral infection, inflammation, or chemical stress)[4,86-88].

The main cellular mechanisms by which host autophagy shapes the gut microbiota (including pathosymbionts) are described below.

Clearance of pathogens

Autophagy mediates the bulk or selective lysosomal degradation of cellular components. In selective autophagy, selective autophagy receptors (SARs) recognize and bind specific cargoes to promote phagophore formation around them, ultimately leading to their degradation into a mature autolysosome. These specific cargoes can be for instance mitochondria (mitophagy), lipid droplets (lipophagy), protein aggregates (aggrephagy), or peroxysomes (pexophagy)[89]. A selective form of autophagy termed xenophagy is dedicated to the elimination of intracellular pathogens (*e.g.*, bacteria, viruses, fungi, or protozoa) and is supported by the expression of several SARs including NDP52, Optineurin, p62, TAX1BP1, Galectin 8, and TECPR1[90]. Xenophagy has been shown to restrict or avoid the intracellular persistence and the replication of various human pathogenic or pathosymbiotic bacteria, residing either in damaged vacuoles [*e.g.*, *Salmonella* Typhimurium or adherent-invasive *Escherichia coli* (AIEC)] or free in the host cytosol (Group A *Streptococcus*)[91-93]. Thus, by limiting the dissemination of invasive pathogens from the gut lumen to extra-intestinal sites, autophagy also restrains their persistency in the gut microbiota[94,95]. Defects in xenophagy are thought to contribute to the etiology of Crohn's disease (CD) an inflammatory bowel disease (IBD) characterized by chronic and severe intestinal inflammation associated with dysbiosis[96]. In particular, a coding polymorphism (Thr300Ala) in the autophagy-related gene *ATG16L1* that confers an increased risk for the development of CD has been shown *in vitro* and *in vivo* to alter the xenophagy process, thus favoring persistency of the CD-associated AIEC bacteria[92,97,98]. CD risk polymorphisms have also been identified in other autophagy-related genes, including core autophagy genes (*IRGM*, *ULK1*, *ATG4a*, and *ATG4d*) and genes involved more specifically in xenophagy (*NOD2* and *NDP52*)[99-101].

One important point is that, besides xenophagy, non-canonical autophagy such as LC3-associated phagocytosis (LAP) can also contribute to the clearance of intracellular pathogens. This specific form of phagocytosis requires an important set of core autophagy proteins (UVRAG, BECN1, VPS34, LC3, ATG3, ATG4, ATG5, ATG7, ATG12, and ATG16L1), but some other proteins involved in canonical autophagy remain dispensable (ATG14, ULK1, FIP200, and AMBRA1). LAP also distinguishes from canonical autophagy by the formation of single-membrane vacuoles called LAPosomes[102]. Efficiency of LAP to increase clearance of pathogens such as *Listeria monocytogenes* or *Aspergillus fumigatus* has been shown[103,104].

Mucus layer maintenance

A mucus layer composed of highly glycosylated proteins (mucins) overlays the gut epithelium and represents an important physical barrier limiting the contact of luminal microbes with the epithelium, thus avoiding their potential translocation into underlying tissues[105]. The mucus layer differs between the small and large intestine in terms of physicochemical properties (*e.g.*, thickness, density, and composition) and it is under the influence of numerous factors, including the gut microbiota and the diet [106-108]. Whereas in the small intestine the mucus is non-attached and constitutes a discontinuous layer, it is organized in two layers - the inner and outer mucus layers - in the large intestine. Compared to the intestinal lumen, only few bacterial species are able to live and to persist in the mucus layer. This is partly due to the important amount of various antimicrobial compounds (*e.g.*, IgA, lysozyme, defensins, REG3γ, and phospholipase A2-IIA) found in the mucus layer, particularly in the small intestine. However, some commensal bacteria are molecularly equipped to bind, degrade the mucus glycans, and/or harvest the oligosaccharides, giving them a selective advantage in colonizing this particular ecological niche[109]. Among others, mucin-degrading specialists include species belonging to the genera *Bacteroides* (*e.g.*, *B. thetaiotaomicron* and *B. fragilis*), *Ruminococcus* (*e.g.*, *R. gnavus* and *R. torques*), and *Akkermansia* (*e.g.*, *A. muciniphila*). Interestingly, *A. muciniphila*, a bacterial species belonging to the phylum Verrucomicrobia, is considered as a healthy marker of the intestine since its presence in high abundance is associated with a healthy mucosa whereas reduction of its abundance is associated with intestinal disorders (*e.g.*, obesity and IBD)[110,111]. Studies suggest that the composition of mucus-associated microbiota differs depending on the intestinal segment or the mucus layer (outer or inner layer) that is considered[105]. Bacteria belonging to the phylum Firmicutes have been found in higher abundance in the mucus layer than Bacteroidetes, both in

humans and in rodents[105].

Mucus plays a critical role in the maintenance of the symbiotic relationship between the host and the gut microbiota[112]. Deletion of the *Muc2* gene in mice results in changes in the gut microbiota composition characterized in particular by an increase in the abundance of potential pathobionts (*e.g.*, *Desulfovibrio*, *Escherichia*, and *Erysipelotrichaceae*), and the reduction of beneficial bacteria (*e.g.*, *Lactobacilli*) and *Lachnospiraceae* [112]. In addition to ensuring an habitat and energy sources for a specific part of the gut microbiota, the mucus constitutes a protective layer against pathogen invasion and infection, although some pathogenic bacteria have developed efficient strategies to colonize this special environment and reach the intestinal epithelium (*e.g.*, *Shigella flexneri* and AIEC)[113,114]. Thus, modifications in mucus layer structure or composition by genetic and environmental factors, such as diet, can modify the gut microbiota[105]. These changes can be beneficial when they strengthen the mucus barrier properties, but they can also be deleterious by favoring emergence of pathobionts, by bringing harmful bacteria closer to the epithelial barrier and by destabilizing the symbiotic relationship between the gut microbiota and the host, at the gut mucosa as well as at systemic levels[107].

Mucus secretion into the gut lumen is achieved by specialized secretory cells, the goblet cells. Mucins, the proteins forming the mucus, are packed into secretory granules that are localized on the apical side of the goblet cells and constitutively secreted by fusion of the granules with the plasma membrane. Proteins belonging to the core autophagy machinery (ATG5, ATG7, and LC3B) are critical in mice for the release of these secretory granules by supporting the generation of reactive oxygen species[115].

The NLRP6 inflammasome has been identified, among others roles, as a key factor involved in autophagy-induced regulation of goblet cell secretory functions[116,117]. NLRP6-deficient mice exhibit defective autophagy in intestinal cells including in goblet cells, a phenotype that is associated with impaired mucus layer formation. This mucus alteration may contribute, together with the other NLRP6-related defects, to modulating the composition of the gut microbiota and abnormally bring microbes closer to the epithelial barrier in NLRP6-deficient mice. Analyses of the gut microbiota in NLRP6-deficient mice revealed an abnormal representation of the bacterial phyla Bacteroidetes (*Prevotellaceae*) and Saccharibacteria (formerly known as TM7)[116]. In addition, alteration of the mucus layer in NLRP6-deficient mice enables *Citrobacter rodentium*, a mouse-specific pathogen, to penetrate deeper into the crypts and be more invasive[117]. The role of autophagy in shaping the gut microbiota through the regulation of mucus layer maintenance is also supported by observations made in *Atg7*-deficient mice. Secretion of mucins from goblet cells was diminished in colonic-epithelial cell-specific *Atg7* knock-out mice[82]. This phenotype was associated with an abnormal composition of the gut microbiota characterized in particular by an increased abundance of *Clostridia* and *Prevotellaceae* in *Atg7*-deficient mice. In addition, those authors observed an increased bacterial burden in the colon, a phenotype that could contribute to the exacerbated sensitivity to experimental colitis observed in *Atg7* knock-out mice. Interestingly, stimulation of the autophagy-related process, either by a beneficial bacterial strain (*Bifidobacterium dentium*) or by a polyphenol (oxyresveratrol), has been shown to enhance mucin production by goblet cells in *in vivo* and *in vitro* models[118,119].

Secretion of antimicrobial compounds in the gut lumen

Autophagy and autophagy-related proteins can also affect the composition of the gut microbiota by regulating the secretion of some antimicrobial compounds released into the gut lumen by enterocytes, Paneth cells, or immune cells. Among them, immunoglobulins of the A class (IgAs) are daily released in huge amount (several grams per day) into the gut lumen and shape the composition of the gut microbiota. Alterations of the gut microbial ecosystem have been reported in the absence of hypermutated intestinal IgA in mice with deficiency of activation-induced cytidine deaminase[120-122]. Changes in the gut microbiota were particularly characterized by expansion of anaerobic bacteria in the small intestine, with a domination by segmented filamentous bacteria[121]. Several other studies in mouse models support the role of IgAs in regulating the diversity and composition of microbiota[123,124]. Data obtained in humans showed that selective IgA-deficiency (sIgAd) is associated with a mild intestinal dysbiosis, characterized by expansion of pro-inflammatory bacteria (*e.g.*, *E. coli*, *Prevotella*), reduction of anti-inflammatory commensals (*e.g.*, *Faecalibacterium*), and perturbation of bacterial dependency association network[125]. In addition, Catanzaro and colleagues reported also a trend toward a decreased alpha diversity and shifts in the relative abundance of some taxa (*e.g.*, increase in *Eubacterium dolichum* and *Rumino-*

coccus bromii and decrease in *Paraprevotellaceae*) in human sIgA subjects compared to controls[126]. IgAs are produced by gut-resident antibody-secreting plasma cells (PCs) that display important metabolic adaptations and endoplasmic reticulum expansion to cope with the stress of producing very large amounts of IgAs[127]. Some studies suggest that autophagy is required for sustainable production of immunoglobulins by PCs since mice with conditional deficiency of *Atg5* in B cells had defective antibody responses, with an increased sensitivity of PCs to cell death[128]. In addition, mice deficient for *Atg5* in B cells harbored a decreased number of IgA-secreting PCs isolated from the gut-associated lamina propria, Peyer's patches, and mesenteric lymph nodes in comparison to control mice[129].

Another important antimicrobial compound to which commensal bacteria are directly exposed in the gut lumen is the lysozyme secreted by Paneth cells, which are secretory epithelial cells located at the bottom of the crypts in the small intestine. This antimicrobial protein is also produced by macrophages and neutrophils in the lamina propria. Three types of lysozyme have been described so far across the animal kingdom[130]. Lysozyme causes bacterial lysis by hydrolyzing bacterial cell wall PGN, but it can also induce cationic killing of bacteria by inserting into and forming pores into the lipid bilayer of the bacterial cell membrane. This is the case with c-type lysozyme expressed in human[130]. Not all bacteria are equally sensitive to lysozyme and some pathogenic bacteria have developed strategies to escape its antimicrobial activity[130]. The contribution of lysozyme in shaping the gut microbiota is illustrated by the dysbiosis observed in lysozyme-deficient mice (*Lyz1*^{-/-} mice) that is characterized by the expansion of some mucolytic bacteria such as *Blautia gnavus* (formerly known as *Ruminococcus gnavus*)[130,131]. No change in luminal bacterial load and alpha-diversity was observed in the cecum- and mucosal-associated bacteria in the ileum and the colon of *Lyz1*^{-/-} mice[131]. However, changes occurred in the composition of the fecal microbiota (expansion of *Dorea formicigenerans* and reduction of *Candidatus Arthromitus*) as well as the ileal microbiota (expansion of *B. gnavus* and *D. formicigenerans* and reduction of *C. Arthromitus*) in *Lyz1*^{-/-} mice[131].

Alpha-defensins (also called crypt defensins or cryptdins) are another example of antimicrobial factors that are produced by Paneth cells, whose roles in host defense against enteric pathogens and regulation of the composition of the gut indigenous microbiota have been described[132]. Interestingly, abnormal packaging and secretion of antimicrobial compounds by Paneth cells have been reported in mice harboring Paneth cells deficient for the autophagy-related genes *Atg5*, *Atg7*, and *Atg16l1* and in patients with CD-associated *NOD2* and *ATG16L1* variants[133-135]. Of note, this defect in lysozyme packaging in autophagy-deficient mice required an infectious (viral or bacterial) trigger[136,137].

Even if canonical autophagy is considered as a degradative process, some infectious agents such as *Salmonella* Typhimurium can trigger a secretory autophagy resulting in the formation of LC3-positive, double-membraned lysozyme granules[136]. These autophagosome-like vacuoles are not directed for the fusion with the lysosomes but instead reach the plasma membrane for the release of their content into the gut lumen. Thus, the autophagy machinery participates in the unconventional protein secretion of lysozyme, thereby affecting the composition of the gut microbiota by counter-selecting the lysozyme-sensitive bacteria. In this context, it has been suggested that vitamin D, via binding to the vitamin D receptor expressed by Paneth cells, can sustain autophagy activities in these cells[138]. To note, several studies suggest that expression and secretion of other antimicrobial peptides than lysozyme, such as the defensins and cathelicidins, would be regulated by autophagy. However, the exact molecular mechanisms remain to be determined[82,139].

Modulation of inflammation

Cell stimulation by microorganisms (*e.g.*, invasive pathogens) or danger signals (*e.g.*, extracellular ATP, uric acid, or HMGB1) are usually associated with the triggering of inflammatory processes through the release of cytokines and chemokines. Inflammation is a protective response that results in tissue repair. However, this response needs to be tightly regulated in order to avoid excessive and/or chronic inflammation that could be detrimental for host tissues. In the gut mucosa, immune tolerance toward the resident gut microbiota should be maintained to avoid chronic gut inflammation and sustain homeostasis[140]. Unbalanced inflammatory responses can also alter the gut microbiota as shown in mouse models of colitis that mimic human IBD, in which inflammation induces microbial dysbiosis[141,142]. Chronic inflammatory state was also suggested to contribute to dysbiosis in IBD patients[143]. This inflammation-driven bacterial dysbiosis is commonly characterized by an overall decrease in bacterial diversity, especially in Firmicutes (*Clostridium* groups) and an overgrowth of

species belonging to *Enterobacteriaceae*[143,144].

Autophagy machinery and autophagy-related proteins are key contributors to the regulation of the inflammatory processes. Thus, one could assume that modulation of inflammation by autophagy could influence the composition of the gut microbiota. Autophagy is usually considered as an anti-inflammatory process, particularly since it controls activation of inflammasomes that are multimeric protein complexes involved in the maturation of pro-inflammatory cytokines[145]. Mice deficient for *Atg16l1* in haematopoietic cells have been shown to be highly sensitive to chemically-induced colitis and produce increased levels of IL-1 β and IL-18, two cytokines processed by inflammasomes[146]. *Atg16l1*-deficient macrophages that were stimulated by LPS also produced higher amounts of these cytokines compared to wild-type macrophages. Autophagy can alleviate activation of inflammasomes, at least by removing stimuli that induced them (*e.g.*, intracellular infectious agents) and by degrading some inflammasome components (*e.g.*, NLRP1, NLRP3, AIM2, or pro-CASP1)[147]. Interestingly, alterations of the gut microbiota (*e.g.*, increased abundance of Bacteroidetes) as well as enhancement of the local Th1 and Th17 immune responses have been reported in mice with dextran sodium sulfate (DSS) colitis that express the CD risk allele *ATG16L1* T300A - a genetic context known to impair some autophagy-related functions - compared to DSS-treated wild-type mice[81]. Similar observations have been made in gnotobiotic mice expressing the CD risk allele *ATG16L1* T300A and inoculated with human stools from active CD patients[81]. These data illustrated how a subtle polymorphism in an autophagy-related gene could deeply impact the equilibrium between immune responses and the gut microbiota.

Autophagy is also able to modulate signaling of interferons, notably by degrading key players of type-I interferon responses (*e.g.*, RIG-I, STING, MDA5, IRF3, MAVS, and cGAS)[148]. Abnormal regulation of interferon signaling can lead to alterations of the gut microbiota as described in knock-out mice and viral infection models[149]. Interestingly, the gut microbiota has been described to stimulate intestinal autophagy *via* the induction of the type-II interferon, and this microbiota-mediated activation of autophagy has been shown to protect the host against infection by the protozoan parasite *Toxoplasma gondii* by limiting the deleterious production of the pro-inflammatory cytokine TNF- α [150]. Autophagy has also been described to limit the production and the secretion of various cytokines including TNF- α , IL-1 β , IL-23, IL-6, TGF- β , and MIF[151,152]. However, the molecular mechanisms by which autophagy regulates their expression remain elusive. In many cases, autophagy reduces secretion of cytokines by simply alleviating cellular stress that triggers the inflammatory responses.

CONCLUSION

Given its crucial role in regulating homeostasis at both cell and tissue levels, it is not surprising that alterations of autophagy are connected to a large number of disorders (*e.g.*, IBD, cancers, and neurodegenerative diseases). To assume its various functions, autophagy activation is tightly regulated and the gut microbiota has recently emerged as a contributor in its regulatory networks in both the gut mucosa and other tissues. This advance in the understanding of the molecular mechanisms supporting this highly integrated cellular process that tip the balance between health and disease offers new opportunities to develop preventive or therapeutic tools. Indeed, the gut microbiota appears as a promising target to restore functional autophagy or to prevent its alterations in various disease conditions. The growing interest that was aroused from the discovery of such a hub position occupied by the gut microbiota in maintaining physical and mental health status has led to the conceptualization, development, and/or examination of various tools to manipulate the gut microbiota (probiotics, prebiotics, synbiotics, postbiotics, FMT, Crispr/Cas9, diet...). In the era of personalized medicine, such a toolbox could constitute a key element that could be integrated in the therapeutic strategies. However, further explorations of the interplay between the gut microbiota and autophagy are needed. Important advances have been made in understanding the local dialogue between the gut microbiota and autophagy at the level of the gut mucosa, but less is known about how and in which extent they communicate at the systemic level. Bi-directionality of the interactions between the gut microbiota and the autophagy network, plasticity and complexity of the gut microbiota and its multiple effects on host, as well as pleiotropy of the functions of autophagy are all factors that increase the level of complexity of the system. Better characterization of the cellular and molecular actors from both sides - the gut

microbiota and autophagy - that contribute and regulate the framework of their interactions to maintain homeostasis constitutes a prerequisite to propose new preventive and therapeutic tools in pathological conditions associated with dysbiosis and/or autophagy dysfunction.

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