

# Aging Effects on Gut Microbiota in SAMP8 Mice <sup>†</sup>

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**Abstract:** We have studied the effects of aging on the fecal microbiota composition in the senescence-accelerated prone mice SAMP8 strain. We compared animals two, four, and six months old. Feces were collected at the end of each period and a genomic study was carried out on fecal DNA using the Illumina MiSeq analyzer. The Shannon diversity index showed similar values along this period and the number of species was neither affected by aging. The phylum Verrucobacteria went up with age, showing a seven-fold increase at six months, compared to two-month old mice. At the family level, changes observed between two and six months of age involved significant increases in Bacteroidaceae ( $q < 0.001$ ) and strong reductions in Lactobacillaceae ( $q < 0.0001$ ) and Prevotellaceae ( $q < 0.05$ ); at the genus level, there was a significant reduction in probiotic *Lactobacillus*. At the species level, we observed an age-related reduction in *Lactobacillus hayakitensis*, a species involved in mucosal immune homeostasis, and in *Blautia hansenii*, which provides protection against *Clostridium difficile* infection. Interestingly, aging increases *Parabacteroides goldsteini*, which is involved in the regulation of the TLR4 pathway. These results support the view that aging results in the proliferation of bacterial species that are associated with the immune deterioration of the gut mucosa.

**Keywords:** senescence; microbiota; aging; mice model of accelerated senescence

## 1. Introduction

The gut microbiota is a highly complex and diverse community of bacteria that closely interacts with the epithelium and the underlying immune cells in the gut [1]. The bacterial phyla that dominate the gut microbiota of different mammalian species are Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria [2,3]. In recent years, it has become clear that the gut microbiota has a major impact on the immune system, metabolism, and even behavior of the host [4]. Moreover, an imbalance in gut microbiota composition (dysbiosis) has been associated with several immunological, metabolic, and mental disorders [5].

Changes in the composition of the gut microbiota along aging likely contribute to immunosenescence and to the development of a pro-inflammatory phenotype [6]. In fact, the elderly is more susceptible to infections and show a weaker immune protection after vaccination. Moreover, homeostasis between pro-inflammatory and regulatory responses is lost, which results in a state of low-grade chronic systemic inflammation, which is called inflammaging [7]. This age-related chronic inflammation likely contributes to the pathology of several diseases typically associated with aging such as dementia, stroke, and cardiovascular diseases. In addition, advanced age has been reported to increase intestinal permeability in rodents and non-human primates, and may subsequently enhance translocation of luminal bacterial products and induce inflammation [8,9].

Inflammaging can impair brain function by an increase in inflammatory cytokine expression and elevated oxidative stress, blood–brain barrier breakdown, peripheral immune cell infiltration, and glial cell activation. Our results with the SAM model support this view because we have demonstrated that this mouse model shows impaired intestinal immune response [10] as well as certain neurodegeneration that impairs memory function [11].

Aging-associated alterations in gut physiology (i.e., gastric motility disorders, hypochlorhydria, degenerative changes in enteric nervous system, etc.) have profound effects on the diversity, composition and functional features of intestinal microbiome [12]. These age-related differences can appear, in particular, because long-term stimulation of immune system may cause immunosenescence. With that appears inflammaging, which accompanies many aging-associated diseases, including both gastrointestinal and non-gastrointestinal pathological conditions such as atherosclerosis, frailty, cancer, fatty liver disease, metabolic syndrome, type 2 diabetes, neurodegenerative diseases, etc. [12–14].

In previous studies performed with SAMP8 mice model, we have observed that at 6 months of age, mice exhibit an impaired immune response in the intestinal mucosa characterized by nonspecific gut-associated lymphoid tissue activation and a weak specific immune response [10]. In addition, in the same mice model, we have observed a cognitive impairment associated to increased brain inflammatory state and with a reduced blood-brain barrier [11].

Since this mouse model shows several characteristics of the deterioration associated with aging, both at the intestinal and cerebral levels, our aim in this work has been to determine whether these changes could be associated with an alteration in the profile of the microbiota.

## 2. Materials and Methods

### 2.1. Animals

Experiments were done in mice prone to senescence (SAMP8 strain), which were maintained and grown in the animal facility of the Facultat de Farmàcia i Ciències de l'Alimentació of the Universitat de Barcelona. Protocols used in this study were approved by the Animal Experimentation Ethics Committee (CEEA) of the Universitat de Barcelona, in accordance with the Generalitat de Catalunya's guidelines for the Care and Use of Laboratory Animals (DAAM: 7939 and 9272).

### 2.2. Aging Effect on Microbiome Composition

We used SAMP8 mice two, four, and six months old (groups named 2M, 4M, and 6M, respectively). Animals were maintained in conventional housing (3–4 animals per cage) until the age of two months, and from then they were kept in individual cages.

### 2.3. Sample Obtention

Feces were collected at three time points (two months, four months, and six months) in clean conditions and immediately frozen in liquid N<sub>2</sub>. Samples were maintained at –80 °C until use. DNA was extracted following Moretó et al. [15].

### 2.4. 16S rDNA Gene Analysis

The extracted genomic DNA was processed and the variable V3 and V4 regions of the 16S rRNA gene were amplified. The primers to detect 16S rRNA gene were: 16S forward 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and reverse 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'. High-through sequencing was done using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) at the Genomics and Bioinformatics Service, Universitat Autònoma de Barcelona (Bellaterra, Spain).

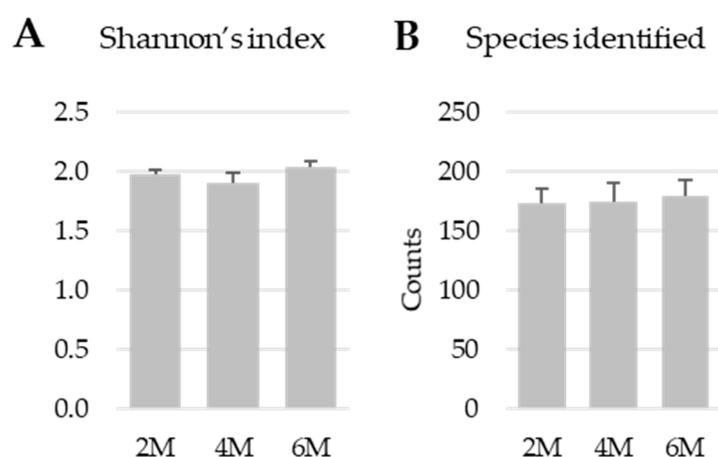
### 2.5. Statistics Analysis

Results are presented as means  $\pm$  SEM. The statistical analysis was performed using GraphPad Prism<sup>®</sup> software v 8.02 (GraphPad Software, Inc., La Jolla, CA, USA). Grubb’s test was performed to determine outliers and the Shapiro–Wilk test was used to check the normality of data distribution. To compare 2M, 4M, and 6M groups, the ANOVA test was used when data were normally distributed; otherwise the non-parametric Kruskal–Wallis test was carried out. All *p*-values were corrected for multiple testing using the false discovery rate (FDR) correction (Benjamini–Hochberg). Statistical differences were considered significant at  $q < 0.05$ .

## 3. Results

### 3.1. Shannon Diversity Index and the Number of Species

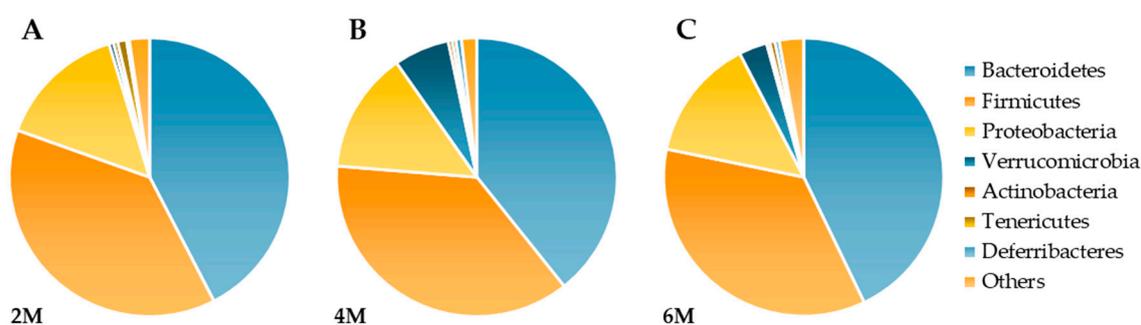
The Shannon diversity index, which is commonly used to characterize species diversity in a community, was not changed by aging, nor was the number of species present in feces (Figure 1).



**Figure 1.** Microbial Shannon’s index (A), number of species (B). Results are expressed as mean  $\pm$  SEM (*n* = 11 mice).

### 3.2. Phylum Level

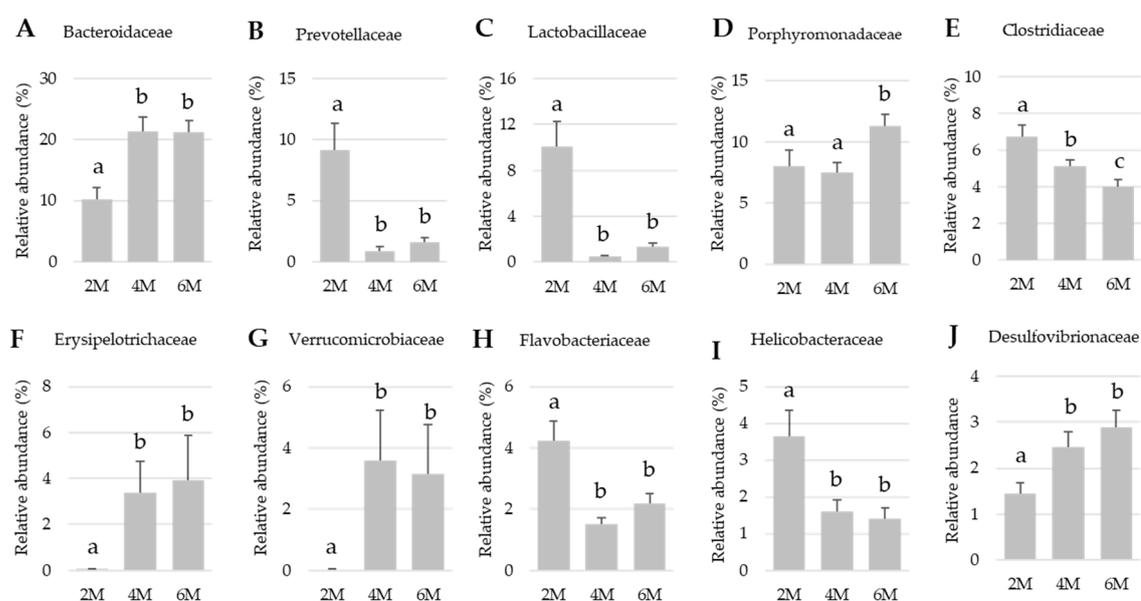
The most represented phyla were Firmicutes (F) and Bacteroidetes (B) and this did not change with age (Figure 2). Therefore, the F/B ratio was also similar in the three age groups, with mean values of 1.07 (2M), 0.98 (4M), and 0.82 (6M). However, the phylum Verrucomicrobia increased several-fold with aging respect to 2M (4M  $q = 0.004$ ; 6M  $q = 0.045$ ).



**Figure 2.** Effects of aging on fecal bacterial composition (phylum level) at 2M (A), 4M (B), and 6M (C). Results are expressed as percent of the total population at phylum level (mean) from 11 mice.

### 3.3. Family Level Families

At this taxonomic level there were changes in the relative presence of some families between two, four, and six months of age (2M, 4M, and 6M, respectively; Figure 3). Four- and six-month old mice showed increased relative abundance in Bacteroidaceae, Erysipelotrichaceae, and Verrucomicrobiaceae (all  $q < 0.02$ ). In contrast, both aged mice (four and six month old) showed a pronounced reduction in the abundance of Prevotellaceae, Lactobacillaceae (both  $q < 0.001$ ). Other families were also modified by aging, smaller but significant reductions in Clostridiaceae and Helicobacteriaceae were also observed (all  $q < 0.05$ ).



**Figure 3.** Effects of aging on fecal bacterial composition at family level. Results are expressed as percent of the total population at family level (mean  $\pm$  SEM;  $n = 10$ – $11$  mice). Statistical differences were considered significant at  $q < 0.05$  (corrected  $p$  values).

### 3.4. Genus Level

There were important changes with age in the abundance of a number of genera, most of which showed a relative reduction in their abundance (*Lactobacillus*, *Prevotella*, *Flavobacterium*, *Helicobacter*, *Natronincola*, *Odoribacter*, *Pedobacter*, *Olivibacter*, just to mention those with a 1% presence of the genus level; all  $q < 0.05$ ; Table 1). Few genera (e.g., *Bacteroides*, *Parabacteroides*, *Elysipelothrix*, *Butyricomonas*, *Akkermansia*) showed an age-dependent increase ( $q < 0.05$ ).

**Table 1.** Effects of aging on fecal bacterial composition at genus level. Results are expressed as percent of the total population at genus level (mean  $\pm$  SEM;  $n = 10$ – $11$  mice). Means in a row with superscripts without a common letter differ,  $q < 0.05$  (corrected  $p$  values).

Phylum	Family	Genus	2M	4 M	6M
Bacteroidetes	Bacteroidaceae	<i>Bacteroides</i>	11.1 $\pm$ 2.0 <sup>a</sup>	23.0 $\pm$ 2.5 <sup>b</sup>	22.9 $\pm$ 2.1 <sup>b</sup>
	Sphingobacteriaceae	<i>Pedobacter</i>	1.75 $\pm$ 0.11 <sup>a</sup>	0.93 $\pm$ 0.08 <sup>b</sup>	1.00 $\pm$ 0.10 <sup>b</sup>
<i>Olivibacter</i>		1.36 $\pm$ 0.19 <sup>a</sup>	0.77 $\pm$ 0.06 <sup>b</sup>	0.73 $\pm$ 0.09 <sup>b</sup>	
Bacteroidetes	Odoribacteraceae	<i>Odoribacter</i>	1.95 $\pm$ 0.31 <sup>a</sup>	0.15 $\pm$ 0.02 <sup>b</sup>	0.14 $\pm$ 0.02 <sup>b</sup>
		<i>Butyricimonas</i>	0.40 $\pm$ 0.07 <sup>a</sup>	1.32 $\pm$ 0.26 <sup>b</sup>	1.59 $\pm$ 0.28 <sup>b</sup>
	Prevotellaceae	<i>Prevotella</i>	9.98 $\pm$ 2.40 <sup>a</sup>	0.98 $\pm$ 0.39 <sup>b</sup>	1.75 $\pm$ 0.40 <sup>b</sup>
	Porphyromonadaceae	<i>Parabacteroides</i>	3.48 $\pm$ 0.45 <sup>a</sup>	3.44 $\pm$ 0.47 <sup>a</sup>	6.57 $\pm$ 0.89 <sup>b</sup>
	Flavobacteriaceae	<i>Flavobacterium</i>	3.95 $\pm$ 0.65 <sup>a</sup>	1.10 $\pm$ 0.19 <sup>b</sup>	1.69 $\pm$ 0.24 <sup>b</sup>
Firmicutes	Lactobacillaceae	<i>Lactobacillus</i>	8.85 $\pm$ 1.99 <sup>a</sup>	0.84 $\pm$ 0.34 <sup>b</sup>	1.15 $\pm$ 0.29 <sup>b</sup>



#### 4. Discussion

Senescence is characterized by an increase in the basal inflammatory state, which contributes to the development and progression of various diseases associated with aging. This increase in the inflammatory state leads to a decrease in the functionality of the immune system and a reduced capacity to respond to the presence of infections, is known as immunosenescence [16]. In addition, this decrease in the immune system functionality is accompanied by low-intensity inflammation sustained over time, known as inflammaging, which is characterized by increased production of pro-inflammatory mediators and cytokines [17,18]. Dysbiosis observed during aging, not only includes changes in the abundance of certain species and/or genera, which alone may already alter intestinal physiology, and the appearance of pathogens [19].

In the microbiota of six-month-old SAMP8 mice, the genera *Bacteroides* and *Parabacteroides* doubled in abundance. These results show the same pattern as that observed in a study of young and elderly individuals, where both genera accounted for 8–27% of the microbiome in a younger cohort, but more than 50% in the over 65 [20]. In addition, several studies have shown that the abundance of the genus *Lactobacilli*, which has generally been considered beneficial for intestinal health, decreases in the elderly [21]. In fact, *Lactobacillus* loss is associated with frailty in the elderly [22]. In the six-month SAMP8 mice from this study, a considerable loss in the abundance of different members of this genus was also observed, in line with studies performed in humans [20].

In previous studies, we have shown that at six months of age, SAMP8 mice exhibit an impaired immune response in the intestinal mucosa characterized by nonspecific gut-associated lymphoid tissue activation and a weak specific immune response [10]. The gastrointestinal tract of the elderly is particularly susceptible to infectious diseases, and this is associated with a lower responsiveness of the mucosal immune system [23]. In addition, several studies have shown that aging is associated with an alteration in the composition of the intestinal microbiota, which can aggravate intestinal inflammation and in turn increase intestinal permeability [24]. In fact, the microbiota of older adults is less diverse [22], and immunosenescence and physiological changes at the intestinal level due to age can destabilize the microbiota [25]. Recently, it has been observed that an ‘aged’ microbiota promotes immune dysfunction, including increased systemic inflammation and phagocytosis of macrophages, which can be partially restored by transferring the microbiota from young mice to aged mice [26].

SAMP8 mice at 6-month-old showed an impaired intestinal permeability that in turns promote intestinal inflammation. Moreover, SAMP8 mice showed increased abundance of *Akkermansia* and Proteobacteria that have been associated with intestinal inflammation and permeability [27]. In addition, increased abundance of *Akkermansia* and *Desulfovibrio* (that belongs to proteobacteria phylum) are associated intestinal inflammation induced by DSS [28]. Further studies indicate that *Desulfovibrio* can contribute to intestinal inflammation comes from observations that these bacteria are associated with the pathogenesis of inflammatory bowel disease [29,30]. In fact, it is not surprising that these two genera have been associated to intestinal inflammation since both altered mucus structure that was more penetrable by bacteria leading to increased intestinal immune infiltration [31,32].

Research in recent years has linked intestinal epithelial barrier dysfunction to various neurological disorders. In fact, increased age-related intestinal inflammation and permeability are thought to contribute to the pathogenesis of these neurological disorders [33]. In this sense, the intestinal-brain axis and the intestinal microbiota appear as key actors, as they form an integrated network in which the microbiota and the central nervous system communicate through endocrine, immune, and neuronal signaling pathways [34]. Several translational studies show that microbiota transfer from patients with neurological disorders, including autism, multiple sclerosis, and Parkinson’s disease, can influence behavior, motor dysfunction, and immune responses in different animal models [35–37].

It has been noted in the gut of Alzheimer disease patients there is a decrease in the population of Firmicutes and an increase in the level of Bacteroidetes [38]. We did not observe a significant reduction in Firmicutes phylum, but we have observed a considerable reduction in several genera and species belonging to this phylum. In a similar manner, we also observed a very large increase in

several species of *Bacteroides* that belongs to the Bacteroidetes phyla. These changes can be related to the increase in phosphorylated NF- $\kappa$ B, a transcription factor that promotes inflammatory responses, observed in senescent SAMP8 mice, inducing neuroinflammation and memory loss [11]. In fact, Zhao and Lukiw [39] showed that LPS secreted by *Bacteroides fragilis* can activate the pro-inflammatory transcription factor NF $\kappa$ B in human primary microglial cells. Moreover, Cox et al., [34] found that *Bacteroides* correlates with aging and amyloid beta plaque deposition in brain of Tg 2576 mice, a model of Alzheimer disease.

SAMP8 mice, at six months of age, have also shown a sharp reduction in the Prevotellaceae family, which could also be related to the cognitive decline observed in these animals [11]. Several authors have also observed a similar reduction in this family in aged mice, which has been associated with neuropathology and Alzheimer's disease [40]. In fact, a reduction of Prevotellaceae can alter mucin synthesis and, therefore, increase mucosal permeability, which would allow local and systemic exposure to bacterial endotoxin, which can lead to the accumulation of  $\alpha$ -synuclein in the colon [41]. Aggregation-prone proteins such as  $\beta$ -amyloid and  $\alpha$ -synuclein can propagate from the intestine to the brain and contribute to the pathogenesis of Parkinson's disease, Alzheimer's disease, and other neurodegenerative disorders [42].

On the other hand, SAMP8 mice showed a sharp reduction in the abundance of several species and genera belonging to Firmicutes phylum, such as *Lactobacillus* or *Blautia*, which are the main members of the Lactobacillaceae and Lachnospiraceae families, respectively. These two genera have been found to be reduced in the elderly and this reduction has been linked to the development of Alzheimer's disease [43,44]. Different strains of *Lactobacillus* produce acetylcholine and aminobutyric acid ( $\gamma$ -Aminobutyric acid, GABA) and improve learning and memory in aged rats suffering from D-galactose-induced Alzheimer's disease [45], and the genus *Blautia* is positively correlated with a low inflammatory state and high cognitive scores [46].

Thus, we can conclude that in SAMP8 mice at six months of age, a reduction of probiotic species is observed, that may modify the synthesis and secretion of neurotrophic factors, while the pro-inflammatory species of the intestinal microbiota increase, which are able to secrete bacterial amyloids and lipopolysaccharides, that are considered neurotoxic [47]. In these mice we have previously described a deterioration in the barrier function of the intestinal wall and in the blood-brain barrier. Therefore, these endotoxins can penetrate from the intestinal cavity into the bloodstream and into brain tissue, where they would have a negative effect on the structure and functions of the central nervous system and promote neuroinflammation and neurodegeneration.

## 5. Conclusions

Six-month-old SAMP8 mice already show fecal microbiota dysbiosis consistent with the reduced intestinal barrier integrity and impaired intestinal immune response previously observed. Both the dysbiosis observed in this work and the intestinal permeability could be involved in the neuroinflammation and blood-brain-barrier reduction that would lead to the cognitive decline observed in these mice.

**Author Contributions:** Conceptualization and design of the experiments: L.M., C.A., J.P., A.P.-B. and M.M.; methodology: L.M., C.A. and A.P.-B.; contribution with essential reagents and materials: J.P.; data analysis: L.M., A.P.-B. and M.M.; writing—original draft preparation: A.P.-B. and M.M.; writing—review and editing: C.A., A.P.-B. and M.M. All authors have read and agreed to the published version of the manuscript.

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## References

1. Hooper, L.V.; Littman, D.R.; Macpherson, A.J. Interactions between the microbiota and the immune system. *Science* **2012**, *336*, 1268–1273, doi:10.1126/science.1223490.
2. Leser, T.D.; Amenuvor, J.Z.; Jensen, T.K.; Lindecrona, R.H.; Boye, M.; Møller, K. Culture-independent analysis of gut bacteria: The pig gastrointestinal tract microbiota revisited. *Appl. Environ. Microbiol.* **2002**, *68*, 673–690, doi:10.1128/AEM.68.2.673-690.2002.
3. Ley, R.E.; Bäckhed, F.; Turnbaugh, P.; Lozupone, C.A.; Knight, R.D.; Gordon, J.I. Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11070–11075, doi:10.1073/pnas.0504978102.
4. Rescigno, M. Intestinal microbiota and its effects on the immune system. *Cell. Microbiol.* **2014**, *16*, 1004–1013, doi:10.1111/cmi.12301.
5. Carding, S.; Verbeke, K.; Vipond, D.T.; Corfe, B.M.; Owen, L.J. Dysbiosis of the gut microbiota in disease. *Microb. Ecol. Health Dis.* **2015**, *26*, 26191, doi:10.3402/mehd.v26.26191.
6. Vaiserman, A.M.; Koliada, A.K.; Marotta, F. Gut microbiota: A player in aging and a target for anti-aging intervention. *Ageing Res. Rev.* **2017**, *35*, 36–45, doi:10.1016/j.arr.2017.01.001.
7. Franceschi, C.; Capri, M.; Monti, D.; Giunta, S.; Olivieri, F.; Sevini, F.; Panourgia, M.P.; Invidia, L.; Celani, L.; Scurti, M.; et al. Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. *Mech. Ageing Dev.* **2007**, *128*, 92–105, doi:10.1016/j.mad.2006.11.016.
8. Mullin, J.M.; Valenzano, M.C.; Verrecchio, J.J.; Kothari, R. Age- and diet-related increase in transepithelial colon permeability of Fischer 344 rats. *Dig. Dis. Sci.* **2002**, *47*, 2262–2270, doi:10.1023/A:1020191412285.
9. Tran, L.; Greenwood-Van Meerveld, B. Age-associated remodeling of the intestinal epithelial barrier. *J. Gerontol. A Biol. Sci. Med. Sci.* **2013**, *68*, 1045–1056, doi:10.1093/gerona/glt106.
10. Miró, L.; Garcia-Just, A.; Amat, C.; Polo, J.; Moretó, M.; Pérez-Bosque, A. Dietary animal plasma proteins improve the intestinal immune response in senescent mice. *Nutrients* **2017**, *9*, 1346, doi:10.3390/nu9121346.
11. Garcia-Just, A.; Miró, L.; Pérez-Bosque, A.; Amat, C.; Polo, J.; Pallàs, M.; Griñán-Ferré, C.; Moretó, M. Dietary Spray-Dried Porcine Plasma Prevents Cognitive Decline in Senescent Mice and Reduces Neuroinflammation and Oxidative Stress. *J. Nutr.* **2020**, *150*, 303–311, doi:10.1093/jn/nxz239.
12. Konturek, P.C.; Haziri, D.; Brzozowski, T.; Hess, T.; Heyman, S.; Kwiecien, S.; Konturek, S.J.; Koziel, J. Emerging role of fecal microbiota therapy in the treatment of gastrointestinal and extra-gastrointestinal diseases. *J. Physiol. Pharmacol.* **2015**, *66*, 483–491.
13. Bischoff, S.C. Microbiota and aging. *Curr. Opin. Clin. Nutr. Metab. Care* **2016**, *19*, 26–30, doi:10.1097/MCO.0000000000000242.
14. Pérez Martínez, G.; Bäuerl, C.; Collado, M.C. Understanding gut microbiota in elderly's health will enable intervention through probiotics. *Benef. Microbes* **2014**, *5*, 235–246, doi:10.3920/BM2013.0079.
15. Moretó, M.; Miró, L.; Amat, C.; Polo, J.; Manichanh, C.; Pérez-Bosque, A. Dietary supplementation with spray-dried porcine plasma has prebiotic effects on gut microbiota in mice. *Sci. Rep.* **2020**, *10*, 2926, doi:10.1038/s41598-020-59756-z.
16. Larbi, A.; Pawelec, G.; Wong, S.C.; Goldeck, D.; Tai, J.J.; Fulop, T. Impact of age on T cell signaling: A general defect or specific alterations? *Ageing Res. Rev.* **2011**, *10*, 370–378, doi:10.1016/j.arr.2010.09.008.
17. Franceschi, C.; Salvioli, S.; Garagnani, P.; de Eguileor, M.; Monti, D.; Capri, M. Immunobiography and the Heterogeneity of Immune Responses in the Elderly: A Focus on Inflammaging and Trained Immunity. *Front. Immunol.* **2017**, *8*, 982, doi:10.3389/fimmu.2017.00982.
18. Larbi, A.; Franceschi, C.; Mazzatti, D.; Solana, R.; Wikby, A.; Pawelec, G. Aging of the immune system as a prognostic factor for human longevity. *Physiology* **2008**, *23*, 64–74, doi:10.1152/physiol.00040.
19. Pickard, J.M.; Zeng, M.Y.; Caruso, R.; Núñez, G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. *Immunol. Rev.* **2017**, *9*, 70–89, doi:10.1111/imr.12567.
20. Claesson, M.J.; Cusack, S.; O'Sullivan, O.; Greene-Diniz, R.; de Weerd, H.; Flannery, E.; Marchesi, J.R.; Falush, D.; Dinan, T.; Fitzgerald, G.; et al. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc. Natl. Acad. Sci. USA* **2011**, *108* (Suppl. 1), 4586–4591, doi:10.1073/pnas.1000097107.
21. Hopkins, M.J.; Sharp, R.; Macfarlane, G.T. Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. *Gut* **2001**, *48*, 198–205, doi:10.1136/gut.48.2.198.

22. Claesson, M.J.; Jeffery, I.B.; Conde, S.; Power, S.E.; O'Connor, E.M.; Cusack, S.; Harris, H.M.; Coakley, M.; Lakshminarayanan, B.; O'Sullivan, O.; et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature* **2012**, *488*, 178–184, doi:10.1038/nature11319.
23. McDonald, K.G.; Leach, M.R.; Huang, C.; Wang, C.; Newberry, R.D. Aging impacts isolated lymphoid follicle development and function. *Immun. Ageing* **2011**, *8*, 1, doi:10.1186/1742-4933-8-1.
24. Rehman, T. Role of the gut microbiota in age-related chronic inflammation. *Endocr. Metab. Immune Disord. Drug Targets* **2012**, *12*, 361–367, doi:10.2174/187153012803832620.
25. Odamaki, T.; Kato, K.; Sugahara, H.; Hashikura, N.; Takahashi, S.; Xiao, J.Z.; Abe, F.; Osawa, R. Age-related changes in gut microbiota composition from newborn to centenarian: A cross-sectional study. *BMC Microbiol.* **2016**, *16*, 90, doi:10.1186/s12866-016-0708-5.
26. Thevaranjan, N.; Puchta, A.; Schulz, C.; Naidoo, A.; Szamosi, J.C.; Verschoor, C.P.; Loukov, D.; Schenck, L.P.; Jury, J.; Foley, K.P.; et al. Age-Associated Microbial Dysbiosis Promotes Intestinal Permeability, Systemic Inflammation, and Macrophage Dysfunction. *Cell Host Microbe* **2017**, *21*, 455–466, doi:10.1016/j.chom.2017.03.002.
27. Fransen, F.; van Beek, A.A.; Borghuis, T.; Aidy, S.E.; Hugenholtz, F.; van der Gaast-de Jongh, C.; Savelkoul, H.F.J.; De Jonge, M.I.; Boekschoten, M.V.; Smidt, H.; et al. Aged Gut Microbiota Contributes to Systemic Inflammation after Transfer to Germ-Free Mice. *Front. Immunol.* **2017**, *8*, 1385, doi:10.3389/fimmu.2017.01385.
28. Håkansson, Å.; Tormo-Badia, N.; Baridi, A.; Xu, J.; Molin, G.; Hagslätt, M.L.; Karlsson, C.; Jeppsson, B.; Cilio, C.M.; Ahm, S. Immunological alteration and changes of gut microbiota after dextran sulfate sodium (DSS) administration in mice. *Clin. Exp. Med.* **2015**, *15*, 107–120, doi:10.1007/s10238-013-0270-5.
29. Rowan, F.; Docherty, N.G.; Murphy, M.; Murphy, B.; Calvin Coffey, J.; O'Connell, P.R. Desulfovibrio bacterial species are increased in ulcerative colitis. *Dis. Colon Rectum* **2010**, *53*, 1530–1536, doi:10.1007/DCR.0b013e3181f1e620.
30. Mukhopadhyay, I.; Hansen, R.; El-Omar, E.M.; Hold, G.L. IBD-what role do Proteobacteria play? *Nat. Rev. Gastroenterol. Hepatol.* **2012**, *9*, 219–230, doi:10.1038/nrgastro.2012.14.
31. Jakobsson, H.E.; Rodriguez-Pineiro, A.M.; Schutte, A.; Ermund, A.; Boysen, P.; Bemark, M.; Sommer, F.; Bäckhed, F.; Hansson, G.C.; Johansson, M.E. The composition of the gut microbiota shapes the colon mucus barrier. *EMBO Rep.* **2014**, *16*, 164–177, doi:10.15252/embr.201439263.
32. Ottman, N.; Davids, M.; Suarez-Diez, M.; Boeren, S.; Schaap, P.J.; Martins Dos Santos, V.A.P.; Smidt, H.; Belzer, C.; de Vos, W.M. Genome-scale model and omics analysis of metabolic capacities of *Akkermansia muciniphila* reveal a preferential mucin-degrading lifestyle. *Appl. Environ. Microbiol.* **2017**, *83*, 1014–1017, doi:10.1128/AEM.01014-17.
33. Walrath, T.; Dyamenahalli, K.U.; Hulsebus, H.J.; McCullough, R.L.; Idrovo, J.P.; Boe, D.M.; McMahan, R.H.; Kovacs, E.J. Age-related changes in intestinal immunity and the microbiome. *J. Leukoc. Biol.* **2020**, doi:10.1002/JLB.3RI0620-405RR.
34. Cox, L.M.; Schafer, M.J.; Sohn, J.; Vincentini, J.; Weiner, H.L.; Ginsberg, S.D.; Blaser, M.J. Calorie restriction slows age-related microbiota changes in an Alzheimer's disease model in female mice. *Sci. Rep.* **2019**, *9*, 17904, doi:10.1038/s41598-019-54187-x.
35. Berer, K.; Gerdes, L.A.; Cekanaviciute, E.; Jia, X.; Xiao, L.; Xia, Z.; Liu, C.; Klotz, L.; Stauffer, U.; Baranzini, S.E.; et al. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 10719–10724, doi:10.1073/pnas.1711233114.
36. Sampson, T.R.; Debelius, J.W.; Thron, T.; Janssen, S.; Shastri, G.G.; Ilhan, Z.E.; Challis, C.; Schretter, C.E.; Rocha, S.; Gradinaru, V.; et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* **2016**, *167*, 1469–1480, doi:10.1016/j.cell.2016.11.018.
37. Sharon, G.; Cruz, N.J.; Kang, D.W.; Gandal, M.J.; Wang, B.; Kim, Y.M.; Zink, E.M.; Casey, C.P.; Taylor, B.C.; Lane, C.J.; et al. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. *Cell* **2019**, *177*, 1600–1618.e17, doi:10.1016/j.cell.2019.05.004.
38. Cattaneo, A.; Cattane, N.; Galluzzi, S.; Provasi, S.; Lopizzo, N.; Festari, C.; Ferrari, C.; Guerra, U.P.; Paghera, B.; Muscio, C.; et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiol. Aging* **2017**, *49*, 60–68, doi:10.1016/j.neurobiolaging.2016.08.019.
39. Zhao, Y.; Lukiw, W.J. Bacteroidetes Neurotoxins and Inflammatory Neurodegeneration. *Mol. Neurobiol.* **2018**, *55*, 9100–9107, doi:10.1007/s12035-018-1015-y.

40. Tran, T.T.T.; Corsini, S.; Kellingray, L.; Hegarty, C.; Le Gall, G.; Narbad, A.; Müller, M.; Tejera, N.; O'Toole, P.W.; Minihane, A.M.; et al. APOE genotype influences the gut microbiome structure and function in humans and mice: Relevance for Alzheimer's disease pathophysiology. *FASEB J.* **2019**, *33*, 8221–8231, doi:10.1096/fj.201900071R.
41. Forsyth, C.B.; Shannon, K.M.; Kordower, J.H.; Voigt, R.M.; Shaikh, M.; Jaglin, J.A.; Estes, J.D.; Dodiya, H.B.; Keshavarzian, A. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS ONE* **2011**, *6*, e28032, doi:10.1371/journal.pone.0028032.
42. Crews, L.; Tsigelny, I.; Hashimoto, M.; Masliah, E. Role of synucleins in Alzheimer's disease. *Neurotox. Res.* **2009**, *16*, 306–317, doi:10.1007/s12640-009-9073-6.
43. Albenberg, L.G.; Wu, G.D. Diet and the intestinal microbiome: Associations, functions, and implications for health and disease. *Gastroenterology* **2014**, *146*, 1564–1572, doi:10.1053/j.gastro.2014.01.058.
44. Zhuang, Z.Q.; Shen, L.L.; Li, W.W.; Fu, X.; Zeng, F.; Gui, L.; Lü, Y.; Cai, M.; Zhu, C.; Tan, Y.L.; et al. Gut Microbiota is Altered in Patients with Alzheimer's Disease. *J. Alzheimers Dis.* **2018**, *63*, 1337–1346, doi:10.3233/JAD-180176.
45. Junges, V.M.; Closs, V.E.; Nogueira, G.M.; Gottlieb, M.G.V. Crosstalk between gut microbiota and the central nervous system: A focus for Alzheimer's disease. *Curr. Alzheimer Res.* **2018**, *15*, 1179–1190. 10.2174/1567205015666180904155908.
46. Bajaj, J.S.; Hylemon, P.B.; Ridlon, J.M.; Heuman, D.M.; Daita, K.; White, M.B.; Monteith, P.; Noble, N.A.; Sikaroodi, M.; Gillevet, P.M. Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2012**, *303*, G675–G685, doi:10.1152/ajpgi.00152.2012.
47. Askarova, S.; Umbayev, B.; Masoud, A.R.; Kaiyrykyzy, A.; Safarova, Y.; Tsoy, A.; Olzhayev, F.; Kushugulova, A. The Links Between the Gut Microbiome, Aging, Modern Lifestyle and Alzheimer's Disease. *Front. Cell. Infect. Microbiol.* **2020**, *10*, 104, doi:10.3389/fcimb.2020.00104.

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