The Aging Gut Microbiota

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Abstract Researchers have detailed changes in host–intestinal microbe homeostasis in elderly humans, but it is not clear whether gut microbiota influence these changes, or if maintaining intestinal homeostasis would support overall health with age. Insight into age-related changes in hosts and their microbiota has been gained by studying vertebrate models such as mice, rats, and African turquoise killifish, and invertebrates, including *Drosophila melanogaster* and *Caenorhabditis elegans*. Studies using aged, germ-free models show that intestinal microbiota do not initiate all age-related pathologies, suggesting that host-specific changes may be a factor in declining host–intestinal microbe homeostasis with age. Although it is not clear how model-based host–intestinal microbe research applies to the elderly, understanding the interplay between aging hosts and gut microbiota will be critical toward the design of therapeutic interventions. Since research on aging microbiota systems is an emerging field, further developments may come through attempts to translate model findings to humans.

Keywords Aging microbiome · Inflammaging · Intestinal permeability · Healthy aging · Age-associated dysbiosis · Model organisms

Introduction

With a growing population of longer-living people, the promotion of healthy aging is an increasingly urgent task. Our intestinal microbiota has gained attention because of the notable changes in host–microbe homeostasis in aged hosts, though there is great difficulty in distinguishing the physiological changes associated with the

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aging host from that of microbe-driven pathologies. Being able to make those distinctions will be of clinical importance, especially in the promotion of healthy aging.

The gut is a highly complex organ system with a tremendous surface area. It not only serves as a barrier against luminal macromolecules and microbes, but it is also involved in immune function, digestion, and nutrient assimilation. Like any other organ system, gut aging is accompanied by physiological changes that lead to impaired function, with ultimately far-reaching consequences on health. For example, age-related changes in intestinal transit time (Woodmansey 2007) and in gut function can impact nutritional intake and absorption (Lovat 1996), potentially exacerbating diet-related influences on intestinal and organismal physiology.

Evidence from invertebrate and vertebrate model organisms suggests that gut barrier integrity is compromised with age (Tran and Greenwood-Van Meerveld 2013; Tricoire and Rera 2015; Dambroise et al. 2016; Gelino et al. 2016; Rera et al. 2018); in mice and the invertebrate model *Drosophila melanogaster*, this change in intestinal permeability is thought to allow the translocation of bacteria or bacterial products from the lumen into circulation (Li et al. 2016; Thevaranjan et al. 2017). The host is then thought to mount an inflammatory response against the leaked microbial signatures. Although it is not yet known if a 'leaky gut' is a natural occurrence in the elderly, aged humans do show increased inflammation. Further, the ability to resolve inflammation may be impaired with advancing age (Sarkar and Fisher 2006). When the homeostatic balance of the immune system is no longer in check, an age-associated inflammatory state, dubbed 'inflammaging', may ensue, resulting in a chronic, low state of inflammation (Franceschi et al. 2000, 2007). Chronic, low-grade inflammation may contribute to a range of comorbidities, accentuating the aging phenotype.

There are documented examples of age-associated effects on components of the intestinal barrier and immune system in humans, but what about the gut microbiota? Here, we overview studies showing that aging is accompanied by changes in the composition of the gut microbiota, but the extent to which these changes are causes or consequences of aging gut physiology remains uncertain.

What Is a 'Healthy' Gut Microbiota?

The gut was believed to be sterile up until birth, although recent studies point to the highly debated possibility of *in utero* colonization (Jimenez et al. 2005; Rautava et al. 2012; Collado et al. 2016; de Goffau et al. 2019; Martin et al. 2016; Perez-Munoz et al. 2017). As delineated earlier in this book, mode of delivery and feeding influence the early intestinal microbiota composition, with possible consequences for immune system development (Hallstrom et al. 2004; Rutayisire et al. 2016). The early life microbiota may be an important factor in health outcomes, as Cesarean births are sometimes associated with higher incidences of immune-related disorders in early life (Negele et al. 2004). Indeed, microbes have been found to influence the developing immune system and have an impact on mucosal and systemic immune

tissues (Macpherson and Harris 2004; Malamitsi-Puchner et al. 2005; Hooper et al. 2012; Tamburini et al. 2016).

With the introduction of solid foods, the composition of gut microbiota is diversified and at around age 3 begins to resemble that of the adult, after which differences resulting from the mode of delivery and breast or formula feeding are less pronounced (Koenig et al. 2011; Yatsunenko et al. 2012; Duncan and Flint 2013). The gut microbiota continues to diversify with age up through adulthood, although there are individual differences in this maturation process (Odamaki et al. 2016). Microbes are housed throughout the intestinal tract, continually increasing in abundance and found in the highest amount in the large intestine (O'Hara and Shanahan 2006). The precise composition of microbiota in the adult gut varies between individuals and even among siblings, although there is thought to be a core, shared microbiota amongst different people (Qin et al. 2010; Yatsunenko et al. 2012). It is thought that greater than 20% of the observed interindividual variation in microbiota composition is due to diet and other environmental factors as opposed to host genetics (Rothschild et al. 2018). And though the gut microbiota is responsive to environmental perturbations, the microbial communities inhabiting an individual remain relatively stable (Costello et al. 2009).

The characterization of a healthy microbiota might assist in the diagnosis and intervention of health conditions associated with alterations in gut microbes. Identifying what constitutes a healthy microbiota, however, has proven difficult despite a number of population-scale studies that have set out to do so (Human Microbiome Project Consortium 2012). The microbial composition of subjects ranging in age and geography have been measured to establish common microbial features (Turnbaugh et al. 2007) and have generally focused on searching for taxa abundant in healthy guts (Lloyd-Price et al. 2016). *Faecalibacterium prausnitzii*, a member of Firmicutes, is one of the most abundant species in a healthy intestine and has anti-inflammatory properties; a loss in abundance has been observed in various intestinal disorders such as irritable bowel disease (Mueller et al. 2006; Sokol et al. 2008; Miquel et al. 2013; Cao et al. 2014). Not all microbes are ubiquitous across humans, and a low prevalence of certain microbial groups does not indicate the lack of functionality. Lesser-represented groups in some Western populations, such as methanogenic archaea, are important despite their low relative abundance; methanogens are useful for energy harvest from ingested food (Walker 2007).

Attempts to identify imbalances that reflect disease states are complicated by interindividual diversity and by the existence of a range of possible 'healthy' microbiota configurations (Lloyd-Price et al. 2016). An 'unhealthy' gut may be defined by a disproportionate amount of pathogenic bacteria, for example, *Clostridium difficile*, or when disease phenotypes manifest in the host as a result of an unbalanced intestinal microbiota, or intestinal dysbiosis (Bien et al. 2013; Henderson and Nibali 2016). Alternatively, attempts to characterize a 'healthy' microbiome can use a metagenomic approach that focuses on the functionality of genes present (Lozupone et al. 2012; Rosen and Palm 2017). An analysis of fecal metagenomes (the genetic material isolated from fecal samples) of humans from different countries found that 12 genetic biomarkers correlate with increasing age, including an elevation of

digestive enzymes that degrade starch (Arumugam et al. 2011). These findings point to a potential use of microbial biomarkers for the detection of an 'aging' microbiota (Arumugam et al. 2011).

A high degree of diversity in an ecosystem is sometimes considered better for adaptation to environmental stresses. Similarly, the diverse gut microbiota is somewhat malleable, responding to dietary changes to the potential benefit of the host (David et al. 2014; Biagi et al. 2017). A system of checks and balances may render a diverse microbiota ecosystem less susceptible to disease (Candela et al. 2012). Therefore, instead of defining a healthy microbiota by a set of taxa known to support health, it may be more informative to identify a set of general characteristics such as microbe diversity, stability, and plasticity (Backhed et al. 2012; Lloyd-Price et al. 2016).

The Elderly Gut Microbiota

Though diversity and stability of the gut microbiome increases with age beginning at birth and throughout early life (Palmer et al. 2007; Koenig et al. 2011; Yatsunenko et al. 2012; Rodriguez et al. 2015), the general trajectory for the aging gut is a loss of biodiversity (Woodmansey 2007; Biagi et al. 2016), compromised stability, and greater individual variation (Claesson et al. 2011, 2012). Although increased inter-individual variation makes it difficult to make generalizations, there are some broad trends and commonalities that are worth mentioning. A study focused on humans ranging in age from adulthood to centenarians and beyond identified a core set of shared microbes that were found to change in abundance over time. The dominant core microbiota was mostly comprised of Ruminococcaceae, Lachnospiraceae, and Bacteroidaceae (Biagi et al. 2016). While this dominant core shrank in representation in increasingly aged humans, subdominant groups increased in abundance (Biagi et al. 2016). Across multiple studies, facultative anaerobes, streptococci, staphylococci, enterococci, and enterobacteria were among the microbial groups elevated with age (Candela et al. 2014). However, the same intestinal microbiota shifts are not always common across studies (Magrone and Jirillo 2013).

Studies on aging humans are often focused on distinct populations and are therefore not always broadly applicable. As such, there are differences found in studies across various groups of aging humans, and the conflicting observations are at least partially due to differences in diet and other environmental factors between cohorts (Magrone and Jirillo 2013). Further, the experimental design, microbe sampling method (Biagi et al. 2012), and targeted populations in clinical trials can lead to varying results between studies and lower the translatability of datasets. That said, it is possible to identify similarities across distinct human populations. A study focused on multiple European countries found that enterobacteria levels were increased in the elderly sampled across countries; between-cohort differences, however, were also noted in the *Bifidobacterium* group within the same study (Mueller et al. 2006). For these reasons, we focus largely on one dataset that used a unique

study design to capture the interplay between gut microbiota and the environment of aging humans.

The ELDERMET project was designed to identify links between health and gut microbiota structure within elderly Irish subjects. The ongoing project has been enacted in phases; one study tracked the elderly across different living situations and care facilities to provide insight into the interaction between health status, diet, lifestyle, and microbiota composition in aged humans. Subjects were categorized as one of the following: individuals living in the community, making out-patient hospital visits, receiving short term care (<6 weeks) for rehabilitation, or residing long-term in residential care. The study identified several shifts in fecal microbiota associated with residence, which also closely associated with diet (Claesson et al. 2012). The extent to which diet is a controllable factor to modulate age-related disease remains a major line of current research to determine if specialized diets can delay the onset of age-related illness.

Most of the long-stay subjects reported a diet that was moderate to high in fat and low in fiber, and their fecal metabolites revealed higher levels of glucose, glycine, and lipids. Microbiota from long-term care residents was composed of a higher proportion of Bacteroidetes over Firmicutes and was associated with *Parabacteroides*, *Eubacterium*, *Anaerotruncus*, *Lactonifactor*, and *Coprobacillus* genera. In contrast, the majority of community dwellers reported diets classified as low to moderate in fat, and high in fiber. Community dwellers had higher levels of the metabolites glutarate, butyrate, acetate, propionate, and valerate. Further, their microbiota showed more abundant *Coprococcus* and *Roseburia* at the genus level, and Lachnospiraceae was among the most prominent of associated families. Those that ate a diet classified as low fat/high fiber not only had the most diverse diet but also had the most diverse intestinal microbiota (Claesson et al. 2012). It is possible that those living at home had more exposure to a variety of foods. These findings may indicate that care facilities can benefit from diversified food menus that promote intestinal microbiota diversity.

The shifts observed in elderly gut microbiota were reflective of changes in health as measured by a number of indices, including mental state, inflammatory markers, and functional independence. A loss of certain community-associated microbes was correlated with increased measures of frailty (Claesson et al. 2012). Frailty can be a useful indicator of health deficit, and studies have shown that reduced microbiota diversity is associated with increased frailty (Jackson et al. 2016). There are reported differences in fecal microbiota composition between elderly persons with low and high frailty scores. For example, Lactobacilli, *Bacteroides*/*Prevotella,* and *F. prausnitzii* were decreased, while Enterobacteriaceae were increased in the high-frailty subjects (van Tongeren et al. 2005). These microbial changes associated with frailty may represent diagnostic targets to monitor as individuals age.

Longitudinal studies following humans across their lifespan are not readily attainable; these studies are more feasible in shorter-lived animal models. Some human studies are semi-longitudinal over a brief portion of the human lifespan, however most are cross-sectional, whereby representative groups are sampled at one point in time. Although longitudinal studies may be ideal to record age-related

trajectories, cross-sectional studies have provided insight into broad differences across age, health, and lifestyle cohorts. Numerous studies not covered here detected shifts in the intestinal microbiota of aged humans (Hopkins et al. 2001; Hopkins and Macfarlane 2002; Hayashi et al. 2003; Woodmansey et al. 2004; Mariat et al. 2009; Biagi et al. 2010; Rampelli et al. 2013; Odamaki et al. 2016; Buford 2017), including other ELDERMET consortium studies (Claesson et al. 2011; Jeffery et al. 2016). It is worth reiterating that the variation in gut microbiota composition and the specificity of human studies can complicate attempts to reveal common associations between microbes and host age; the degree to which observed changes are due to dietary or lifestyle factors, or are part of the natural aging process, is not always clear. Future research may benefit from an integrative approach to reveal how environmental factors impact a broader range of human populations.

Age-Related Changes in the Host–Microbiota System

Existing studies have detected age-related changes in the composition of gut microbial populations (Buford 2017), leading to an interest in detailing causative factors. One proposed causal factor is a change in nutrition in older adults (Lu and Wang 2018), which can be driven by natural processes, age-related illnesses, or behavioral and lifestyle changes (Nagpal et al. 2018; Riaz Rajoka et al. 2018). Beyond changes in physiologic systems, external factors including the environment and how an individual responds to their environment can also influence the intestinal microbiota in an age-dependent manner. Elderly humans show a higher threshold for sweet, salty, sour, and bitter tastants, indicating that taste is altered with age (Fukunaga et al. 2005), potentially contributing to changes in food intake. Since changes in nutrition correlate with fecal microbiota composition in healthy (Wu et al. 2011) and elderly humans (Claesson et al. 2012), the factors that alter dietary intake with age may interact to influence intestinal microbiota during the aging process. It is not yet clear if specific diets can prevent aging-related microbial changes. However, it was proposed that achieving optimal levels of protein, fiber, and fat may support intestinal and immune health in the elderly (Clements and Carding 2018).

In addition to changes in nutrition, antibiotic use in elderly patients can reduce or eradicate certain microbial species in fecal microbiota (Bartosch et al. 2004). The rising antibiotic use in residential care facilities (Lim et al. 2014) and in older United States residents (Lee et al. 2013, 2014) represents an increasingly influential factor on intestinal microbes. Additionally, living conditions can impact how intestinal microbiota respond to antibiotic treatment (Jeffery et al. 2016), making it difficult to perform controlled analyses on human populations. Given the myriad of factors that influence intestinal microbiota in humans, it follows that laboratory models are commonly used for a more controlled approach to researching aging host–microbe systems. The use of model systems has produced some of the most informative data to date on age-associated changes in hosts and their intestinal microbes (Maynard and Weinkove 2018).

Humans and model organisms experience age-associated changes in systemic and intestinal immunity (Man et al. 2014); models are useful to study aging host–microbe systems because altered immune regulation can impact microbial symbionts, and vice versa. Advancing studies use models to pursue a century-old hypothesis generated by Elie Metchnikoff: maleffects of old age stem from changes in intestinal microbiota and a restoration of host–microbe homeostasis can improve age-related illnesses (Metchnikoff 1908). To test this idea, researchers have begun detailing aging guts to determine if altered host–microbe homeostasis underlies broader age-related maladies.

One commonality of interest is the increase in chronic, systemic inflammation with age (i.e., inflammaging). An age-related increase in inflammation is seen in a range of organisms from insects (Rera et al. 2012; Clark et al. 2015; Li et al. 2016) to mice (Conley et al. 2016) and humans. The inflammation status of elderly humans is thought to be an indicator of disease and mortality risk (Franceschi and Campisi 2014). Although associative changes are known to occur along with inflammaging, the definitive cause of age-related inflammation remains mostly unknown. As a starting point for investigations of age-related inflammation, some research has focused on the innermost layer of the intestine, the mucosa.

The mucus layer coats the inner lining of the intestinal tract and comes into contact with luminal microbes; deterioration of this interface may be a source for the homeostatic breakdown between host and microbes. In rodents, the density of the colonic mucus layer varies both vertically and longitudinally, and this viscosity gradient can impact the distribution of colonic microbes (Swidsinski et al. 2007b). Although the mucus layer thickness varies across species, many animals (Varum et al. 2012) including humans have two colonic mucus layers (Matsuo et al. 1997) with a relatively dense inner layer adjacent to epithelial cell surfaces, and a less-dense outer layer exposed to the intestinal luminal contents. The inner layer is expected to be absent of microbes, whereas the outer layer is colonized with microbes, suggesting that microbes are typically partitioned from the epithelium by the dense inner mucosal layer (Johansson et al. 2008, 2011).

Since the mucus layer covering the intestinal epithelium acts as a barrier for those epithelial cells (Johansson 2014), a malfunctioning mucus layer is associated with translocation of bacteria into intestinal crypts and an increase in intestinal inflammation (Johansson et al. 2008; Johansson 2014). The mucus layer of the mouse colon declines with age (van Beek et al. 2016). Aged mice show a diminishing mucus layer and bacterial translocation into the mucus or even into the intestinal epithelium. These changes are associated with a change in microbiota composition and activation of the intestinal immune response (Elderman et al. 2017).

As in other organisms, the human colonic mucus layers largely prevent contact between intestinal microbes and the epithelium. An investigation of normal and inflamed colons from human subjects found an association with decreased mucus layer thickness and increased inflammation, as well as a migration of bacteria into the mucosa (Swidsinski et al. 2007a). Further, upon some intestinal insults, a diminished mucus layer is associated with increased intestinal epithelial permeability in rats (Qin et al. 2011; Fishman et al. 2013). Thus, once this protective mucosal layer

is diminished with age or with illness, the cellular barrier of the intestine may be compromised and an associated increase in inflammation can occur.

It has been proposed that a compromised intestinal barrier function upon age may allow gut microbes, or microbial products, to leak into non-tolerant areas (Franceschi and Campisi 2014); a translocation of microbial signatures then sparks a subsequent inflammatory response against exogenous products that hosts encounter in circulation. Indeed, aged mice show increased intestinal permeability—specifically, the colonic region shows higher paracellular permeability, indicating that the increased intestinal permeability is due to compromised passage between intercellular spaces (Thevaranjan et al. 2017). Aged mice also show higher levels of a bacterial cell-wall product called muramyl dipeptide outside of the intestinal lumen, which may indicate that displaced microbes or microbial products are indeed circulating systemically (Thevaranjan et al. 2017).

Mice null for TNF, a pro-inflammatory cytokine, have been used to determine how inflammation influences these age-associated changes. Aged TNF mutants do not show heightened systemic inflammation, compromised intestinal barrier function, or increased microbial signatures in circulation (Thevaranjan et al. 2017). Further, these mutants appear to have less prominent age-associated microbial adjustments, and anti-TNF therapy has the capacity to modulate microbial diversity (Thevaranjan et al. 2017). These results indicate that TNF-mediated inflammation may influence aging phenotypes related to altered gut barrier function and microbial composition.

Aged germ-free mice also lack some of the aforementioned age-related symptoms perhaps because of the absence of intestinal microbiota. Old germ-free mice do not show a decline in intestinal barrier function or increased systemic inflammation. Co-housing germ-free mice with young or old conventional mice exposed germ-free mice to a conventional, or 'standard', microbiota. Young germ-free mice exposed to aged donor mice demonstrate an increase in intestinal permeability and systemic inflammation (Thevaranjan et al. 2017). These results are consistent with a model suggesting that microbiota from aged individuals can drive these intestinal and systemic symptoms. However, no causal changes within the microbiota from aged mice have been identified. Thus, specific dysbiotic changes in the microbiota composition or quantity remain unknown. Interestingly, aged germ-free mice show increased TNF when exposed to microbes from both young and old mice, suggesting that older mice may also possess sensitivities to intestinal microbiota that are absent in younger mice (Thevaranjan et al. 2017). Sensitivity to microbiota upon age could potentially compound age-related symptoms.

In a similar study, fecal microbiota were transferred from young or old conventional mice into young, germ-free mice by oral gavage. This process exposed formerly germ-free mice to conventional youthful or aged mouse gut microbiota. After 4 weeks, recipients of 'old' microbes showed systemic immune activation along with an upregulation of several immune pathways in the small intestine (Fransen et al. 2017). These changes were not detected in young mice, or recipients of 'young' microbiota (Fransen et al. 2017). A bioinformatics analysis suggested that

lipopolysaccharides, molecules found on the outer membrane of some bacteria, induce the immune modulatory effect of 'old' microbiota (Fransen et al. 2017).

Further, cell culture-based tests indicated that sera from recipients of 'old' microbiota, but not from 'young' microbiota, may contain immune-stimulatory factors, a proxy measurement for microbial signatures (Fransen et al. 2017). This is consistent with a model in which the transfer of 'old' microbiota into young mice may lead to translocation of immune-activating bacterial moieties systemically. More definitive tests should be performed, however, since sera from conventional aged mice show no increased signatures of bacterial components when compared to young conventional mice. Changes in a few groups of bacteria including decreases in *Akkermansia* and increases in TM7 and Proteobacteria are associated with older mice or recipients of 'old' microbiota. These changes in microbiota composition are dynamic over a month-long period and it is not known if shifts in any of these groups are causative to the observed age-related outcomes (Fransen et al. 2017).

Many of the current studies on the interaction between microbiota and the aging host are associative. Further research is necessary to approach the status of clear, causal evidence. Overall, these studies show that age-related changes occur in both the host and microbiota across a range of animals, and that these changes are associated with negative health outcomes. As a result, there is great interest in finding ways to prevent or delay ailments of old age by treating both the host and intestinal microbiota.

Preventing Age-Related Deterioration by Genetically Manipulating the Host

Studies on *D. melanogaster* were some of the first to provide detailed information on the homeostatic changes in intestinal, commensal, and host physiology in aged animals. Some of the benefits of the fly model are its genetic tractability, relatively short lifespan (typically ranging from 30 to 80 days), and the similarities between mammalian and fly intestinal biology (Buchon et al. 2013; Marianes and Spradling 2013). These benefits allow rapid studies on the connection between host–commensal physiology, and organismal aging and longevity. A properly functioning intestinal barrier is influential on longevity in *D. melanogaster*. Further, the general status of fly–microbe homeostasis is indicative of intestinal barrier integrity and host mortality (Rera et al. 2012; Clark et al. 2015; Li et al. 2016). As flies age, they demonstrate changes in the configuration and numbers of intestinal microbes, and flies also show diminished barrier function (Guo et al. 2014; Clark et al. 2015). A recent focus on the etiology of intestinal and commensal maleffects has identified genetic manipulations that can impede or lessen these age-related breakdowns in host–microbe homeostasis, ultimately extending life.

The fly intestine normally comprises ten or more compartments (Buchon et al. 2013, Marianes and Spradling 2013) that are involved in the localization of luminal

microbes along the intestinal tract (Li et al. 2016). An acidic region of the gut is formed by the 'copper cells'. pH alterations of the acidic region can modulate microbiota levels, suggesting this distinct compartment has a regulatory role over gut microbes (Overend et al. 2016). When the acidic region is intact, microbes are most commonly housed within the anterior gut. When the copper cell region is genetically ablated, however, the quantity of luminal microbes increases throughout the intestinal tract (Li et al. 2016). As these changes occur, systemic inflammation elevates as measured by the activity of conserved pathways that control inflammation in mammals (Li et al. 2016). It is possible that this inflammatory response occurs as the fly responds to translocated microbial factors, similar to what was suggested in the mouse model (Fransen et al. 2017; Thevaranjan et al. 2017).

The copper cell region of aging flies undergoes metaplastic changes as copper cells are replaced with cell types typically found in other intestinal compartments; these changes are also demonstrated by germ-free animals (Li et al. 2016). Aged flies, even when germ-free, experience changes in septate junction protein localization that may negatively impact intestinal barrier integrity (Byri et al. 2015; Resnik-Docampo et al. 2017, 2018; Salazar et al. 2018). The occurrence of these cellular alterations in germ-free flies indicates that intestinal microbiota do not initiate these age-related intestinal pathologies. This is consistent with the possibility that age-related changes in the copper cell region and intercellular junctions may drive changes in host–microbe homeostasis. Importantly, these results suggest that genetic manipulations of the aging fly host could help pinpoint the onset of age-related malfunctions in fly–microbe homeostasis.

To determine the etiology of age-related intestinal pathologies, researchers selectively focused on the JAK/Stat pathway, which can control inflammatory-like responses against infection and is deregulated with age (Guo et al. 2014). Further analysis detected heightened JAK/Stat pathway activity in the intestine of aged flies. Importantly, intestinal JAK/Stat activation causes metaplastic changes in the copper cell region, comprised of both mis-differentiated and trans-differentiated cells (Li et al. 2016). These results indicate that JAK/Stat activation can impair intestinal partitions, one of the hallmarks of declining host–microbe homeostasis in the fly.

In further supportive studies, knocking-down JAK/Stat activity in the intestinal copper cell region counteracts negative health parameters in aged flies; these flies harbor lower counts of intestinal bacteria and showed cellular characteristics of younger flies (Li et al. 2016). Interestingly, these animals also have an increased lifespan even when germ-free, suggesting that JAK/Stat misregulation in the copper cell region generates negative health outcomes in aging flies, and inhibiting JAK/Stat activity reverses some of those symptoms to extend life. Further, the longer lifespan and intestinal compartment preservation in axenic flies with decreased JAK/Stat signaling adds evidence that changes in intestinal microbiota alone do not explain all of the ailments of age. Further studies are required to determine why intestinal decompartmentalization drives negative effects with age, and how these effects may influence host–microbe homeostasis.

Although there are some noted similarities between models and humans, such as late-life shifts in microbiota (Claesson et al. 2011; Guo et al. 2014; Clark et al.

2015; Conley et al. 2016; Li et al. 2016; Fransen et al. 2017; Thevaranjan et al. 2017) and increased inflammation (Franceschi and Campisi 2014; Li et al. 2016; Thevaranjan et al. 2017), whether the remaining age-related disturbances occur in humans is unknown. Therefore, while there are similarities between the age-related pathologies across mice and flies, the implication of these findings for humans is not definitive. Still, model systems have contributed valuable insight into potential mechanisms leading to age-related inflammation and intestinal decline. Continued work may enhance the translational power of models. Recent *Drosophila* studies suggest that microbes can influence host nutritional status or act as a nutritional resource (Ridley et al. 2012; Broderick et al. 2014; Wong et al. 2014; Chaston et al. 2016) to impact fly lifespan under certain conditions (Yamada et al. 2015; Bing et al. 2018; Keebaugh et al. 2018). It may be interesting to consider passive versus active microbial effects in an aging fly model, potentially by differentiating between microbes that stably colonize the fly intestine (Obadia et al. 2017; Pais et al. 2018) versus those that pass through during meals. Ultimately, modern studies with various models may provide a deeper understanding of the physiological alterations influencing host–microbe homeostasis and whether these changes impact longevity. Future research might also investigate how interventions targeting the aging intestine can influence host–microbiota outcomes.

Treating Age-Related Symptoms with Probiotics

Some of the intestinal microbial species that decrease in aged humans can be beneficial for preventing inflammatory responses. Whether compositional changes impacting these species influence increased age-related inflammation remains unknown (Rehman 2012). Research suggests that the immunomodulatory effect of some microbial strains is impacted by aging (You and Yaqoob 2012). Because of the potential link between microbiota, aging, and immune regulation, there is an interest in treating aging symptoms by promoting beneficial microbes.

Live microbes that promote health benefits when adequately consumed are generally referred to as probiotics (see chapter on "Probiotics and Prebiotics"). There are different ways probiotics can be administered, including as foods or as supplements, and probiotics can have a range of beneficial effects on hosts (Hill et al. 2014). Fermented foods have also been found to have beneficial effects in older adults (Turchet et al. 2003; Beausoleil et al. 2007; Fukushima et al. 2007; Hickson et al. 2007; Guillemard et al. 2010) although not all fermented foods can be considered probiotics given their unquantified amount of microbes. Further, it is not always straightforward to differentiate the benefits of microbes within fermented foods with those associated with the food item itself (Hill et al. 2014). That said, studies have found that dietary supplementation or fermented drinks with quantified levels of *Bifidobacterium* can increase the levels of these microbes in fecal samples (Ahmed et al. 2007; Lahtinen et al. 2009), and is correlated with an increase in

cellular immune function (Gill et al. 2001) and potentially beneficial shifts in pro- and anti-inflammatory cytokines in elderly subjects (Ouwehand et al. 2008).

Although some of the individual symptoms of age may be treated with probiotics, there is no current probiotic or fermented food regimen to prevent the suite of aforementioned age-related pathologies in host–microbe homeostasis. Most of the evidence for age-related probiotic treatments is largely produced in mice or in accelerated-aging mouse models. There is some evidence for specific microbial strains that can improve the intestinal permeability and longevity of aged mice. Middle-aged mice gavaged with *Bifidobacterium animalis* strain LKM512 three times a week show decreased colon permeability and improved survival rates over the 11-month dosing period (Matsumoto et al. 2011). Further, mice show suppressed systemic and colonic inflammation at 45 weeks of treatment (Matsumoto et al. 2011). Currently, the relevance of these findings in humans is unknown.

Many studies, including some using human subjects, focus on the bacterial species *Lactobacillus plantarum. L. plantarum* is a fermentative lactic acid bacterium that is found in a variety of food products and in the intestines of multiple animals (Ahrne et al. 1998; de Vries et al. 2006). A study testing the adherence capacity of different *L. plantarum* strains found that a majority of tested strains have the capacity to bind to a human-derived colonic cell line via what appears to be a mannose-specific mechanism, suggesting that some *L. plantarum* strains adhere to mannose-containing receptors within the intestine (Ahrne et al. 1998). Certain strains of *L. plantarum* from a fermented diet can survive the gastrointestinal tract and become associated with the intestinal mucosa in both healthy (Johansson et al. 1993) and ill patients (Klarin et al. 2005), although the capacity for *L. plantarum* to colonize the human intestinal tract varies (Johansson et al. 1993; Vesa et al. 2000). Since constant exposure is required for persistence of some strains (Vesa et al. 2000), recent attempts to identify 'persisting' *L. plantarum* strains are focusing on strains derived from healthy human guts as opposed to other sources (Suryavanshi et al. 2017). Such strains that are sustained within the intestine may be more suited for probiotic applications. To date, various *L. plantarum* strains have been tested for probiotic effects in human trials on patients harboring a diverse range of illnesses (Darby and Jones 2017); of potential interest for the aging population, *L. plantarum* strain 299v has the potential to attenuate systemic inflammation in ill patients (McNaught et al. 2005; Jones et al. 2013).

Recently, a mouse model of accelerated aging was used to test for the effects microbes have on aging intestines, since little is known about the impact of specific microbial strains on the aging gut. Accelerated-aging mutant mice and their wild-type littermates were exposed to *L. plantarum* strain WCFS1 by gavage three times per week for a 10-week period. The WCFS1 strain impedes the thinning of the colonic mucus barrier, which is a normal occurrence in the accelerated-aging mutants. Interestingly, there are no noted effects of WCFS1 supplementation in wild-type littermates, suggesting that the beneficial effects of this strain may be specific to aged animals (van Beek et al. 2016). As *L. plantarum* strain WCFS1 has a sequenced and annotated genome, it may provide a powerful system to investigate the mechanisms underlying beneficial effects in aging mice (Kleerebezem et al. 2003).

Conclusion and Future Directions

The expanse of recent gut microbiota research details a complex relationship between hosts and their associated microbes. It is increasingly evident that the maintenance of intestinal homeostasis may contribute to the overall health status of aged individuals. With a growing population of elderly people, understanding how an aging microbiota might accelerate or slow the pathophysiology of aging is of particular interest and may lead to novel therapeutics or dietary interventions that can restore intestinal homeostasis and support health.

It is currently unclear the degree to which aging gut physiology is a cause or consequence of the microbiota shifts accompanying age. Numerous studies on the elderly have detected changes in gut microbiota composition as well as increased levels of inflammation, but it is not known whether microbiota drive inflammaging. Although we do not yet understand the underlying etiological mechanisms in their entirety, we know that there are a number of factors that may compromise our homeostatic relationship with gut microbes, possibly tipping the scale toward a dysbiotic ecology. Although the appealing idea to enterically treat the suite of age-related gut and microbe alterations has no current support in humans, studies have demonstrated that certain microbes may have the ability to modify the host phenotype in ways that pertain to host health.

Diet is a somewhat controllable factor by which to manipulate gut microbiota, and a diverse, healthy diet is associated with a diverse gut microbiota. Modern approaches may help in the development of dietary interventions for aging humans. Researchers are investigating long-lived models of 'healthy aging' to identify lifestyle and dietary habits that might support the maintenance of microbial diversity and health with age (Kong et al. 2016, 2018; Franceschi et al. 2018), and considering biological markers of aging as opposed to chronological age to better understand the interaction of diet, aging, and the microbiota (Kim and Jazwinski 2018). These studies, in combination with longitudinal approaches (Santoro et al. 2018) and new genome-scale metabolic modeling methods (Kumar et al. 2016), may eventually reveal how physiological changes upon age impact nutritional intake and microbiota composition and reveal nutritional means by which aging humans can maintain health.

Researchers are responding to the mounting knowledge on aging intestinal microbiota with attempts to develop food-based or probiotic treatments. A downstream initiative from the ELDERMET studies, referred to as ELDERFOOD, is identifying food ingredients that support a healthy microbiota and overall health in the elderly. As researchers continue to catalog specific functions performed by particular microbial strains, we may see an increase in targeted therapeutic probiotics. Fermented foods are another abundant source of microbes, some of which are part of traditional diets. Future studies may focus on aging human subjects to infer beneficial effects of specific microbial strains or fermented foods. However, mechanistic investigations into age-related changes are likely to be restricted to genetically tractable model organisms.

Most of the aforementioned treatment-focused studies rely on model organisms, and they would not be possible without the prior progress made by aging model research. Fly and mouse research provided premier details on the interrelated, age-associated changes in host intestines, microbiota, and systemic immune regulation. Although research has found correlative changes between intestinal microbiota and age, causal roles that distinct microbial strains play in age-related changes have not yet been detailed. Subsequent work using models may focus on identifying specific dysbiotic changes that influence, or are characteristic of, age-related pathologies. Identifying specific dysbiotic shifts across animals may help to identify health- or age-associated microbes that may ultimately support direct probiotic developments.

There is still more to come from research pertaining to aging and gut microbiota across animal systems. The nematode *Caenorhabditis elegans* has been used to identify pro-longevity variants in *Escherichia coli* mutant libraries (Han et al. 2017); downstream efforts from this study may aid in the development of pro-longevity probiotics. *C. elegans* research has also demonstrated that intestinal microbes can influence drug efficiency (Garcia-Gonzalez et al. 2017; Scott et al. 2017), and recent studies in mice indicate that the microbiome can contribute greatly to drug metabolism (Zimmermann et al. 2019). Future work on modeling host–gut microbe–drug interactions may be important for aging humans because of the increasing polypharmacy observed with age (Charlesworth et al. 2015).

Aged African turquoise killifish lose gut microbe diversity during aging and live longer when colonized with microbiota from younger fish (Smith et al. 2017). This suggests that negative changes occur in killifish microbiota with age, and restoring microbiota to a more youthful state is beneficial to older fish. Model organisms have unique attributes and limitations (Douglas 2018). Although innate differences in gut anatomy or microbiota partitioning may interfere with translating findings from study organisms to humans (Nguyen et al. 2015; Keebaugh and Ja 2016), animal models will continue to be valued for their use in uncovering molecular mechanisms and in developing host- or microbe-targeted interventions.

Researchers have only scraped the surface in terms of aging microbiota research. In particular, microbial populations outside of the intestine are lesser-studied and may have significance for aged humans. For example, it has been suggested that toxic proteases from *Porphyromonas gingivalis,* a bacterium associated with periodontal disease, are found in higher levels in the brains of Alzheimer's patients; small-molecule inhibitors of those proteases reduced Alzheimer's-like disease pathology in the mouse brain and are now being tested in human trials (Dominy et al. 2019). Further interesting developments may come as researchers continue to compile and analyze data across species, and attempt to translate findings from model organisms to the human system.

References

Ahmed, M., Prasad, J., Gill, H., Stevenson, L., & Gopal, P. (2007). Impact of consumption of different levels of *Bifidobacterium lactis* HN019 on the intestinal microflora of elderly human subjects. *The Journal of Nutrition, Health & Aging, 11*(1), 26–31.

Ahrne, S., Nobaek, S., Jeppsson, B., Adlerberth, I., Wold, A. E., & Molin, G. (1998). The normal Lactobacillus flora of healthy human rectal and oral mucosa. *Journal of Applied Microbiology, 85*(1), 88–94.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., Leclerc, M., Levenez, F., Manichanh, C., Nielsen, H. B., Nielsen, T., Pons, N., Poulain, J., Qin, J., Sicheritz-Ponten, T., Tims, S., Torrents, D., Ugarte, E., Zoetendal, E. G., Wang, J., Guarner, F., Pedersen, O., de Vos, W. M., Brunak, S., Dore, J., Meta, H. I. T. C., Antolin, M., Artiguenave, F., Blottiere, H. M., Almeida, M., Brechot, C., Cara, C., Chervaux, C., Cultrone, A., Delorme, C., Denariaz, G., Dervyn, R., Foerstner, K. U., Friss, C., van de Guchte, M., Guedon, E., Haimet, F., Huber, W., van Hylckama-Vlieg, J., Jamet, A., Juste, C., Kaci, G., Knol, J., Lakhdari, O., Layec, S., Le Roux, K., Maguin, E., Merieux, A., Melo Minardi, R., M'Rini, C., Muller, J., Oozeer, R., Parkhill, J., Renault, P., Rescigno, M., Sanchez, N., Sunagawa, S., Torrejon, A., Turner, K., Vandemeulebrouck, G., Varela, E., Winogradsky, Y., Zeller, G., Weissenbach, J., Ehrlich, S. D., & Bork, P. (2011). Enterotypes of the human gut microbiome. *Nature, 473*(7346), 174–180.

Backhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., Versalovic, J., Young, V., & Finlay, B. B. (2012). Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. *Cell Host & Microbe, 12*(5), 611–622.

Bartosch, S., Fite, A., Macfarlane, G. T., & McMurdo, M. E. (2004). Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Applied and Environmental Microbiology, 70*(6), 3575–3581.

Beausoleil, M., Fortier, N., Guenette, S., L'Ecuyer, A., Savoie, M., Franco, M., Lachaine, J., & Weiss, K. (2007). Effect of a fermented milk combining *Lactobacillus acidophilus* CL1285 and *Lactobacillus casei* in the prevention of antibiotic-associated diarrhea: A randomized, double-blind, placebo-controlled trial. *Canadian Journal of Gastroenterology, 21*(11), 732–736.

Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucci, L., Pini, E., Nikkila, J., Monti, D., Satokari, R., Franceschi, C., Brigidi, P., & De Vos, W. (2010). Through ageing, and beyond: Gut microbiota and inflammatory status in seniors and centenarians. *PLoS One, 5*(5), e10667.

Biagi, E., Candela, M., Fairweather-Tait, S., Franceschi, C., & Brigidi, P. (2012). Ageing of the human metaorganism: The microbial counterpart. *Age, 34*(1), 247–267.

Biagi, E., Franceschi, C., Rampelli, S., Severgnini, M., Ostan, R., Turroni, S., Consolandi, C., Quercia, S., Scurti, M., Monti, D., Capri, M., Brigidi, P., & Candela, M. (2016). Gut microbiota and extreme longevity. *Current Biology, 26*(11), 1480–1485.

Biagi, E., Rampelli, S., Turroni, S., Quercia, S., Candela, M., & Brigidi, P. (2017). The gut microbiota of centenarians: Signatures of longevity in the gut microbiota profile. *Mechanisms of Ageing and Development, 165*(Pt B), 180–184.

Bien, J., Palagani, V., & Bozko, P. (2013). The intestinal microbiota dysbiosis and *Clostridium difficile* infection: Is there a relationship with inflammatory bowel disease? *Therapeutic Advances in Gastroenterology, 6*(1), 53–68.

Bing, X., Gerlach, J., Loeb, G., & Buchon, N. (2018). Nutrient-dependent impact of microbes on *Drosophila suzukii* development. *MBio, 9*(2), e02199.

Broderick, N. A., Buchon, N., & Lemaitre, B. (2014). Microbiota-induced changes in drosophila melanogaster host gene expression and gut morphology. *MBio, 5*(3), e01117–e01114.

Buchon, N., Osman, D., David, F. P., Fang, H. Y., Boquete, J. P., Deplancke, B., & Lemaitre, B. (2013). Morphological and molecular characterization of adult midgut compartmentalization in Drosophila. *Cell Reports, 3*(5), 1725–1738.

Buford, T. W. (2017). (Dis)Trust your gut: The gut microbiome in age-related inflammation, health, and disease. *Microbiome, 5*(1), 80.

Byri, S., Misra, T., Syed, Z. A., Batz, T., Shah, J., Boril, L., Glashauser, J., Aegerter-Wilmsen, T., Matzat, T., Moussian, B., Uv, A., & Luschnig, S. (2015). The triple-repeat protein anakonda controls epithelial tricellular junction formation in *Drosophila*. *Developmental Cell, 33*(5), 535–548.

Candela, M., Biagi, E., Maccaferri, S., Turroni, S., & Brigidi, P. (2012). Intestinal microbiota is a plastic factor responding to environmental changes. *Trends in Microbiology, 20*(8), 385–391.

Candela, M., Biagi, E., Brigidi, P., O'Toole, P. W., & De Vos, W. M. (2014). Maintenance of a healthy trajectory of the intestinal microbiome during aging: A dietary approach. *Mechanisms of Ageing and Development, 136–137*, 70–75.

Cao, Y., Shen, J., & Ran, Z. H. (2014). Association between *Faecalibacterium prausnitzii* reduction and inflammatory bowel disease: A meta-analysis and systematic review of the literature. *Gastroenterology Research and Practice, 2014*, 872725.

Charlesworth, C. J., Smit, E., Lee, D. S. H., Alramadhan, F., & Odden, M. C. (2015). Polypharmacy among adults aged 65 years and older in the United States: 1988-2010. *Journals of Gerontology Series A-Biological Sciences and Medical Sciences, 70*(8), 989–995.

Chaston, J. M., Dobson, A. J., Newell, P. D., & Douglas, A. E. (2016). Host genetic control of the microbiota mediates the *Drosophila* nutritional phenotype. *Applied and Environmental Microbiology, 82*(2), 671–679.

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., Stanton, C., van Sinderen, D., O'Connor, M., Harnedy, N., O'Connor, K., Henry, C., O'Mahony, D., Fitzgerald, A. P., Shanahan, F., Twomey, C., Hill, C., Ross, R. P., & O'Toole, P. W. (2011). Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proceedings of the National Academy of Sciences of the United States of America, 108*(Suppl 1), 4586–4591.

Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'Connor, E. M., Cusack, S., Harris, H. M. B., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G. F., Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen, D., Wallace, M., Brennan, L., Stanton, C., Marchesi, J. R., Fitzgerald, A. P., Shanahan, F., Hill, C., Ross, R. P., & O'Toole, P. W. (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nature, 488*(7410), 178–184.

Clark, R. I., Salazar, A., Yamada, R., Fitz-Gibbon, S., Morselli, M., Alcaraz, J., Rana, A., Rera, M., Pellegrini, M., Ja, W. W., & Walker, D. W. (2015). Distinct shifts in microbiota composition during *Drosophila* aging impair intestinal function and drive mortality. *Cell Reports, 12*(10), 1656–1667.

Clements, S. J., & Carding, S. R. (2018). Diet, the intestinal microbiota, and immune health in aging. *Critical Reviews in Food Science and Nutrition, 58*(4), 651–661.

Collado, M. C., Rautava, S., Aakko, J., Isolauri, E., & Salminen, S. (2016). Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Scientific Reports, 6*, 23129.

Conley, M. N., Wong, C. P., Duyck, K. M., Hord, N., Ho, E., & Sharpton, T. J. (2016). Aging and serum MCP-1 are associated with gut microbiome composition in a murine model. *PeerJ, 4*, e1854.

Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., & Knight, R. (2009). Bacterial community variation in human body habitats across space and time. *Science, 326*(5960), 1694–1697.

de Vries, M. C., Vaughan, E. E., Kleerebezem, M., & de Vos, W. M. (2006). *Lactobacillus plantarum*- survival, functional and potential probiotic properties in the human intestinal tract. *International Dairy Journal, 16*(9), 1018–1028.

de Goffau, M. C., Lager, S., Sovio, U., Gaccioli, F., Cook, E., Peacock, S. J., Parkhill, J., Charnock-Jones, D. S., Smith, G. C. S. (2019). Human placenta has no microbiome but can contain potential pathogens. *Nature, 572*(7769), 329–334.

Dambroise, E., Monnier, L., Ruisheng, L., Aguilaniu, H., Joly, J. S., Tricoire, H., & Rera, M. (2016). Two phases of aging separated by the Smurf transition as a public path to death. *Scientific Reports, 6*, 23523.

Darby, T. M., & Jones, R. M. (2017). Beneficial influences of *Lactobacillus plantarum* on human health and disease. In Y. Ringel & W. A. Walker (Eds.), *The microbiota in gastrointestinal pathophysiology* (pp. 109–117). Boston: Academic.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A., Biddinger, S. B., Dutton, R. J., & Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature, 505*(7484), 559–563.

Dominy, S. S., Lynch, C., Ermini, F., Benedyk, M., Marczyk, A., Konradi, A., Nguyen, M., Haditsch, U., Raha, D., Griffin, C., Holsinger, L. J., Arastu-Kapur, S., Kaba, S., Lee, A., Ryder, M. I., Potempa, B., Mydel, P., Hellvard, A., Adamowicz, K., Hasturk, H., Walker, G. D., Reynolds, E. C., Faull, R. L. M., Curtis, M. A., Dragunow, M., & Potempa, J. (2019). Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. *Science Advances, 5*(1), eaau3333.

Douglas, A. E. (2018). Which experimental systems should we use for human microbiome science? *PLoS Biology, 16*(3), e2005245. https://doi.org/10.1371/journal.pbio.2005245.

Duncan, S. H., & Flint, H. J. (2013). Probiotics and prebiotics and health in ageing populations. *Maturitas, 75*(1), 44–50.

Elderman, M., Sovran, B., Hugenholtz, F., Graversen, K., Huijskes, M., Houtsma, E., Belzer, C., Boekschoten, M., de Vos, P., Dekker, J., Wells, J., & Faas, M. (2017). The effect of age on the intestinal mucus thickness, microbiota composition and immunity in relation to sex in mice. *PLoS One, 12*(9), e0184274.

Fishman, J. E., Levy, G., Alli, V., Sheth, S., Lu, Q., & Deitch, E. A. (2013). Oxidative modification of the intestinal mucus layer is a critical but unrecognized component of trauma hemorrhagic shock-induced gut barrier failure. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 304*(1), G57–G63.

Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 69*(Suppl 1), S4–S9.

Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., & De Benedictis, G. (2000). Inflamm-aging. An evolutionary perspective on immunosenescence. *Annals of the New York Academy of Sciences, 908*, 244–254.

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panouraia, M. P., Invidia, L., Celani, L., Scurti, M., Cevenini, E., Castellani, G. C., & Salvioli, S. (2007). Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. *Mechanisms of Ageing and Development, 128*(1), 92–105.

Franceschi, C., Ostan, R., & Santoro, A. (2018). Nutrition and Inflammation: Are centenarians similar to individuals on calorie-restricted diets? *Annual Review of Nutrition, 38*, 329–356.

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., Faas, M. M., & de Vos, P. (2017). Aged gut microbiota contributes to systemical inflammaging after transfer to germ-free mice. *Frontiers in Immunology, 8*, 1385.

Fukunaga, A., Uematsu, H., & Sugimoto, K. (2005). Influences of aging on taste perception and oral somatic sensation. *The Journals of Gerontology: Series A, 60*(1), 109–113.

Fukushima, Y., Miyaguchi, S., Yamano, T., Kaburagi, T., Iino, H., Ushida, K., & Sato, K. (2007). Improvement of nutritional status and incidence of infection in hospitalised, enterally fed elderly by feeding of fermented milk containing probiotic *Lactobacillus johnsonii* La1 (NCC533). *The British Journal of Nutrition, 98*(5), 969–977.

Garcia-Gonzalez, A. P., Ritter, A. D., Shrestha, S., Andersen, E. C., Yilmaz, L. S., & Walhout, A. J. M. (2017). Bacterial Metabolism Affects the *C. elegans* Response to Cancer Chemotherapeutics. *Cell, 169*(3), 431–441. e438.

Gelino, S., Chang, J. T., Kumsta, C., She, X., Davis, A., Nguyen, C., Panowski, S., & Hansen, M. (2016). Intestinal autophagy improves healthspan and longevity in *C. elegans* during dietary restriction. *PLOS Genetics, 12*(7), e1006135.

Gill, H. S., Rutherfurd, K. J., Cross, M. L., & Gopal, P. K. (2001). Enhancement of immunity in the elderly by dietary supplementation with the probiotic Bifidobacterium lactis HN019. *The American Journal of Clinical Nutrition, 74*(6), 833–839.

Guillemard, E., Tondu, F., Lacoin, F., & Schrezenmeir, J. (2010). Consumption of a fermented dairy product containing the probiotic *Lactobacillus casei* DN-114001 reduces the duration of respiratory infections in the elderly in a randomised controlled trial. *The British Journal of Nutrition, 103*(1), 58–68.

Guo, L., Karpac, J., Tran, S. L., & Jasper, H. (2014). PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. *Cell, 156*(1–2), 109–122.

Hallstrom, M., Eerola, E., Vuento, R., Janas, M., & Tammela, O. (2004). Effects of mode of delivery and necrotising enterocolitis on the intestinal microflora in preterm infants. *European Journal of Clinical Microbiology & Infectious Diseases, 23*(6), 463–470.

Han, B., Sivaramakrishnan, P., Lin, C. C. J., Neve, I. A. A., He, J. Q., Tay, L. W. R., Sowa, J. N., Sizovs, A., Du, G. W., Wang, J., Herman, C., & Wang, M. C. (2017). Microbial genetic composition tunes host longevity. *Cell, 169*(7), 1249–1262.

Hayashi, H., Sakamoto, M., Kitahara, M., & Benno, Y. (2003). Molecular analysis of fecal microbiota in elderly individuals using 16S rDNA library and T-RFLP. *Microbiology and Immunology, 47*(8), 557–570.

Henderson, B., & Nibali, L. (2016). *The human microbiota and chronic disease: Dysbiosis as a cause of human pathology*. Hoboken, NJ: Wiley Blackwell.

Hickson, M., D'Souza, A. L., Muthu, N., Rogers, T. R., Want, S., Rajkumar, C., & Bulpitt, C. J. (2007). Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: Randomised double blind placebo controlled trial. *BMJ, 335*(7610), 80–83.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews. Gastroenterology & Hepatology, 11*(8), 506–514.

Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *Science, 336*(6086), 1268–1273.

Hopkins, M. J., & Macfarlane, G. T. (2002). Changes in predominant bacterial populations in human faeces with age and with *Clostridium difficile* infection. *Journal of Medical Microbiology, 51*(5), 448–454.

Hopkins, M. J., Sharp, R., & Macfarlane, G. T. (2001). Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. *Gut, 48*(2), 198–205.

Human Microbiome Project Consortium (2012). Structure, function and diversity of the healthy human microbiome. *Nature, 486*(7402), 207–214.

Jackson, M. A., Jeffery, I. B., Beaumont, M., Bell, J. T., Clark, A. G., Ley, R. E., O'Toole, P. W., Spector, T. D., & Steves, C. J. (2016). Signatures of early frailty in the gut microbiota. *Genome Medicine, 8*(1), 8.

Jeffery, I. B., Lynch, D. B., & O'Toole, P. W. (2016). Composition and temporal stability of the gut microbiota in older persons. *The ISME Journal, 10*(1), 170–182.

Jimenez, E., Fernandez, L., Marin, M. L., Martin, R., Odriozola, J. M., Nueno-Palop, C., Narbad, A., Olivares, M., Xaus, J., & Rodriguez, J. M. (2005). Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Current Microbiology, 51*(4), 270–274.

Johansson, M. E. (2014). Mucus layers in inflammatory bowel disease. *Inflammatory Bowel Diseases, 20*(11), 2124–2131.

Johansson, M. L., Molin, G., Jeppsson, B., Nobaek, S., Ahrne, S., & Bengmark, S. (1993). Administration of different Lactobacillus strains in fermented oatmeal soup: In vivo colonization of human intestinal mucosa and effect on the indigenous flora. *Applied and Environmental Microbiology, 59*(1), 15–20.

Johansson, M. E. V., Phillipson, M., Petersson, J., Velcich, A., Holm, L., & Hansson, G. C. (2008). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proceedings of the National Academy of Sciences of the United States of America, 105*(39), 15064–15069.

Johansson, M. E. V., Larsson, J. M. H., & Hansson, G. C. (2011). The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 4659–4665.

Jones, C., Badger, S. A., Regan, M., Clements, B. W., Diamond, T., Parks, R. W., & Taylor, M. A. (2013). Modulation of gut barrier function in patients with obstructive jaundice using probiotic LP299v. *European Journal of Gastroenterology & Hepatology, 25*(12), 1424–1430.

Keebaugh, E. S., & Ja, W. W. (2016). Microbes without borders: Decompartmentalization of the aging gut. *Cell Host & Microbe, 19*(2), 133–135.

Keebaugh, E. S., Yamada, R., Obadia, B., Ludington, W. B., & Ja, W. W. (2018). Microbial quantity impacts *Drosophila* nutrition, development, and lifespan. *iScience, 4*, 247–259.

Kim, S., & Jazwinski, S. M. (2018). The gut microbiota and healthy aging: A mini-review. *Gerontology, 64*(6), 513–520.

Klarin, B., Johansson, M. L., Molin, G., Larsson, A., & Jeppsson, B. (2005). Adhesion of the probiotic bacterium *Lactobacillus plantarum* 299v onto the gut mucosa in critically ill patients: A randomised open trial. *Critical Care, 9*(3), R285–R293.

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O. P., Leer, R., Tarchini, R., Peters, S. A., Sandbrink, H. M., Fiers, M. W. E. J., Stiekema, W., Lankhorst, R. M. K., Bron, P. A., Hoffer, S. M., Groot, M. N. N., Kerkhoven, R., de Vries, M., Ursing, B., de Vos, W. M., & Siezen, R. J. (2003). Complete genome sequence of *Lactobacillus plantarum* WCFS1. *Proceedings of the National Academy of Sciences of the United States of America, 100*(4), 1990–1995.

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., Angenent, L. T., & Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America, 108*(Suppl 1), 4578–4585.

Kong, F., Hua, Y., Zeng, B., Ning, R., Li, Y., & Zhao, J. (2016). Gut microbiota signatures of longevity. *Current Biology, 26*(18), R832–R833.

Kong, F., Deng, F., Li, Y., & Zhao, J. (2018). Identification of gut microbiome signatures associated with longevity provides a promising modulation target for healthy aging. *Gut Microbes, 10*(2), 210–215.

Kumar, M., Babaei, P., Ji, B., & Nielsen, J. (2016). Human gut microbiota and healthy aging: Recent developments and future prospective. *Nutrition and Healthy Aging, 4*(1), 3–16.

Lahtinen, S. J., Tammela, L., Korpela, J., Parhiala, R., Ahokoski, H., Mykkanen, H., & Salminen, S. J. (2009). Probiotics modulate the bifidobacterium microbiota of elderly nursing home residents. *Age (Dordrecht, Netherlands), 31*(1), 59–66.

Lee, G. C., Daniels, K., Lawson, K. A., Attridge, R. T., Lewis, J., & Frei, C. R. (2013). Age-based outpatient antibiotic prescribing in the United States from 2000 to 2010. *Value in Health, 16*(3), A78–A78.

Lee, G. C., Reveles, K. R., Attridge, R. T., Lawson, K. A., Mansi, I. A., Lewis, J. S., & Frei, C. R. (2014). Outpatient antibiotic prescribing in the United States: 2000 to 2010. *BMC Medicine, 12*, 96.

Li, H., Qi, Y., & Jasper, H. (2016). Preventing age-related decline of gut compartmentalization limits microbiota dysbiosis and extends lifespan. *Cell Host & Microbe, 19*(2), 240–253.

Lim, C. J., Kong, D. C. M., & Stuart, R. L. (2014). Reducing inappropriate antibiotic prescribing in the residential care setting: Current perspectives. *Clinical Interventions in Aging, 9*, 165–177.

Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine, 8*(1), 51.

Lovat, L. B. (1996). Age related changes in gut physiology and nutritional status. *Gut, 38*(3), 306–309.

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature, 489*(7415), 220–230.

Lu, M., & Wang, Z. (2018). Linking gut microbiota to aging process: A new target for anti-aging. *Food Science and Human Wellness, 7*(2), 111–119.

Macpherson, A. J., & Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system. *Nature Reviews. Immunology, 4*(6), 478–485.

Magrone, T., & Jirillo, E. (2013). The interaction between gut microbiota and age-related changes in immune function and inflammation. *Immunity & Ageing, 10*(1), 31.

Malamitsi-Puchner, A., Protonotariou, E., Boutsikou, T., Makrakis, E., Sarandakou, A., & Creatsas, G. (2005). The influence of the mode of delivery on circulating cytokine concentrations in the perinatal period. *Early Human Development, 81*(4), 387–392.

Man, A. L., Gicheva, N., & Nicoletti, C. (2014). The impact of ageing on the intestinal epithelial barrier and immune system. *Cellular Immunology, 289*(1–2), 112–118.

Marianes, A., & Spradling, A. C. (2013). Physiological and stem cell compartmentalization within the *Drosophila* midgut. *eLife, 2*, e00886.

Mariat, D., Firmesse, O., Levenez, F., Guimaraes, V. D., Sokol, H., Dore, J., Corthier, G., & Furet, J. P. (2009). The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiology, 9*, 123.

Martin, R., Makino, H., Yavuz, A. C., Ben-Amor, K., Roelofs, M., Ishikawa, E., Kubota, H., Swinkels, S., Sakai, T., Oishi, K., Kushiro, A., & Knol, J. (2016). Early-life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. *PLoS One, 11*(6), e0158498.

Matsumoto, M., Kurihara, S., Kibe, R., Ashida, H., & Benno, Y. (2011). Longevity in mice Is promoted by probiotic-induced suppression of colonic senescence dependent on upregulation of gut bacterial polyamine production. *PLoS One, 6*(8), e23652.

Matsuo, K., Ota, H., Akamatsu, T., Sugiyama, A., & Katsuyama, T. (1997). Histochemistry of the surface mucous gel layer of the human colon. *Gut, 40*(6), 782–789.

Maynard, C., & Weinkove, D. (2018). The gut microbiota and ageing. *Sub-Cellular Biochemistry, 90*, 351–371.

McNaught, C. E., Woodcock, N. P., Anderson, A. D., & MacFie, J. (2005). A prospective randomised trial of probiotics in critically ill patients. *Clinical Nutrition, 24*(2), 211–219.

Metchnikoff, E. (1908). *The prolongation of life: Optimistic studies*. New York and London: GP Putnam's Sons.

Miquel, S., Martin, R., Rossi, O., Bermudez-Humaran, L. G., Chatel, J. M., Sokol, H., Thomas, M., Wells, J. M., & Langella, P. (2013). *Faecalibacterium prausnitzii* and human intestinal health. *Current Opinion in Microbiology, 16*(3), 255–261.

Mueller, S., Saunier, K., Hanisch, C., Norin, E., Alm, L., Midtvedt, T., Cresci, A., Silvi, S., Orpianesi, C., Verdenelli, M. C., Clavel, T., Koebnick, C., Zunft, H.-J. F., Doré, J., & Blaut, M. (2006). Differences in fecal microbiota in different European study populations in relation to age, gender, and country: A cross-sectional study. *Applied and Environmental Microbiology, 72*(2), 1027–1033.

Nagpal, R., Mainali, R., Ahmadi, S., Wang, S., Singh, R., Kavanagh, K., Kitzman, D. W., Kushugulova, A., Marotta, F., & Yadav, H. (2018). Gut microbiome and aging: Physiological and mechanistic insights. *Nutrition and Healthy Aging, 4*(4), 267–285.

Negele, K., Heinrich, J., Borte, M., von Berg, A., Schaaf, B., Lehmann, I., Wichmann, H. E., Bolte, G., & L. S. Group. (2004). Mode of delivery and development of atopic disease during the first 2 years of life. *Pediatric Allergy and Immunology, 15*(1), 48–54.

Nguyen, T. L. A., Vieira-Silva, S., Liston, A., & Raes, J. (2015). How informative is the mouse for human gut microbiota research? *Disease Models & Mechanisms, 8*(1), 1–16.

O'Hara, A. M., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO Reports, 7*(7), 688–693.

Obadia, B., Guvener, Z. T., Zhang, V., Ceja-Navarro, J. A., Brodie, E. L., Ja, W. W., & Ludington, W. B. (2017). Probabilistic invasion underlies natural gut microbiome stability. *Current Biology, 27*(13), 1999–2006. e1998.

Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J. Z., Abe, F., & Osawa, R. (2016). Age-related changes in gut microbiota composition from newborn to centenarian: A cross-sectional study. *BMC Microbiology, 16*, 90.

Ouwehand, A. C., Bergsma, N., Parhiala, R., Lahtinen, S., Gueimonde, M., Finne-Soveri, H., Strandberg, T., Pitkala, K., & Salminen, S. (2008). Bifidobacterium microbiota and parameters of immune function in elderly subjects. *FEMS Immunology and Medical Microbiology, 53*(1), 18–25.

Overend, G., Luo, Y., Henderson, L., Douglas, A. E., Davies, S. A., & Dow, J. A. (2016). Molecular mechanism and functional significance of acid generation in the *Drosophila* midgut. *Scientific Reports, 6*, 27242.

Pais, I. S., Valente, R. S., Sporniak, M., & Teixeira, L. (2018). *Drosophila melanogaster* establishes a species-specific mutualistic interaction with stable gut-colonizing bacteria. *PLoS Biology, 16*(7), e2005710.

Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the human infant intestinal microbiota. *PLoS Biology, 5*(7), e177.

Perez-Munoz, M. E., Arrieta, M. C., Ramer-Tait, A. E., & Walter, J. (2017). A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: Implications for research on the pioneer infant microbiome. *Microbiome, 5*(1), 48.

Qin, J. J., Li, R. Q., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J. H., Xu, J. M., Li, S. C., Li, D. F., Cao, J. J., Wang, B., Liang, H. Q., Zheng, H. S., Xie, Y. L., Tap, J., Lepage, P., Bertalan, M., Batto, J. M., Hansen, T., Le Paslier, D., Linneberg, A., Nielsen, H. B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H. M., Yu, C., Li, S. T., Jian, M., Zhou, Y., Li, Y. R., Zhang, X. Q., Li, S. G., Qin, N., Yang, H. M., Wang, J., Brunak, S., Dore, J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J., Bork, P., Ehrlich, S. D., Wang, J., & MetaHIT Consortium (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature, 464*(7285), 59–65.

Qin, X. F., Sheth, S. U., Sharpe, S. M., Dong, W., Lu, Q., Xu, D. Z., & Deitch, E. A. (2011). The mucus layer Is critical in protecting against ischemia-reperfusion-mediated gut injury and in the restitution of gut barrier function. *Shock, 35*(3), 275–281.

Rampelli, S., Candela, M., Turroni, S., Biagi, E., Collino, S., Franceschi, C., O'Toole, P. W., & Brigidi, P. (2013). Functional metagenomic profiling of intestinal microbiome in extreme ageing. *Aging (Albany NY), 5*(12), 902–912.

Rautava, S., Luoto, R., Salminen, S., & Isolauri, E. (2012). Microbial contact during pregnancy, intestinal colonization and human disease. *Nature Reviews. Gastroenterology & Hepatology, 9*(10), 565–576.

Rehman, T. (2012). Role of the gut microbiota in age-related chronic inflammation. *Endocrine, Metabolic & Immune Disorders Drug Targets, 12*(4), 361–367.

Rera, M., Clark, R. I., & Walker, D. W. (2012). Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America, 109*(52), 21528–21533.

Rera, M., Vallot, C., & Lefrancois, C. (2018). The Smurf transition: New insights on ageing from end-of-life studies in animal models. *Current Opinion in Oncology, 30*(1), 38–44.

Resnik-Docampo, M., Koehler, C. L., Clark, R. I., Schinaman, J. M., Sauer, V., Wong, D. M., Lewis, S., D'Alterio, C., Walker, D. W., & Jones, D. L. (2017). Tricellular junctions regulate intestinal stem cell behaviour to maintain homeostasis. *Nature Cell Biology, 19*(1), 52–59.

Resnik-Docampo, M., Sauer, V., Schinaman, J. M., Clark, R. I., Walker, D. W., & Jones, D. L. (2018). Keeping it tight: The relationship between bacterial dysbiosis, septate junctions, and the intestinal barrier in *Drosophila*. *Fly (Austin), 12*(1), 34–40.

Riaz Rajoka, M. S., Zhao, H., Li, N., Lu, Y., Lian, Z., Shao, D., Jin, M., Li, Q., Zhao, L., & Shi, J. (2018). Origination, change, and modulation of geriatric disease-related gut microbiota during life. *Applied Microbiology and Biotechnology, 102*(19), 8275–8289.

Ridley, E. V., Wong, A. C., Westmiller, S., & Douglas, A. E. (2012). Impact of the resident microbiota on the nutritional phenotype of *Drosophila melanogaster*. *PLoS One, 7*(5), e36765.

Rodriguez, J. M., Murphy, K., Stanton, C., Ross, R. P., Kober, O. I., Juge, N., Avershina, E., Rudi, K., Narbad, A., Jenmalm, M. C., Marchesi, J. R., & Collado, M. C. (2015). The composition of the gut microbiota throughout life, with an emphasis on early life. *Microbial Ecology in Health and Disease, 26*, 26050.

Rosen, C. E., & Palm, N. W. (2017). Functional classification of the gut microbiota: The key to cracking the microbiota composition code: Functional classifications of the gut microbiota reveal previously hidden contributions of indigenous gut bacteria to human health and disease. *BioEssays, 39*(12), 1700032.

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., Costea, P. I., Godneva, A., Kalka, I. N., Bar, N., Shilo, S., Lador, D., Vila, A. V., Zmora, N., Pevsner-Fischer, M., Israeli, D., Kosower, N., Malka, G., Wolf, B. C., Avnit-Sagi, T., Lotan-Pompan, M., Weinberger, A., Halpern, Z., Carmi, S., Fu, J., Wijmenga, C., Zhernakova, A., Elinav, E., & Segal, E. (2018). Environment dominates over host genetics in shaping human gut microbiota. *Nature, 555*, 210–215.

Rutayisire, E., Huang, K., Liu, Y., & Tao, F. (2016). The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: A systematic review. *BMC Gastroenterology, 16*(1), 86.

Salazar, A. M., Resnik-Docampo, M., Ulgherait, M., Clark, R. I., Shirasu-Hiza, M., Jones, D. L., & Walker, D. W. (2018). Intestinal snakeskin limits microbial dysbiosis during aging and promotes longevity. *iScience, 9*, 229–243.

Santoro, A., Ostan, R., Candela, M., Biagi, E., Brigidi, P., Capri, M., & Franceschi, C. (2018). Gut microbiota changes in the extreme decades of human life: A focus on centenarians. *Cellular and Molecular Life Sciences, 75*(1), 129–148.

Sarkar, D., & Fisher, P. B. (2006). Molecular mechanisms of aging-associated inflammation. *Cancer Letters, 236*(1), 13–23.

Scott, T. A., Quintaneiro, L. M., Norvaisas, P., Lui, P. P., Wilson, M. P., Leung, K. Y., Herrera-Dominguez, L., Sudiwala, S., Pessia, A., Clayton, P. T., Bryson, K., Velagapudi, V., Mills, P. B., Typas, A., Greene, N. D. E., & Cabreiro, F. (2017). Host-Microbe Co-metabolism Dictates Cancer Drug Efficacy in *C. elegans*. *Cell, 169*(3), 442–456. e418.

Smith, P., Willemsen, D., Popkes, M., Metge, F., Gandiwa, E., Reichard, M., et al. (2017). Regulation of life span by the gut microbiota in the short-lived African turquoise killifish. *Elife. 6*.

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L. G., Gratadoux, J.-J., Blugeon, S., Bridonneau, C., Furet, J.-P., Corthier, G., Grangette, C., Vasquez, N., Pochart, P., Trugnan, G., Thomas, G., Blottière, H. M., Doré, J., Marteau, P., Seksik, P., & Langella, P. (2008). *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proceedings of the National Academy of Sciences of the United States of America, 105*(43), 16731–16736.

Suryavanshi, M. V., Paul, D., Doijad, S. P., Bhute, S. S., Hingamire, T. B., Gune, R. P., & Shouche, Y. S. (2017). Draft genome sequence of *Lactobacillus plantarum* strains E2C2 and E2C5 isolated from human stool culture. *Standards in Genomic Sciences, 12*, 15.

Swidsinski, A., Loening-Baucke, V., Theissig, F., Engelhardt, H., Bengmark, S., Koch, S., Lochs, H., & Dorffel, Y. (2007a). Comparative study of the intestinal mucus barrier in normal and inflamed colon. *Gut, 56*(3), 343–350.

Swidsinski, A., Sydora, B. C., Doerffel, Y., Loening-Baucke, V., Vaneechoutte, M., Lupicki, M., Scholze, J., Lochs, H., & Dieleman, L. A. (2007b). Viscosity gradient within the mucus layer determines the mucosal barrier function and the spatial organization of the intestinal microbiota. *Inflammatory Bowel Diseases, 13*(8), 963–970.

Tamburini, S., Shen, N., Wu, H. C., & Clemente, J. C. (2016). The microbiome in early life: Implications for health outcomes. *Nature Medicine, 22*(7), 713–722.

Thevaranjan, N., Puchta, A., Schulz, C., Naidoo, A., Szamosi, J. C., Verschoor, C. P., Loukov, D., Schenck, L. P., Jury, J., Foley, K. P., Schertzer, J. D., Larche, M. J., Davidson, D. J., Verdu, E. F., Surette, M. G., & Bowdish, D. M. E. (2017). Age-associated microbial dysbiosis promotes intestinal permeability, systemic inflammation, and macrophage dysfunction. *Cell Host & Microbe, 21*(4), 455–466. e454.

Tran, L., & Greenwood-Van Meerveld, B. (2013). Age-Associated Remodeling of the Intestinal Epithelial Barrier. *Journals of Gerontology Series A-Biological Sciences and Medical Sciences, 68*(9), 1045–1056.

Tricoire, H., & Rera, M. (2015). A new, discontinuous 2 phases of aging model: Lessons from *Drosophila melanogaster*. *PLoS One, 10*(11), e0141920.

Turchet, P., Laurenzano, M., Auboiron, S., & Antoine, J. M. (2003). Effect of fermented milk containing the probiotic *Lactobacillus casei* DN-114001 on winter infections in free-living elderly subjects: A randomised, controlled pilot study. *The Journal of Nutrition, Health & Aging, 7*(2), 75–77.

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature, 449*(7164), 804–810.

van Beek, A. A., Sovran, B., Hugenholtz, F., Meijer, B., Hoogerland, J. A., Mihailova, V., van der Ploeg, C., Belzer, C., Boekschoten, M. V., Hoeijmakers, J. H., Vermeij, W. P., de Vos, P., Wells, J. M., Leenen, P. J., Nicoletti, C., Hendriks, R. W., & Savelkoul, H. F. (2016). Supplementation with *Lactobacillus plantarum* WCFS1 prevents decline of mucus barrier in colon of accelerated aging *Ercc1(-/Delta7)* mice. *Frontiers in Immunology, 7*, 408.

van Tongeren, S. P., Slaets, J. P., Harmsen, H. J., & Welling, G. W. (2005). Fecal microbiota composition and frailty. *Applied and Environmental Microbiology, 71*(10), 6438–6442.

Varum, F. J. O., Veiga, F., Sousa, J. S., & Basit, A. W. (2012). Mucus thickness in the gastrointestinal tract of laboratory animals. *The Journal of Pharmacy and Pharmacology, 64*(2), 218–227.

Vesa, T., Pochart, P., & Marteau, P. (2000). Pharmacokinetics of *Lactobacillus plantarum* NCIMB 8826, *Lactobacillus fermentum* KLD, and *Lactococcus lactis* MG 1363 in the human gastrointestinal tract. *Alimentary Pharmacology & Therapeutics, 14*(6), 823–828.

Walker, A. (2007). Genome watch—Say hello to our little friends. *Nature Reviews. Microbiology, 5*(8), 572–573.

Wong, A. C., Dobson, A. J., & Douglas, A. E. (2014). Gut microbiota dictates the metabolic response of *Drosophila* to diet. *The Journal of Experimental Biology, 217*(Pt 11), 1894–1901.

Woodmansey, E. J. (2007). Intestinal bacteria and ageing. *Journal of Applied Microbiology, 102*(5), 1178–1186.

Woodmansey, E. J., McMurdo, M. E., Macfarlane, G. T., & Macfarlane, S. (2004). Comparison of compositions and metabolic activities of fecal microbiotas in young adults and in antibiotic-treated and non-antibiotic-treated elderly subjects. *Applied and Environmental Microbiology, 70*(10), 6113–6122.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F. D., & Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science, 334*(6052), 105–108.

Yamada, R., Deshpande, S. A., Bruce, K. D., Mak, E. M., & Ja, W. W. (2015). Microbes promote amino acid harvest to rescue undernutrition in *Drosophila*. *Cell Rep, 10*(6), 865–872.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., Knight, R., & Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature, 486*(7402), 222–227.

You, J. L., & Yaqoob, P. (2012). Evidence of immunomodulatory effects of a novel probiotic, *Bifidobacterium longum bv. infantis* CCUG 52486. *FEMS Immunology and Medical Microbiology, 66*(3), 353–362.

Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R., & Goodman, A. L. (2019). Separating host and microbiome contributions to drug pharmacokinetics and toxicity. *Science, 363*(6427), eaat9931.