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# AGEING AND THE MICROBIOME: AN AVENUE FOR INTERVENTION

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## SUMMARY

Progress in ageing research has been impressive in recent years, leading to an increased understanding of the biological determinants of ageing and the identification of a number of interventions that might slow ageing in humans. Given that ageing is the largest risk factor for the chronic diseases that are increasing in prevalence globally, this raises the possibility that through the use of these interventions it will be feasible to slow ageing and prevent the onset of many diseases simultaneously. However, ageing is not generally considered as a disease and any intervention to slow ageing will necessitate a high safety profile to be used in healthy individuals. For this reason, it is important to consider non-pharmaceutical interventions, and modification of the microbiome is a promising avenue for exploration. Here I discuss how manipulation of the microbiome may impact ageing, highlighting data from animal models and human studies.

## INTRODUCTION

Demographics are rapidly changing, leading to an increasing percentage of the population over the age of 65. In some countries that percentage will approach 40% in the near future. With ageing comes functional decline and disease onset. Most individuals over the age of 65 have one or more chronic diseases (e.g. neurologic, cardiovascular and metabolic syndromes, as well as cancers). While each of these diseases have identified risk factors, they pale in comparison to the risks associated with ageing itself (*Kennedy* et al., 2014). Targeting risks factors has proven effective in disease prevention; lowering cholesterol and glucose reduces onset and progression of cardiovascular and metabolic disease, respectively. What would happen if the biggest risk factor, ageing, was targeted?

Efforts to understand the molecular determinants of ageing have been underway for nearly a century and great progress has been made in the last few decades, largely through the use of non-vertebrate models such as yeast, worms and flies. Prior to these studies, many researchers proposed that ageing, due to its complexity, was highly difficult to modulate. Working with these organisms, however, researchers identified hundreds of genes that impact ageing and proposed several mechanisms to explain their action (*Longo* et al., 2012; *Uno* and *Nishida*, 2016; *Piper*

and *Partridge*, 2018). A surprising finding was that ageing pathways and mechanisms are likely conserved between these organisms, even though they are often quite divergent from an evolutionary perspective (*Smith* et al., 2008). Further extending this observation, more recent reports indicate that many of these same genes impact mammalian, and even possibly human, ageing (*Tian* et al., 2017).

Calorie (or dietary) restriction was shown to slow ageing in mice and rats over eight decades ago, and remains a hot topic for research today (*Balasubramanian* et al., 2017). The pathways modulated by nutrient limitation, including insulin/IGF and mTOR signalling, also modulate ageing and recent evidence has suggested that reducing mTOR activity may impact aspects of human ageing (*Mannick* et al., 2014). Interestingly, these interventions have been shown to alter the microbiome as well, raising the question as to whether their effects are modulated through the gut microbiota.

Here links between the microbiome and ageing are discussed using three short perspectives, with a focus on the gut. First, I detail changes in the gut microbiome that occur with human ageing, findings that might point to targeted modulation. Second, I outline the links between known interventions affecting ageing and alterations in the microbiome. Finally, I discuss the prospects of using targeted interventions to modulate microbiome composition as a means of slowing ageing. The intersection of two major fields of study, microbiome and ageing, offers great promise for novel discoveries and more importantly an avenue toward extending human healthspan, the disease free and highly functional period of life.

## MICROBIOME ALTERATIONS DURING AGEING

Perhaps not surprisingly, there are extensive microbiome changes during the ageing process (*Kundu* et al., 2017). Diversity between individuals also increases with ageing (*Claesson* et al., 2011). One surprising finding was that the microbiome of residential dwelling elders was significantly different from those in long-term residential care (*Claesson* et al., 2011). Community dwelling elders have a more diverse microbiome, correlated with a diet higher in fibre and reduced levels of age-associated inflammatory factors, such as IL-6, TNF-$\alpha$ and C-reactive protein. Metagenomic analysis further indicated higher metabolism of butyrate and short-chain fatty acids in community dwellers, who were also healthier by several parameters. Microbiome changes are also associated with several diseases of ageing, including neurodegenerative conditions (*Kundu* et al., 2017). Finally, a recent study suggests that semi-supercentenarians (105-109 years) have a microbiome associated with health promotion, for instance enriched for taxa such as *Bifidobacterium*, *Christensenellaceae*, and *Akkermansia* (*Biagi* et al., 2010). None of these studies demonstrate a causal role for the microbiome in mediating ageing, they certainly raise that possibility. How easy it will be to modify the microbiome of elders, however, remains unclear.

Microbiome-related changes are also evident in ageing mice, with notable similarities and differences. One genus that declines in both human and mice is *Akkermansia*, which is associated with protection from a range of diseases (*Langille* et al., 2014). Germ-free mice are also a major tool for research and a

recent study suggests that these mice have enhanced lifespan (*Thevaranjan* et al., 2017), similar in some respects to that of *C. elegans* exposed to dead bacteria as a food source (*Thevaranjan* et al., 2017) or adult worms on plates deprived of bacteria entirely (*Kaeberlein* et al., 2006). Germ-free mice also experienced lower inflammation, which was increased when these mice were co-housed with old conventional animals (*Thevaranjan* et al., 2017). Numerous other changes have been detected, although some discrepancies exist between different mouse studies. By continuing to exploit the mouse model to understand the relationship between microbiome composition, ageing, and disease, it is almost certain that insights will be gained regarding human ageing and interventional strategies developed.

## AGEING INTERVENTIONS AND THE MICROBIOME

A major step forward in ageing research has been the identification of dietary and small molecule-based interventions that slow ageing and extend healthspan in animal models. Among the most prominent of these are calorie restriction, metformin and rapamycin, all of which have been linked to changes in the microbiome. An advantage of the animal models is that it is easier to study the relationship between interventions that extend lifespan and microbiome changes. For instance, calorie restriction as expected is associated with significant changes in the microbiome, including an increase in bacterial species associated longer lifespan. Interestingly, both calorie restriction and intermittent fasting, which mimics the effects of calorie restriction, have been reported to extend lifespan in flies and this is associated with a reduced gut bacterial load (*Regan* et al., 2016; *Catterson* et al., 2018). The effect is more prominent in females, which are more prone to gut deterioration with age. Further analysis of the effects of calorie restriction and fasting will be important as these interventions enhance longevity and promote healthspan across a wide range of species.

Metformin is a widely used drug used to treat hyperglycaemia in the context of a range of metabolic conditions and has also been reported to extend lifespan in several animal models (*Barzilai* et al., 2016). Intriguingly, retrospective studies in humans suggest that diabetic patients taking metformin have a lower-than-expected mortality rate and moreover metformin may be protective for a range of other chronic conditions (*Bannister* et al., 2014). These findings together have suggested that metformin may deserve even more widespread use to extend human healthspan. A surprising finding regarding metformin's ageing effects came from *C. elegans*, where it was shown that lifespan extension by the drug required live bacteria as a food source (*Cabreiro* et al., 2013; *Heintz* and *Mair*, 2014). Axenic worms or those grown on dead bacteria do not respond to the drug. The mode of action was reported to be an inhibition of folate metabolism in *E. coli*, leading to reduced methionine. This is consistent with lifespan extension by methionine reduction in a variety of animal models, including worms. Several studies suggest that metformin affects the mammalian microbiome (*Forslund* et al., 2015; *Wu* et al., 2017; *Bauer* et al., 2018), but this relates to lifespan extension in mammals remains unknown.

Inhibition of the mTOR pathway is associated with lifespan and healthspan

extension in a range of animal models (*Kennedy* and *Lamming*, 2016), and preliminary data using rapalogs [a class of very specific mTOR inhibitors in which rapamycin is the founder (*Lamming* et al., 2013)] suggests similar effects may be possible in humans (*Mannick* et al., 2014, 2018). Acute rapamycin treatment in old mice impacts the faecal microbiome, with a notable increase in segmented filamentous bacteria. An earlier study in middle-aged mice reported modest effects of rapamycin on gut bacterial composition (*Hurez* et al., 2015). Whether these changes are important for the longevity effects of rapamycin remains unknown. A number of other drugs have been reported to extend lifespan in mice and they may have affected microbiome composition as well. For instance, acarbose, an α-glucosidase inhibitor that extends lifespan in male mice (*Strong* et al., 2016), alters microbiome and in turn its efficacy as an antidiabetic treatment may be dependent on microbiome composition (*Su* et al., 2015; *Gu* et al., 2017).

## MICROBIOME INTERVENTIONS AND AGEING

Drugs are promising interventions for targeting ageing, but as stated this approach is fraught with regulatory hurdles. By contrast, microbiome-mediated interventions are likely less subject to regulatory concerns but research must be performed to determine to optimize the efficacy of any such approach (*Kundu* et al., 2017). Faecal microbiota transplants (FMTs) are perhaps the most promising to date, although the long-term effects of this approach remain controversial. At first blush, it would seem that to delay ageing, any intervention must be durable, or administered relatively frequently. Other approaches, including postbiotics and dietary approaches to modify bacterial populations, are probably even more primitive to date.

In particular, metagenomic studies that identify metabolite deficiencies in the gut of ageing people and corresponding changes in systemic metabolism offer great promise as it may be possible to correct these deficiencies with supplements or other approaches. For instance, healthier community dwelling elders were found to have enriched bacterial species for short chain fatty acid production, which may promote more youthful metabolism (*Claesson* et al., 2012). It should be noted that molecular changes associated with ageing are not always detrimental, and in some cases might be compensatory. A good example is testosterone, which declines with ageing in the male population. Testosterone supplementation to restore the hormone to youthful levels is not necessarily beneficial, and in fact is associated with increased risk of disease in some contexts (*Sansone* et al., 2017). Together, these observations call for more intensive studies in animal models and humans to (1) identify candidate microbiome-associated interventions that might extend healthspan and (2) test them effectively.

## CONCLUSION

The possibility to slow human ageing and extend healthspan is a relatively recent occurrence, as this has only been relatively recently achieved in animal

models. The potential benefits of preventing multiple chronic diseases simultaneously and improving function later in age demand that this approach be explored. Meanwhile, the importance of gut bacterial species in human health and disease has emerged almost concurrently. To what extent do our residents age us, or keep us young? Merging these two concepts, ageing and microbiome research, offers great promise and requires intense investigation. Possibly we can live longer and healthier by training our residents to behave, or perhaps helping them train us to adopt healthier lifestyles?

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