Microbiome: Effects of Ageing and Diet

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Abstract

The microbial community inhabiting our intestine, known as 'microbiota', and the ensemble of their genomes (microbiome) regulate important functions of the host, being essential for health maintenance. The recent development of next-generation sequencing (NGS) methods has greatly facilitated the study of the microbiota and has contributed to evidence of the strong influence exerted by age and diet. However, the precise way in which the diet and its components modify the functionality of the intestinal microbiome is far from being completely known. Changes in the intestinal microbiota occur during ageing, frequently accompanied by physiological changes of the digestive tract, modification of dietary patterns and impairment of the immune system. Establishing nutritional strategies aiming to counterbalance the specific alterations taking place in the microbiota during ageing would contribute to improved health status in the elderly. This review will analyse changes appearing in the intestinal microbiota from adulthood to old age and their association with dietary patterns and lifestyle factors.

Introduction

The microbiota is the pool of commensal microorganisms colonizing our body and includes bacteria, archaea, viruses and unicellular eukaryotes. The largest microbial population is found in the human gut; in particular, the colon is the most densely populated area, with a total microbial content estimated as about 10^{14} cells (Hooper and Gordon, 2001). The ensemble of genes contained by this microbiota is known as the 'intestinal microbiome' and they encode for multiple functions, some of which strongly influence the host physiology

and metabolism. The gut microbiota and its metabolites interact with the host at different levels and its correct composition and functionality is essential for maintaining a 'healthy status' through life. The advent of culture-independent techniques, particularly the more recent development and cost affordability of DNA next-generation sequencing (NGS) and the omics technologies (metatranscriptomics, metaproteomics and metabolomics), have made possible the study of the microbiota composition and its functional capacity. The latest scientific advances have evidenced that the microbiota is strongly influenced by diet and age, whilst some important aspects of its functionality still remain unknown. Short-chain fatty acids (SCFA; acetate, propionate and butyrate) are the most abundant metabolites produced by the microbiota as end products from the fermentation of indigestible carbohydrates and proteins in the colon. It is known that their production is influenced by dietary patterns. These compounds affect the physiology of the colon, contribute to shape the intestinal microbial environment, serve as energy source to host cells and influence the host metabolism and physiology (Rios-Covian *et al.*, 2016).

The gut microbiota colonization starts early after birth and even a primary fetal colonization may occur. The diversity, stability and complexity of this microbiota evolves until the approximate age of three years, remains without noticeable variations in healthy adults and becomes unstable again in the elderly. Changes in the intestinal microbiota occurring during ageing are concomitant with a general physiological decline, anatomical modifications, cognitive impairment and a deregulation of the immune system. This, together with variations in dietary habits promoted by social changes (solitude, living in long-term residential institutions, etc.), anatomical impairment and lower energy requirements due to changes in the whole body composition, increases the risk of nutritional deficiencies and malnutrition in the elderly. However, there still are a limited number of studies describing the composition of the microbiota in elderly individuals and even less about its functionality.

Lifespan is increasing all over the world, with this trend being more pronounced in industrialized countries. According to a recent report from the United Nations (2015), the population aged over 60 years is the fastest growing, and more than 20% of the world's population will be over the age of 60 years by 2050. The increase in life expectancy is one of the most relevant social and medical achievements of the last two centuries. However, the rise in health-care costs associated with recurrent disease and disability frequently occurring in the elderly represents an economic burden and a sustainability challenge for our current social structure. Therefore, it is urgent that scientists and medical professionals seek an understanding of how to maintain health throughout the ageing process whereas social and economic policies should be implemented in order to translate scientific advances to the general population, with the final perspective of contributing to healthy ageing and ultimately to a sustainable welfare society. Besides physical activity and lifestyle, nutrition is one of the most important factors playing a key role in healthy ageing. The possibility of shaping the microbiota through diet in order to beneficially affect host health is a priority within this multidisciplinary task.

The present review aims to analyse from a global perspective the current state of knowledge about the effect of age and nutritional status on the gut microbiota during the ageing process and the nutritional and lifestyle strategies that contribute to favour healthy ageing of the body and its microbiota. This analysis is approached from the perspective of the most recent techniques available for the study of the composition and functionality of

the intestinal microbiota, considering the physiological, immune and nutritional states that influence the global health status of the individual.

The physiology and the immune status in the elderly

With the advancement of age there is a normal and continuous decline in physiological functions, which constitutes the so-called 'senescence' process. This natural process, not a disease, is multifactorial and several inter-related body functions are affected, including metabolic alterations, inflammation, macromolecular damage and reduced protein homeostasis, among others (Kennedy *et al.*, 2014). The impairment of these functions renders the individuals more prone to different conditions such as infectious and digestive diseases, bone injuries or mental health problems. The incidence of these chronic diseases represents a serious societal concern related to the ageing of the population. As an example, mental health-related problems, such as dementia, are expected to double in the next three decades (Dolgin, 2016), giving an idea of the extraordinary magnitude of the challenges associated with an aged population and underlining the need for immediate action. It is thus of great importance to differentiate between normal physiological decline and the changes related to pathological processes with high incidence in aged populations. In this context, the term 'senescence' is often used to refer to non-pathological biological and physiological processes strictly dependent on age, whereas 'ageing' would refer to changes, physiological and pathological, associated with the passage of time (Rowe, 1997; Troen, 2003).

Different body functions are affected by the ageing process, among them immune deregulation (Mazari and Lesourd, 1998), and changes in the intestinal microbiota (Odamaki *et al.*, 2016) and gastrointestinal physiology (Remond *et al.*, 2015) are well recognized. The age-associated changes in the immune function are multifaceted and involve both cellular and humoral immunity. These changes include a reduced activity of immune cells, such as natural killer cells and T cells, a defective response to mitogen-induced proliferative responses, or an increased level of circulating pro-inflammatory cytokines (Jing *et al.*, 2007; Candore *et al.*, 2008, 2010; Vallejo, 2011; Salazar *et al.*, 2013). These alterations, often referred to as 'immunosenescence' (Larbi *et al.*, 2008; Monti *et al.*, 2017), may contribute to explain the increased disease risk observed in elderly people. Moreover, these changes in immune functions may promote neurological disorders. As an example, the existence of a pro-inflammatory status in senior population, which includes also neuroinflammation (Layé *et al.*, 2011), may lead to cognitive decline and neurodegenerative diseases (Wyss-Coray, 2016). This potential primary role of inflammation in different age-related diseases has led to the proposal of the 'inflammaging' theory (Franceschi *et al.*, 2000).

Concomitantly with the above-mentioned immune changes, senescence is also related to difficulties in maintaining physiological and metabolic homeostasis. At the gastrointestinal tract level, changes from mouth to colon occur and go from reduced production of saliva (xerostomia) and other digestive secretions to impaired absorption of nutrients and slower gastrointestinal transit (Culross, 2008; Gonsalves *et al.*, 2008; Rémond *et al.*, 2015). These gastrointestinal changes, together with the loss of taste, induce changes in food preferences and dietary patterns, increasing the risk of nutritional deficiencies and malnutrition (Salazar *et al.*, 2017). As a consequence, there is a decrease of lean and bone mass, a phenomenon known as 'sarcopenia' (Beaufrère and Morio, 2000), which further compromises the homeostasis of the individual.

To summarize, the ageing process is linked to several changes in dietary patterns, immune senescence, impaired gastrointestinal physiology and a concomitant decline in cognitive function. This process is also related to changes in microbiota functionality and composition (dysbiosis), all together contributing to frailty. Given the key role that the gut microbiota plays in important body functions, changes in this human microbial ecosystem may reduce colonization resistance and lead to immune changes resulting in increased disease risk. Therefore, the gut microbiota has often been considered a target to improve health in the senior population (Valdés *et al.*, 2017), together with the modulation of immune parameters, such as reducing the age-related pro-inflammatory status (Salazar *et al.*, 2017).

High-throughput techniques for the study of the microbiome

In the early era of microbiology, the study of the gut microbiota community commonly involved the isolation and cultivation of individual microbes from faeces; this allowed the discovery of coliforms by Teodor Escherich, who isolated the first gastrointestinal bacterium, *Bacterium coli commune*, later named *Escherichia coli* (Escherich, 1989). Following that, other major gastrointestinal bacterial members, such as *Bacteroides*, *Bifidobacterium* and *Bacillus*, were isolated (Rajilić-Stojanović and de Vos, 2014). However, efforts in this way were neglected by the fact that most of the microorganisms inhabiting in the gastrointestinal tract are obligate anaerobes and most of them were uncultivable. A significant advance in gut microbiota characterization was the introduction of the anaerobic culture techniques by Hungate (1969). Although cultivation-based approaches continue to be very important for the study of the gut microbiota, it was not until the emergence of the independent-culture techniques that the gut microbiota could be studied in detail without omitting uncultivable microbes. In addition, microbial taxonomy and phylogenetic relationships between organisms started to be established (Woese *et al.*, 1990).

DNA-based molecular techniques, such as denaturing gradient gel electrophoresis (DGGE), were used to identify gut dysbiosis associated with Crohn's disease, suggesting changes in *Clostridium* spp. and *Bacteroides* spp. (Martinez-Medina *et al.*, 2006; Scanlan *et al.*, 2006). This technique was also used to identify gut dysbiosis associated with cystic fibrosis, where lower abundance and temporal instability of bifidobacteria and *Clostridium* cluster XIVa in the faecal microbiota of patients with cystic fibrosis was observed (Duytschaever *et al.*, 2013). In a similar way, quantitative PCR (qPCR) was employed to compare the gut microbiota of healthy and hospitalized elderly, observing a reduction in the number of bacteria and the complete elimination of certain bacterial communities in hospitalized elderly individuals submitted to antibiotic treatment, as well as the proliferation of the opportunistic species *Enterococcus faecalis* (Bartosch *et al.*, 2004). Similarly, differences in gut microbiota composition were evidenced by this technique between a group of institutionalized elderly and a group of seniors (Salazar *et al.*, 2013). qPCR also evidenced a profound impact on the gut microbiota establishment of premature babies with respect to full-term neonates, with a lower number of anaerobes such as *Bifidobacterium* or *Bacteroides*, a higher number of pathogens such as *Klebsiella pneumoniae* and significantly reduced gut microbiota diversity occurring in preterm infants (Arboleya *et al.*, 2012). Finally, a study using the terminal-restriction fragment length polymorphism (T-RFLP) technique showed significantly reduced numbers of Bacteroidetes and a higher Firmicutes-to-Bacteroidetes ratio in obese subjects compared with non-obese subjects, which was consistent with data

obtained by other methods (Kasai *et al.*, 2015). T-RFLP was used as well to analyse gut biopsy tissues in inflammatory bowel disease, showing an increment in species richness from control to ill but non-inflamed tissues, followed by a declined in fully inflamed tissues (Sepehri *et al.*, 2007).

Despite the fact that the association between microorganisms and host health was known previously to the advent of high-throughput sequencing techniques (Miller *et al.*, 1957), the emergence and fast progress of DNA sequencing technology has completely revolutionized human microbiota research in the last decade. These techniques are relatively fast and cost affordable and capable of generating billions of sequence reads, such as with Illumina HiSeq platforms, or providing long read lengths, such as with PacBio (Reuter *et al.*, 2015). This advance allows generating a big overview of the microbial members present in a sample, thus bypassing the need for culturing. Moreover, with the PCR-based sequencing approaches, enrichment of DNA templates with initial low concentration led to the identification of minority populations within complex communities. During the last decade, international research initiatives such as the Metagenomics of the Human Intestinal Tract consortium (MetaHIT) (Li *et al.*, 2014) or the Human Microbiome Project (HMP) (Human Microbiome Project Consortium, 2012) have taken advantage of those giant steps forward in the microbiome research and have generated many data and much knowledge. The last study from HMP has been published, updating body-wide metagenomics profile of the human microbiome, from the analyses of 1631 new samples, concluding that the Bacteroidetes to Firmicutes ratio should not be used as a defining attribute of an individual's gut microbiome (Lloyd-Price *et al.*, 2017). This rapidly moving advance in the sequencing platforms is producing a large number of data that far surpasses the ability to analyse them, creating in some cases a bottleneck in the interpretation of results. The bioinformatics field is growing fast as well and new tools are emerging to provide interpretation of data generated from NGS platforms.

Woese and Fox (1977) found that the ribosomal RNA (rRNA) gene could be used as marker gene to establish phylogenetic associations between organisms. Nowadays, the high-throughput sequencing of the small subunit rRNA gene is the most common method for gut microbiota characterization at compositional level. After total DNA isolation, PCR amplification with primers targeted towards highly conserved regions of the gene, 16S for prokaryotes and archaea and 18S rRNA for eukaryotes (including fungi) (Eckburg *et al.*, 2005; Amaral-Zettler *et al.*, 2009), generates a pool of amplicons derived from the different microbes present in a sample. Next, they are massively sequenced and clustered into operational taxonomic units (OTUs) by sequence similarity. This technique, also called 'marker gene survey', provides a broad census of the microbial species present in a sample. High-throughput amplicon sequencing has provided a large number of data on human microbiome diversity and composition, showing that Bacteroidetes, Firmicutes and Proteobacteria are the dominant taxa in the human gut (Eckburg *et al.*, 2005; Human Microbiome Project Consortium, 2012; Huse *et al.*, 2012; Yatsunenko *et al.*, 2012; Lloyd-Price *et al.*, 2017). The rRNA gene sequencing has also generated insight into host–microbe interactions, showing a direct correlation between gut microbiota, diet and health and yielding hypotheses about microbiota-based disease mechanisms (Turnbaugh *et al.*, 2009b; Muegge *et al.*, 2011; Claesson *et al.*, 2012). However, this technique presents some limitations, such as the biases that primer efficiency, PCR amplification and bioinformatics pipelines, among others, can introduce to results (Sharpton, 2014). The same principle, but focused on

specific functional genes, was carried out to identify novel butyrate and propionate bacteria producers (Walker *et al.*, 2014; Louis *et al.*, 2010; Reichardt *et al.*, 2014). In other studies, amplifications of the RNA polymerase β-subunit (*rpoB*) gene were performed for the differentiation of *Bifidobacterium* species in infant samples (Kim *et al.*, 2010; Murphy *et al.*, 2015).

Cataloguing members of the gut microbiota has developed into routine; however, the foundational understanding of the functionality of each individual member in their ecosystem still remains a challenge. With this purpose, meta-omics technics focused not only on DNA, but also on RNA, proteins or small molecules, with these providing huge progress in the comprehension of the intricate crosstalk among gut bugs and gut bugs and the host.

Shotgun metagenomics sequencing extends the information provided by 16S/18S rRNA amplicon sequencing to study the entire genomic content of all microbes, cultured or uncultured, present in a sample. This approach avoids previous sequencing limitations as all the genomic DNA is trimmed into tiny fragments that are all individually sequenced, instead of sequencing only a specific amplicon. It will provide both taxonomic and biological function information encoded by the genome (Sharpton, 2014). This technique has evolved to address the questions of 'who is present?' and 'what they can do?' (Oulas *et al.*, 2015). Moreover, it allows the identification of bacteriophages, members of the gut virome, which modulate the bacterial community and interact with the host, playing an important role in host health (Reyes *et al.*, 2010, 2015). There are, however, some important limitations to the use of shotgun metagenomics such as its higher cost, complexity and difficulty of data analyses, as well as the unwanted host DNA that usually contain (Prakash and Taylor, 2012; Sharpton, 2014). The first human intestinal metagenome study, dating from 2006 (Gill *et al.*, 2006) and carried out with samples from two healthy adult volunteers, revealed an enrichment in metabolic pathways related to carbohydrates, amino acids and xenobiotics. Modifications in the gut microbiota and its link with human metabolism on ageing have been corroborated with this technique in a study carried out by Rampelli *et al.* (2013). A loss of genes for SCFA production and an overall decrease of the saccharolytic potential in the intestinal metagenome of elderly with respect to younger adults were observed. Shotgun sequencing has been used to define functional profiles of gut microbiota in the context of several diseases (Qin *et al.*, 2010; Morgan *et al.*, 2012; Zeller *et al.*, 2014).

Whereas metagenomics focuses on all of the functions present in a community (the functional *potential*), metatranscriptomics describes the functional activity of the gut microbiota expressed at a given time and depending on the specific environmental conditions: 'what they are doing?'. This technique involves isolation of RNA to be converted to a cDNA library, which is subsequently sequenced in a high-throughput platform. Next, reads are mapped to a reference genome or transcripts, or assembled *de novo* (Wang *et al.*, 2009). Metatranscriptome data indicated that the gut microbiota contributes to reinforce and increase the metabolic potential of the host, as almost 50% of genes expressed by this microbial community are involved in transport and metabolism of carbohydrates, amino acids, lipids, nucleic acids and energy production (Gosalbes *et al.*, 2011), which corroborates findings obtained with other omics techniques (Gill *et al.*, 2006). Metatranscriptomics have been used to detect microbial metabolic perturbations and to show the influence of environmental factors on gut microbiome functions, evidencing differences between the functional potential and the activity of the gut microbiome (Franzosa *et al.*, 2014). It also allowed the analysis of the gut virome (Santiago-Rodriguez *et al.*, 2015) and the study of microbiota–host interactions (Camp *et al.*, 2014). Moreover, *Fusobacterium nucleatum*

has been discovered to be involved in colorectal cancer by this technique (Castellarin *et al.*, 2012). In spite of this, metatranscriptomic usually becomes more challenging than metagenomics because of the need for conversion of mRNA to cDNA, the lack of reference genomes or databases available and the short-half-life of mRNA molecules (Walker, 2016).

Metaproteomics, defined as the large-scale profiling of the complement of proteins produced by a complex microbial ecosystem (Wilmes and Bond, 2009), presents some advantages over metatranscriptomics because the study of proteins provides a more representative overview of the final functional activity of the community (Walker, 2016). Despite its limitations (Walker, 2016), this techniques has been largely employed to characterize functional roles of gut microbiota in health and disease conditions (Lee *et al.*, 2017).

Together with metaproteomics, metabolomics closes the entire pathway from genes to the functionality of the gut microbiota. The study of the final products (metabolites) of bacterial metabolism present in a sample provides a better knowledge of the functionality and physiology (Ursell *et al.*, 2014). Metabolomics involves the identification and quantification of the metabolites (Fiehn, 2002) and it can be performed in a targeted or untargeted way. The targeted method is based on the analysis of different classes of previously defined compounds, such as amino acids, lipids, carbohydrates or fatty acids, whereas the non-targeted approach provides a global profile picture of the metabolic diversity of the sample (Smirnov *et al.*, 2016). Different methodology and analytical platforms are employed in metabolomics, including gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) or proton nuclear magnetic resonance spectroscopy (H-NMR), among others (Vernocchi *et al.*, 2016). The use and combination of more than one methodology is necessary to analyse the complete metabolome of a sample, because of the diversity in properties and concentrations of the metabolites (Smirnov *et al.*, 2016). Some limitations are linked to this technique, such as the lack of good and complete reference databases, the resolution limits or the difficulty of assigning the production of particular metabolites to specific microbes in an accurate way (Walker, 2016). The number of metabolomic studies in gut microbiota have increased in the last years (Smirnov *et al.*, 2016), evidencing a gut microbiota modulation related to diet and ageing. De Filippis *et al.* (2016) analysed the effects of a Mediterranean diet on the human gut metabolome profile and the effects of synbiotic foods on gut metabolome were studied by Vitali *et al.* (2010). Age-related metabolic changes were found in urine and faeces from mice (Calvani *et al.*, 2014), as well as in Italian centenarians, by using an H-NMR approach (Collino *et al.*, 2013).

The high-throughput techniques for the gut microbiome study mentioned above have their 'pros' (advantages) and 'cons' (limitations). The integration of the information generated by all of them could provide an extensive and more comprehensive snapshot of the gut microbiota and its relationship with the host, in addition to a definition of biomarkers and interventional targets in different conditions and for different populations (Abram, 2015; Zhang and Zhao, 2016). Despite this, to determine which particular bacteria has a specific function, or how its function is affecting the host, the research still has to come back to the 'the microscope' (Troponi *et al.*, 2017) and the culturing approach. In the last years, Raoult's research group, through a high-throughput culturing approach, so-called 'culturomics', has 'broken the records' and increased the number of species isolated and cultured from the human gastrointestinal tract to over 1000 (Lagier *et al.*, 2012, 2015, 2016). They were able to cultivate organisms corresponding to sequences previously not assigned by metagenomics. Culturomics combines high-throughput culture conditions and matrix-assisted laser

desorption/ionization-time of flight (MALDI-TODF) or 16S rRNA sequencing for the identification of growing colonies (Lagier *et al.*, 2016). This rebirth technique improves the capture of gut diversity, improving our understanding of the gut environment.

The evolution of the intestinal microbiota through ageing

Traditionally, the establishment of the intestinal microbiota and the microbial colonization of the gastrointestinal tract have been considered to occur during and after delivery; however, the presence of microorganisms in placenta and amniotic fluid (Collado *et al.*, 2016) suggests a primary fetal colonization. The classic pattern of colonization at the beginning of life involves mainly facultative anaerobes, which are replaced over time by strict anaerobic microorganisms due to the consumption of oxygen by the first colonizers (Fanaro *et al.*, 2003). The initial gut microbiota composition is simple, dynamic and very unstable and undergoes marked fluctuations. Some of the most influential factors on the nascent microbiota appear to be the mode of delivery, feeding practices, maternal weight or lifestyle and the use of antibiotics (Nogacka *et al.*, 2017, 2018). Accumulating evidence indicates that the establishment and development of the microbiota in early life and its cross-talk with the host is especially critical in the developmental programming of adult health and disease. Moreover, there is increasing concern about the potential long-term effects of microbiota alterations in the neonatal early life. This early microbiota seems to provide a stimulus required for an adequate development of the gut and immune functions (Nogacka *et al.*, 2018). The microbiota development process determines to some extent the predisposition to develop diseases in early and later life including metabolic and immune pathologies such as necrotizing enterocolitis, obesity and type 2 diabetes, chronic intestinal inflammation, or allergy, among others (Goulet, 2015).

The classical microbiota development begins from low diversity and complexity and evolves until reaching a complex structure that stabilizes at 2–3 years of age (Yatsunenko *et al.*, 2012). The adult-like intestinal microbiota is characterized by a set of microorganisms that mainly inhabit the gastrointestinal tract, and have co-evolved with humans in a commensal way, reaching its highest concentration in the colon (about 10^{14} cells) (Arumugam *et al.*, 2011). A ratio between intestinal bacteria and total human cells was estimated as $10:1$, although considering only the nucleated human cells (excluding erythrocytes) the approximate ratio is $1.3:1$ (Sender *et al.*, 2016). The composition of adult intestinal microbiota at the phylum level is represented almost entirely by two major phyla, Bacteroidetes and Firmicutes. The ratio between them is frequently used as an indicator of alterations and bacterial shifts, although, based on data generated in the HMP project about the human microbiome, researchers conclude that the Bacteroidetes-to-Firmicutes ratio should not be used as a defining attribute of an individual's gut microbiome (Lloyd-Price *et al.*, 2017). The remaining minority phyla of the human microbiota in descending order are Actinobacteria, Proteobacteria, Verrucomicrobia and Euryarchaeota (Arumugam *et al.*, 2011).

The microbiome is usually recognized as our second genome (Aagaard *et al.*, 2013) and contributes extensively to our physiology and metabolism (Qin *et al.*, 2010). There are some well-established functions attributed to the intestinal microbiota, among which the following are noteworthy: (i) the maintenance of the intestinal epithelium integrity and functionality, contributing to enhance the intestinal barrier, to nutrients supply and to the protection against pathogens, (ii) inhibition of pathogen adhesion to intestinal surfaces,

(iii) modulation and maturation of the immune system, (iv) degradation of non-digestible carbon sources and (v) production of different metabolites such as vitamins and SCFA (Tojo *et al.*, 2014). The intestinal microbiota is relatively stable throughout adulthood, although, as a dynamic ecosystem, it moderately fluctuates and is susceptible to some variations owing to stress, antibiotics, diet and lifestyles (Rodríguez *et al.*, 2015).

In the elderly, an inverse process occurs that resembles a mirror image of the neonatal gut colonization and the microbiota becomes unstable again and dysbiosis frequently appears (Salazar *et al.*, 2017). Age-related changes in the gut microbiota are associated with physiological changes in the gastrointestinal tract, as well as in dietary patterns, with a concomitant decline in the normal function of the immune system that may contribute to increased risk of infection and frailty (Claesson *et al.*, 2011, 2012; Odamaki *et al.*, 2016). An imbalanced diet frequently occurs because of problems in the smell sense, accompanied by difficulties in swallowing and masticatory dysfunction. In addition, the reduced intestinal motility leads to faecal impaction and constipation and increases the intestinal transit time. Besides, a slower intestinal transit times leads to altered nutrient availability and absorption, reduced stability and diversity of microbial communities, thinning of the mucosal lining and subsequent dysfunction of the intestinal barrier, and increased inflammation, all of them being common manifestations of ageing of the gastrointestinal tract (Biagi *et al.*, 2012). These factors inevitably affect the functionality of the microbiota and are linked with alterations of microbiota composition and metabolism.

There is no currently agreed definition of old age-specific gut microbiota profile due to the high inter-individual variability, differences in diet and lifestyle and the unclear definition of the term 'elderly'. In addition, there are a limited number of studies in the literature that describe the composition of the microbiota of elderly individuals, which restricts our ability to establish cause and effects relationships. Briefly, the elderly's gut microbiota is characterized by a reduced bacterial diversity, shifts in the dominant species, a decline in beneficial microorganisms, increase of facultative anaerobic bacteria, decreased levels of total SCFA and changes in the ratios among the different SCFA with respect to that found in younger adults (Claesson *et al.*, 2012; Salazar *et al.*, 2013, 2014). Opportunistic pathogenes also appear more frequently at old age (O'Toole and Claesson, 2010; Kumar *et al.*, 2016; Salazar *et al.* 2017). Moreover, the microbiota composition is also influenced by the well-established driving force, which is the geographical factor (Yatsunenko *et al.*, 2012). Therefore, there are numerous confounding data about the changes due to age in several microbial groups. Thus, for example, a comparative study of the microbial composition in four European countries indicated age-related structural differences that differ from country to country (Mueller *et al.*, 2006). Nevertheless, some trends are repetitively observed such as the decrease in bifidobacteria in the elderly population, confirmed by several studies using different methodologies (Gavini *et al.*, 2001; Hopkins *et al.*, 2001; Biagi *et al.*, 2012). The gut microbiota analysis in old population showed a clear shift to a *Clostridium* cluster IV-dominated community in the elderly Irish population with respect to younger adults (Claesson *et al.*, 2011) but this observation was the inverse in Austrian elders (Zwielehner *et al.*, 2009) and in a Finnish population (Mäkivuokko *et al.*, 2010). This fact may be due to the inclusion of the genus *Faecalibacterium*, which shows a country-specific pattern, with higher levels observed in French, German, Swedish (Mueller *et al.*, 2006) and Irish (Claesson *et al.*, 2011) populations and lower levels in Italian (Mueller *et al.*, 2006; Biagi *et al.*, 2016), Spanish (Salazar *et al.*, 2013) and Chinese (Wang *et al.*, 2015) cohorts. *Clostridium* cluster

XIVa has also been found to be associated with a country–age relationship. In German seniors increased populations have been observed (Mueller *et al.*, 2006) whereas decreased populations in Spanish (Salazar *et al.*, 2013), Italian (Mueller *et al.*, 2006; Biagi *et al.*, 2016) and Japanese (Hayashi *et al.*, 2003) elders, as compared with their respective adult cohorts, have been reported for these bacterial groups. Similar country-age discordances occur with *Bacteroides – Prevotella* group. That cluster is under-represented in Italian (Mueller *et al.*, 2006; Claesson *et al.*, 2011; Biagi *et al.*, 2016), English (Woodmansey *et al.*, 2004) and Spanish (Salazar *et al.*, 2013) cohorts and is over-represented in German, Swedish (Mueller *et al.*, 2006), Austrian (Zwielehner *et al.*, 2009), Finnish (Mäkivuokko *et al.*, 2010), Irish (Claesson *et al.*, 2011) and Japanese (Odamaki *et al.*, 2016) cohorts. Nevertheless, in spite of discrepancies existing among studies in different countries, there is a rather general agreement that the variations in the gut microbial composition at senescence frequently involve lower levels of *Blautia coccoides* (also known as *Clostridium* XIVa group), *Bifidobacterium* and *Faecalibacterium* groups as compared with adults (Hopkins *et al.*, 2001; Biagi *et al.*, 2010; Claesson *et al.*, 2011, 2012; Salazar *et al.*, 2013, 2017; Odamaki *et al.*, 2016). Other relevant intestinal microbial populations such as the phylum Bacteroidetes and the *Lactobacillus* group remain controversial; although some authors have reported lower levels in aged subjects (Hopkins *et al.*, 2001; Hayashi *et al.*, 2003; Bartosch *et al.*, 2004; Woodmansey, 2007; Biagi *et al.*, 2010; Tiihonen *et al.*, 2010; Claesson *et al.*, 2011; Salazar *et al.*, 2013), others have observed increases at older ages (Mäkivuokko *et al.*, 2010; Tiihonen *et al.*, 2010; Salazar *et al.*, 2013; Odamaki *et al.*, 2016). However, it is still unclear whether the variability in the results obtained for certain microbial groups is related to actual population differences or to methodological issues, such as the different techniques used for determining microbial abundancies. As expected from the differences in microbiota observed between elderly and younger adults, the production of bacterial metabolites, mainly SCFA, is also altered at old age.

Recent studies comparing the trajectory of the microbiota from adults to elderly, centenarians and long-lived semi-supercentenarians (105–110 yeasr) evidenced a cumulative abundance decreasing of some symbiotic bacterial taxa (mainly belonging to the Ruminococcaceae, Lachnospiraceae and Bacteroidaceae families) with age. In extreme longevity, the microbial diversity increases again: facultative anaerobes and allochthonous opportunistic bacteria occur, but at the same time some new microorganisms, not found in younger adults and elder, seem to emerge, whereas a higher prevalence than in younger individuals occur for some health-associated bacterial groups (e.g. *Akkermansia*, *Bifidobacterium* and Christensenellaceae) (Biagi *et al.*, 2016). Regarding the Firmicutes to Bacteroidetes ratio, frequently used in the scientific literature as an indicator of bacterial shifts, variable and controversial results have been reported (Salazar *et al.*, 2017). However, it is not currently possible to determine if these features observed in extremely old people were already present in the same individuals at a younger age and/or if they are associated with their past lifestyle (Biagi *et al.*, 2016).

Gastrointestinal infections in the elderly

In addition to structural and functional changes, increase in oxidative stress and inflammation of the intestinal mucosa naturally occurring in senescence (Soenen *et al.*, 2016), other

factors such as antibiotic and non-steroidal anti-inflammatory drug treatments, changes in diet and/or institutionalization contribute to pathological alterations of the intestinal microbiota (Claesson *et al.*, 2012; Tarnawski *et al.*, 2014). *Clostridium difficile* is the most frequent pathogen in bacterial infections associated with health care and ageing (Magill *et al.*, 2014), followed by *Helicobacter pylori* and, to a lesser proportion and depending on the cohort, *Bacteroides fragilis*, *Klebsiella oxytoca* and enteropathogenic *Escherichia coli* (Magill *et al.*, 2014; Kullin *et al.*, 2015; Luo *et al.*, 2017).

C. difficile is an anaerobe, spore-forming, Gram-positive bacterium found in the intestinal tracts of both animals and humans (Elliott *et al.*, 2017). Institutionalization is one of the main risk factor for *C. difficile* infection (CDI), this fact contributing to increases up to 21% of carriage rate in individuals under short- or long-term care in hospitals, in comparison with 1.6% in the community (Rea *et al.*, 2012). It has been indicated that *C. difficile* can be acquired by patients with diarrhoea caused by other microorganisms after some days of stay in hospital (Rand *et al.*, 2015). Antibiotic treatment is another main factor that facilitates infection by this pathogen. In fact, different antibiotic treatments lead to different intestinal microbiota modifications and, as a result, different susceptibilities to CDI (Willing *et al.*, 2011; Lewis *et al.*, 2015). A decrease in bacterial diversity occurs during CDI in elderly patients, with health-promoting bacteria including *Faecalibacterium* and *Bifidobacterium* being under-represented in those patients (Rea *et al.*, 2012). A recent study comparing CDI patients with healthy controls that were not under antibiotic treatment revealed a decrease in alternative health-related bacteria, as *Akkermansia*, and in some groups of commensal bacteria, as members of the families Ruminococcaceae and Lachnospiraceae, along with members of the genera *Bacteroides* and *Alistipes* (Milani *et al.*, 2016). In the two last studies mentioned above, authors found an increase in some recognized or opportunistic pathogens, such as *Enterococcus*, *Staphylococcus*, *Klebsiella*, *Escherichia* or *Helicobacter* members, among others (Milani *et al.*, 2016). Residents in nursing houses with CDI had more comorbidities than non-CDI residents, including diabetes, urinary incontinence or chronic obstructive pulmonary disease (Zarowitz *et al.*, 2015). Additionally, residents with CDI acquired before institutionalization were less likely to carry a burden of illness than residents who acquired CDI in the nursing house (Zarowitz *et al.*, 2015). Common antibiotic treatments against CDI, including vancomycin, metronidazole and fidaxomicin, show a relatively high recurrence rate (Kociolek and Gerding, 2016); however, promising results are being obtained with the novel therapy consisting of faecal microbiota transplantation, which reaches an 83–94% response rate in recurrent CDI (Bagdasarian *et al.*, 2015). As comorbidities frequently occur in elderly people suffering CDI, faecal microbiota transplantation should be considered case by case; experts stress the importance of prevention and combination of antimicrobial and non-antimicrobial complementary treatments as first-line options in CDI (Asempa and Nicolau, 2017).

H. pylori is a microaerophilic, Gram-negative, spiral-shaped and flagellated bacterium found in the gastrointestinal tract (Tomb *et al.*, 1997). *Helicobacter* infections are more frequent as the population gets older, starting to rise at the age of 40–55 years and keeping the same levels of prevalence in the elderly (Genta *et al.*, 2017). *H. pylori* infection (HpI) can lead to Barrett's oesophagus, peptic ulcers, intestinal metaplasia and stomach cancer (Reva *et al.*, 2015). Some studies have related the presence of *H. pylori* in the elderly with changes in plasma levels of ghrelin and in the ghrelin-to-obestatin ratio, which are known

to play an important role in appetite regulation (Gao *et al.*, 2009a; Kim *et al.*, 2017). In particular, Kim *et al.* (2017) found that the reduction of ghrelin levels in plasma, and not the presence of *H. pylori per se*, were related to the intestinal metaplasia associated with infection by this bacterium. As one of the main risk factors of infections in the elderly is malnutrition, the impairment in ghrelin production during HpI can contribute to a reduction in appetite and food intake, thus worsening even more the state of malnutrition and increasing the risk of subsequent infections (Soenen *et al.*, 2016). The eradication of *H. pylori* contributes to improve several symptoms associated with HpI, such as dyspepsia or peptic ulcers. In those areas with high levels of resistance to the preferentially used antibiotics clarithromycin and metronidazole, the treatment of HpI with bismuth quadruple therapy is recommended (Malfertheiner *et al.*, 2017).

Norovirus is a single-stranded RNA virus (Zheng *et al.*, 2006) that, together with *C. difficile*, was the cause of most of the gastroenteritis deaths that occurred during 1999–2007 (83%) in the US elderly population (Hall *et al.*, 2012). The most common population hospitalized because of norovirus infection are children and the elderly, with infection being more severe in the former group (Yi *et al.*, 2016). Treatment with proton pump inhibitors, a very common drug used among elderly to alleviate digestive discomfort, has been correlated with higher rates of norovirus infection (Prag *et al.*, 2017). Norovirus are clustered in seven genogroups and the majority of the outbreaks in humans have been caused by the genotype GII.4. Although some vaccines are under trial, the duration of protection after administration is still undetermined (Aliabadi *et al.*, 2015).

An integrated approach to changes on the intestinal microbiota, dietary patterns and lifestyle factors: from adulthood to senescence

The first evidence of the influence of food and exercise in health appears in the Hippocratic corpus in the fifth century bc. Currently, there is solid scientific evidence about the importance of adequate nutritional status for the maintenance of good health in all stages of life, and particularly among the elderly, in order to prevent the appearance and progression of several chronic diseases. Nutritional requirements vary throughout life as a consequence of the modification of a plethora of physiological parameters such as body composition, basal metabolic rate or nutrient absorption, which take place together with other factors very common in the elderly, such as a sedentary lifestyle, loneliness or depression. Most of these age-related changes are intrinsically linked to the functioning of the digestive system and to the modifications of dietary patterns that occurs from adulthood to senescence. For example, the appearance of dysgeusia and xerostomia or the deterioration in dentition may impact the appetite in many ways and alter food choices, which can lead long term to malnutrition (Clemente *et al.*, 2012; Amarya *et al.*, 2015) and greatly influence the gut microbial composition, diversity and functionality (Flint, 2012). As gut microorganisms are pivotal for homeostasis in the intestine, the regulation of the immune system (Garrett *et al.*, 2010; Li *et al.*, 2016) and the prevention of major chronic non-communicable inflammatory and metabolic disorders (Shanahan *et al.*, 2017), diet could be one of the environmental factors with a greater impact on the future health of the elderly population.

Health implications of changes on the intestinal microbiota through age

Sorting out the type of microbiota is the first step in the design of functional foods specifically focused on the elderly. Nevertheless, this is a difficult task considering the high inter-individual variability existing in the intestinal microbial composition and the pool of factors that influence its establishment and composition along life. Different studies have reported changes in the gut microbial composition at senescence (see 'The evolution of the intestinal microbiota through ageing'). We have analysed differences in the nutritional status, intestinal microbial composition and intestinal microbial metabolites in a sample cohort of 114 volunteers from northern Spain, with non-declared pathology, who were stratified by age in three groups: adults (19–55 years), seniors (56–65 years) and elderly (65–95 years) (Table 1.1). Similarly to those reported by other authors, we have also found some differences in the faecal microbial composition related to age (Table 1.2). In our cohort, individuals older than 65 years showed lower levels of *Bacteroides*, *Bifidobacterium*, *Bl. coccoides* and *Faecalibacterium* together with a lower faecal concentration of the major SCFAs. The loss of the adult-associated microbiota and a reduced diversity in elderly subjects have been repeatedly reported to be associated with frailty (Claesson *et al.*, 2011; Jackson *et al.*, 2016) and have been related to a pro-inflammatory status. This pro-inflammatory status could be a possible driver of the ageing process (Biagi *et al.*, 2010), contributing to increased frailty and comorbidities and with the nutritional status (Lynch *et al.*, 2014).

There is not a specific age that determines the changes from adult to an elderly microbiota; this transition usually occurs gradually, is dependent on external factors and is different from one individual to another. It seems therefore that the maintenance of a healthy microbiota could have benefits to maintain a good quality of life associated with old age. In general, a high microbial diversity is important for intestinal homeostasis, favouring the tolerance of the microbial environment to the modification in external factors such as dietary ones (Turnbaugh *et al.*, 2009b; Lozupone *et al.*, 2012). Several studies have reported that some members of the *Bl. coccoides* group (belonging to *Clostridia* cluster XIVa) can impact human health by diverse mechanisms including the production of butyrate, which

Table 1.1 General characteristics of the study sample (northern Spanish healthy population) by age group

Characteristic	Elderly ($n=40$)	Senior ($n=37$)	Adult ($n=37$)
Age (years)	82 ± 6*	61 ± 3*	41 ± 8*
BMI (kg/m^2)	29.15 ± 4.14	25.91 ± 3.39*	29.42 ± 4.51
Energy intake (kcal/day)	1687.03 ± 393.97*	1929.93 ± 562.10	1867.32 ± 510.20
Protein intake (g/day)	82.54 ± 20.59	94.53 ± 29.33*	77.25 ± 28.09
Lipid intake (g/day)	73.68 ± 21.34	77.14 ± 22.67	73.10 ± 26.03
Carbohydrate intake (g/day)	171.61 ± 41.76	198.97 ± 83.86	220.48 ± 59.22*
Fibre intake (g/day)	15.96 ± 5.06	23.02 ± 9.14*	17.84 ± 5.37

*Significant differences ($P < 0.05$).
The results are presented as the mean ± standard deviation. Statistical analyses among groups were carried out using an analysis of variance (ANOVA) test.

Table 1.2 Levels of the major intestinal microbial groups (log no. of cells per gram of faeces) obtained by qPCR and short-chain fatty acids (mM) determined by gas chromatography

	Elderly ($n=40$)	Senior ($n=37$)	Adult ($n=37$)
Microbial group:			
Akkermansia	6.55 ± 2.84a	6.26 ± 2.12a	5.26 ± 1.43a
Bacteroides	7.78 ± 2.82a	9.31 ± 0.80b	7.93 ± 1.06a
Bifidobacterium	6.85 ± 2.64a	7.84 ± 1.47a,b	8.38 ± 0.95b
Bl. coccoides	5.54 ± 2.44a	7.51 ± 1.56b	8.94 ± 0.58c
Lactobacillus	6.40 ± 2.80a	5.85 ± 1.27a	6.32 ± 0.99a
Faecalibacterium	6.14 ± 1.64a	7.07 ± 0.74b	8.08 ± 0.90c
SCFA:			
Acetic acid	20.97 ± 13.41a	30.47 ± 9.88b	51.22 ± 16.49c
Propionic acid	8.05 ± 6.15a	13.59 ± 6.10b	15.73 ± 7.71b
Butyric acid	7.03 ± 6.78a	12.39 ± 9.46b	9.48 ± 3.77a,b
Total SCFAs (acetic, propionic and butyric acid)	36.06 ± 25.37a	56.44 ± 24.10b	76.44 ± 25.4

The results are presented as mean ± deviation. Different letters (a, b, c) indicate significant differences among age group ($P<0.05$) (statistical analysis: ANOVA and least significant difference *post hoc* test).

influences host carbohydrates and lipids metabolism (Louis and Flint, 2009; Rios-Covian *et al.*, 2016), favouring the production of secondary bile acids (Kitahara *et al.*, 2000) and inducing formation of T-regulatory cells (Atarashi *et al.*, 2013). In addition, *F. prausnitzii* is one of the most abundant butyrate producers in the human colon and has been proposed to have anti-inflammatory properties (Chun *et al.*, 2007).

Consequently to microbiota alterations in the elderly, the faecal SCFA profile is also modified. The concentrations of SCFA in the colon are critically important for immunoregulation (Maslowski and Mackay, 2011) and for maintaining gut and overall health (Gao *et al.*, 2009b; Peng *et al.*, 2009; Fukuda *et al.*, 2011). Therefore, the lower excretion of all the evaluated SCFA in our elderly Spanish cohort is in good agreement with that previously reported in the literature (Claesson *et al.*, 2011; Salazar *et al.*, 2013) and may also be related to imbalances in host health.

Dietary patterns as related to age and microbiota

It has been estimated that between 2% and 16% of institutionalized elderly people present an inadequate intake of protein and calories (Smeedings, 2001), which could increase the risk of malnutrition and exert an important impact on gut microbiota. In this way, it has been demonstrated that in adults the major microbial 'enterotypes' (Arumugan *et al.*, 2011) were clearly determined by the long-term dietary patterns of an individual: although diets with a high content of protein and animal fat were associated with a *Bacteroides* enterotype, those with a higher proportion of carbohydrates were dominated by *Prevotella* (Wu *et al.*, 2011). The sample cohort from northern Spain that we have examined in the present article presents a moderate reduction in the calorie intake with advancing age, linked to

a decrease in the carbohydrates and proteins from diet. It was, however, enough to satisfy the requirements of the sample, taking into account the reduction of the basal metabolic rate inherent to the ageing process. In addition, protein intake accounts for approximately 19% of the total energy, satisfying the standard recommendations. Protein under-nutrition usually arises associated with weight loss, which does not happen in our sample whose body mass index (BMI) is above 29 kg/m². This value equates to an overweight BMI category (Salas-Salvado *et al.*, 2007), so not pointing to significant deficiencies in the intake of macro-nutrients in the elderly group from our cohort.

The maintenance of a high dietary variety is essential for an overall optimal nutritional status and health outcomes in frail elderly people (Bernstein *et al.*, 2002). In spite of this, foods with low palatability, such as vegetables and fruits, and those difficult to chew or to digest, like meats, are often excluded from the diet or are reduced with ageing. Based on this premise, changes that take place drastically with age could have a major impact on the intestinal microbial ecosystem. To examine whether there is a change in the dietary pattern in our sample associated with age we analysed the intake of the major food groups in our sample according to age. Significant differences in the intake of sugar and sweets, alcoholic and non-alcoholic beverages, cereals, fruits, eggs, milk and milk products, fish and seafoods, roots and vegetables were observed according to age (Fig. 1.1). Interestingly, our data show a striking reduction in the intake of cereals, fruits and vegetables at the age of 65 years. Thus, despite the absence of a consensus about a cut-off point to define the beginning of old age, it seems possible that, from a nutritional point of view, the age of 65 years marks a change in the feeding pattern.

As we commented previously, foods may not only provide the energy and structural components, but also contain carbon and nitrogen sources that are essential elements for the growing and metabolism of the intestinal microbiota (Li *et al.*, 2017), and bioactive compounds with a demonstrated effect as microbiota modulators. From them, fibres, defined by the Association of Cereal Chemists as 'the edible parts of plants or analogous
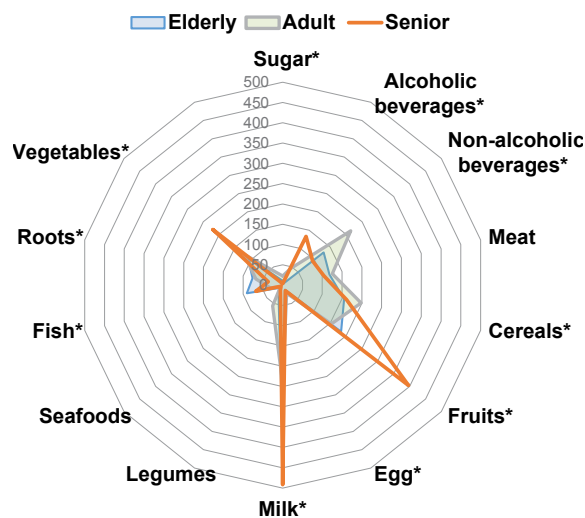

Figure 1.1 Mean intake of major food groups (grams/day) according to age.

carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine' (AACC, 2001), seem to be a key dietary component in the general population with a special focus on aged people. It has been reported that only about 40% of subjects above 71 years have dietary intakes above the adequate level of this dietary component (IOM, 2010); in line with these data, the intake in our sample is far from the 25–30 g/day recommended for protecting against heart disease, the prevention of several metabolic conditions and the proper maintenance of intestinal health (IOM, 2010). The reduction of carbohydrates accessible to gut microbes from fibre-containing foodstuffs may result in a long-term reduction of microbiota diversity and in the appearance of 'unhealthy' microbiomes (Sonnenburg *et al.*, 2005, 2016; Yatsunenko *et al.*, 2012). In this regard, some types of fibres, such as inulin, fructo-oligosaccharides or resistant starch, have shown a prebiotic effect promoting the grow of colonic bifidobacterial (Yen *et al.*, 2011; Petry *et al.*, 2012; Yang *et al.*, 2013).

Fruits, vegetables and cereals are food groups containing different mixtures of fibres and phytochemicals that may have shown a positive modulatory effect on some intestinal bacterial populations (Lee *et al.*, 2006; Vendrame *et al.*, 2011; Massot-Cladera *et al.*, 2012; Queipo-Ortuño *et al.*, 2012; Guglielmetti *et al.*, 2013), contributing to the maintenance of a well-balanced microbiota in the elderly (Gutiérrez-Díaz *et al.*, 2017; Salazar *et al.*, 2017). Specifically, there is recent evidence on a positive association between the frequency of consumption of fruits and vegetables and some intestinal microorganisms such as *Lactobacillus*, *Bl. coccoides* and *Prevotella* (La-ongkham *et al.*, 2015). Although it is not possible to establish causal relationships, vegetables and fruits were the major dietary sources of pectins in a population of Spanish healthy adults (Cuervo *et al.*, 2015). Pectins can be fermented by certain colonic bacteria such as *Bacteroides* resulting in the production of SCFA (Topping and Clifton, 2001), which contributes to increase the production of SCFA in the colon. Besides, fruit and vegetables contain sources of vitamin C and carotenoids, compounds that have known anti-inflammatory activity and also provide other bioactive compounds, such as polyphenols, that are involved in the regulation of some metabolic conditions linked to inflammation and oxidative stress (Shen *et al.*, 2012). Thus, the identification of novel nutritional and biological biomarkers of ageing will be a promising target in future ageing research.

Restoring a balanced microbiome through diet and lifestyle

The increased susceptibility to disease in the elderly, which is frequently related to malnutrition, a pro-inflammatory status of the immune system and the impaired intestinal microbiota functionality, provides the rationale for developing dietary strategies targeting the intestinal microbiota, the immune system and the specific nutritional needs in old age.

Nutritional needs in the elderly can differ from those of middle-aged adults (McCarty and DiNicolantonio, 2015). Although there is not a consensus on the nutritional deficiencies in the elderly and these can vary from one human group to another, some nutrients have been linked to a better immune response: monounsaturated fatty acids (MUFA), beta-carotene, vitamins A, B_6, C and D, and bioactive compounds. In addition, in the elderly some macro- and micro-nutrients fall frequently below the recommended intake levels (Trumbo *et al.*, 2002): proteins, fibre, iron, B group vitamins (including folic acid) and vitamin D (Salazar *et al.*, 2017).

It is currently clear that the impact of diet on the microbiota is more important in the elderly than in young adults (Claesson *et al.*, 2012; Jeffery *et al.*, 2016). It is worth remarking that not all changes occurring in the microbiota as age advances may necessarily have a detrimental health effect, as functional redundancy exists in this microbial ecosystem. However, the reduction in microbial diversity, the shift in dominant species and the reduced levels of some groups of bacteria such as those producing butyrate, the increase of facultative anaerobic bacteria, the decreased levels and imbalanced production in the gut of SCFA, and increased levels of lactate, methane and branched-chain fatty acids are generally considered relevant targets for intervention (Salazar *et al.*, 2017). In this respect, it seems that in the elderly the loss of the community-associated microbiota, considered as the group of microorganisms shared by the individuals from a given social group, has been correlated with a decrease in microbial diversity and with increased frailty (Claesson *et al.*, 2011; Jackson *et al.*, 2016; Jeffery *et al.*, 2016).

Intervention targets with regard to the intestinal microbiota and the immune system may differ among elderly groups; in fact, nutritional profiles and the characteristics of the intestinal microbiota and the immune system can vary depending on the geographical location and dietary and social patterns (Mueller *et al.*, 2006; Turnbaugh *et al.*, 2009a; Claesson 2012). On the other hand, there is not a specific age determining the shift from an adult to an elderly microbiota but it depends on the external factors just commented, and possibly some others (O'Toole and Jeffery, 2018). Therefore, prior to designing nutritional interventions it is necessary to identify the specific action targets for specific human groups. The microbiota of healthy subjects is considered the reference for comparison, in order to define targets for restoring a balanced microbiota. However, a 'healthy microbiota profile' cannot be defined 'a priori'. Thus, human groups of reference must have a socio-economic status (geographical location, social habits, historical past, dietary habits) as close as possible to that of the population under study. This is especially important in the elderly in whom prolonged exposure to different environments could make it difficult to identify variations specifically due to ageing and those due to other factors. The study of centenarians and super-centenarians and their offspring deserves special mention, as a model to disentangle genetic and environmental lifestyles that determine healthy ageing and the characteristics of their microbiota in comparison to septuagenarians and octogenarians (Santoro *et al.*, 2018). In long-term planning, longitudinal studies along years of the intestinal microbiota, dietary patterns, lifestyle factors and health status over ample cohorts will provide the most complete information about changes in the intestinal microbiota with ageing, the influence of external factors, such as diet, on these changes and, ultimately, how these variables determine healthy ageing.

A moderate enhancement of the colonic fermentation of dietary fibre could be considered beneficial in the elderly as it could contribute to reinforce the intestinal barrier, to the increase in intestinal motility, to reduce inflammation and to increase the butyrate production by cross-feeding mechanisms with members of the intestinal microbiota able to produce this compound.

Relevant nutritional strategies for improving intestinal function include the design of diets adapted to the elderly as well as the use of selected probiotics, prebiotics, synbiotics (combined use of pro- and prebiotics), supplements and bioactive compounds. Probiotics are defined as 'live microorganisms, which when administered in adequate amounts confer health benefit to the host' (FAO/WHO, 2006). Most of the common probiotics are strains

from different species of *Bifidobacterium* and *Lactobacillus*. Probiotics can act by inhibiting enteric pathogens or sequestrating their toxins (Valdés-Varela *et al.*, 2016), interacting with some members of the intestinal microbiota through cross-feeding mechanisms in the presence of prebiotic substrates (Rios-Covián *et al.*, 2015) and modulating the immune system directly or through their interaction with members of the gut microbiota (Hevia *et al.*, 2015). Prebiotics are 'selectively fermented ingredients resulting in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits on host health' (Gibson *et al.*, 2017). Most prebiotics are complex carbohydrates or oligosaccharides from fruits, wholegrain s and other vegetables or are produced industrially. Prebiotics are not digested in the small intestine and they reach the colon unabsorbed, where they are selectively fermented by some members of the intestinal microbiota, resulting in beneficial changes in microbiota composition and its metabolic activity.

In spite of the increasing interest of the industry in the market of dietary products for the elderly, there is still a scarcity of studies demonstrating the efficacy of probiotics and prebiotics. Intervention studies carried out in the last years for demonstrating the efficacy of probiotic *Lactobacillus* and *Bifidobacterium* strains were mainly focused on ameliorating gastrointestinal disorders, such as constipation and diarrhoea associated with antibiotics or *C. difficile* infection, and few of them were carried out with the aim of improving the composition and functionality of the whole gut microbiota; nevertheless, some bifidobacteria and lactobacilli showed beneficial changes on specific groups of the intestinal microbiota and improved the profile of immune mediators (Bjorklund *et al.*, 2012; Salazar *et al.*, 2014, 2017; Spaiser *et al.*, 2015). Among prebiotics, galacto-oligosaccharides (GOS) were the most studied until now. A defined commercial mixture of trans-GOS, named as Bimuno or B-BOS, showed an improvement in the faecal microbiota composition and immune parameters in the elderly (Vulevic *et al.*, 2008, 2015). More human intervention studies are needed on the efficacy of alternative prebiotics such as xylo-oligosaccharides, beta-glucan, pectin, malto-oligosaccharides and others. Synbiotic combinations of *Lactobacillus* and/or *Bifidobacterium* strains with prebiotic polysaccharides, oligosaccharides or polyols have been also tested to counteract health-related microbiota changes and impaired physiological functions in the elderly (Bartosch *et al.*, 2005; Bjorklund *et al.*, 2012; Macfarlane *et al.*, 2013). Polyphenols have been proven to promote beneficial changes in the intestinal microbiota and to exert health benefits on the host; however, the specific use in the elderly and the relationship between their metabolism by the intestinal microbiota and general host health parameters have been scarcely explored (Guadamuro *et al.*, 2017; Gutiérrez-Díaz *et al.*, 2017).

There is a need for more studies exploring the influence of diet and specific nutrients and supplements on microbiota functionality in the elderly. These studies should be performed with high number of individuals from different geographical locations in order to reach robust conclusions on the efficacy of nutritional interventions in the elderly.

Concluding remarks

The spectacular advance in recent years of DNA NGS technologies and its cost affordability for the analysis of complex microbial ecosystems has provided knowledge of the composition of the human intestinal microbiota, from its establishment in the early stages of life to the most advanced ages. Moreover, other omics technologies targeting mRNA, proteins and metabolites are allowing the metabolic potential of these microorganisms and

their extraordinary influence on host health to be disentangled. The elderly population is increasing over the world and particularly in industrialized countries. The physiological, immunological and cognitive decline that occurs in old age is strongly influenced by lifestyle, social habits and nutritional status. Ageing is often accompanied by disease and disability. In addition, modification in eating patterns frequently occur, because of physiological and physical changes, which frequently involve a relative state of malnutrition that, in its turn, exerts a negative impact on the gut microbiota, the immune system and the cognitive state. All this results in a vicious circle that is difficult to leave. Current technological and scientific advances are bringing us the opportunity to understand the mechanisms underlying the beneficial or detrimental action of specific nutrients on the human intestinal microbiota and to know the influence of such microorganisms on physiological, immunological and cognitive function. It is therefore urgent that nutritional strategies are developed that are able to positively modulate the composition and functionality of the intestinal microbiota in order to promote healthy ageing that allow to dignify life in the elderly and to manage health-care costs.

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