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REVIEW

Nutrition and the gut microbiome in the elderly

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ABSTRACT

The gut microbiota is the assembly of microorganisms living in our intestine and their genomes are known as the microbiome. The correct composition and functionality of this microbiome is essential for maintaining a "healthy status." Aging is related to changes in the gut microbiota which are frequently associated with physiological modifications of the gastrointestinal tract, as well as, to changes in dietary patterns, together with a concomitant decline in cognitive and immune function, all together contributing to frailty. Therefore, nutritional strategies directed at restoring the microbiota in the elderly have to be addressed from a global perspective, considering not only the microbiota but also other extra-intestinal targets of action. The present review aims at summarizing the current knowledge on intestinal microbiota alterations and other functions impaired in the elderly and to analyze tools for implementing nutritional strategies, through the use of probiotics, prebiotics or specific nutrients in order to counterbalance such alterations.

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Introduction

During the past 2 centuries lifespan has been increasing by approximately 2 y per decade in developed countries. A quarter of European population will be older than 65 y by the middle of the present century.[1] While this increase of life expectancy represents an extraordinary social and medical achievement, the healthcare costs associated with recurrent disease and disability that frequently occurs in the elderly, constitute an economic burden and a sustainability challenge for the current social structure of industrialized countries. The elderly population frequently suffers from an impairment of several biological functions that are associated with important changes of their intestinal microbiota. It is increasingly clear that nutrition contributes to shape the microbiota, which opens the possibility of interacting through diet with this microbial community inhabiting our body. This is especially important in those situations where other extra-intestinal disorders are also associated with microbiota alterations, as occurs in the elderly. Thus, advancing our knowledge on the interactions between nutrition and gut microbiota structure and function in older individuals could help to improve the general health status of this population through the design on individualized nutritional strategies.

The gut microbiome: Composition, functionality and evolution across the lifespan

The gut microbiota is the wide, complex and diverse collection of microorganisms living in our intestine, and includes bacteria, archaea, viruses and some unicellular eukaryotes that have co-evolved with humans in a commensal way. The gut microbiota represents the largest number and concentration of microorganisms in the human body, reaching levels of 10^{14} cells in the colon.[2] This microbiota contains a vast genetic catalog, the so called "intestinal microbiome." Overall almost 10 million different genes have been found in the human microbiome so far,[3] representing an important genetic resource. A number of 1000 to 1500 bacterial species have been reported to be widespread in the human gut, each person harboring around 150 different species,[4] which all together contain more genes than the human genome.[4] The microbiome is usually recognized as our second genome[5] and

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contributes extensively to our physiology and metabolism.[4]

There is an increasing interest by the research community to decipher the role of the microbiota in maintaining human health, and several projects have been launched with this aim across the globe.[6] In the last few years, the development of high-throughput analytical tools and "meta-omics" technologies has allowed more detailed studies on the gut microbiome composition and functionality. Despite this progress, what really constitutes a "healthy" gut microbiota remains still unclear.

The microbial colonization of the gastrointestinal tract (GIT) starts immediately after birth, and this process determines to some extent the predisposition to develop diseases in early and later life.[7,8] The colonizing microbiota community is initially unstable and suffers a succession phenomenon that involves a first colonization by facultative anaerobes, which creates a more reduced environment for the subsequent colonization by strict anaerobes.[9] From an initial low diversity and complexity, the intestinal microbiota evolves until reaching a diverse, complex, and stable population about the age of 3 y.[10,11] At the phylum taxonomic level, the dominant bacteria into healthy adults are Firmicutes, and Bacteroidetes, which constitute between 80–90% of the total microbiota, with representatives of Proteobacteria, Fusobacteria, Cyanobacteria, Verrucomicrobia and Actinobacteria phyla, among others often found at considerably lower levels.[10] Furthermore, in spite of the high inter-individual variability, 3 specific microbial enterotypes, each one being dominated by a particular bacterial genus (*Bacteroides, Prevotella* or *Ruminococcus)*, that appear to be independent of nationality, sex, age, or body mass index, have been proposed.[12] These enterotypes have been associated with the long-term dietary pattern[13] and are influenced by short-term diets.[14,15] Nevertheless, the classification of human-associated bacteria in enterotypes is a controversial concept and some disparities have been found, due to the lack of consistency in the methodologies employed.[16,17] The adult-like intestinal microbiota is regarded as relatively stable throughout adulthood although it is susceptible to variations owing to stress, antibiotics and as a consequence of diet and lifestyles.[18] At senescence, an inverse process occurs that resembles a mirror image of the neonatal gut colonization and the microbiota becomes unstable again.

The intestinal microbiota at advanced age

Age-related changes in the gut microbiota are associated with physiological changes in the GIT, 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.[19-21] The gut microbiota of elderly subjects is characterized by a reduced bacterial diversity, shifts in the dominant species, a decline in beneficial microorganisms, increase of facultative anaerobic bacteria and a decrease in the availability of total short chain fatty acids.[11] More specifically, when comparing the microbiota of elderly with that of younger adults, lower levels of Firmicutes, mainly *Clostridium* cluster XIVa and *Faecalibacterium prausnitzii*, and Actinobacteria (mainly bifidobacteria), and increased populations of Proteobacteria have been found[19-24] (Table 1). With regard to other relevant intestinal microbial populations such as the phylum Bacteriodetes, the results are more variable, with some studies reporting lower levels[19,23-27] while others have indicated increases of this microbial group in elderly subjects.[21] Similarly, variable results have also been observed for lactobacilli, with some studies reporting a reduction[22,28] and others an increase in the levels of these microorganisms at older ages.[24,27,29] It is still unclear whether this 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 (Table 1).

As expected from the differences on microbiota observed between elderly and younger adults and different dietary patterns between the groups of age, the production of bacterial metabolites is also altered at old-age. The levels of short chain fatty acids (SCFA), the main bacterial metabolites in the colon, are lower in elderly and the ratios among the different fecal SCFA also vary with respect to healthy younger adults.[20,24] These changes in the production of bacterial metabolites have been suggested to be related with variations in the colonic bacterial metabolism, which shifts from the predominantly saccharolytic metabolism normally observed in adults toward a predominantly putrefactive metabolism.[30]

It is important to highlight that a change in the gut microbiota may not necessarily mean a detrimental health effect. Functional redundancy is known to exist in the gut microbiota and, thus, not all replaces in

Table 1 Main studies employing molecular techniques to analyze the gut microbial composition in elderly. nF, number females; nM, number males; NSAID, non-steroidal anti-inflammatory drugs; Ref, reference; U, unknown;

Subjects, country, age (nF, nM)	Gut microbiota alterations identified	Molecular technique	Ref.
• Healthy elderly, Japan, 74-94y (5F,1M)	↓ *Clostridium* cluster XIVa	16S DNA libraries, T-RFLP	25
• Healthy adults (**HY**), United Kingdom, 19-35y, (9F, 3M) • Healthy elderly (**HE**), United Kingdom 67-75y (6F) • Elderly hospitalized receiving antibiotics (**AE**), United Kingdom 73-101y (2F,8M)	↓*Bacteroides*, Bifidobacteria, species diversity (**HE**, **AE**) ↑Fusobacteria, Clostridia, Propionibacteria **AE** *vs* **HE, HY** ↑*Enterobacteriaceae* (**HE**, **AE**)	16S rRNA qPCR	30
• Adult group (**A**): France (**F**), Germany (**G**), Italy (**I**), Sweden (**S**) 20-50y (85, U) • Elderly group (**E**): France (**F**), Germany (**G**), Italy (**I**), Sweden (**S**)>60y (145, U)	↑Enterobacteria (**EF, EI, EG, ES**) ↓*Bacteroides* (**EI**), ↑*Bacteroides (***EG**, **ES**) ↑*Clostridium* cluster XIVa (**EG**), ↓*Clostridium* cluster XIVa, *F. prausnitzii* (**EI**) ↑*F. prausnitzii* (**EF**, **EG**, **ES**)	16S rRNA FISH	35
• Infant group, U, 3weeks-10 months (21, U) • Adult group, U, 25-45y (21,U) • Elderly group, U, 70-90y (20, U)	↓Firmicutes/ Bacteroidetes ratio in elderly *vs* adults ↑*Escherichia coli* in elderly vs adults	16S rRNA qPCR	108
• Institutionalized elderly, Austria, 78-94y (9F,8M) • Young volunteers, Austria, 18-31y (9F,8M)	↑*Bacteroides* in elderly ↓*Bifidobacterium*, *Clostridium* cluster IV, total bacteria and *Clostridium* cluster IV diversity in elderly	16S rRNA qPCR, PCR-DGGE,	109
• Young adults (**Y**), Italy, 25-40y (9F,11M) • Elderly: Group **E**, Italy, 63-76y (11F,11M)+ offspring of centenarians (Group **F**), Italy, 59-78y (11F,11M) • Centenarians (**C**). Italy, 99-104y (20F, 1M)	↓*Clostridium* cluster XIVa (**C**) ↓*F. prausnitzii* and relatives, species diversity (**C**) ↑*Eubacterium limosum* and relatives, Proteobacteria, Bacilli (**C**)	HITChip, 16S rRNA qPCR	23
• NSAID elderly, Finland, 77–85y (6F,3M) • No NSAID elderly, Finland 70–83y (6F,3M) • Young adults, Finland, 21–39y (5F,9M)	↓ *Ruminococcus*, *Roseburia*, *Coprobacillus*, *Dialister* in No NSAID elderly vs adults ↑*Lactobacillus*, *Streptococcus, Bacteroides* in No NSAID elderly *vs adults* ↑*Lactobacillus*, *Collinsella* in No NSAID elderly *vs* NSAID elderly	% G+C content, 16S rDNA sequencing	29
• Healthy elderly, Ireland, >65y (161, U) • Healthy adult, Ireland, 28-46y (9,U)	*Bacteroidetes* dominate Irish elderly ↑*Clostridium cluster IV* (*Faecalibacterium, Sporobacter, Ruminococcus)* in elderly	16S rRNA gene sequencing	19
• Institutionalized elderly, Spain, 77–95y (7M, 31F) • Middle-Aged adults, Spain, 57–67y (11M, 27F)	↓*Faecalibacterium, Bacteroides, Clostridium cluster XIVa* in elderly ↑*Lactobacillus* group in elderly	16S rRNA qPCR	24
• Group RC, China, 100-108y (5F,3M) • Group RE, China, 85-99y (5F,3M) • Group CE , China, 80-92y (4F, 4M)	↑*Roseburia, Escherichia* in centenarians ↓*Lactobacillus*, *Faecalibacterium*, *Parabacteroides*, *Butyricimonas*, *Caprococcus*, *Megamonas*, *Mitsuokella*, *Sutterela*, *Akkermansia* in centenarians	16S rRNA gene sequencing	110
• Young adult (**Y**), Italy, 22-48y (8F, 7M) • Young elderly (**E**), Italy, 65-75y (7F,8M) • Supercentenarians (**S**), Italy, 105-109y (18F, 6M) • Centenarians (**C**), Italy, 99-104y(14F , 1M)	↓ by age: *Bacteroidaceae*, *Lachnospiraceae*, *Ruminococcaceae* *Coprococcus, Roseburia, Faecalibacterium: (-) correlation with age* *Oscillospira, Odoribacter, Butyricimonas: (+) correlation with age* ↑*by age: Eggerthela, Akkemansia, Anaerotruncus, Synergistaceae, Bilophila, Christensenellaceae* ↓*Bifidobacterium **E**, **C** but ↑**S***	16S rRNA gene sequencing	111
• Healthy volunteers, Japan, 0-104y (213F,158M): elderly volunteers, Japan >60y (53F, 36M)	↓by age: Actinobacteria ↑ Bacteroidetes, Betaproteobacteria, Deltaproteobacteria in elderly ↑*Porphyromonas*, *Treponema*, *Fusobacterium*, *Pseudoramibacter* in elderly	16S rRNA gene sequencing, qPCR	21

microbial composition are going be translated into a functional deficiency. However, in the case of elderly subjects, an altered microbiota has been repeatedly reported to be associated with frailty.[19,31] In this way, some microorganisms, such as *F. prausnitzii*, and a reduced microbial diversity, have been associated negatively, whereas other microorganisms were associated positively with frailty. Therefore, there is indication of

potential detrimental effects of the microbiota changes associated with aging. It is also important to consider that the above mentioned shifts in the gut microbiota do not initiate at a defined age, and they are rather a gradual process,[32] likely related with the physiological decline of the individual. Moreover, not surprisingly, life-style and diet have been found as the most important drivers for these microbiota changes[20] pointing out at the need to promote healthy dietary habits among the elderly population.

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. This is especially relevant in this human population since aging is related with dietary changes and a subsequent increase in the risk of malnutrition, as well as, with changes in the immune system.[24,33,34] All these alterations considered together, may explain the higher susceptibility of elderly people to disease. Besides, differences among elderly from different geographical locations have been also observed.[35]

Clostridium difficile-*associated diarrhea*

Clostridium difficile (*C. difficile*) is an obligate anaerobic, gram-positive and spore-forming bacterium that is often present in the large intestine of healthy adults.[36,37] However, it is able to proliferate and produce toxins and disease in a susceptible host, when there is an event that causes a disruption of the intestinal microbiota.[38] *C. difficile* spores are resistant to high temperatures, ultraviolet light and harsh chemicals, and can survive for long periods of time in hospitals and health care facilities, thus favoring its dissemination.[39,40] Furthermore, spores are also resistant to antibiotics and can persist in the GIT and potentially contribute to recurrent disease following treatment against the vegetative form.[39]

C. difficile infection (CDI) is the most common cause of nosocomial diarrhea in the industrialized world.[41] It generally affects elderly hospitalized patients who have received a broad-spectrum antimicrobial treatment.[42] The incidence, severity and mortality of CDI have significantly increased over the last decade. These epidemiologic changes in CDI incidence have been partially explained by the emergence of hyper-virulent strains, such as *C. difficile* BI/NAP1/027;[40] infection by this strain in patients between 60 and 90 y of age has been related to an increased probability of CDI-related death.[38]

The most important risk factors for the development of CDI, mortality and recurrence of the disease are advanced age (≥65 years of age) and exposure to antibiotics. The disproportionate burden of CDI in elderly individuals may be related to the increase of exposure to healthcare environments, the raise of comorbidities, use of drugs including antibiotics, and age-related changes in the immune system and the intestinal microbiota of the host.[41] Moreover, antibiotic exposure alters the intestinal microbiota, which facilitates the overgrowth of *C. difficile*. The most commonly antibiotics associated to CDI are clindamycin, aminopenicilins, second- and third-generation cephalosporins and fluoroquinolones.[37]

The main mechanism of virulence in *C. difficile* is related to the production of 2 enterotoxins, TcdA and TcdB, which cause the intestinal injury and activate an inflammatory cell response. Therefore, CDI is a toxin-mediated disease of the colon with clinical manifestations ranging from asymptomatic colonization or mild, self-limited diarrhea, to fulminant pseudomembranous colitis, toxic megacolon, colonic perforation, sepsis, shock and death.[43] It is well known that, both the characteristics of *C. difficile* strain and the host's immune response, influence CDI severity, recurrence risk and mortality.[40]

Vancomycin and metronidazole have been used as effective treatment for CDI for over 30 y. However, a high CDI recurrence rates in patients who respond to initial treatment with these 2 antibiotics has been observed. For this reason, new therapeutic strategies are now under investigation for the prevention and treatment of CDI.[44]

In spite of ethical concerns and practical issues that should be solved and adequately managed, fecal microbiota transplantation is currently emerging as a highly effective therapy for intestinal multidrug resistant pathogens, with particular efficacy against recurrent CDI.[45] This therapy aims to restore normal gut microbiota by instillation of donor stool into the gastrointestinal tract of patients with CDI.[44] However, concerns of donor infection transmission to patients has limited its use. In spite of this, a recent systematic and meta-analysis indicates that adverse events after fecal microbiota transplantation such as autoimmune disease and infectious disease was not significant in a follow up of 90 d after transplantation, whereas

authors identified old age of patients (>65 years) as a risk factor for the primary cure failure and early recurrence.[46] Beyond this, it should be possible to isolate a fecal cocktail of defined bacterial multi-species and/or design synthetic mixtures that could be administered for correcting intestinal dysbiosis, thus avoiding the risk of transmission of potentially harmful microorganisms from donors to recipients.[47]

Physiological and immune status in the elderly

It is important to differentiate between those physiological, organic and structural changes occurring as a result of the advance of time and those derived from pathological processes. In this context, the term "senescence" refers mainly to non-pathological biological and physiological processes strictly dependent of age, while aging, a more generic term, would refer to changes, physiological and pathological, associated to the passage of time.[48,49] Age-related changes include a physiological general decline that involves all organs and functions, such as glomerular filtration rate, maximum heart rate, vital capacity or immune response.

One of the most recognized effects of aging is the decline in immune function, or more precisely the age-associated immune deregulation,[50] that includes a decrease in the proliferative response to mitogens, low activity of natural killer (NK) cells,[51,52] a progressive involution of the thymus together with lower number and reduced response of immune T cells,[53] and increased levels of pro-inflammatory cytokines.[24,33,34] This imbalance, commonly called "immunosenescence", may contribute to explain the high susceptibility of elderly people to disease.

Recently, a link between immune aging and some neurological disorders associated with senescence has been proposed, suggesting that microglia, the innate immune cells of the brain, could undergo this process.[54] In turn, senescence affects *per se* neurological functions and neuronal plasticity[55] which leads frequently to cognitive impairment and, in extreme cases, to brain diseases such as dementia or Alzheimer, affecting the independence and quality of life.[56]

With advancing age, it becomes more difficult for the organism to maintain homeostasis, especially under stress conditions.[49,57] Multiple changes take place at different levels of the GIT: a decrease in saliva production (xerostomy) and in gastric acid secretion, a lower absorption of nutrients such as iron and vitamin B_{12},[58] slower gastrointestinal motor function and food transit, and a reduced chemical food digestion and nutrient absorption.[59] As age increases there is a natural trend to decrease food intake, resulting in the anorexia of aging.[60] Some authors hypothesize that this lack of appetite could be the result of a physiological adaptation to the decrease in calorie requirements, as a consequence of the changes in body composition and reduction of physical activity.[61] Multiple factors could explain the reduction in the appetite in seniors: among homeostatic factors, there is a clear deregulation in the secretion and response to some of principal hormones related with the control of food intake such as cholecystokinin, ghrelin or insulin.[59] This dysregulation together with a decrease in smell and taste perception, modifies the appetence and food preferences, increasing the risk of malnutrition. Within non-homeostatic factors, life-style factors such as social isolation, low incomes, geographical location, social support, illness, the use of multiple drugs, or depression, could compromise food intake and the quality of life in the elderly.[62] Also, the presence of oral problems and xerostomy modifies the foods choice; especially those foods with hard and sticky consistency tend to be avoided, leading to changes in dietary patterns.

Concerning body composition, the proportion of fat mass in human body increases significantly over time, as a consequence of the decrease in lean mass and bone mass, phenomenon known as "sarcopenia."[63] This is related with a decrease in the plasma concentration of albumin, a biochemical risk factor for cardiovascular disease and mortality,[64,65] and with a reduction in the total body water content.[66] Also, the relative increase in body fat reduces the energy requirements as a consequence of the decline in the basal metabolic rate. However, maintaining a nutrient-dense diet is of critical importance since the consumption of nutrients below the recommendations is associated with higher risk of mortality,[61] whereas an over-consumption is associated with higher risk of obesity and related diseases.

Nutritional status in the elderly

It has been traditionally considered that a healthy diet requires an appropriate balance of energy, macro- and micronutrients, and water. While maintaining correct dietary habits is important for individuals of all ages,

this is particularly relevant for older adults, who as stated above are more vulnerable to malnutrition. The nutritional needs of the elderly are not substantially different from those of younger adults with similar anthropometric and physiological characteristics, and caloric expenditure.[24] However, the efficiency of nutrient absorption may be impaired, which, together with common chewing difficulties and loss of appetite, may alter the nutritional status of seniors.[24]

Due to the lack of standard tools for the nutritional assessment of elderly people, there is no consensus in the prevalence of malnutrition, and results from different studies are difficult to compare. The percentage of malnourished people is variable among countries, but, it is generally well accepted that this proportion is higher in hospitalized elderly than in institutionalized ones, followed by the free living population.[67] From macronutrients, the third National Health and Nutrition Examination Survey (NHANES III 1991–1994) NHANES revealed that approximately 30% of people over 50 y do not meet the Recommended Dietary Allowances (RDA) for protein,[68] one of the main factors limiting muscle synthesis in the elderly.[69,70] Another nutrient for which intake falls short of the recommended intake is dietary fiber, important for maintaining intestinal health and protecting against cardiovascular disease.[71] Also, in almost every dietary survey conducted over the past few decades, older adults have inadequate intakes of iron, vitamin B_6, vitamin B_{12}, folic acid and vitamin D.[72]

Iron deficiency is common for different reasons: an inadequate intake of heme iron (present in animal products), a reduced protein intake, the high presence in the diet of components that reduce iron absorption as phytates or oxalates, and some gastrointestinal disturbances frequent in old age (gastritis, intestinal atrophy, ulcers or other digestive disorders).[73] Data from NHANES revealed a prevalence of anemia in 20% of subjects older than 85 years, associated with functional decline and other adverse outcomes.[74] Also, low levels of vitamin D are frequent in seniors and especially in those subjects with low sun exposure, this being associated with a higher risk of fractures and osteoporosis.[75] The association of B-group vitamins with neurological disorders has been recently discussed.[76] The role of other nutrients at this age remains to be elucidated.

It is well-known that an adequate nutritional status is of great importance for maintaining proper immune system functionality and preventing frailty in the elderly, but the components in foods that improve immune functions in elderly are still far from fully understood.[77] Protein-calorie malnutrition is one of the main causes of immune deficiency in elderly.[78] An inadequate protein intake may lead to a decrease in the amino acids, compromising the immune system functioning.[79] In addition, monounsaturated fatty acids, β-carotene and vitamins A, B_6, C, and D, and bioactive compounds have been linked to a better immune response.[80-82]

Thus, the correction or improvement of all these age-related changes constitutes a target for the development of nutritional intervention strategies focused to this population group (Fig. 1).

Nutritional strategies for restoring a balanced composition and functionality of the microbiota in the elderly

The higher susceptibility to disease in the elderly population, frequently related with malnutrition, impairment of the intestinal microbiota functionality and a pro-inflammatory status of the immune system, provide a rationale for developing nutritional strategies and functional foods aiming at microbiota and immune modulation in this population group. Prior to designing nutritional interventions for specific human populations, it is necessary to identify the specific action targets with those precise groups. The microbiota of healthy subjects is considered the reference for comparison, in order to restore a balanced microbiota. At the beginning of life, for newborns, the microbiota of full-term vaginally-delivered breast-fed babies is considered as the gold standard of a healthy microbiota. However, the identification of human populations bearing a "healthy microbiota" becomes less evident as life progresses. The human groups of reference must have had a socio-economic status the most similar as possible to the population to be studied. This is especially important in the elderly, where the prolonged exposure to different environments could make it difficult to identify variations specifically due to aging.[20,24] In this case, the microbiota of a population of younger healthy adults, sharing similar characteristics in terms of diet, geographical location, social habits, historical past etc. with the elderly population under study, could be considered the healthy microbiota of reference.[11,24]

Intervention targets on the intestinal microbiota and the immune system may differ among elderly groups from different environments or geographical locations. This is especially relevant as differential effects of probiotics in different population groups have been previously demonstrated[83] suggesting that the targets on the gut microbiota may also be different depending on location. Loss of the community-associated microbiota, defined as the group of microorganisms shared by the individuals from a given social group, has been correlated with increased frailty.[19,31,84] The reduced microbial diversity, low levels of butyrate producing bacteria, decreased levels and imbalanced proportions of SCFA, the incidence of CDI, and higher levels of lactate, methane and branched chain fatty acids (valeric, isovaleric, isobutyric, and caproic acids) are generally considered as relevant targets for intervention.[19,24,30,37,85-88] As mentioned previously, changes in the intestinal microbiota composition and functionality in the elderly are usually accompanied by alterations in the physiology and function of the digestive tract, and by nutritional deficiencies related with lower intakes of

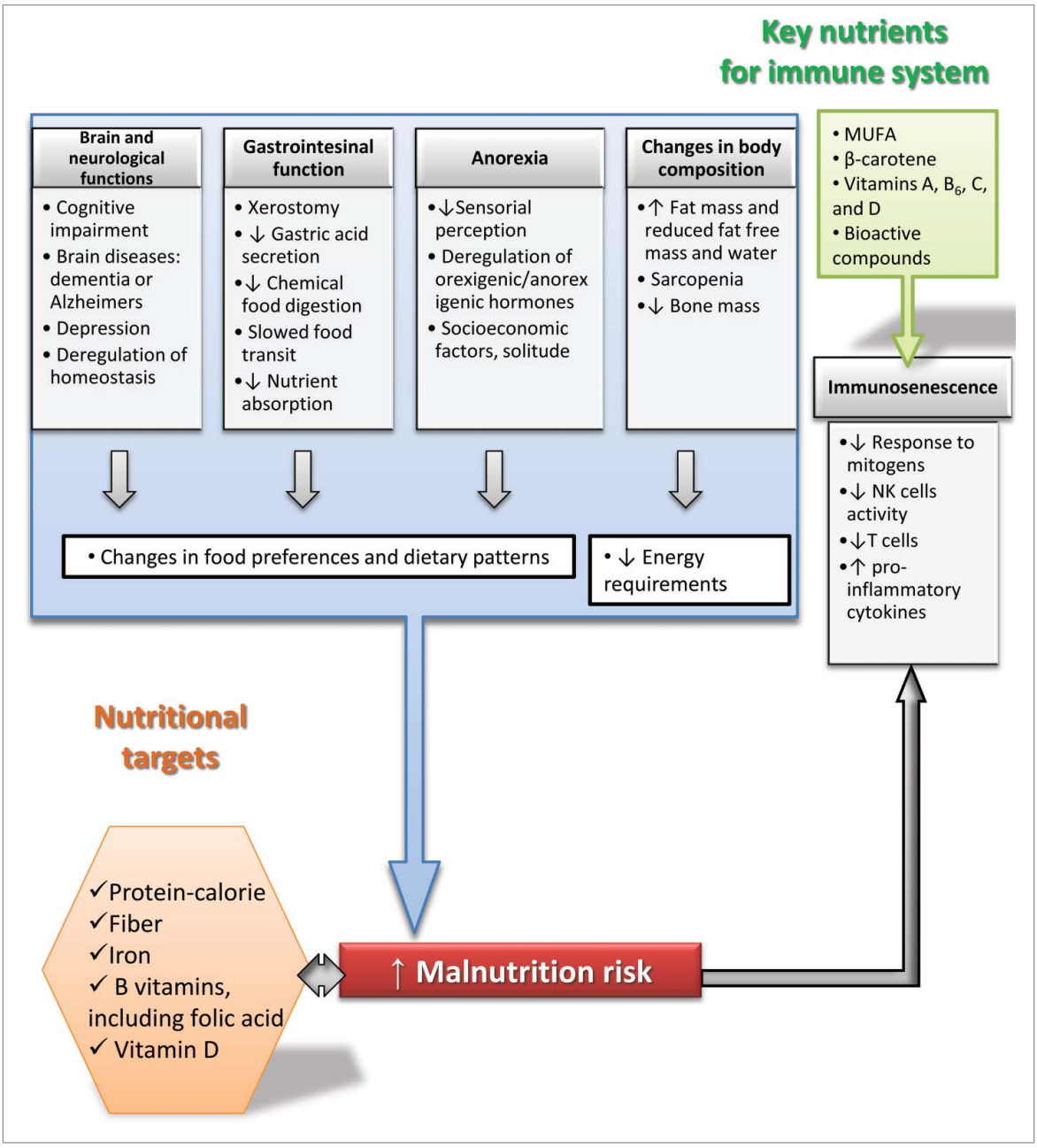

Figure 1. Schematic representation of physiological, nutritional and immune targets of intervention generally identified for elderly people. MUFA: mono-unsaturated fatty acids; NK: natural killer cells.

specific nutrients that are important for maintaining the immune and gastrointestinal functions.[89] Additionally, the actual nutritional needs of the older population can differ from those of the middle-age.[90] Therefore, nutritional strategies in the elderly should be addressed from a holistic perspective, considering the intestinal microbiota, the immune system as well as nutritional deficiencies and needs as a whole (Fig. 2).

In general, the moderate enhancement of the colonic fermentation of dietary fiber could be considered as beneficial in the elderly. This will strengthen the intestinal barrier against pathogens, increasing the intestinal motility and helping to reduce the underlying pro-inflammatory status (immunosenescence), thus contributing to the general well-being. Of particular relevance is the enhancement of the intestinal butyrate production through the promotion of microbial interactions by cross-feeding mechanisms involving members of the intestinal microbiota able to produce butyrate (*Blautia coccoides* and *Clostridium leptum* groups, among the most relevant).[91,92]

The design of adapted diets and the specific use of selected probiotics, prebiotics or synbiotics (combined use of probiotics and prebiotics) are relevant nutritional strategies for improving the intestinal homeostasis. Prebiotics are "selectively fermented ingredients resulting in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host health."[93] Most prebiotics are complex carbohydrates present in whole grains, fruits and vegetables or are produced industrially. These compounds are undigested and unabsorbed in the small intestine and reach the colon where their selective fermentation promotes changes in the composition and metabolic activity of the intestinal microbiota. Probiotics are defined as "live microorganisms, which when administered in adequate amounts confer health benefit to the host."[94] The use and scope of the term probiotic has been recently discussed by an expert panel from the International Scientific Association for Probiotics and Prebiotics.[95] Probiotics may inhibit enteric pathogens, interact with the intestinal microbiota and modulate the immune

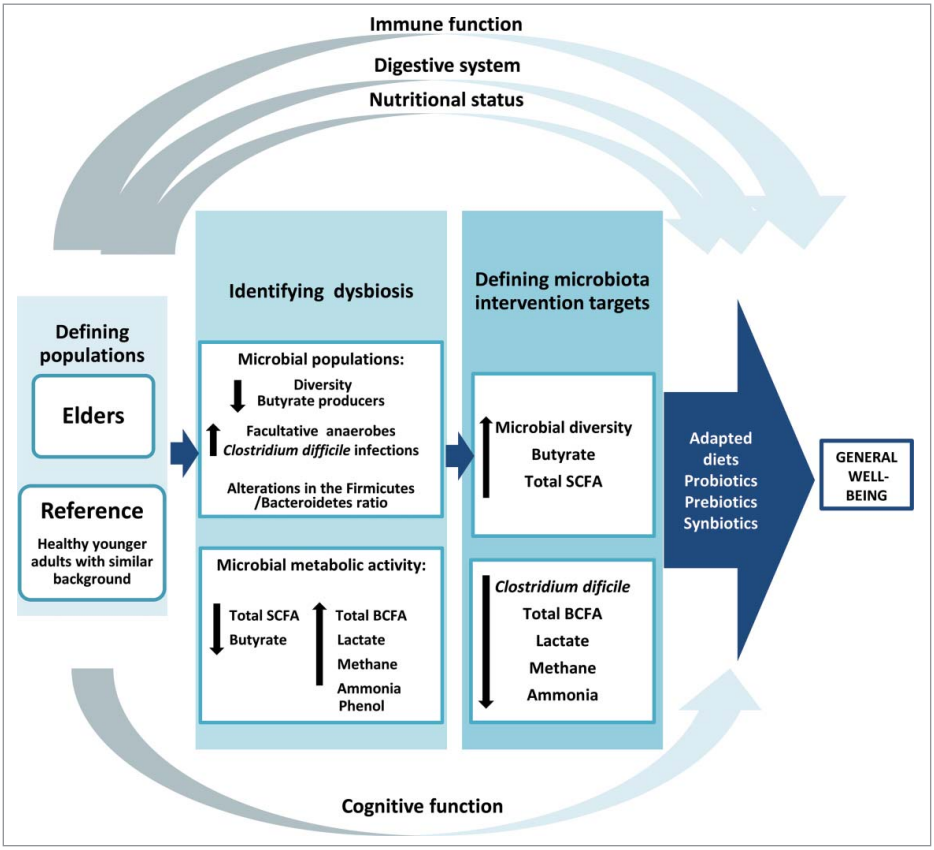

Figure 2. General strategy proposed for designing nutritional interventions in the elderly. The intestinal microbiota and its functionality have been considered the central axis, clustering around them the other intestinal and extra-intestinal possible targets. SCFA: short chain fatty acids; BCFA; branched chain fatty acids.

Table 2. Intervention studies showing the impact of probiotics, prebiotics or synbiotics on the gut microbiota in elderly people. The general treatment performed (probiotics, prebiotics or synbiotics) is indicated with uppercase bold letters. D, day; w, week; m, month; y, year.

Subjects, country, age (n)	Dietary intervention (period)	Main gut microbiota changes	Reference
	PROBIOTICS		
Healthy elderly, Finland, 76-92y (66)	Randomized, double blinded placebo controlled study: fermented oat drink with *Bifidobacterium longum* 46 and *B. longum* 2C (BIF) *vs* non fermented placebo oat drink (6 m)	**BIF**:↑bifidobacteria, *Bifidobacterium catenulatum*, *Bifidobacterium bifidum*, *Bifidobacterium breve*	[112]
Healthy elderly, Italy, 71-78y (32)	Randomized double-blinded placebo-controlled study: biscuit with *Bifidobacterium longum* Bar33 and *Lactobacillus helveticus* Bar13 (PRO) *vs* placebo biscuit (1 m)	**PRO**:↓ opportunistic pathogens: *Clostridium cluster XI*, *Clostridium difficile* and *Clostridium perfringens*, *Enterococcus faecium* and *Campylobacter*	[113]
Healthy elderly, Italy, France, Germany, 65-85y (62)	Open label, randomized, multicentre study: RISTOMED diet alone (RD) *vs* RD + probiotic VSL#3 (VSL) (8 w)	**VSL**:↑ bifidobacteria.	[114]
Healthy elderly (>60y) (80)	Randomized double-blinded placebo-controlled study: reconstituted skimmed milk with *Bifidobacterium lactis* HN019 at low dose (L), *B. lactis* medium dose (M), *B. lactis* high dose (H) *vs* placebo (4w and 2w wash out)	**L, M, H:**↑ bifidobacteria, lactobacilli, enterococci **L, M, H:**↓ enterobacteria	[115]
	PREBIOTICS		
Healthy elderly, England, (64-79y) (44)	Randomized, double blinded placebo controlled, cross over: galacto-oligosaccharides mixture (BGOS) *vs* placebo (10w and 4w wash out)	**BGOS**:↑ bifidobacteria, lactobacilli, Clostridia Cluster XIVa ↓bacteroides, *Clostridium histolyticum* group, *Escherichia coli*, and *Desulfovibrio* spp	[116]
Healthy elderly, England, (>50y) (37)	Randomized, double blinded placebo controlled, cross over: juice containing galacto-oligosaccharides (GOS) *vs* placebo juice (3w and 3w wash out)	**GOS**:↑ bifidobacteria	[107]
Healthy elderly, England, (65-80y) (40)	Randomized, double blinded placebo controlled, cross over: galacto-oligosaccharides mixture(BGOS) *vs* placebo (10w and 4w wash out)	**BGOS**:↑ bifidobacteria, bacteroides ↑ lactic acid	[106]
	SYNBIOTICS		
Healthy elderly, Scotland, (>62 y) (18)	Double blinded placebo controlled: mixture of *Bifidobacterium bifidum* BB-02 + *Bifidobacterium lactis* BL-01 + inulin (BB+BL+IN) *vs* placebo (5w and 3w wash out)	**BB+BL+IN**:↑ bifidobacteria, *B. bifidum*, lactobacilli	[117]
Healthy elderly, Finland, (>65y) (51)	Double blinded placebo controlled: mixture of *Lactobacillus acidophilus* NCFM+ lactitol (LB+L) *vs* placebo (4w and 3w wash out)	**LB+L**:↑ bifidobacteria, lactobacilli	[118]
Healthy elderly, Scotland, (65-90y) (43)	Randomized, double blinded placebo controlled, cross over: mixture of *Bifidobacterium longum* + inulin (BL+IN) *vs* placebo (4w and 4 w washout)	**BL+IN**:↑ bifidobacteria, phyla Actinobacteria and Firmicutes ↑butyrate ↓*Proteobacteria*	[119]

system directly or through the modification of the gut microbiota. The most commonly used probiotics in foods are bifidobacteria and lactobacilli species. Some *in vitro* studies confirm the participation of potentially probiotic *Bifidobacterium* strains in cross-feeding interactions with intestinal microorganisms producing butyrate in the presence of prebiotic substrates, as inulin-type fructans and fructo-oligosaccharides.[96-100]

Regarding CDI, in a recent study Vincent et al.[101] observed differences in the microbiota between elderly developing infection and those colonized by the microorganism but remaining healthy. Moreover, the authors identified potentially protective microorganisms. These sort of data suggest both, microbial markers for an increased risk of infection as well as targets of microbiota modulation for disease prevention in the elderly. To this regard, potentially probiotic strains and prebiotic substrates have been selected on the basis of their ability to counterbalance the specific microbiota alterations associated to old-age.[102] Recent *in vitro* studies also demonstrated the ability of some *B. longum* and *B. breve* strains to inactivate the toxins released by *C. difficile* and to partially inhibit the growth of this pathogen, also reducing its toxicity in the presence of some prebiotic substrates.[103,104]

There is a growing interest of the food industry in the elderly population. Despite this, there is a scarcity

of intervention studies demonstrating the effectiveness of specific prebiotic substrates for the elderly population, and no specific probiotic products are available in the market for old people. Several intervention studies with commercial strains from *Lactobacillus* and *Bifidobacterium* genera have been performed in the elderly population during the last 10 y. Only few of them were made with the aim of testing the effect of probiotics on the gut microbiota composition (Table 2) but mainly to assess the efficacy of these strains in gastrointestinal disorders associated with age, such as constipation, and diarrhea due to antibiotics consumption or associated with CDI.[105] Some of these probiotics have shown to be efficient in promoting the increase of bifidobacteria or other microorganisms such as lactobacilli and enterococci, or to cause a decrease of certain opportunistic pathogens (i.e. *C. difficile, Enterococcus faecium, Clostridium perfringens* or *Campylobacter*) and enterobacteria. Regarding the prebiotic approach, the use of galacto-oligossaccharides has been successful in correcting microbiota changes and for improving the immunological state associated with the elderly[106,107] (Table 2), however intervention studies with alternative prebiotics (pectin, xilo-oligosaccharides, malto-oligosaccharides, etc) are lacking. Synbiotics have been also recently tested to improve age-related changes in the gut microbiota, with some positive effects on specific intestinal microbial populations (Table 2). In spite of this, very few studies have addressed the influence on the metabolic functionality of the intestinal microbiota in elders (i.e., through the production of SCFA or organic acids); in addition, the low number of individuals recruited and the different specific geographical locations of individuals participating in interventions, mainly in the case of prebiotics and synbiotics, prevents the formulation of firm conclusions on the efficacy of these functional dietary ingredients in the elderly population. Therefore, there is still a need for a joint effort by clinicians and scientists to establish standardized, reproducible and comparable intervention protocols that would enable investigation into the efficacy of probiotics, prebiotics and adapted diets in different groups of the elderly population.

Conclusions

Over the past 5 years, advancements in high throughput technologies and the associated reduction in costs has enabled investigations into the composition and functionality of complex microbial species across the lifespan. More studies are needed in elderly populations, particularly those with the most advanced ages (>90 years of age), to better understand how the microbiota shifts overtime and how it is associated with dietary changes and associated comorbidities. Understanding the mechanisms underlying the beneficial actions of prebiotics, prebiotics, as well as interactions between specific nutrients and the microbiota, is needed to provide the scientific support for the rational design of specific food products for the elderly. Ideally, it would be possible to assess the disease risk of specific susceptible subpopulations and to design cost-effective personalized treatments and products.

Abbreviations

CDI	Clostridium difficile infection
GIT	Gastrointestinal Tract
NHANES	National Health and Nutrition Examination Survey
NK	Natural Killer
RDA	Recommended Dietary Allowances
SCFA	Short Chain Fatty Acids
TcdA	Toxin A produced by *Clostridium difficile*
TcdB	Toxin B produced by *Clostridium difficile*

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