TITLE

Effects of probiotics on cognitive and emotional functions in healthy older adults: protocol for a double-blind randomised placebo-controlled crossover trial

ABSTRACT

Ageing is a process that includes changes in cognitive and emotional functions, as well as changes in the diversity and integrity of gut microbiota. Probiotic treatments have recently been studied as a potential new therapeutic approach to alleviate a wide range of problems in other populations; however, clinical studies in older adults remain insufficient and limited. Thus, the aim of this project is to evaluate the efficacy of a multi-species probiotic formulation as a therapeutic strategy for attenuating the emotional and cognitive decline associated with ageing in adults over the age of 55. This is a double-blind randomised placebo-controlled crossover trial involving at least 32 older adults and comparing two conditions: (a) probiotic, providing a multi-species probiotic for 10 weeks (*Lactobacillus rhamnosus* and *Bifidobacterium lactis*); and (b) placebo, receiving a harmless substance (potato starch). Despite the increasing use of probiotics for the treatment of cognitive and emotional problems, no study has yet focused on this group, to the best of our knowledge. Therapeutic strategies of the kind outlined in this protocol will help to shed light on the current state of knowledge about this topic, as well as promote health programs tailored to this population, which would encourage active ageing and healthy lifestyles. Not only do we expect improvements in the emotional dimension in terms of anxiety, stress, depression, and sleep quality, we also expect improvements in the cognitive dimension in terms of attention, memory, and decreased impulsivity.

Keywords: aging; clinical trial; older adult; gut microbiota; probiotics

INTRODUCTION

The number of middle-aged and older adults is on the rise worldwide, with people aged 55 and over representing a large percentage of the population due to increasing life expectancy (Partridge et al., 2018; Sanderson & Scherbov, 2014). According to the World Health Organisation (WHO), the proportion of older adults is expected to be more than 12% in 2030, 16% in 2050, and nearly 23% in 2100, posing both challenges and opportunities for societies and their healthcare systems (Department of Economic and Social Affairs, 2019). The global cost of some types of age-related disease care, such as dementia, has been estimated to be a trillion dollars in 2018, with a projected two-fold increase by 2030 (Patterson, 2018).

Ageing is a normal and complex biological process characterised by a progressive decline in some physiological functions of the organism, resulting in both physical and cognitive deterioration and predisposing to the development of various pathological disorders (Juan & Adlard, 2019). Different biological, environmental, and lifestyle factors modify and alter nearly all body tissues and organs during the ageing process, with the brain being one of the most affected structures (Harman & Martín, 2019). Anatomical-physiological changes in the brain affect some aspects of cognition such as processing speed, working memory, and executive functions (Reuter-Lorenz & Park, 2014). It is estimated that 30% of adults over the age of 65 will suffer from cognitive impairment (CI) at some point in their lives (Romo-Araiza & Ibarra, 2020), increasing the risk of developing neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD) (Pettigrew & Soldan, 2019). Similarly, while affective disorders such as anxiety and depression are very common in older adults (John et al., 2019), both emotional and

cognitive diseases have a direct impact on the quality of life of the older adult population and have become one of our major societal problems (Beard et al., 2015).

BACKGROUND

In recent years, the gut microbiota (GM) has emerged as an important target for studying emotional and cognitive diseases, particularly in populations with altered GM, such as the older adults (Bana & Cabreiro, 2019). The GM is a constantly changing ecosystem comprised of a collection of live microorganisms that inhabit the human gastrointestinal tract and are influenced by a variety of factors such as dietary habits, alcohol and tobacco use, medications, physical activity, and body composition (Gallè et al., 2019; Lee et al., 2018; Maier & Typas, 2017; Mörkl et al., 2017; Rinninella et al., 2019; Wang et al., 2020). Several studies have found that the aging process alters the variety and integrity of GM (Bander, Nitert, & Mousa, 2020; Maynard & Weinkove, 2018), resulting in a reduction in the diversity of species such as *Lactobacillus*, *Bifidobacterium*, *Firmicutes,* and *Bacteroidetes* (Bischoff, 2016; Claesson et al., 2011). Maintaining the diversity of GM is essential because they promote nutrient absorption, protect the host from pathogen invasion, produce metabolites related in energy homeostasis, and play an important role in immune system modulation (Marshall-Jones et al., 2006; Tanner et al., 2016; Wrzosek et al., 2013). Ageing alters the integrity of the intestinal barrier, allowing microbes and microbial particles to pass through the intestinal epithelial cell lining (Bana & Cabreiro, 2019). This decreased GM diversity induces overgrowth of different microbes and metabolic instability, inducing an abnormal immune response and chronic inflammation (Wilms et al., 2020), which in turn appear to be associated with a variety of alterations in functional health, including frailty, impaired cognition, and depression (Claesson et al., 2011, 2012).

Evidence supports the claim that the microbiota influences some stages of cognitive impairment through the gut-brain axis (GBA) (Brüssow, 2013). The GBA is a bidirectional communication pathway between the gastrointestinal tract and the brain that is regulated by multiple neural, hormonal, and immunological pathways (Cryan et al., 2019). Changes in the gut metagenome, a set of intestinal microbial genes, appear to be also connected to cognitive function, brain iron deposition, and some ageing-related factors like oxidative stress and inflammation (Blasco et al., 2017; Heyck & Ibarra, 2019). Given this evidence, some authors have suggested that GM modulation could provide beneficial effects in cognitive and emotional processes (Cryan et al., 2019; Lew et al., 2019).

Gut microbiota composition in conditions of cognitive impairment

In terms of cognitive process, low levels of *Firmicutes* and *Bifidobacterium* phyla, as well as a decrease in microbial diversity and an increase in *Bacteroidetes,* have been detected in faecal samples from AD patients (Vogt et al., 2017). Another study identified a connection between an increase in the abundance of proinflammatory species including *Escherichia* and *Shigella* in the gut, brain amyloidosis, and behavioural impairment (Cattaneo et al., 2017). Similarly, short chain fatty acid (SCFA)-producing bacteria from the genera *Butyrivibrio*, *Eubacterium,* and *Faecalibacterium* are also present in lower proportions in AD patients, resulting in intestinal disruption, because compounds like butyrate exert anti-inflammatory effects on the mucosa (Haran et al., 2019; Ling et al., 2021); and reductions in the *Faecalibacterium* genus and its most well-known species, *Faecalibacterium prausnitzii*, have been found to be positively correlated with MMSE scores (Leblhuber et al., 2017; Ling et al., 2021). The presence of species from the *Lactobacillaceae*, *Enterobacteriaceae,* and *Enterococcaceae* families, as well as genera

from the *Porphyromonadaceae* family, defines the composition of the PD gut microbiota; additionally, PD patients with CI showed lower faecal abundance of the genera *Blautia* and *Ruminococcus* (Ren et al., 2020).

Likewise, certain components belonging the outer membrane of gram-negative bacteria, such as lipopolysaccharides (LPS), appear to have a role in CI, as they have been found in high concentrations in the brain and plasma of CI patients (Zhan et al., 2018). LPS increases NF-kappaB (NF-KB) activity and hence initiates inflammatory and immunological processes. Since elevated quantities of these compounds have been detected in brain parenchyma and vessels, it has been postulated that LPS transit from the gut to the brain across the blood-brain barrier occurs in AD patients (Zhan, 2017; Zhou et al., 2014). The data suggest that patients with CI have a shift in the composition of their faecal microbiota, which may result in a proinflammatory environment and increased intestinal permeability due to changes in its regulating proteins and tight junction proteins such as occludins and zonulins (Clairembault et al., 2015; Forsyth et al., 2011; Leblhuber et al., 2018; Y. Wang et al., 2020). As a result, there has been a surge in interest in the relationship between GM and ageing in recent years, with the aim of fostering new therapeutic strategies to prevent and treat cognitive and emotional decline in the older adults (Sun et al., 2020).

Numerous studies have been conducted to investigate the role of different GM modulating strategies in altering GM status and improving host health, i.e., modulating age-related changes (Sherwin et al., 2016). These strategies include faecal microbial transplantation, dietary changes, or the consumption of probiotics, prebiotics, or synbiotics, (Cryan et al., 2019; Roman et al., 2018). This study will focus on probiotics, which are defined as live microorganisms that provide a health benefit to the host when administered in appropriate

levels, and require regular use to generate positive effects since they do not often become resident in the gut (FAO/WHO, 2001). Probiotics have been shown to address or prevent age-related dysbiosis, reducing or preventing intestinal permeability and associated inflammation, inhibiting the generation of harmful and/or toxic metabolites, and promoting the production of beneficial bacterial components (Cancello et al., 2019). Several research studies have been conducted to better understand the role of probiotic administration in the modulation of GM composition and its association with CI (Akbari et al., 2016; Leblhuber et al., 2018; Roman et al., 2018; Xiao et al., 2020); a summary of this research is presented below.

Probiotics modulation of gut microbiota in cognitive impairment

A recent meta-analysis found that probiotic consumption improves cognition in AD or CI, presumably through lowering inflammatory and oxidative biomarkers while raising brain-derived neurotrophic factor (BDNF) levels (Ruiz-Gonzalez et al., 2021). Adults aged 52–75 with CI who consumed *Lactobacillus rhamnosus* GG improved their total cognition score more than those in the placebo group, as shown by Sanborn and collaborators (2020). Comparably, administering *Bifidobacterium breve* A1 to CI older adults for 16 weeks or a probiotic-fermented milk supplement to AD patients for 90 days have shown to improve memory, visual-spatial/abstraction abilities, executive/language functions, and decreased several cytokine markers of inflammation and markers of oxidative stress (Xiao et al., 2020). Probiotic supplementation with different *Lactobacillus* and *Bifidobacterium* species for 28 days also decreased zonulin levels in the faeces, which correlated negatively with MMSE scores in AD patients (Leblhuber et al., 2018). In addition, it has been reported that probiotic supplementation raises BDNF levels, implying a reduction in dementia (Ng et al., 2019). Since then, probiotics

containing *Bifidobacterium bifidum* BGN4 and *Bifidobacterium longum* BORI have been shown to improve BDNF levels in healthy adults over the age of 65 (Kim et al., 2021), just as *Lactobacillus plantarum* C29 treatment for 12 weeks enhanced serum BDNF levels and improved cognitive functions related to memory and attention in people with mild CI (Hwang et al., 2019).

Considering the increasing use of probiotics for the treatment of cognitive and emotional problems, clinical studies are still insufficient and limited, and more large-scale, long-term, randomised controlled trials (RCT) are needed (Deng et al., 2020). Besides, no study has yet focused on this group of healthy older adults, to the best of our knowledge. Thus, the aim of this study is to determine the efficacy of a multiprobiotic formulation as a therapeutic strategy for attenuating the cognitive decline associated with ageing and promoting emotional dimension in adults over the age of 55. Our hypothesis is that providing a multi-species probiotic for 10 weeks will slow and/or improve the age-related decline in cognitive functions and emotional function. We will particularly analyse the impact on depression, anxiety, and general cognitive status, as well as the cognitive functions of working memory, planning ability, problem-solving ability, selective attention, cognitive flexibility, response inhibition, motor impulsivity, and inhibitory response control.

METHODS

Design

A randomised, double-blind, placebo-controlled crossover trial was designed in accordance with the recommendations of the Consolidated Standards of Reporting Trials

(CONSORT) standards for randomised trials (Moher et al., 2012). This study has been registered at ClinicalTrials.gov (NCT04828421).

This study will compare two conditions: (a) probiotic, a daily capsule containing a multi-species probiotic (3.3 billion *Lactobacillus rhamnosus* and *Bifidobacterium lactis*); and (b) placebo, a harmless substance (potato starch) encapsulated by the same manufacturer under identical conditions as the multi-species probiotic to ensure study masking. Both the probiotic and the placebo will be administered after breakfast for ten weeks under identical conditions. The probiotic strains selected have shown to be effective in similar populations or in cognitive function (Agahi et al., 2018; Czajeczny et al., 2021; Kelly et al., 2017; Mehrabadi & Sadr, 2020; Oh et al., 2020; Patel et al., 2020; Sanborn et al., 2020; Yang et al., 2020), and the time duration has been predicted based on previous studies exploring the influence of probiotics on cognitive and emotional functions (Ruiz-Gonzalez et al., 2021). Assessments will be conducted in three stages: at the start of the trial, following the intervention (10 weeks), and at study end (20 weeks). Figure 1 depicts the study flowchart.

[INSERT FIGURE 1 ABOUT HERE]

Study participants

Participants will be recruited from the seniors' university programme at the University of Almeria. The average student profile is of an urban 60–70-year-old person with Spanish nationality and a higher education level than the general public. People aged 55 and above have access to this university programme for six academic years, with around 100 students per year. The inclusion criteria for the study will be as follows: (i) being 55 years or older, (ii) voluntarily agreeing to participate in the study in accordance with the

Declaration of Helsinki, and (iii) not being involved in another study that could interfere with the results. Conversely, participants will be excluded if they: (i) have any serious mental illness, (ii) have a score below 10 on the Mini-Mental State Examination (MMSE) (severe cognitive impairment), (iii) are taking medications that affect cognition, microbiome or gastrointestinal motility, for example antipsychotics, anxiolytics, antibiotics, anti-inflammatory/secretory drugs or opioids, or (iv) have another serious illness (e.g., cancer, Parkinson's or Alzheimer's).

Sample size and power

The G*power software (v.3.1.9) has been used to calculate the sample size based on previous studies (Akbari et al., 2016; Marotta et al., 2019), with a confidence level of 95%, power of 80%, and an expected dropout rate of 25%. The Mini-Mental State Examination (MMSE) for the cognitive dimension and the State Trait Anxiety Inventory (STAI) for the emotional dimension were considered our primary outcome measures. A target sample of 19 participants was identified for the MMSE, with significant differences for dependent samples in the questionnaire of 1.1 for the mean and 1.3 for the standard deviation. A target sample of 32 participants was identified for the STAI, with an effect size of 0.25. Taking these two main variables into account, a sample size of 32 participants was considered adequate.

Measures

Sociodemographic variables will be assessed using an ad-hoc questionnaire that will include the following questions: age, sex, marital status, employment status, educational level, digestive problems, tobacco use, elimination pattern (constipation, normal and

diarrhoea), digestive diseases, nutritional supplements/components use, regular medication and antibiotic use in the previous month.

Emotional status

State and trait anxiety will be measured using the reliable and valid State-Trait Anxiety Inventory (STAI) (Creighton et al., 2018; Spielberger et al., 1970). Each subscale consists of a total of 20 items in a 4-point Likert-type response system according to intensity (0= al-most never/not at all; 1= somewhat/sometimes; 2= quite often; 3= very much/almost always). The total score for each of the subscales ranges from 0 to 60 points.

Depression will be measured using the reliable and valid Beck Depression Inventory (BDI-I) (Beck et al., 1961; Gatewood-Colwell et al., 1989). the BDI consists of a 21-item self-administered questionnaire with four response options (numerical scale from 0 to 3 points). The total score in this questionnaire ranges from 0 to 63 points, with the following cut-off points accepted for grading the intensity and/or severity of the symptoms (Sanz-Fernández, 2013): no depression (0–9 points), mild depression (10-15 points), moderate depression (16–23 points) and severe depression (more than 24 points).

Cognitive Status

General cognitive status in five areas: orientation, fixation, concentration and calculation, memory and language, and construction will be measured using the Mini-Mental State Examination (MMSE) (Lobo et al., 1979, 1999). A total of 35 points can be obtained, with less than 24 points indicating cognitive impairment in those over 65, and less than 28 points indicating cognitive impairment in those 65 and younger.

Cognitive functions will be assessed using the Psychology Experiment Building Language (PEBL) Test Battery software (Mueller & Piper, 2014). This battery includes the Corsi task, the Tower of London test, the Wisconsin Card Sorting test, Stroop task, Trail Making test, Go/No-Go test, and Iowa Gambling task.

Working memory will be assessed using the Digit and Corsi tasks from the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1955). In the Digit task, the experimenter reads aloud, and in order, a series of numbers that the participant is asked to repeat in the same order (direct condition) or in reverse order (reverse condition). The test ends when two attempts at a given level are failed. In the Corsi task, the participant must recall the order in which the differing figures on the screen are illuminated with increasing difficulty. The performance of the participant determines when each block ends, that is, when they fail two attempts at a given level.

Planning ability will be assessed using the Tower of London test (Robinson et al., 1980). Participants in this test must place tokens of various colours in the same order and position as the reference, with limited time and movement.

Problem-solving ability will be assessed using the Wisconsin Card Sorting test (Shallice, 1982), with participants attempting to sort cards into different groups based on the shape, colour, and number of figures. Participants will attempt to sort in the correct sequence as directed by a randomly varied sorting order.

Selective attention, cognitive flexibility, and response inhibition will be assessed using the Stroop task (Stroop, 1935). This test measures the interference that occurs when a subject performs a test in which he or she is required to indicate the colour in which a word is written that does not correspond to its meaning. A computerised version of the

Trail Making test will be used to assess visual attention and the ability to switch tasks (Reitan & Wolfson, 1995), in which the subject must connect circles containing numbers, letters, or a combination of both. The participant has 20 seconds to complete the task.

Motor impulsivity or inhibitory response control will be assessed using the Go/No-Go task, a computerised test that investigates the ability to inhibit an inappropriate predominant response (Casey et al., 1997). In this task, the subject must press the mouse button when the letter "P" appears on the screen while avoiding pressing the button when the letter "R" appears. The test is then repeated in reverse.

Choice impulsivity or impulsive decision-making will be assessed using the Iowa Gambling task (Bechara et al., 1994), a task in which participants must choose between four different piles of cards in order to win as much money as possible. Piles A and B produce further gains than C and D, but also more losses, resulting in less virtual money earned. Thus, options A and B are disadvantageous, whereas options C and D are advantageous.

Potential confounding variables

Potential confounding variables such as food habits, weight, body composition, alcohol use, physical activity will be assessed to ensure that the results are not influenced by factors unrelated to the intervention. A description of the different scales, questionnaires, and tests is presented below.

Dietary habits will be measured using a "24-hour recall" questionnaire. This method entails precisely recalling, describing, and measuring the intake of meals and beverages consumed in the 24-hour period preceding, or on the day preceding, the interview, beginning with the initial intake in the morning and ending with the last foods or

beverages consumed at night (Salvador-Castell et al., 2015). The ingredients used, weight in home measurements, and method of preparation will all be described (Pannucci et al., 2018).

Hazardous drinking and active alcohol use disorders will be measured using the Alcohol Use Disorders Identification Test (AUDIT) (Pérula-de Torres et al., 2009; Saunders et al., 1993). The AUDIT is a simple ten-question test with five alternative answers, with a score ranging from 0 to 40 points, whereby a higher score indicates a higher degree of dependence.

Body composition will be measured using the OMRON BF511 bioimpedance meter (Mulasi et al., 2015), a safe, objective, and non-invasive technique for assessing numerous biomedical parameters (Park et al., 2018).

Physical activity will be measured using the International Physical Activity Questionnaire (IPAQ), including frequency, duration, and intensity of activity performed in the last seven days (Craig et al., 2003; Tomioka et al., 2011). This questionnaire consists of 7 questions and classifies the level of activity in three categories (low, moderate and high).

Type and consistency of bowel movements will be measured using the Bristol Scale (Lewis & Heaton, 1997; Parés et al., 2009). This scale classifies human stool form into 7 categories: constipation (types 1 and 2), ideal stools (types 3 and 4) and diarrheic stools (types 5, 6 and 7). Multiple reports associate stool characteristics to the composition of the GM (Kwon et al., 2019; Takagi et al., 2019; Vandeputte et al., 2016).

Sleep quality will be measured using the Pittsburgh Sleep Quality Index Questionnaire (PSQI) (Beaudreau et al., 2012; Buysse et al., 1989). This questionnaire consists of 24 questions, the first four of which are answered in their own words, and the remaining

twenty are answered on a four-grade ordinal scale. The sum of the scores obtained in each of the partial components yields a total score ranging from 0 to 21, with a total score of 5 indicating good sleep quality and a score of 5 or less indicating poor sleep quality.

Stress levels will be measured using the Perceived Stress Questionnaire (PSQ) (Levenstein et al., 1993; Sanz-Carrillo et al., 2002) and the Gastrointestinal Symptom Rating Scale (GSRS) (Kulich et al., 2008; Svedlund et al., 1988). The first questionnaire contains 30 items distributed across six dimensions, with each item answered on a Likert-type scale with four response options (from 1 “almost never” to 4 “almost always”). The higher the score, the more stress the participant perceives. The GSRS scale has five subscales (re-flux, diarrhoea, constipation, abdominal pain, and indigestion), with the mean subscale score ranging from 1 (no discomfort) to 7 (great discomfort). Higher scores indicate a greater burden of gastrointestinal symptoms.

Procedure

Researchers will inform all students from the seniors’ university programme about the study after one of their face-to-face lessons and via email. First, individual eligibility criteria will be evaluated for each individual who indicates an interest in participating. Potential participants who meet eligibility criteria will be given a document containing detailed information about the study and will be offered the opportunity of participating voluntarily. Those who accept the invitation to participate will be required to sign written informed consent. Participants will be assessed at baseline, given one of the conditions (placebo/probiotic) for 10 weeks, and then switched to the other condition (probiotic/placebo) for 10 weeks more. Participants will be given a 4-week washout period between each condition. Participants will be given instructions for taking the probiotic or placebo at home and regular contact will be made to verify that the scheduled

procedures are followed appropriately throughout the intervention period. The treatment will be administered in a staggered manner, with each participant receiving blister packs of capsules twice for each condition (approximately at week 5 of probiotic or placebo), in order to ensure therapeutic adherence to the medication and quantify the number of capsules used. Additionally, none of the participants, assignors, or outcome assessors will be aware of individual intervention status the during the course of the study.

Data collection will take place in two stages at the University of Almeria. First, participants will take baseline questionnaires (approximately 3 hours). The following day, they will use a computer to complete virtual testing. Table 1 shows the variables measured by questionnaires as well as a summary of each questionnaire; all questionnaires have been validated in Spanish.

[INSERT TABLE 1 ABOUT HERE]

Statistical analysis

Continuous variables will be expressed as mean and standard deviations or median, range (maximum and minimum values) based on their distribution, and categorical variables will be expressed using a table of frequencies and percentages. The Kolmogorow-Smirnov test will then be applied to each continuous variable to determine its normality. For both means and proportions, 95% confidence intervals will be obtained.

In order to compare the results of both conditions in terms of quantitative variables, repeated measures ANOVA and pairwise-tests using post hoc Fisher's least significant difference (LSD) or non-parametric equivalents (Friedman and Wilcoxon respectively) will be used. Categorical variables will be compared with the $\chi 2$ or Fisher's exact test. Finally, measures of effect sizes ($\eta 2$) will be generated for each analysis that reaches

statistical significance, providing information beyond mere statistical significance. More broadly, it will allow us to determine the proportion of the variability in the results that can be attributed to our experimental manipulations. All the statistical analyses will be conducted with IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA).

Ethical considerations

This study was approved by the Ethics Committee from the University of Almeria (UALBIO2020/001) and adheres to the fundamental principles stated in the Declaration of Helsinki. All participants will be informed about the length and nature of the study, the voluntary nature of their participation, and the option to withdraw at any time. Any questions potential participants may have about the study will be answered thoroughly, and they will be given an informed consent document to sign if they wish to participate in the study. Participants will also be informed that all data gathered during the investigation will be treated confidentially in accordance with current data protection legislation. Participants will be provided a customised calendar that includes the most important research dates, such as the date of the initial assessment and the date of the post-treatment evaluation. Additionally, given the sheer number of measurements, the assessment sessions would be arranged by experienced evaluators across at least two days to reduce participant fatigue. Participants will not be compensated for their participation in the study.

DISCUSSION

Age-related changes in physical, emotional, and cognitive function have a direct impact on daily activities, independence, and quality of life (S. Lee et al., 2019). Some of these

biological processes are connected to the development of chronic diseases, such as dementia or depression (Sengoku, 2020; Wahl et al., 2019), which have health and social consequences, including increased health costs associated with demanding therapies, high drug consumption, hospitalisations, or home health services (Mangiola et al., 2018). Exhaustive research has demonstrated that changes in the gut microbiome (GM) of older adults occur as they age (Claesson et al., 2011; Coman & Vodnar, 2020), and that these changes are tied to the pathophysiological processes which cause functional impairment (Badal et al., 2020; Mangiola et al., 2018; Vaiserman et al., 2017). This research study will provide pertinent data on the efficacy of a multi-species probiotic formulation as a therapeutic strategy aimed at improving the emotional and cognitive decline associated with ageing in adults over the age of 55 years. Thus, we anticipate that this intervention will result in an improvement or strengthening of cognitive and affective health in older persons in a less advanced stage of ageing, or a halting of decline in these spheres when organic modifications are more visible. If the study verifies the efficacy and sustainability of the intervention, a novel strategy for promoting healthy ageing and reducing the health-cost impact associated with this biological process will be available.

Progress to date

The recruitment process for participants began in early 2020, with a high level of interest among the target population (N=120). Notwithstanding that the COVID-19 home confinement drove many interested older adults to decline, causing the recruitment process to be prolonged until mid-2021, the current sample (n=35) fulfils the required sample size for the study. The initial evaluation of participants is ongoing, and we plan to conclude the crossover study in the first half of 2022. The findings are expected to be completed by the end of 2022.

Challenges encountered

The current societal environment, as a result of the COVID-19 pandemic, has been a significant hurdle to recruiting volunteers and organising face-to-face meetings, extending the evaluation and research processes. The recruitment process was initially planned to be carried out in person at the university buildings. Yet, potential participants had to be reached both by email (which was not widely used) and by phone as a consequence of the pandemic. The assessment process in RCTs of this sort is usually organised in a group setting, with each assessor working with one participant. As a consequence of the pandemic, however, all assessment sessions had to be planned on an individual basis. All hygienic measures and safety standards were followed during the assessment and treatment delivery process; in general, social distance measures were maintained, continuous ventilation of the room was ensured, and surfaces were disinfected before and after each use. In addition, because the older adult population often has a wide range of medical disorders and therapies, the fear of infection has been a significant impediment.

Lessons learned

We have learned lessons from this project that will help us improve our future work. Adapting the assessment and follow-up procedure to the target population is critical in order to acquire results that are in conformity with the capacities and characteristics of the group to be studied. Although many of the questionnaires were self-administered and could be completed by participants at home during the assessment process, we found it was preferable to complete the questionnaires in person in order to resolve any questions and facilitate understanding. In this sense, the computer tasks chosen were those that had previously been used in populations with similar sociodemographic characteristics, and

instructions were reinforced by the researchers' guidance to facilitate comprehension. Another observation was the importance of being in regular contact with the participants and performing a fortnightly follow-up assessment of their status in order to detect slight difficulties that could hamper the procedure. Instant messaging apps, social media, and networks (e.g., Telegram, WhatsApp, Skype, Google Meet, etc.) have been used in other populations with whom we have previously worked with; yet, many of our participants do not have these forms of technology or have difficulty using them. For this reason, a regular telephone contact with the participants was provided in order to ensure adherence and address any further queries or concerns. Finally, in this project, collaboration with other researchers who are conducting studies with similar populations are fundamental, which aided in the recruitment of new participants and broadened our perspective. For example, we benefit from other researchers who are already familiar with a significant proportion of our participants from previous studies and can clarify the meaning of our study while also contributing in the allocation of suitable appointments (time and place) to conduct their assessments. These aspects are crucial for the development of the research and should be considered in future studies with a population group with similar characteristics.

Limitations

This study is restricted by the project budget in order to assess and select specific probiotic species for our participants, as well as to investigate changes in the gut microbiome as a secondary analysis over time. A direct measurement of GM would have reported more information about probiotic modulation. The multispecies probiotic selected, however, is commercially available, and its ability to restore gut flora balance has previously been demonstrated (Poutsiaka et al., 2017). Indeed, evidence has shown that these species have

a positive effect on GM as well as health benefits (Koning et al., 2008). The determination of biochemical and inflammatory biomarkers may provide significant information for the diagnosis of CI (Akiyama et al., 2000; Johnson et al., 2016), however funding is limited. The most validated biomarkers in cerebrospinal fluid (CSF) include β-amyloid peptide (Aβ1-42), total tau protein (T-tau), and phosphorylated tau (P-tau) (Andreasen et al., 2003), as well as the relationship between these three proteins (Humpel, 2011). Currently, different researchers advocate for the use of CSF biomarkers in addition to clinical measurements in the study of CI (Johnson et al., 2016).

SUMMARY

Physiologic changes within the older population, including changes in gut microbiota, are related to the ageing process. These changes have an impact on the well-being and functional ability of older adults, with cognitive function decline and emotional alterations modulating of quality of life, independence, and social development. We are conducting a randomised, double-blind, placebo-controlled crossover trial of a multi-species probiotic as modulators of cognitive and emotional deterioration in this population. We expect that administration of *Lactobacillus rhamnosus* and *Bifidobacterium lactis* for 10 weeks will reduce the cognitive and emotional decline of older adults. Specifically, we expect improvements in the emotional dimension of anxiety, stress, depression, and sleep quality and in the cognitive dimension of attention, memory, and impulsivity. These improvements will support individual independence and active ageing.

CONFLICT OF INTERESTS

The authors declare no conflict of interest. The manufacturer had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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