Emerging Research

The Ageing Gut-Brain Study: Exploring the role of the gut microbiota in dementia

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Running head: Ageing-Gut Brain study

Abstract

Up to than 90% of people with dementia will experience behavioural and psychological symptoms of dementia (BPSD) as part of their illness, and nearly two thirds of those living with dementia in care homes will experience BPSD. BPSD describes the disturbed perceptions, thought content, moods or behaviours that frequently occur in patients with dementia. There is increasing evidence that the gut microbiota plays a role in the interaction between specific nutrients and brain function. The *Ageing Gut-Brain* study described here is based on the hypothesis that the gut microbiota, and microbial metabolites, impact upon the gut-brain axis and thereby on behaviour, including BPSD. In the absence of available cures for Alzheimer's disease and its symptoms, if evidence in support of the gut-brain axis hypothesis is found, diet/nutritional interventions comprising important modifiable component/s may have significant impact on the management of BPSD.

Keywords: Dementia, Diet, Gut, Microbiota, Brain, Alzheimer’s disease

Introduction

With a growing number of ageing populations across the world, healthy life expectancy is a key area of research. One of the major health challenges in recent years is the alarming rise in the prevalence of non-communicable diseases including dementia. In Europe, the age-adjusted prevalence of dementia of any kind among people aged 65 years and older is 6.4% and of Alzheimer's disease (AD) specifically 4.4% (Lobo *et al.* 2000; McVeigh & Passmore 2006). It has been estimated that 36 million people have dementia worldwide (Prince *et al.* 2013) and that there are 4.6 million new cases of dementia every year (Ferri *et al.* 2005). Epidemiological evidence supports the hypothesis that modifiable lifestyle-related factors are associated with cognitive decline, which opens new avenues for prevention (Solfrizzi *et al.* 2008).

Alzheimer's disease is the commonest cause of dementia in older people, accounting for 60–70% of all dementia cases when using traditional diagnostic criteria for dementia subtypes (Fratiglioni *et al.* 1999; Blennow *et al.* 2006). There are no available cures for AD; an alternative approach is to use strategies that delay disease progression at an early stage (Lobo *et al.* 2000). Optimal brain function results from highly complex interactions between numerous genetic and environmental factors, including food intake, physical activity, age and stress (Solfrizz *et al.* 2008). Future studies linking nutrition with advances in neuroscience and 'omics' technologies might provide novel approaches to the prevention of cognitive decline, and treatment of dementia and AD. Diet in particular has become the object of intense research in relation to cognitive ageing and neurodegenerative diseases.

Behavioural and psychological symptoms of dementia

During the course of dementia the vast majority of people will experience some form of behavioural or psychological symptoms of dementia (BPSD). BPSD are central to dementia syndromes and seen in between 50% and 90% of patients with dementia at some point during their lifetime (Ballard & Waite 2006; Cerejeira *et al.* 2012), with higher prevalence in hospital and long-term residential care facilities in comparison to community dwelling settings. These symptoms can result in significant reduction in quality of life, are a major source of caregiver stress and increase financial burden through the requirement for institutional care. BPSD include agitation, aggression, calling out repeatedly, sleep disturbance, and lack of interest and motivation. Numerous studies have reported that BPSD can be a major source of distress for family and caregivers and are an important predictor of family caregiver depression, burden and care home admission (Porter *et al.* 2016). The degree and presentation of BPSD varies, depending on dementia severity, brain-damaged state and aetiology of the dementia syndrome (Cerejeira *et al.* 2012). It has been suggested that the physiological basis for BPSD relates to an imbalance of neurotransmitters including acetylcholine, dopamine, noradrenaline, serotonin and gamma-Aminobutyric acid (GABA) (Perry *et al.* 2003). Differences in the presentation of BPSD between pathological subtypes of dementia are likely to be partially explained by the different neurophysiological changes associated with each subtype (Cerejeira *et al.* 2012).

Many dementia syndromes have no modern therapeutic treatments, and there is little evidence that treatments that are given help to manage symptoms. Psychosocial therapies and antipsychotic medications are commonly used in an attempt to manage symptoms, with over 40% of patients with dementia in institutional care receiving antipsychotic medications (Maust *et al.* 2015). There is a risk of harm from antipsychotic drug use: falls and drowsiness

are common. More serious adverse effects include accelerated cognitive decline, and increased risk of arrhythmia and stroke. The use of antipsychotics in patients with dementia has also been shown to be associated an increased mortality risk of up to 3.8% (Maust *et al.* 2015).

In the absence of available cures for dementia syndromes, and with a lack of safe and effective treatments for the neuropsychiatric symptoms associated with the advanced stages of dementia, there is increased interest in whether dietary modifications can offer benefits. However, there is currenly little knowledge about the role of diet and dietary supplements in management of clinical symptoms of dementia. It has been hypothesised that the gut-brain axis plays a central role in BPSD, with emerging research suggesting a relationship between cognitive ageing and neurodegenerative diseases and the gut microbiota (Mariat *et al.* 2009; Claesson *et al.* 2012).

Gut microbiota in the older adult

The physiology and functioning of the gastrointestinal (GI) tract and diet change with age, and this impacts on the composition of the gut microbiota (O'Toole & Jeffery 2015). Fraility, rather than chronological age, has been found to correlate with changes in the microbiota in community-dwelling older adults (Fried *et al.* 2001). Maintaining a 'younger-adult' diverse microbiota, as a result of a diverse diet, may protect against fraility and poor health. For example, Italian semi-supercentenarians (those aged 105 – 109), with a lower incidence of chronic diseases that generally affect the elderly, were found to have different but still diverse microbiota in comparison to in comparison to adults, elderly adults, and centenarians (Biagi *et al.* 2016). Two studies that profiled the gut microbiota in the elderly, comparing free-living and care-home residents, demonstrated significant relationships between microbiome

profiles and indices of frailty and poor health (Jackson *et al.* 2016, Jeffery *et al.* 2016). Changes in dietary composition and diversity were considered the main drivers of the shifts in gut bacteria profile. As these studies are correlational, evidence from randomised controlled trials (RCTs) is required to understand whether a diverse microbiota is a cause or consequence of frailty and poor health in the elderly (Caracciolo *et al.* 2014). Age-related changes in the gut microbiota can contribute to the onset and progression of inflammation associated with ageing by increasing the production of a number of pro-inflammatory mediators, or lowering production of those that are anti-inflammatory, thus tilting the equilibrium toward inflammation (O'Toole & Jeffery 2015). The studies comparing the microbial profile of frail elderly to that of healthy elderly show that bacterial species associated with inflammation are more prevalent in the former group (O'Toole 2012).

Research focussed specifically on AD has shown that increases in levels of neurotoxic proteins in the brain (amyloid formation) and circulating pro-inflammatory cytokines correlate with a lower abundance of the butyrate–producing anti-inflammatory bacterium *Eubacterium rectale* and higher levels of pro-inflammatory *Escherichia coli/Shigella* (Cattaneo *et al.* 2017). As neuro-inflammation is associated with cognitive decline (Solas *et al.* 2017), this is an important finding but research is needed to understand the direction fo the relationship between neuro-inflammation and the composition of the gut microbiota.

There are numerous potential pathways through which the activity of the gut microbiota may influence the brain (sumarised in Figure 1). These include direct effects of gut microbiota metabolites [*e.g.* short-chain fatty acids (SCFA), 5-hydroxytryptamine (5-HT) acetylcholine, gamma-aminobutyric acid (GABA), serotonin], neural routes (vagus and enteric nervous systems), the adrenal axis (cortisol), and cytokines via their effect on immune cells. Any change to the balance of the gut microbiome could therefore alter the signals received

by the brain, potentially affecting emotions, mood and behaviour. Under conditions of stress, the brain can also send signals to the gut via the adrenal axis and vagus nerve.

Dietary modification as a potential strategy for the treatment of Alzheimer's disease

A number of human intervention studies have shown rapid changes in the gut microbiota composition following dietary modification, with concomitant changes in production of bacterial metabolites. For example, reduced carbohydrate diets have been linked with lower bacterial production of SCFAs, particularly butyrate, and a reduction in the numbers of butyrate producing bacteria (Duncan *et al.* 2007; Walker *et al.* 2011). However, there is a need to understand the underlying mechanisms which could potentially link diet-induced microbiota changes to changes behaviour, such as BPSD.

The degenerative diseases associated with ageing are frequently linked to oxidative stress and inflammation, and inflammation in the gut is linked to changes in microbiota composition (see review by Vaiserman *et al.* 2017). Many probiotic bacteria are lactic acid bacteria, some of which have antioxidant and immune regulatory activities (Lee *et al.* 2016). In a mouse model of ageing, oxidative stress (measured by monitoring levels of hepatic anti-oxidant enzymes in the liver) was reduced, and the microbial composition and activity restored to that of the control group, by adding *Lactobacillus helveticus* by daily oral gavage for 8 weeks (Li *et al.* 2018). Several other small animal studies have explored brain function following probiotic supplementation, with improvements demonstrated for different aspects of memory impairments following different triggers (*e.g.* Beilharz *et al.* 2018; Chunchai *et al.* 2018, O'hagan *et al.* 2017), but limited research has been conducted in humans. In one study, a mixture of three lactobacilli strains plus a *Bifidobacterium bifidum* strain consumed daily in probiotic milk for 12 weeks improved cognitive function in AD patients (Akbari *et al.* 2016),

but had little effect on markers of oxidative stress and inflammation, only serum high sensitivity C-reactive protein (hs-CRP) and malondialdehyde (MDA) decreased. In another study, the abundance of bifidobacteria in elderly individuals was increased following bifidobacteria administration, with a concomitant lowering in the levels of pro-inflammatory cytokines and increases in anti-inflammatory cytokines (Ouwehand *et al.* 2008). The study assessed only the levels of specific cytokines and the bifidobacteria component of the microbiota, and did not include any cognitive or behavioural outcome measures. A recent review that focussed on the role of the gut microbiota in neurogenerative disorders (Sarkar & Banerjee 2019) concluded that while research indicate a potential role for probiotics in AD, cognition and inflammation, more research is required to understand the mechanisms.

Other studies have focussed on using prebiotic strategies to modulate the microbiota with the aim of increasing the bacterial diversity and altering parameters associated with ageing. For example, daily supplementation with a mixture of five prebiotics (at 20 g/ day) for 6 months did not have the expected effect of increased bacterial diversity in a study comparing cohorts of healthy adults, elderly adults (70+ years) and elderly adults in long-stay environments (80+ years), although small specific effects on some bacterial families and a decrease in the inflammatory cytokine CCL11 were observed following the prebiotic intervention (Tran *et al.* 2019). The authors noted that it was difficult to achieve compliance in the elderly cohort due to the perceived negative side effects associated with increased fibre consumption. Compliance is likely to be an issue that all dietary studies in the elderly will have to negotiate. In another prebiotic intervention trial in older individuals without dementia (70+ years) living in carehomes, consumption of a fructo-oligosaccharide/inulin supplement for 13 weeks resulted in a reduction in frailty levels, as assessed by a combination

score, in participants with the highest initial levels of frailty. In this study, there was no assement of the effects of the intervention on the particpants' microbiota profile or inflammatory markers (Theou *et al.* 2019).

Two ongoing studies are investigating the effects of dietary interventions on cognition in older adults. A trial in the US in 200 adults aged 55 – 75 years is exploring the effect of a probiotic *Lactobacillus* species (specifically LGG) on mood and cognitive functioning , as well as the gut microbiota composition and blood markers of inflammation (Sanborn*et al.* 2018). In an Australian trial with 400 older participants aged 60 – 75 years, the effects of daily consumption of two different herbal supplements and a placebo on cognitive performance are being compared. The study will include neuroimaging and assessment of the participants' faecal microbiota composition (Stough *et al.* 2012; Simpson *et al.* 2019).

Overview of Ageing-Gut Brain Interactions Study

Potential mechanisms underpinning the hypothesised bi-directional signalling between the brain and gut are outlined in Figure 1. At present it is unclear if modifying the gut microbiota profile through dietary changes can help prevent or treat neurological conditions, such as dementia. There has been no published work that we are aware of to examine the gut microbiota profile in patients with AD with associated BPSD. With increasing evidence that the gut microbiota may mediate the interaction between nutrition and brain function and that the composition of the microbiota correlates with diet and health in the elderly, the *Ageing Gut-Brain* project is designed to explore the role of the gut microbiota in BPSD in AD.

The *Ageing Gut-Brain* project has been funded by Tenovus Scotland, the NHS Grampian Research and Endowments Fund, and the Scottish Government as part of the Strategic Research Programme at the Rowett Institute. The project commenced in March 2018 and is expected to be completed at the end of 2019. The main aim of this proof-of-concept study is to explore whether gut microbiota profiles, assessed from faecal samples, differ among three participant groups: (1) dementia with BPSD, (2) dementia without BPSD and (3) healthy age-matched controls. Studying those living only in a care home environment means there is likely to be less dietary diversity between participants, a confounding factor affecting the gut microbial composition. Data collected as part of the *Ageing Gut-Brain* study will inform a future dietary intervention study to tease out the complex relationship between diet, the gut microbiome and BPSD in AD. Our ultimate ambition is to develop evidence-based dietary and lifestyle recommendations for people with AD plus BPSD, to reduce the burden of this condition on patients, family and society, and create safer environments for carers and society generally.

Ageing Gut-Brain project: Hypothesis and aims

The *Ageing Gut-Brain* proof-of concept study will test the hypothesis that differences exist in the gut microbiota profiles and activity of its metabolites between normal healthy ageing participants and those with dementia, with a focus on those with challenging behaviour consistent with BPSD. In addition, the study will gather information on the feasibility and acceptability of a future nutritional intervention trial in this population group. The project aims are summarised in Figure 2 and sample collection protocol summarised in Figure 3.

The five study aims are:

- **Aim 1**: To assess care homes' willingness to participate in a dietary intervention to reduce the burden of BPSD in AD.
- **Aim 2**: To test the feasibility of recruitment in older populations with or without dementia in residential care facilities, including the practicalities of faecal sample collection and working in the care home environment.
- **Aim 3**: To test the hypothesis that the gut microbiota profile is different among three groups of older people with (1) no dementia, (2) with AD and BPSD and (3) with AD but without BPSD, living in the same environment.
- **Aim 4**: To assess the willingness of older people with AD (with or without BPSD) and their carers (formal or informal) to participate in the proposed future nutritional intervention trial and the practical feasibility of assessing quality of life in this cohort.
- **Aim 5:** To measure dietary intake in the study cohort.

Ageing Gut-Brain project: Methods of research

To achieve Aim 1, we carried out a survey using Survey Monkey. The survey link was distributed via email to all Scottish care homes known to the 'Neuroprogressive and Dementia Network', and remained open for a month in June 2018. In summary, there were 105 responses representing approximately 95% of Scottish postcode areas. Eighty one percent of respondents completing the full survey and 83% of responses were from care home managers. The median % of care home residents with any type of dementia was 70.2% (interquartile range: 51.3-84.7%), and the median % of those with dementia with challenging behaviours was 34.8% (interquartile range: 17.4-50.0%). The usual immediate step taken by care home staff to help residents exhibiting challenging behaviour are shown in Figure 4. Challenging behaviours are recorded on 'ABC' (Antecedent, Behaviour, Consequences) charts

in 80% of care homes. Care home staff were positive about researchers approaching their care home to conduct a dietary intervention in those with challenging behaviours, with 62% of those responding ≥80% likely to say yes to the research being conducted. More than 90% of care homes stated that they would require information on staff time and staff training requirements before commiting to become a study site. Care homes have great potential for hosting research into dietary components affecting AD progression. While most respondents showed a high level of support for research in their care homes, researchers will need to design studies with the high demands on care home staff time in mind (Johnstone & Donaldson, 2019).

To achieve aims 2 and 3, 20 volunteers from each of the three study groups (60 in total) will provide two faecal samples, at least a week apart for validation purpose (to examine the within-subject variation for this cohort). These will be analysed for microbial composition and metabolic activity by assessing SCFA profiles. Faecal samples will be collected using a pot on a toilet/commode, or incontinence pad, which will be sealed and processed in the laboratory within 16 hours. To confirm group allocation, care home staff will be asked to log any episodes which require staff intervention due to challenging behaviours. We will collect additional information to mitigate the confounding effects of medication (especially antibiotic use), personal characteristics, co-morbidities, and active GI disease.

To achieve aim 4, we will collect information on the following to explore the practical feasabilities and willingness of older people with AD and their carers to participate in a future intervention study:

- proportion of eligible older people with dementia with or without challenging behaviors (BPSD);

- proportion of eligible participants willing to participate;
- proportion of participants who are able to provide samples in each group;
- proportion of participants (or nearest relative, welfare guardian or welfare attorney) who are able to provide study data /information; and
- quality of life for those with dementia using a validated questionnaire.).

We intend to survey staff, next of kin and participants (whenever feasible) to explore their experiences with the proof-of-concept study and issues of acceptability.

The assessment of dietary intake in elderly participants with dementia, even in the care home environment, is challenging as the usual self-report restrospective recall methods (via food diaries, food frequency questionnaires) are not appropriate for use in this population group. Therefore, to achieve aim 5, the *Ageing Gut-Brain* study will assess dietary intakes by obtaining care-home menu records, accepting there are a number of major limitations with this approach.

Conclusions

The *Ageing Gut-Brain* study aims to increase understanding of the relationship between the gut microbiotia composition and BPSD in AD. A future intervention study will explore whether dietary modification can change the gut microbiota and alleviate signs and symptoms of dementia in this vulnerable group, and therefore improve quality of life and reduce carer stress. This work has the potential to open opportunities for lifescience companies and small-to-medium size businesses to exploit mechanistically relevant biomarkers of BPSD in AD, and for industrial markets (from agriculture to food industry) and specialist caterers to develop healthy foods/diets that are tailored to the specific needs of an ageing population.

Cognitive and behavioural problems in dementia can result in reduced appetite, increased physical activity, and the disruption of eating and feeding behaviours, all of which can reduce quality of life, increase risk of hospital admissions, morbidity (*e.g*. falls) and early mortality. Thus, it is expected that a targeted nutritional strategy could result in a number of wider health benefits. In future, we envisage investigating how dietary adjustments could promote healthier ageing in vulnerable older adults who are at risk of dementia due to cognitive deficit but who do not yet have a dementia diagnosis.

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Conflict of Interest

The authors have no conflicts of interest to declare.

Figure 1: Potential mechanisms underpinning the hypothesised bi-directional signalling between the brain and the gut. SCFA, short chain fatty acid; GABA, gamma-Aminobutyric acid ; 5-HT, 5-hydroxytryptamine

Figure 2: Aims of the Ageing Gut-Brain study

Figure 3: Sample collection protocol for the Ageing Gut-Brain study

Figure 4 : Initial response by care home staff to a resident displaying challenging behaviour.

Respondents could select all that apply therefore combined totals exceed 100%

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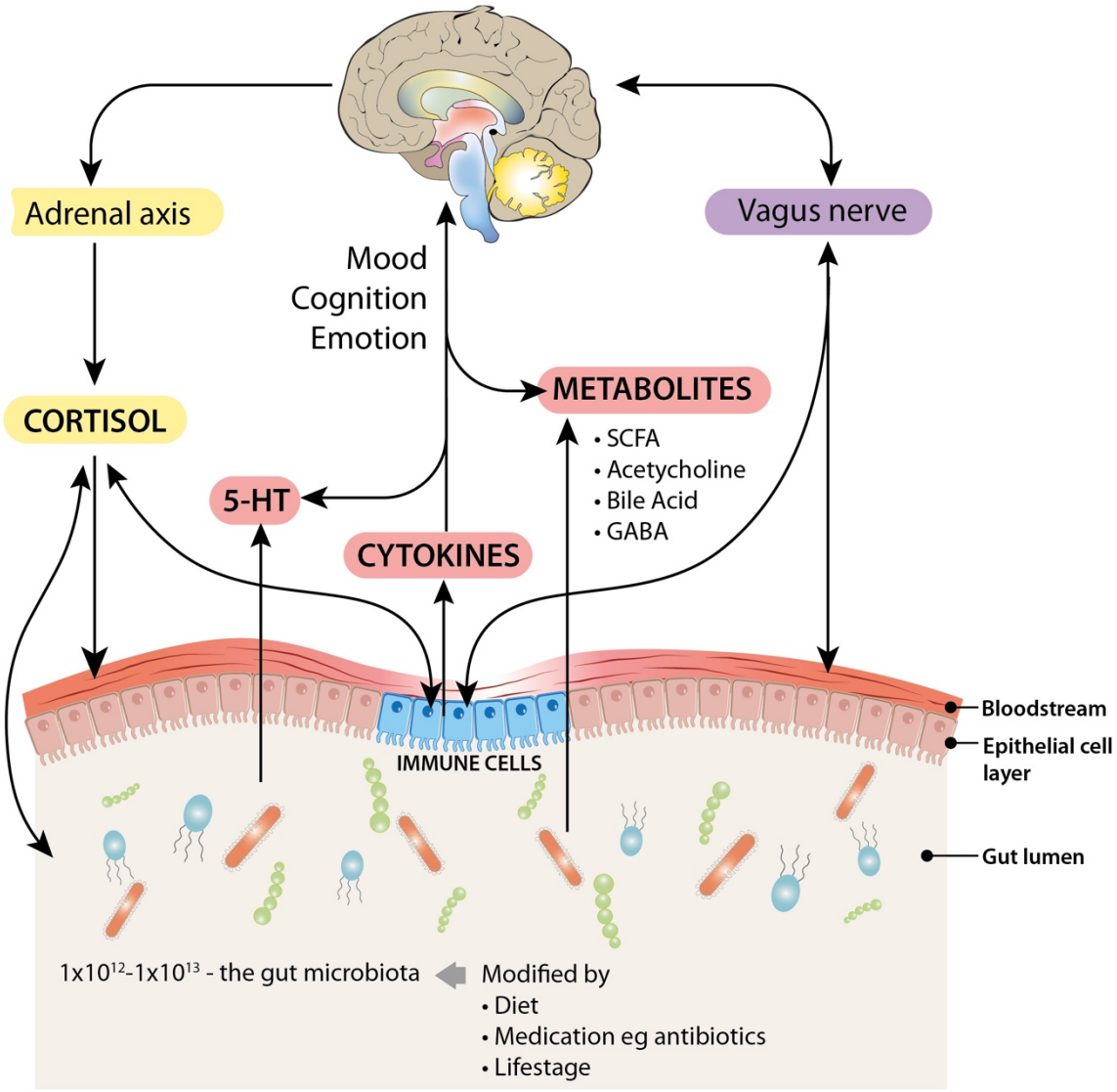
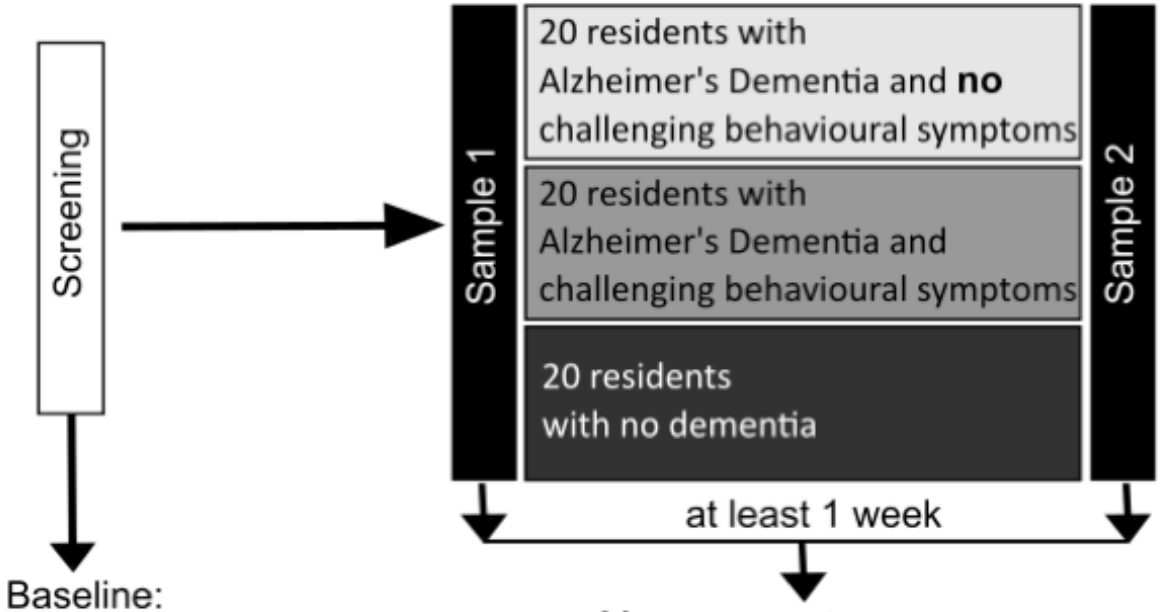

Adrenal axis
Vagus nerve
Mood
Cognition
Emotion
CORTISOL
METABOLITES
• SCFA
• Acetycholine
• Bile Acid
• GABA
5-HT
CYTOKINES
IMMUNE CELLS
Bloodstream
Epithelial cell layer
Gut lumen
1x10¹²-1x10¹³ - the gut microbiota
Modified by
• Diet
• Medication eg antibiotics
• Lifestage

Aim 4:
Exit questionnaire to inform future research

ment

Aim 3:
Analyse faecal samples to test the hypothesis that the gut microbiota profile is different among three groups of older people with and without Alzheimer's Disease

Aim 5:
Assess dietary intake in the study cohort

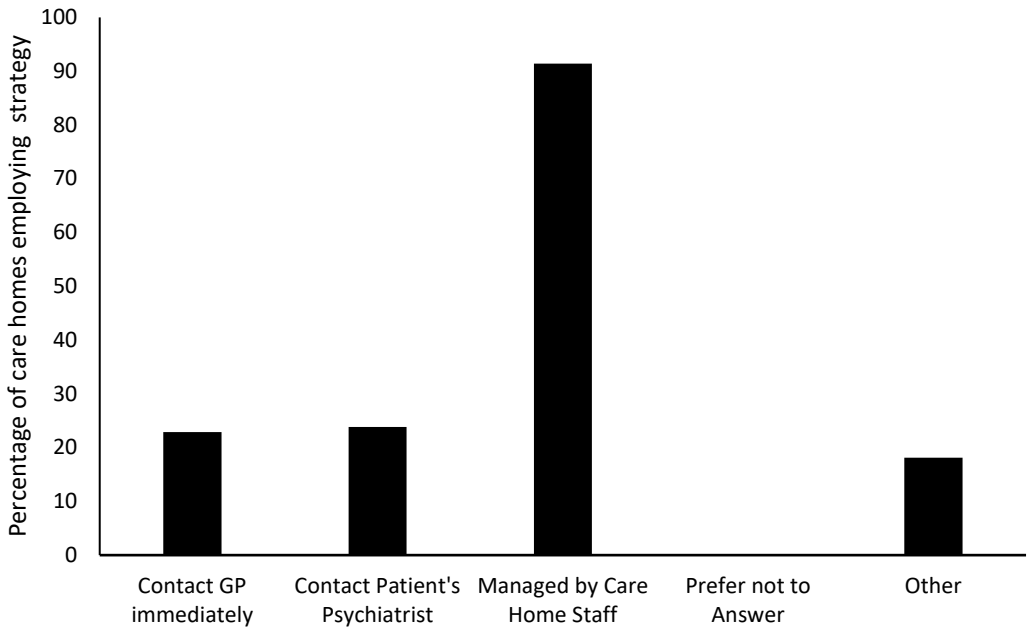


Baseline:

- Consent Paperwork (from participants and for participants by proxy)
- Health Status checked
  - information on medication, personal characteristics and co-morbidities

Measurements:

- Two Faecal Samples,
  - collected at least one week apart
  - processed in the lab within 12hours of collection
- Diet composition by assessment of weekly care home menu
- Record of every episode of challenging behaviour that requires staff intervention
- Quality of Life Questionnaire


Percentage of care homes employing strategy
100
90
80
70
60
50
40
30
20
10
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Contact GP immediately
Contact Patient's Psychiatrist
Managed by Care Home Staff
Prefer not to Answer
Other