The Cognitive Ageing, Nutrition and Neurogenesis (CANN) trial: Design and progress

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Abstract

Introduction: The Cognitive Ageing, Nutrition and Neurogenesis trial hypothesizes that a combined intervention with long-chain n-3 polyunsaturated fatty acids (n-3) and cocoa flavan-3-ols (FLAV) will mitigate the cognitive decline anticipated to naturally occur over 1 year in older adults.

Methods: In a double-blinded, placebo-controlled parallel design, 259 individuals with mild cognitive impairment or subjective memory impairment were randomized to a control or n-3 FLAV group (1.5 g docosahexaenoic acid + eicosapentaenoic acid and 500 mg n-3 FLAV daily) for 12 months. Cognition was measured at 0, 3, and 12 months. The primary end-point is hippocampus-sensitive cognitive function (e.g., number of false-positives on the Picture Recognition Task of the Cognitive Drug Research test battery). Secondary outcomes include additional cognitive measures, brain atrophy and blood flow (assessed by magnetic resonance imaging), vascular function, circulating biomarkers of cardiovascular and cognitive health, gut microflora, red blood cell fatty acid status, and urine flavan-3-ol metabolites.

Results: Screening began in 2015, with all baseline visits completed in March 2017. The intervention was finished in March 2018.

Discussion: Cognitive Ageing, Nutrition and Neurogenesis aims to identify an effective diet-based intervention to prevent or delay cognitive impairment in cognitively at-risk individuals, which could ultimately contribute to a reduced population burden of dementia.
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Keywords: Cognition; Dementia; Mild cognitive impairment; Subjective memory impairment; Eicosapentaenoic acid; Docosahexaenoic acid; Cocoa flavan-3-ols; Hippocampus; Magnetic resonance imaging

1. Background and rationale

Although there is evidence of a decrease in incidence in early old age, the number of dementia cases is set to approximately double every 20 years, increasing to 115 million by 2050 [1]. Lifestyle strategies to preserve or improve memory and cognition would provide significant health, social, and economic benefits. In the UK, delaying dementia onset by 2 or 5 years would result in 19% and 33% reductions in prevalence, respectively, by 2050 with a much lower prevalence of severe dementia [2]. Similarly, it has been estimated that a 2-year delay in onset would reduce the global incidence of dementia by 22% by 2047 [3], resulting in 25 million fewer cases [1,3–5]. Even greater benefits would be observed in “at-risk” and prodromal population subgroups, such as apolipoprotein E4 (APOE4) carriers, and those with existing mild cognitive impairment (MCI) or subjective memory impairment (SMI; self-diagnosed and defined as noticing a decline in memory over the previous 2–3 years) [4–7]. Those with MCI and SMI, although experiencing metabolic brain changes and some loss of function, are unlikely to have yet undergone the same degree of macroscopic neural cell death in brain regions implicated in the pathogenesis of dementias as those with a confirmed diagnosis [8,9], and thus may be more responsive to interventions and experience some reversal of cognitive decline [10].

To date, the reductionist approach to nutritional interventions has often focused on the impact of individual foods, food groups, or dietary components on cognitive function. The neural processes involved in cognitive decline are complex and multifactorial. Therefore, combination treatments with dietary compounds that target multiple physiological and molecular mechanisms are likely to have the most realistic chance of affecting cognition [11,12].

Substantial data from prospective cohort studies and animal models provide evidence for the individual neurophysiological effects of the fish-derived long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as flavan-3-ols (n-3 FLAV; plant bioactives found in cocoa, teas, and berries). In a prospective study with 3.9 years of follow-up, higher total n-3 PUFA and DHA intakes were associated with a lower relative risk of Alzheimer’s disease (AD; relative risk, 0.3; 95% CI, 0.1–0.9, in the highest quintile of DHA intake) [13], with subsequent observations of associations between plasma EPA and DHA status and dementia risk [14,15] and cognitive decline [15,16]. In the Women’s Health Initiative Memory Study, EPA and DHA status was associated with larger total brain and hippocampal volumes, which was confirmed in more recent investigations [17,18]. The limited number of large-scale interventions with LC n-3 PUFA, predominantly in those with incident dementia, has produced mixed findings [19,20] and highlights the need to take a combined intervention approach with a focus on preclinical individuals.

Flavonoids are plant bioactives, with considerable evidence supporting their neurocognitive benefits. In the Personnes Agées QUID cohort, which included 1640 adults free of dementia aged 65 years or older, higher total flavonoid intake was associated with better cognitive performance at baseline and a better performance trajectory over a 10-year period [21]. An association between higher cocoa flavan-3-ol (which represents the focus of the current Cognitive Ageing, Nutrition and Neurogenesis [CANN] trial) consumption and performance on various cognitive instruments has been repeatedly observed [22–24], with Sorond and colleagues [24] also reporting an association with neurovascular coupling, which refers to the close functional and spatial relationship between cerebral blood flow (CBF) and neuronal activity.

Rodent studies provide substantial mechanistic insight [25–28] into the pleiotropic impact of EPA + DHA and n-3 FLAV, and suggest that they likely confer additive and potentially synergistic benefits. Neuronal membranes are particularly enriched in DHA, with DHA supplementation increasing DHA availability for cell membrane synthesis in neurogenesis, neurite outgrowth, and synaptogenesis [26]. Furthermore, DHA decreases neuroinflammation, mediated by the 15-lipoxygenase DHA derivative, neuroprotectin D1 [29]. n-3 FLAV promote neuronal survival, spine density formation, synaptic plasticity, and the production of brain-derived neurotrophic factor [26,27]. They are also anti-inflammatory and reduce the production of amyloid beta (40–42) peptides [30]. Through the enhancement of antioxidant enzyme activities, n-3 FLAV are also likely to indirectly reduce the oxidation of membrane DHA [31]. Finally, EPA + DHA plus n-3 FLAV may confer cognitive benefits through their impact on vascular function and CBF [32–35].

The CANN trial is designed to examine for the first time the impact of LC n-3 PUFA plus n-3 FLAV on cognition, brain atrophy and blood flow, and biomarkers of cognitive and cardiovascular function, over a 1-year period in older
adults with SMI or MCI. It aims to identify an intervention to promote cognitive health and reduce dementia risk, which would contribute to a reduced overall population burden of disease.

2. Design and methodology

2.1. Overview

This study uses a double-blinded, placebo-controlled parallel design. There are two recruitment sites: the University of East Anglia (Norwich, UK) and the Swinburne University of Technology (Melbourne, Australia), with magnetic resonance imaging (MRI) images analyzed centrally at the University of Illinois (Urbana Champaign, Urbana, IL). After a telephone screening, postal questionnaires, and an on-site screening visit (V1), participants are asked to attend three clinical visits (V2, V3, and V4) at 0 (baseline), 3, and 12 months (Fig. 1). At these visits, participants undergo cognitive assessment and provide a number of clinical measures and biological samples.

2.2. Ethical conduct of the study

The conduct, evaluation, and documentation of this study abide by Good Clinical Practice guidelines and the guiding principles of the Declaration of Helsinki. The study was approved by Bellberry Human Research Ethics Committee (Study ID 2015-03-227) and Swinburne University Human Research Ethics Committee (SHR Project 2015-208) for the Swinburne University of Technology site and the National Research Ethics Service Committee (Study ID 14/EE/0189) for the University of East Anglia site. All participants provided informed signed consent before participating.

2.3. Intervention products

Participants are asked to consume 3 × 1 g oil capsules (Captek Soft Gel International, Cerritos, CA) and 33 g of chocolate drops of either test or control products daily for 12 months, and to take the products with the main meal of the day (as there is some evidence of reduced EPA and DHA bioavailability in the fasting state relative to a fat-containing meal [36]). The test capsules provided fish oil (EPAX AS, Aalesund, Norway) delivering 1.1 g DHA and 0.4 g EPA per day. The control capsules contained a blend of 80% palm oil and 20% corn oil (Cargill and Hybco, Los Angeles, CA) that provides a fatty acid (FA) composition typical of a UK or Australian diet. Both the test and control capsules contained 1% lemon oil, to provide a lemon flavor to maintain study blinding, and 1% mixed tocopherols for stability of the study oils. The test chocolate drops (33 g) contained cocoa powder (Acticoa; Barry Callebaut, Lebbeke, Belgium) providing 500 mg of n-3 FLAV, ranging from monomers to decamers (i.e., degrees of polymerization from 1 to 10). The test chocolate drops delivered 158 calories, 262 mg theobromine, and 30 mg caffeine. The control chocolate drops (33 g; Blommer Chocolate Co, Chicago, IL) were similar in size and color and delivered 38 mg n-3 FLAV, 160 calories, 64 mg theobromine, and 5.4 mg caffeine. The chocolates were matched for macronutrient composition. The doses of n-3 FAs and n-3 FLAV have been selected to be (1) physiologically relevant, equivalent to half a portion of oily fish and two portions of enriched cocoa per day, and (2) bioactive based on prospective epidemiologic data, as described previously.

At V2, participants are provided with their intervention products for the next 3 months, and at V3 participants receive the remaining 9 months’ worth of supply. They are provided with a log to record the time at which the intervention products are consumed each day and are asked to return their empty chocolate sachets and oil capsule bottles to the research team to monitor compliance. The threshold for categorization as adherent to the intervention is set at 80%. Participants are contacted on a monthly basis...
by telephone to promote compliance and to check for any adverse events or changes in health status, including medication use.

2.4. Study hypotheses

2.4.1. Primary hypothesis

Participants allocated to the intervention arm will perform significantly better than the control group on hippocampus-sensitive measures of cognitive function, namely the number of false-positives on the Picture Recognition Task of the Cognitive Drug Research (CDR) test battery and performance on the iPosition task.

2.4.2. Secondary hypotheses

1. Participants allocated to the intervention arm will perform better in the composite cognitive domains of attention, working memory, episodic memory, and speed of retrieval from memory, relative to the control intervention.

2. Participants allocated to the intervention arm and carrying the APOE4 allele will show a better cognitive response to intervention relative to APOE4 noncarriers.

3. Cognitive response to intervention will be associated with speciation of the gut microbiota.

4. Cerebrovascular blood flow and hippocampal volume, as assessed by MRI, will be greater in the intervention group compared with the control group at 12 months.

2.5. Participant inclusion criteria

All inclusion and exclusion criteria are detailed in Tables 1 and 2, respectively.

2.6. Sample size

The sample size estimate was based on the predicted impact of 1.5 g LC n-3 PUFA (1.1 g DHA and 0.4 g EPA) and 500 mg cocoa n-3 FLAV administered daily for 12 months. This combination has not been previously used in any intervention study and therefore a power calculation using directly relevant data was not possible. For LC n-3 PUFA, the most relevant previous study [37] administered 1.3 g DHA and 450 mg EPA for 12 months to MCI participants. Effects sizes, estimated from partial ηs, were as follows: for a delayed recall task, $\eta^2 = 0.121$ (approx. $d = 0.74$); for the Digit Span task, $\eta^2 = 0.254$ (approx. $d = 1.17$); and for a visual reproduction task, $\eta^2 = 0.114$ (approx. $d = 0.72$). The computed $d$ for a composite memory score (presented as a $z$-score) was 1.17. Regarding flavonoids, Desideri and colleagues administered 990 or 520 mg of n-3 FLAV, with a Cohen’s $d$ of 1.64 reported for the lower dose, which closely corresponds to that in our trial [22]. The outcome in this study was a composite cognitive score. The consumption of concord grape juice by MCI patients was associated with Cohen’s $f$ of 0.28 for verbal learning, and 0.33 for delayed recall (i.e., medium effect sizes) [38,39].

On the basis of the range of these effect sizes, we conservatively base our power calculation on a medium effect size, that is, Cohen’s $d$ of approximately 0.5, which generates a sample size of 108 per group for a two-arm trial, with 90% power to detect a significant change at the 5% probability level. The group sizes of 120 assumed a 10% attrition rate.

2.7. Study measures

Table 3 lists all the study procedures by visit.

2.8. Screening

The screening process was conducted in two phases.

2.8.1. Telephone screening

The CANN Telephone Screening Questionnaire assesses general adherence to the inclusion and exclusion criteria and provides an assessment of flavonoid, oily fish, and fish oil supplement intake. It also includes the Geriatric Depression Scale–short form (GDS-15) [40], the Modified Telephone

Table 1
Overview of CANN inclusion criteria

| Type                  | Criterion                                                                 |
|-----------------------|---------------------------------------------------------------------------|
| General               | Males and females, aged 55 y and older                                      |
|                       | Diagnosis of MCI or SMI with no indication of clinical dementia or depression |
|                       | Willing and able to provide written informed consent, and verbal informed consent for the telephone screening eligibility, |
|                       | and to comply with all study procedures                                      |
|                       | Fluent in written and spoken English                                         |
|                       | In good general health, including blood biochemical, hematologic, and urinalysis results within the normal range at screening (V1) |
|                       | Normal or corrected-to-normal vision and hearing                             |
|                       | Stable use of any prescribed medication for at least 4 wk before baseline (V2) |
| MRI (50% of cohort)   | Aged from 55 to 85 y                                                       |

Abbreviations: CANN, Cognitive Ageing, Nutrition and Neurogenesis; MCI, mild cognitive impairment; MRI, magnetic resonance imaging; SMI, subjective memory impairment.
Either 160 systolic BP or 100 diastolic BP (mm Hg), the participant was deemed ineligible for trial entry, and advised to consult with their general practitioner. A fasted blood sample was collected for APOE genotyping, red blood cell (RBC) FA analysis, full blood count, and liver and kidney function test (see Appendix A). Individuals with an RBC DHA level of >6% of total FAs, or a high intake of oily fish or flavonoid-rich foods (see Appendix A) were precluded from participating, on the basis that they had a high habitual intake of DHA and flavonoids, which are unrepresentative of the normal population and are unlikely to be responsive to intervention. Further details are given in Appendix A.

2.8.3. Assessment of cognitive status

After a standard breakfast, participants completed a neuropsychological test battery to confirm MCI or SMI status. The following instruments were administered: the Montreal Cognitive Assessment (MoCA) [42], California Verbal Learning Test-II [43], Boston Naming Test [44], Figure Copy task [45], Digit Span task (Forward and Backward) [46], Trail Making Test (A and B) [47], Test of Premorbid Functioning [48], CDR test battery [49], and iPosition task [50,51] (see Appendix B).
For classification of MCI status, criteria developed by the National Institute on Aging-Alzheimer’s Association workgroup [52] are used (Fig. 2). If a respondent feels that (1) their memory has declined in the last 2 to 3 years, and this is of concern to them, a diagnosis of MCI is possible depending on evidence of (2) preservation of independence in functional abilities (Functional Activities Questionnaire score \( \geq 6 \)), (3) absence of dementia (MoCA score \( \geq 21 \)), and (4) depression (GDS-15 score \( < 10 \)). Finally, impairment in one or more cognitive domains that are greater than would be expected given a person’s age and education is required for MCI status (\( \geq 1 \) standard deviation \[SD\] below the mean on any of the following neuropsychological tests: memory, California Verbal Learning Test-II or Logical Memory I or II of the Wechsler Memory Scale–Revised; language, Boston Naming Test; visuospatial function, the Figure Copy task of the Repeatable Battery for the Assessment of Neuropsychological Status; attention, the Digit Span task (Forward or Backward) of the Wechsler Adult Intelligence Scale, third edition; and executive function, the Trail Making Test [A or B] [see Appendix C]). Participants who satisfy aforementioned criteria (1) to (4), but do not score \( \geq 1 \) SD below the mean on any neuropsychological test are classified as SMI.

All eligible participants at V1 are sent the EPIC Food Frequency Questionnaire, the International Physical Activity Questionnaire, and the Profile of Mood States (see Appendix D).

### 2.9. Randomization of participants to groups

In total, 259 participants were recruited (by means of general practitioner surgeries, general advertising, newspaper articles, and radio show participation) and randomized to arm A or arm B (Fig. 3) using a randomization algorithm (Covariate Adaptive Randomization software [53]), with groups stratified by APOE genotype (E4 carrier vs. non-E4 carriers).
Do you feel your memory is worse than 2–3 years ago? (established at telephone screen)

Yes, this worries me  Yes, but this does not worry me  No
ELIGIBLE  INELIGIBLE

Geriatric Depression Scale – short form score < 10 (telephone screen)
Functional Activities Questionnaire score < 6 (Visit 1)
Montreal Cognitive Assessment score > 17 (Visit 1)

Yes to all the above  No to any of the above
ELIGIBLE  INELIGIBLE

Impairment on > 1 cognitive domain greater than would be expected for a person’s age (Visit 2; California Verbal Learning Test-II, Logical Memory I & II, Boston Naming Test, Figure Copy task, Digit Span task, Trail Making Test; see Appendix C for cut-offs)

Yes  No
MCI  SMI

Fig. 2. Flowchart for classifying mild cognitive impairment (MCI) and subjective memory impairment (SMI) status.

carrier), sex (male vs. female), and cognitive status (SMI vs. MCI).

2.9.1. Clinical visits V2 to V4

The neuropsychological test battery for V2 to V4 comprises the CDR test battery, MoCA, Delis-Kaplan Executive Function System, Verbal Fluency Test (V2 and V4 only), and iPosition task (Appendix B). Participants provide an overnight fasted blood sample, a urine sample analyzed for flavan-3-ol metabolite profile, and a fecal sample to determine speciation and metabolism of the gut microbiota. Cardiovascular assessments, including aortic and 24-hour ambulatory BP and carotid-femoral pulse wave velocity were also conducted (for full details see Appendix E). The plasma samples will be analyzed for a number of biomarkers of cardiovascular and cognitive function including triglycerides, total and HDL cholesterol, glucose, insulin, cortisol, brain-derived neurotrophic factor, ApoE, and C-reactive protein (as a measure of inflammation).

2.9.2. MRI and magnetoencephalography (V2 and V4)

We use a variety of magnetic resonance techniques to characterize aspects of the brain that are sensitive to MCI. These include structural imaging, diffusion tensor imaging, magnetic resonance spectroscopy, arterial spin labeling, and magnetoencephalography. Diffusion tensor imaging provides a measure of white matter integrity that is sensitive
to MCI and AD [54]. Magnetic resonance spectroscopy is used to measure the biochemistry of the brain and has been used to obtain measures that are predictive of MCI [55]. In CANN, we will measure N-acetylaspartate, creatine, choline, glutamate, and myo-inositol as biomarkers of neurogenic activity. Arterial spin labeling has previously been used to monitor changes in CBF in patients with AD and MCI [56]. Protocol details for each imaging modality used in CANN are given in Appendix F.

3. Statistical analysis

Data will be analyzed using SAS (ver. 9.4; SAS Institute, Cary, NC) and SPSS software (ver. 22.0; SPSS Inc, Chicago, IL). Summary statistics (n, mean, median, SD, standard error of the mean, range) will be generated and change from baseline scores for treatment arms A and B, on all outcome measures, will be calculated by subtracting the baseline score (V2) from those at V3 and V4. Group comparisons will be performed using independent t tests and analysis of variance as appropriate, and correlation analyses will be conducted using Pearson’s r.

4. Results

Fig. 3 shows the flow of participants throughout the study period. After the initial telephone screening (N = 637), prospective participants were invited to the on-site screen (V1, n = 351) within 3 months; those who were deemed eligible were then allocated to the intervention or control arm (n = 259) and invited back for their first clinical visit (V2) within 3 months. Two hundred forty-six participants completed V2, with a mean (±SD) age and BMI of 66.5 ± 6.5 years and 268.9 ± 4.3 kg/m², respectively. In total, 57% of the cohort was female and 28%...
were APOE4 carriers; there was an MCI/SMI ratio of 104:142 (Table 4).

5. Discussion

CANN will investigate if an intervention with a combination of dietary components can prevent or delay cognitive decline in older adults at increased risk of dementia. The choice of outcome measures has long been a subject of debate for randomized control trials targeting dementia risk. Given that the focus in CANN is on prevention rather than on conversion of SMIMCI to AD, sensitive neurocognitive tests were used to test efficacy with false-positives in the Picture Recognition Task and the iPosition task chosen as highly sensitive indices of hippocampus integrity and function.

The inclusion of structural and functional MRI and magnetoencephalography, along with a range of prognostic cardiovascular assessments, such as 24-hour ambulatory BP and pulse wave velocity, will provide insight into the physiological basis of the impact of intervention on cognition. Cardiovascular health is being increasingly recognized as an important and modifiable determinant of neurocognitive function and dementia risk [57], influencing perfusion, blood-brain barrier function, and brain inflammatory and lipid status. Given the cardiovascular benefits of LC n-3 PUFA [58] and n-3 FLAV [59], improved cardiovascular function is likely to underlie any observed cognitive benefits.

A further design strength is the use of RBC FA status (n-3 index) as an exclusionary criterion. Individuals with a total RBC n-3 index of >6% were ineligible on the basis that they are unrepresentative of the general population. This subgroup constitutes the “health conscious” older adult deciles, with an EPA + DHA intake of about 300 mg per day provided by fish oil supplements or at least one portion of oily fish per week [60, 61]; those with such a high baseline status are unlikely to be responsive to additional intake. Most previous trials have used fish oil supplements (rather than EPA + DHA status) as an exclusionary criterion, with high oily fish consumers often included, which may have contributed to the observed lack of responsiveness to intervention [62].

In the UK, about 60% of the population aged >55 years are on prescribed medications, which increases to over 90% of those aged 85 years and older [63]. The CANN trial took a pragmatic approach to maximize population applicability, with only those on high-dose medications directly targeting neurologic functions precluded from participating. For all other medications, participants were required to have stable use for at least 2 months before the baseline assessment.

APOE genotype is the strongest common genetic risk factor for cognitive decline and AD, with those with APOE3/E4 and APOE4/E4 genotypes at a 4- and 16-fold increased risk of AD relative to the wild-type APOE3/E3 genotype [64]. There is accumulating evidence that part of the neurologic dysfunction in APOE4 carriers is mediated by altered DHA metabolism, which in healthy and prodromal individuals could be mitigated by increased DHA intake and status. Although not fully powered to establish APOE4 × treatment interactions, APOE4 carrier status was used to randomize the participants to the intervention arm, and an exploratory analysis was conducted to assess response to intervention according to the genotype.

Given the ever-increasing population burden of dementias, the lack of effective therapeutics, and the large impact on population prevalence of even modest delays in disease onset, the outcomes of the CANN trial are of substantial public health relevance.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.trci.2018.08.001.
1. Systematic review: Search of PubMed and Web of Science databases for articles on dietary interventions for cognitive decline and/or dementia, including the terms “cognition,” “dementia,” “Alzheimer’s,” “mild cognitive impairment,” “subjective memory impairment,” “omega-3 fatty acids,” “n-3 fatty acids,” “flavan-3-ols,” and so forth. The literature suggests long-chain n-3 polyunsaturated fatty acid and flavonoids may improve cognition but there are important gaps regarding their possible synergistic effects.

2. Interpretation: Our trial is the first to investigate the impact of co-supplementation with marine n-3 fatty acid and cocoa flavan-3-ols on cognition and brain structure/function (by magnetic resonance imaging) in older adults at risk of dementia. Delaying onset by 2 to 5 years would reduce population burden in the UK by 19% to 33% by 2050, respectively.

3. Future directions: Future studies should explore the effects of the timing (within the healthy-to-advanced dementia disease trajectory) of intervention with flavan-3-ols in producing the maximum cognitive benefits and also to examine response to intervention according to APOE genotype status.

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