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TITLE

A randomised controlled trial investigating the effect of n-3 long-chain polyunsaturated fatty acid supplementation on cognitive and retinal function in cognitively healthy older people: the Older People And n-3 Long-chain polyunsaturated fatty acids (OPAL) study [ISRCTN72331636]

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BACKGROUND

The number of individuals with age-related cognitive impairment is rising dramatically in the UK¹ and globally.² Global burden of disease estimates now place dementia above stroke, musculoskeletal disorders, cardiovascular disease and all forms of cancer in terms of the percentage of years lived with disability in people aged 60 years and older.² Defining simple and effective strategies to prevent or delay cognitive impairment among older people is therefore a priority for healthcare and social services.

There has been specific interest in the hypothesis that enhancing the diet of older people may act to slow the progression of cognitive decline. The importance of good nutrition among older people for the maintenance of health has long been advocated, and evidence-based dietary recommendations for older people have been published.³ Interest has recently turned to the potential importance of n-3 long-chain polyunsaturated fatty acids (n-3 LCPs), largely obtained from oily fish, in the maintenance of good cognitive health.

Mechanistic data support the roles of two of the most important n-3 LCPs, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), for vascular and neuronal health respectively.⁴ The high levels of DHA found in the brain⁵ and the recently discovered functions of docosanoids, metabolic products of DHA, in neuronal protection⁶ suggest an important role for n-3 LCPs in cognitive health. Age-related decrease in n-3 LCP level in total brain lipids have been reported in humans, and it has been postulated that this decline is correlated in part with age-related deterioration of functions of the central nervous system.^{7,8} This findings may be particularly relevant in the UK since recent survey data⁹ demonstrates that older people in the UK habitually consume a diet that is low in fish.

Epidemiological observational studies reporting associations of fish or n-3 LCP consumption with cognitive function have shown mixed results; some studies have reported a positive association with higher fish consumption,¹⁰⁻¹⁶ while others have found no association.^{17,18} Studies evaluating the associations of serum levels of n-3 LCP with cognitive function have tended to report a positive association with high blood n-3 LCP concentrations,¹⁹⁻²² although again there is some disagreement.²³ A Cochrane review²⁴ concluded that there was a growing body of evidence from biological, observational and epidemiological studies that suggested a protective effect of n-3 LCPs against dementia, but did not identify a single published randomised controlled trial on which to base recommendations for the use of dietary or supplemental n-3 LCPs for the prevention of cognitive impairment or dementia. One RCT has recently been published to investigate the effect of n-3 LCP supplementation on cognitive function among healthy older people.²⁵ This 6 month

intervention study randomised 302 adults aged 65 years and over to either a low dose supplement (400mg n-3 LCP), a high dose supplement (1800mg n-3 LCP) or a placebo, and did not detect an effect of n-3 LCP supplementation on cognitive function.

Numerous randomised controlled trials have demonstrated that a relative sufficiency of n-3 LCPs results in improved sensitivity of the retina to light in infancy.²⁶ It is thought that this results from the ability of n-3 LCPs (specifically DHA) in rod outer segments of the retina, to enhance photoreceptor signal transduction processing. Relative inadequacy of DHA results in decreased signal transduction, and DHA deficient subjects therefore require greater light stimulation to elicit the same level of photoelectric response. In addition DHA plays a key role in photoreceptor growth and functional development, a role which has recently been demonstrated to be mediated by DHA effects on gene expression.²⁷

A highly significant change occurring in the ageing retina is a decrease in phototransduction efficiency in rods resulting in a decreased sensitivity of rods to light.²⁸ Part of this decreased sensitivity may be associated with reduction in DHA levels, such as also occurs in the ageing brain, and may be susceptible to dietary manipulation. It is hypothesised that increasing dietary DHA intake among older people will result in increased levels of DHA in the brain and retina, and therefore prevent or minimise the age-related reduction in phototransduction efficiency.

The current study is the first trial of its kind aiming to slow the decline of cognitive and retinal function in healthy older people by increasing daily dietary intake of n-3 LCPs.

METHODS

The study was designed as a double-blind randomised placebo-controlled trial. The procedures are illustrated schematically in Figures 1 and 2, and detailed in the text. More details are available in the full protocol.²⁹ The trial aimed to test two primary hypotheses.

Study hypotheses

Cognitive function study – for healthy, cognitively normal adults aged 70-79 years of age, daily supplementation with n-3 LCPs (500mg DHA and 200mg EPA) will slow the rate of cognitive decline.

Retinal function study – for healthy, cognitively normal adults aged 70-79 years of age, daily supplementation with n-3 LCPs (500mg DHA and 200mg EPA) will improve visual function by enhancing rod photoreceptor response to light and visual-cortical integration.

Study recruitment

(i) Centres

20 National Health Service general practices, members of the Medical Research Council General Practice Research Framework (GPRF) were recruited. The retinal function sub-study practices were situated in the Southeast of England to facilitate attendance at Moorfields Eye Hospital.

(ii) Patients

Participating general practices identified all healthy, cognitively-normal adults aged 70-79 years from their practice registers. These potential participants were pre-screened for eligibility by study nurses employed by each practice, using available clinic records and a GPRF computer identifier programme. Individuals diagnosed with either diabetes (Type I or Type II) or dementia were excluded. The exclusion of individuals with diabetes was necessary as these individuals have raised susceptibility to vascular and neural damage and may therefore be less sensitive to the proposed intervention. Individuals with pre-diagnosed dementia were excluded as the intervention was aimed at delaying cognitive decline in people who were currently not demented. The list of potential participants was checked by their General Practitioner to exclude individuals not deemed suitable to take part in the study (e.g. recent bereavement, terminal illness).

Eligible individuals identified by this process each received a letter and information sheet from their general practice outlining the nature and importance of the study. The letter also acted as a screen for potential participants who did not wish to consume fish products (such as vegetarians) or who were already consuming fish-oil supplements. Participants reporting the daily use of fish-oil supplements were excluded from the trial. Potential participants were invited to make an appointment with the research nurse at their local general practice. Participants were offered the

use of a taxi or their travel costs on public transport or mileage in private cars to attend the appointment, up to a maximum of £10. The invitation letter explicitly stated that if they did not wish to take part in the study it would not prejudice the quality of health care provision from their general practice.

On attending their general practice, potential participants were fully informed by the research nurse about the nature and relevance of the trial, and exactly what would be involved if they agreed to take part. All potential participants were told that a prerequisite of their joining the study would be that they agreed not to initiate non-study fish-oil supplement use over the course of the trial. Potential participants who remained interested in taking part in the study, were then asked to give consent to undergo a short cognitive screen, the Mini Mental State Examination (MMSE). The MMSE is an easy to administer test that has been widely validated as a screen for dementia and was used to exclude participants with low cognitive status.³⁰ Participants with an MMSE score of less than 24 (out of a maximum of 30) were not included in the trial, and were offered a referral to their General Practitioner for further monitoring. The cut-off of less than 24 has traditionally been used as a marker for possible dementia in adults. It is possible that individuals with an MMSE of 24 or greater may have mild dementia. However, given the potential public health relevance of this trial, the purpose of the proposed cut-off was to exclude individuals with possible frank dementia rather than select a highly functioning group of individuals. Individuals with an MMSE score below 24 were thanked for their time and co-operation.

Participants who scored MMSE of 24 or greater were invited to give full, informed written consent to take part in the main cognitive function study. Individuals unwilling to participate further were thanked for their time and co-operation.

Individuals giving informed consent were enrolled in the trial. The research nurse telephoned the central randomisation service to register the participant, giving identifying details and the participant's age. Randomisation allowed secure blind allocation of eligible people to one or other arm of the study. Following random allocation a study number was given for each study participant. This was also used to identify the supply of capsules prescribed for each participant. The study number was entered on the participant's entry form. Pre-labelled identical-looking packs of capsules (see *Dietary Intervention* below) were stored securely in the general practice. Minimisation was used to ensure a balance of key prognostic factors using the following two criteria: age group (70-74 and 75-79 years) and general practice.

Participants were then assessed by completing questionnaires and undertaking a series of tests (see *Baseline Data Collection* below). The full set of baseline tests took approximately 60 minutes to complete. Any couples recruited to the study undertook their assessments separately. Participants were provided with the contact details of the study nurses and trial manager. Information was also available from the trial web-site (www.opal-study.org.uk) for those participants who wished to use that method of obtaining information. Participants were thanked for their time and co-operation.

Outcome measures for cognitive function study

Primary outcome

Change in cognitive function at 24 months determined by the California Verbal Learning Test. This is a test of memory of 16 words.

Secondary outcomes

Cognitive performance as measured by immediate and delayed recall of a short story, tests of prospective memory, timed letter search/cancellation task, verbal fluency, digit span forwards and backwards, symbol digit modalities test, simple and choice reaction time, and spatial memory.

Psychological health.

Compliance determined by counting the number of capsules remaining every 3 months, and by measuring the change in n-3 LCP concentration in buccal epithelial cells over 24 months.

Blood pressure.

Change in Body Mass Index (a measure of body size).

Number of hospital admissions for cardiovascular events over 24 months.

Death.

Sample size

The sample size required for this trial is based on a 0.3SD difference between trial arms in long-delay free recall of List A of the California Verbal Learning Test over the 24 months of intervention. To detect a 0.3SD difference between trial arms, with 90% power and 1% significance, 332 individuals are required per treatment group. Allowing for 20% drop-out over 24 months of intervention, the total sample size required for the study is 798 individuals. A difference of 0.3SD is clinically relevant and would be equivalent to individuals in the intervention arm being able to remember one more word out of the 16 in the California Verbal Learning Test than those in the placebo arm.³¹

Recruitment

In order to attain this sample size it was expected that a sample of approximately 4500 individuals, pre-screened for diabetes and dementia, would initially be drawn from the registers of 20 participating general practices. A conservative estimate of the proportion of eligible individuals

who would agree to participate in the trial was 20%. It was estimated that it would take 6 months to enrol the full sample for this trial.

Baseline (pre-intervention) cognitive function data collection

At trial entry to enable randomisation

Initials

Date of birth

Gender

General practice number

MMSE score

Consent

Baseline data collection

1. Psychological health: the 30-item General Health Questionnaire (GHQ-30) was used to assess the affective state of participants.³² The GHQ-30 is quick and easy to administer, and an assessment of mood is important as it may be associated with cognitive performance.
2. Educational achievement: the widely used Burnham Scale of educational achievement was used.³³ This is an important covariate as educational level has been shown to be strongly related to cognitive performance.
3. Blood pressure: this is necessary as hypertensive individuals may have a raised susceptibility to vascular and neural damage. Blood pressure was measured using the automated DYNAMAP device. Two measurements each of systolic and diastolic pressure were taken in the sitting position (the mean of the two measurements was used in analysis); participants had rested for 5 minutes before their blood pressure was measured and there was a minimum of 5 minutes between the two measurements. Normal general practice procedures were followed for the management and treatment of high blood pressure.
4. Cardiovascular health: any history of hospital admission for myocardial infarction (MI) or stroke over the preceding 5 years was abstracted from patient notes.
5. National Health Service number: so that the study organisers did not lose contact with patients should they move address and also to follow up on health status, participants were asked to give their agreement for their names and NHS numbers to be sent to the NHS Central Register.
6. Basic anthropometric measures: height and weight were measured using standardised procedures in order to enable the calculation of the Body Mass Index: a basic marker of body size.
7. Swab of cheek cells: nurses collected a sample of buccal mucosal epithelial cells from each participant. This is a relatively non-invasive procedure in which a sterile wooden spatula is scraped briskly along the inside of the cheek (about 10 strokes on each cheek). The cheek cells were sent to laboratories in sterile containers to be analysed for their n-3 LCP concentration.

8. Fish consumption: two simple questions regarding habitual fish consumption were asked. Firstly, a question regarding the frequency of fish consumption, and second, participants were asked to list the three species of fish they consume most frequently.
9. Cognitive ability: the participants were asked to complete a validated series of cognitive tests. It is currently not known which, if any, cognitive domain will be most affected by n-3 LCP supplementation and therefore a series of tests were selected which cover the major domains (memory, attention, psychomotor speed and executive function):
 - a. Brief assessment of subjective symptoms of cognitive impairment (memory, language/word-finding, concentration).
 - b. Immediate and delayed recall of a short story (from the Wechsler Memory Scale).
 - c. Immediate and delayed recall of a 16-item word list (California Verbal Learning Test).
 - d. Three tests of prospective memory i.e. remembering to carry out instructions without being reminded.
 - e. Timed letter search/cancellation task (attention/psychomotor speed/executive function).
 - f. Verbal fluency – naming animals (word-finding/executive function).
 - g. Digit span forwards and backwards (working memory/executive function) from the Wechsler Adult Intelligence Scale.
 - h. Symbol digit modalities test (attention/psychomotor speed/executive function).
 - i. Simple (psychomotor speed) and choice (decision speed) reaction time.
 - j. Spatial memory (memory/visual spatial function).

Dietary intervention

Following the completion of the series of tests detailed above, those participants not enrolled in the retinal function study were introduced to the dietary intervention. The dietary intervention was a daily dietary supplement in the form of capsules which were identical in size, shape, colour and smell for both the intervention and placebo arms of the trial. Given the lack of trial evidence to support the use of any particular level of dietary supplementation, the dose selected in the OPAL study was based on the following considerations: the UK Food Standards Agency currently recommends that men and post-reproductive age women consume one to four, 140g portions of oily fish a week;³⁴ typical dietary recommendations, such as those of the World Health Organisation fall in the range 0.3-0.5g n-3 LCPs daily;³⁵ the dose of n-3 LCPs generally recognised as safe (GRAS) is 3g per day.

Taking these considerations into account, a pragmatic decision was made on the dose for the OPAL study. The intervention arm was asked to consume two 650mg soft-gel capsules daily containing a total of 500mg DHA and 200mg EPA. This is equivalent to approximately 4.9g of n-3 LCPs a

week (0.7g/day), a level that can be achieved via the consumption of approximately 300g (slightly more than 2 portions) of fatty-fish a week. This level of supplementation falls well below the upper GRAS level and should thus be safe. The placebo arm was asked to consume two identical 650mg capsules containing olive oil daily, this oil is rich in n-9 fatty acids and will thus have a minimal effect on the 18:2 n-6/18:3 n-3 ratio. Changes in the n-6/n-3 ratio may affect prostanoid balance which affects vascular and inflammatory responses. The higher DHA to EPA ratio in the supplement is justified by the rationale of the study, which prioritises the neuroprotective effect of DHA over the vascular and anti-thrombotic effect of EPA.

The research nurses explained the importance of consuming the capsules every day, suggesting that it should become part of their daily routine, for example by always consuming the capsules at breakfast-time. Participants were given a 3-month supply of capsules at the baseline clinic visit, and asked to attend the clinic every 3-months throughout the 24-month course of the trial i.e. at 3, 6, 9, 12, 15, 18 and 21 months. Participants were sent reminders to collect repeat supplies of capsules.

The participants enrolled in the retinal function study were invited back to the general practice after their baseline retinal function testing to be given their dietary supplements.

Data collection at 3, 6, 9, 12, 15, 18, and 21 months

Participants had appointments to meet the research nurse every 3 months throughout the study (8 repeat visits in total including 24 month assessment). During these appointments, participants were reminded of the importance of the study and of the need to comply with the study protocol. The research nurses recorded information volunteered by the participants regarding any discomfort related to the capsules. The recognised side-effects of n-3 LCP capsules include belching, flatulence, abdominal discomfort and loose stools. These discomforts are generally mild and decrease over time, and the research nurses provided reassurance to any concerned participants.

Participants were asked to bring their dietary capsule containers with them to their appointments so that any remaining capsules could be counted as a measure of compliance. Buccal cell swabs were taken during these repeat appointments from a random 20% sample every 6 months (i.e. at 6, 12 and 18 months) as a further measure of compliance. Participant records from the general practice were consulted every 3 months in order to record any hospital admissions for MI or stroke over the intervening 3 month period.

Assessment at 24 months

A final evaluation of the cognitive function of all trial participants was carried out after 24 months of intervention. Participants were invited to attend their general practice and were assessed for:

1. Psychological health: as at baseline.
2. Blood pressure: as at baseline.
3. Cardiovascular health: record of hospital admissions for MI or stroke between the 21 and 24 month appointments.
4. Basic anthropometric measures: as at baseline.
5. Buccal cell DHA concentration: as at baseline. Collected as a marker of the impact of the intervention on buccal cell DHA concentration, and compliance in the trial.
6. Cognitive function: as at baseline.

Participants were offered the use of a taxi, or their travel costs on public transport or mileage in private cars to attend the appointment up to a maximum of £10.

Although initially designed to be as non-invasive as possible, it became clear that the study would be strengthened with the collection of blood samples. Ethical committee approval was given to collect a 10ml fasting blood sample after the 24 months of intervention from participants providing additional consent. These samples were analysed for serum fatty acid profile, and DNA was separated and stored for future analysis of genetic markers of cognitive health.

Data analysis for assessing change in cognitive function

Primary analysis was carried out based upon the groups as randomised (“intention to treat”).

Results have been presented as appropriate effects sizes with a measure of precision (95% confidence intervals). Covariates such age, gender, and age at leaving full time education were adjusted for in the analysis.

The CVLT is the primary outcome of the OPAL study (sum of words recalled at three immediate recalls, and words recalled at long-delayed recall). To avoid multiple statistical testing, data on secondary cognitive outcomes were transformed into z-scores, grouped into the following cognitive domains and standardised for further statistical analysis:

$$\text{Global cognitive function} = (Z_{\text{CVLT sum of words recalled}} + Z_{\text{CVLT delayed recall}} + Z_{\text{prospective memory-test1}} + Z_{\text{prospective memory-test2}} + Z_{\text{prospective memory-test3-item}} + Z_{\text{prospective memory-test3-location}} + Z_{\text{story recall}} + Z_{\text{story recall-delayed}} + Z_{\text{verbal fluency}} + Z_{\text{letter cancellation}} + Z_{\text{location memory}} + Z_{\text{location memory-delayed}} + Z_{\text{symbol-letter substitution}} + Z_{\text{digit span forwards}} + Z_{\text{digit span backwards}} + Z_{\text{simple reaction time}} + Z_{\text{choice reaction time}}) / 17$$

$$\text{Memory} = (Z_{\text{CVLT sum of words recalled}} + Z_{\text{CVLT delayed recall}} + Z_{\text{location memory}} + Z_{\text{location memory-delayed}} + Z_{\text{story recall}} + Z_{\text{story recall-delayed}}) / 6$$

$$\text{Processing speed} = (Z_{\text{letter cancellation}} + Z_{\text{simple reaction time}} + Z_{\text{choice reaction time}} + Z_{\text{symbol-letter substitution}}) / 4$$

$$\text{Executive function} = (Z_{\text{digit span backwards}} + Z_{\text{verbal fluency}}) / 2$$

$$\text{Global delay score} = (Z_{\text{CVLT delayed recall}} + Z_{\text{location memory delayed recall}} + Z_{\text{story recall delayed}}) / 3$$

Outcome measures for retinal function study

Primary outcome

Change in rod sensitivity over 24 months of intervention as measured by electroretinogram.

Secondary outcomes

Colour vision measured by conventional means; colour vision is a good marker of central retinal (macular) function.

Eye health assessed by carrying out a full ophthalmic examination.

Sample size for retinal function study

The calculation for the sample size required to demonstrate a significant difference between the placebo and intervention arms of the trial is based on the decline in rod sensitivity with age.²⁸ In line with previous studies on the effect of DHA during infant development, it was anticipated that an increase in dietary intake of DHA would prevent or reduce the decline in rod sensitivity; increases of the order of +2SD in rod sensitivity have been demonstrated in studies of DHA supplementation in preterm infants.^{36,37} Considering the complexity of the study a biologically significant effect was defined as an increase of 1SD. To detect this effect with 90% power and 5% significance, the sample size required was 22 per group. Allowing for 25% drop-out during the trial, the total sample size required for this sub-study was 56 individuals.

Recruitment for retinal function study

Following the initial selection of 20 general practices to be involved in the trial, 5 of these practices were selected to take part in the retinal function study. Potential participants from these general practices were informed about the retinal function study in addition to the cognitive function study, and were invited to take part in both studies.

During the initial meeting at the general practices, the research nurses fully outlined the nature of the trial to potential participants, and in addition, the nurses described the objectives and methods of the retinal function tests. Potential participants who remained interested in taking part in either the cognitive study or the cognitive and retinal function study, were then asked to give consent to undergo a short cognitive screen, the MMSE (as outlined above).

After the collection of baseline cognitive function data, suitable participants were invited to consider the retinal function study. Participants were free to opt in or out of the retinal function study. It was explicitly stated that refusing to take part in the retinal function study would not prejudice the quality of treatment and support that they would receive in the cognitive function study or from the general practice. Participants expressing an interest in taking part in the retinal

function study were asked for consent to carry-out a simple visual test (measurement of Logmar visual acuity) and responded to a few questions on eye health.

Any participant reporting a personal or family history of genetically-determined ocular or neurological disease, diabetes, glaucoma or age-related macular degeneration was excluded from the retinal function study. Participants on the retinal function study were required to have a Logmar visual acuity of +0.2 or better. Participants reporting poor eye-health or/and those with visual acuity scores below +0.2 were excluded from the retinal function study (but not the trial in general) and offered a referral to their General Practitioner for further management.

Participants with good eye-health and scoring +0.2 or better on Logmar were invited to give full, informed consent to take part in the retinal function study. Following the completion of the baseline cognitive function testing, participants were given an appointment at Moorfields Eye Hospital for baseline retinal function testing. These participants did not receive the dietary intervention until they had completed their baseline retinal function tests.

Baseline (pre-intervention) retinal function data collection

1. Full-field electroretinogram (ERG): the ERG is a mass electrical response of the retina to a luminance stimulus. It is recorded through an electrode placed on the surface of the eye in response to light stimuli provided by a Ganzfeld dome stimulator. The response typically consists of an “a-wave” followed by a “b-wave”. The first 10 milliseconds or so of the a-wave arises in relation to photoreceptor hyperpolarisation, and the slope of the linear portion of the a-wave can be related to the kinetics of phototransduction. The b-wave is generated in the inner nuclear layer of the retina, principally the ON-bipolar cells. Further recording examined the a-wave evoked by a brighter flash specifically to examine the characteristics that can be related to phototransduction.
2. Colour vision: trial participants were tested for colour vision, which is a good measure of central retinal (macular) function. This was performed using a computerised system that enables colour contrast sensitivity assessment by measuring thresholds in the protan and tritan colour confusion axes.
3. Ophthalmic examination: this was conducted in order to determine the “health” of the eye at baseline. Participants were fully informed of the health of their eyes. In addition, fundus photography was performed such that documentary evidence would be available if any changes in the ocular fundus occurred that could be putatively be related to the dietary supplementation.

The duration of the full protocol for eye examination was approximately 2 hours. Although these examinations may cause slight discomfort to some participants, they are generally very well

tolerated. ERG is a standard ophthalmological investigation, and Moorfields Eye Hospital performs more than 2000 ERGs each year to standards in excess of those recommended by the International Society for Clinical Electrophysiology of Vision.³⁸ Participants were offered the use of a taxi, or their travel costs on public transport or mileage in private cars to attend the baseline appointment at Moorfields Eye Hospital up to a maximum of £20.

Dietary intervention

Following the completion of the cognitive function test and the retinal function tests, trial participants were given an appointment to return to the general practice to be introduced to the dietary intervention (see *Dietary intervention* above).

Data collection at 3, 6, 9, 12, 15, 18, and 21 months

Participants involved in the retinal function study in addition to the cognitive function study had appointments to meet the research nurse every 3 months throughout the study in an identical manner to those only involved in the cognitive function study (see above).

Final (post-intervention) retinal function data collection

A final evaluation of the retinal function of trial participants was carried out after 24 months of intervention. Study participants included in the retinal function study were invited to attend Moorfields Eye Hospital where they were assessed as follows:

1. Full electroretinogram: as at baseline.
2. Colour vision: as at baseline.
3. Ophthalmic examination: as at baseline.

Participants were informed of the results of their eye examination, and offered the use of a taxi, or their travel costs on public transport or mileage in private cars to attend the 24 month appointment at Moorfields Eye Hospital up to a maximum of £20.

Data analysis for assessing change in retinal function

Primary analysis was carried out based upon the groups as randomised (“intention to treat”). Results have been presented as appropriate effects sizes with a measure of precision (95% confidence intervals). Covariates such as age and gender were adjusted for in the analysis.

Trial Steering Committee

The overall scientific aspects of the project were managed by a Steering Committee. The Steering Committee included expert independent advisors Professor Sir John Grimley Evans (Chair), Professor Martin Prince, Professor Alan Bird, Dr. Madge Vickers, two members of the public Mrs. Ursula Shine and Mrs. Yvonne Davidson, the principal applicants, and project staff *ex-officio*.

The responsibility of the Steering Committee was to ensure the scientific integrity and quality of the project. To achieve this, the specific responsibilities of the Steering Committee included:

- maintaining adherence to the study protocol
- approving changes to study protocol if required
- reviewing quality assurance indicators
- monitoring study recruitment and the overall study timetable
- advising, as required, on specific scientific items that may arise
- compliance with legislation
- adherence to research governance
- reporting to funders
- approving publication and dissemination strategies.

The Steering Committee met face-to-face on five occasions and received two interim reports.

Data Monitoring Committee

An independent Data Monitoring Committee (DMC) was established. The membership comprised Professor Tom Sanders (Chair), Professor Graham Dunn (statistician) and Dr. Gill Livingston (clinician). The role of the DMC was firstly to check on safety by random allocation. Secondly the DMC looked at on-going compliance data by random allocation to check that a difference in exposure was occurring. Thirdly, the DMC considered data at trial entry by random allocation in order to be able to interpret the compliance and side effects data. The committee met at the start of the trial to agree terms of reference. The DMC met face-to-face on two occasions and reviewed data electronically on a further three occasions.

Project Management Group (PMG)

A Project Management Group (comprising principal investigators, trial manager, senior nurse, database manager and statistician) ran the trial on a day-to-day. The PMG met 27 times over the course of the trial. The responsibilities of the PMG included:

- establishing and monitoring recruitment of participating centres and participants
- distributing and supplying data collection forms and other appropriate documentation for the trial
- distributing and supplying dietary supplements to trial practices
- data collection and management
- data entry and cleaning
- data analysis
- organising and servicing the Data Management Committee
- organising and servicing the Trial Steering Committee.

Ethics committee approvals

The OPAL study received ethics committee approvals from the National Research Ethics Service (04/mre05/31) and the LSHTM ethics committee (no. 2038). The trial was registered on ISRCTN (72331636) and EudraCT (2004-001196-18).

RESULTS

Participant flow

Nearly 14,000 potential participants were identified, of whom 5,309 were sent invitation letters, and 944 were interviewed (Figure 3). Reasons for not being interviewed included reporting fish oil consumption (52%), no interest (38%), no reply/other (10%). At the baseline interview 76 potential participants were excluded for the following reasons (not mutually exclusive): did not attend (n=9); incomplete baseline data (n=27); refused consent (n=25); MMSE<24 (n=8); reporting fish oil consumption (n=5); diagnosed with diabetes or dementia (n=3). One individual was randomised to the study in error leaving a final total of 867 participants in the OPAL study. Withdrawal from the study (49 vs. 53) and deaths (9 vs. 8) over the 24 months of intervention were very similar between the fish oil and placebo arms respectively (Figure 4). Data on 748 participants (86% of randomised) are available after 24 months of intervention.

Compliance

A live survey by nurses of capsules brought to clinics at follow-up appointments conducted in November 2006 provided data on 607 participants and gave an estimate of approximately 70% apparent compliance. Some data are available on capsule return at appointments and will be analysed further.

It was originally agreed that buccal cell analysis would be conducted by MRC Human Nutrition Research Cambridge (MRC-HNR). Unfortunately, well into the study, MRC-HNR withdrew and an alternative laboratory at King's College London (KCL) was identified. KCL pilot tested the procedures and identified some concerns, but agreed to conduct the analyses. 740 swabs from baseline and 573 swabs from 24 months were analysed. The amount of total lipid on the swabs was very small, and thus the measurement of n-3 LCP was unreliable and highly variable. The laboratory also had significant concerns over oxidation products present on the swabs.

Fasting blood samples were available on 242 participants after final 24 month appointments. Sera from 235 (98%) participants were analysed for fatty acid composition. Analysis of the sera identified significant differences after 24 months of intervention (Table 1), with the fish oil arm having significantly higher EPA and DHA levels and significantly lower arachidonic acid levels.

Baseline data cognitive function study

At baseline, study arms were similar in socio-demographic, physical and psychological health variables (Table 2). The study arms were also well matched for the California Verbal Learning Test (the OPAL primary cognitive function outcome) at baseline (Table 3).

Outcome cognitive function study

Change in primary outcome over the course of the study was small in both the fish oil and placebo arms. There was no statistically significant difference in the primary outcome between trial arms at the end of the study (Table 4). The mean difference in total number of words recalled over 3 trials between the intervention and control arms (adjusted for age, sex and age at leaving full-time education) was -0.5 words (95% CI -1.2 to 0.2). The combined z-scores of cognitive function domains all declined slightly over 24 months of intervention (Table 5) and the size of decline did not differ significantly between trial arms.

Participant flow in retinal function study

The system for recruitment of participants into the retinal function study (described above) resulted in a chance imbalance in numbers in the two trial arms (33 fish oil vs. 23 placebo) at the start of the study (Figure 5). There were more losses in the fish oil arm (3 did not complete baseline assessment, 3 withdrew and 2 did not attend final retinal function assessment) compared to one withdrawal in the placebo arm. 87% (47/53) of participants attended their final appointment at Moorfields. Data on primary outcomes were available at the end of the study on 24 participants in the fish oil arm and 19 participants in the placebo arm.

Baseline data retinal function study

The trial arms were well matched in the primary retinal function outcomes at the start of the study (Table 6).

Outcome retinal function study

There was no statistically significant difference between trial arms in the primary retinal function outcomes at the end of the study (Table 7), although the change in amplitude of rod responses was larger in the fish oil than the placebo arms (Tables 7 and 8). In all cases, rod amplitudes in the fish oil arm increased over the course of the study, while those in the placebo arm either declined or remained the same. There was no significant difference between arms in cone responses (Table 8) or in colour vision at the end of the study (data not shown).

INTERPRETATION

There is considerable public and scientific interest in the hypothesis that increased n-3 LCP consumption is beneficial for cognitive function in later life, but the available evidence from RCTs is extremely limited.^{24,25} We report here on the largest RCT ever undertaken to test this hypothesis, conducted among healthy older people aged 70-79 years in the UK.

Participant recruitment, retention and compliance

Participants were screened and recruited at general practices which were part of the MRC GPRF. Individuals with pre-existing diabetes or dementia were not invited to be part of the study. Invited individuals reporting daily fish oil consumption were similarly excluded. This latter group comprised more than 50% of those invited suggesting that older people in the UK are already widely consuming fish oil supplements (for various reasons). The recruitment process was extremely efficient; the sample of 798 required for the cognitive function study was recruited from 20 general practices in approximately seven months. Recruitment continued for a further 6 months in order to randomise sufficient participants to the retinal function study from clinics in the SE of England.

Randomisation of individuals to fish oil or placebo was via a secure automated telephone randomisation service (University of Aberdeen). Treatment allocation was masked to study nurses and investigators. The study treatments were identical in shape, colour and smell. Participants (n=693) were asked at the end of the study if they knew the content of their allocated capsules and there was no evidence of bias in the trial: 217 (31.3%) believed that they were taking placebo, 193 (27.9%) believed that they were taking fish oil, 278 (40.1%) did not know and 5 (0.7%) declined to answer. In terms of the actual drug assignments 132 (38.7%) of those taking placebo guessed correctly and 139 (39.6%) taking fish oil guessed correctly. After the end of the study, the intervention capsules were analysed for their fatty acid content and found to be as prescribed in the protocol.

Participant retention was high, probably as a consequence of the good, repeated and systematic follow-up by research nurses. This age group also expressed interested in research of this kind and seem to have been content to comply with the study protocol.

It should be noted that at least in part due to the exclusion criteria, the study participants were relatively healthy and highly functioning. Median MMSE scores at the start of the study of 29 (out of 30) attest to high cognitive functioning. The ratio of men to women in the study was higher than usual in studies in older individuals. Investigation of participant flow data demonstrated that

women were more likely than men to report daily fish oil consumption; the proportion of women in the sample available for randomisation was therefore lower. All participants in the study were flagged for mortality by ONS and the total number of deaths over 24 months of intervention was 17. Analysis of the 2006 ONS data suggest that for England and Wales in a sample of this age and sex distribution 55 deaths would be expected over 24 months (Prof. Emily Grundy pers. comm.). The healthy, cognitively normal population included in this study is therefore not generalisable to all older people in the UK.

Absolute measures of compliance are not available, but the clear differences (which correspond to hypothesised differences) in serum fatty acid profiles between trial arms at the end of the intervention study attest to different exposures in the two arms of the study, and demonstrate that the intervention resulted in biological changes in fatty acid profiles.

Baseline data

Numerous observational studies have suggested a positive association between fish or n-3 LCP consumption and cognitive function in later life (see Background). We therefore conducted analysis of baseline data to investigate whether there were any cross-sectional associations in this sample between reported fish consumption and cognitive function.³⁹ Strong statistically significant positive associations were demonstrated between increasing levels of fish consumption (graded by type [white vs. oily] and frequency [5 categories from once a month or less to more than once a week]) and the primary and secondary cognitive function outcomes of the OPAL study. The strength of these associations were, however, significantly diminished once adjusted for age, sex, age at leaving full-time education and GHQ-30 score (a marker of psychological health), suggesting that some of the positive association with higher fish consumption were at least in part confounded by the higher education levels and better psychological state of regular oily fish consumers. The randomised controlled trial design used in the OPAL study is the only study design that can adequately remove this potential source of confounding.

Cognitive function outcome

There was very limited change in cognitive function in this cohort of older people over the 24 month course of the study. Results from the study do not provide evidence to enable us to reject the null hypothesis of no effect of fish oil supplementation on cognitive function.

There are various possibilities to explain the lack of effect of fish oil supplementation on cognitive function. It could be that when tested under this rigorous experimental design (as opposed to observational study designs), there is in fact no effect of fish oil on cognitive function. An alternative explanation could be that the population under investigation was too healthy, or already

consumed sufficient n-3 LCP, thereby obviating any effect of n-3 LCP supplementation. To support this proposal, there was no significant decline in cognitive function over the course of the study, while our initial hypothesis was that fish oil would prevent age-related decline. Furthermore, fatty acid analysis of sera at 24 months demonstrated DHA sufficiency in both treatment groups as evidenced by a ratio of DHA to DPA (n-6) of more than 2.

It may be that the study intervention period or follow-up period was too short, and that any effect of n-3 LCP supplementation may only have become evident after several more years of supplementation or follow-up. Indeed, the absence of decline in cognitive function over 24 months in the total sample suggests that a considerably longer follow-up period was probably needed to identify real change in cognitive function, although this would probably lead to an increase in the number of participants being lost to follow-up. Finally, the dose or/and mixture of n-3 LCPs used in the study may have been insufficient. We selected a safe n-3 LCP dose that was within current guidelines and contained greater amounts of DHA, which appears to have specific neuroprotective actions, than EPA. The serum fatty acid analysis demonstrates that n-3 LCP content was statistically significantly higher in the fish oil than placebo arms, suggesting that relevant changes in fatty acid profiles were achieved as a result of intervention.

Retinal function outcome

The study was powered to detect a 1SD difference in rod sensitivity after 24 months of supplementation. There is no evidence of a 1SD effect, but there is a consistent but smaller effect (approx. 0.2SD) of fish oil supplementation on amplitude of a- and b- wave ERG amplitudes.

Implications for practice

There is no evidence from the OPAL study to support the use of fish oil supplementation for the maintenance of cognitive function in healthy older people in the UK. The OPAL study found no evidence of harm from consumption of fish oil supplements over 24 months in this population. Fish oil supplementation is beneficial for other health-related outcomes.³⁴

Implications for further research

Replication of this study in a more socially disadvantaged population with lower levels of fish consumption and lower likelihood of supplementation use, or among individuals with pre-existing cognitive function problems may be appropriate. The psychometric tests used in this study were selected as the best available paper and pencil tests of cognitive function in older people. However, in future studies, it may be necessary to use more sensitive electrophysiological markers of CNS function underlying cognition. There is also potentially the need for a longer-term trial based on the

finding of enhanced a- and b-wave ERG amplitudes which correspond to a greater ability to see in dim light.

It may be that in older people in the UK, there are other nutrients present in insufficient quantities that may be associated with neurocognitive function. To this end, it should be noted that we are currently conducting the Older People and Enhanced Neurocognitive function (OPEN) study which is designed to determine the effect of daily supplementation of 1mg vitamin B12 or placebo on electrophysiological markers of neurocognitive function. Older people are frequently deficient in vitamin B12 and demonstrating that vitamin B12 dependant neurological impairment is present even in individuals without clinical symptoms will have considerable public health significance.

Figure 1: Flow-chart of cognitive function study protocol

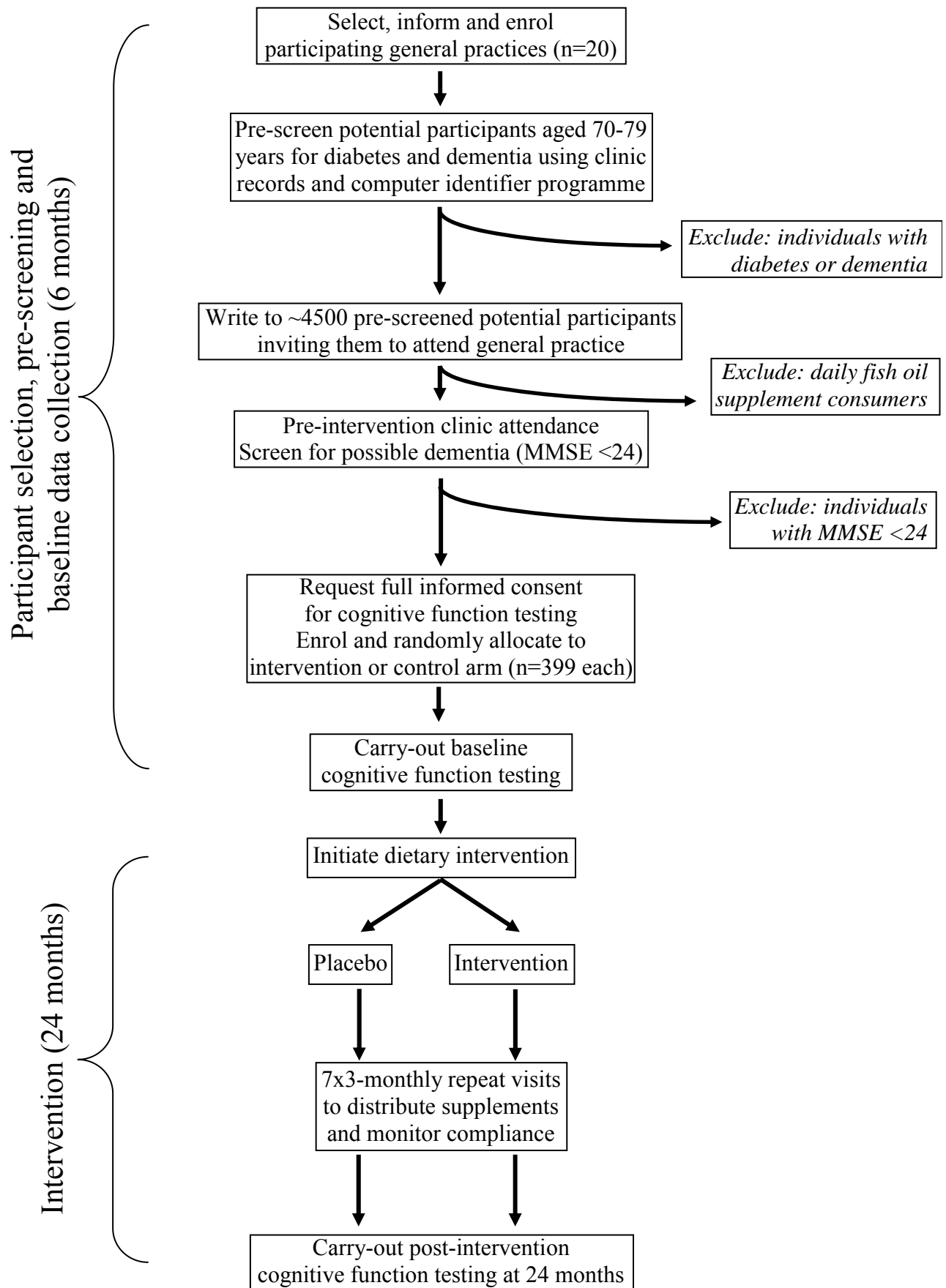


Figure 2: Flow-chart of retinal function study protocol

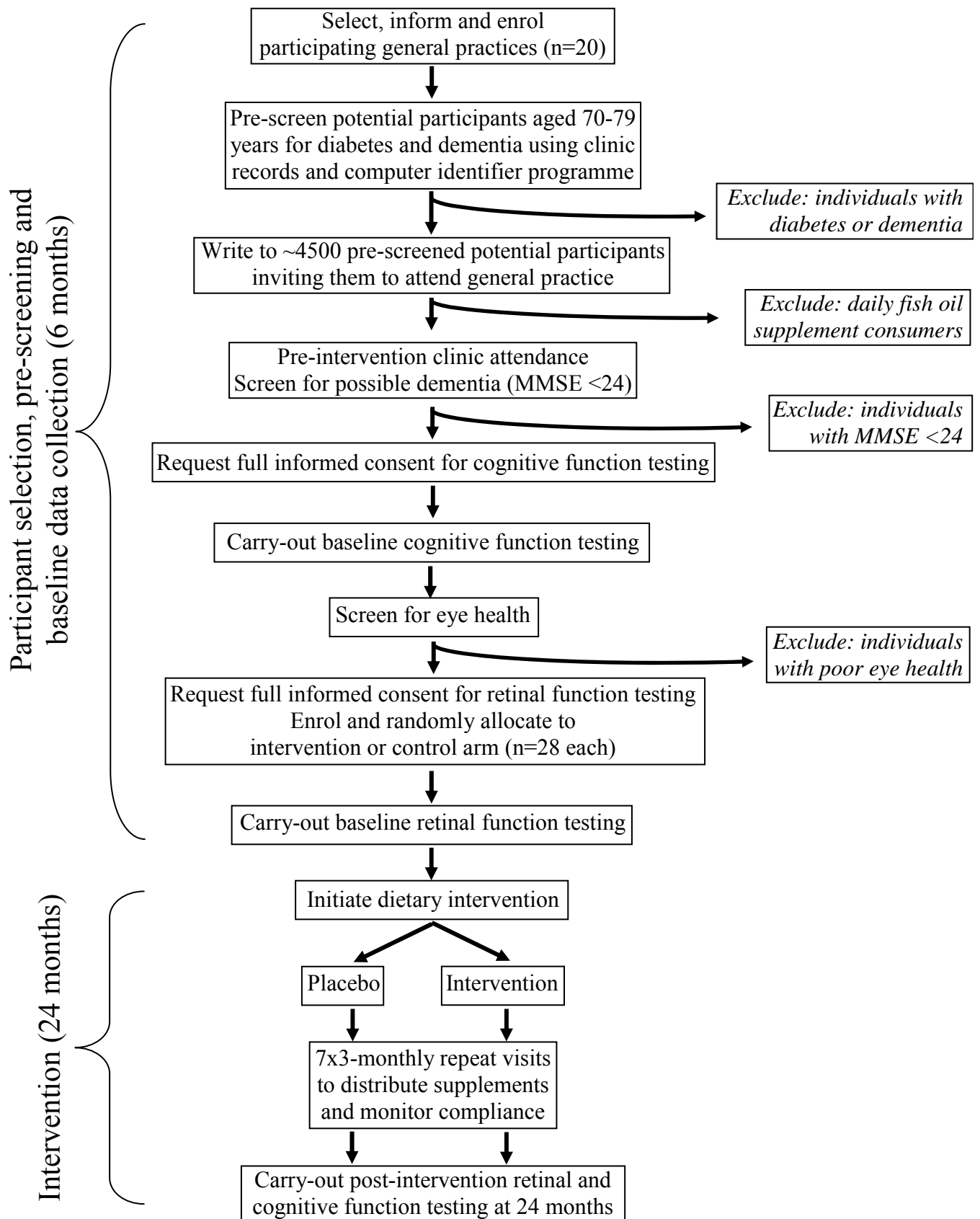


Figure 3: Recruitment flow in the OPAL study

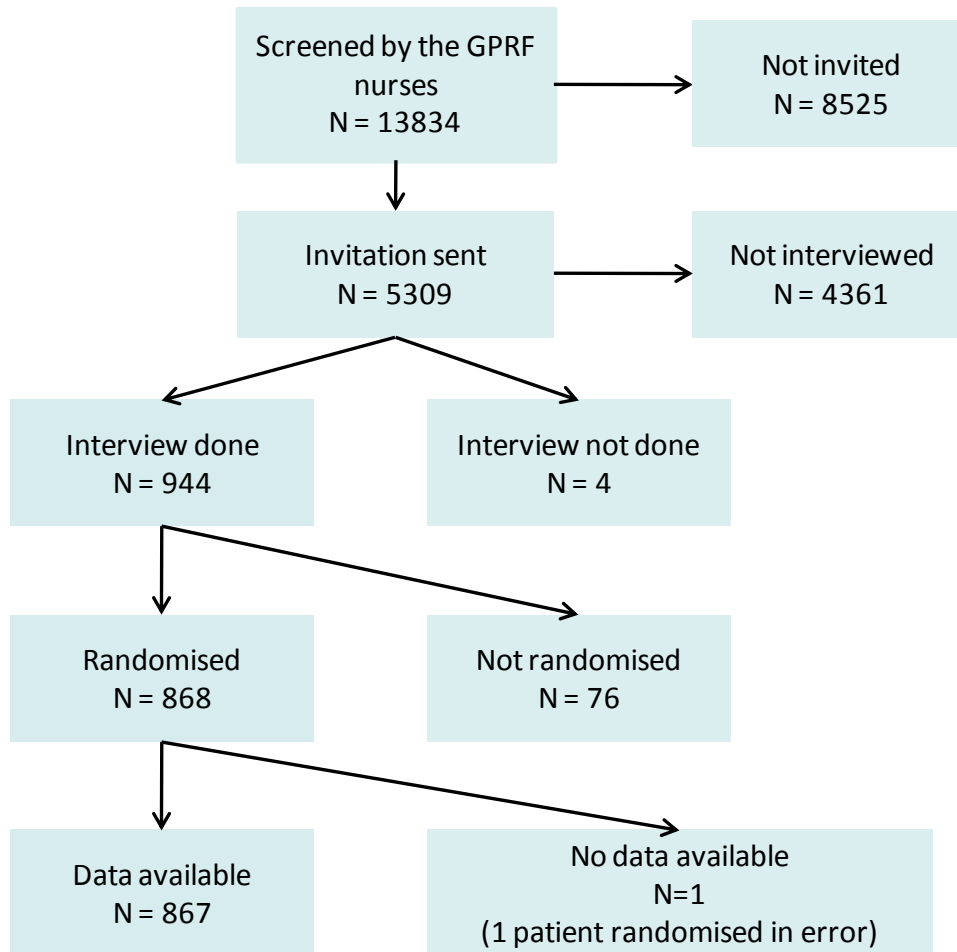


Figure 4: Participant flow in the OPAL study

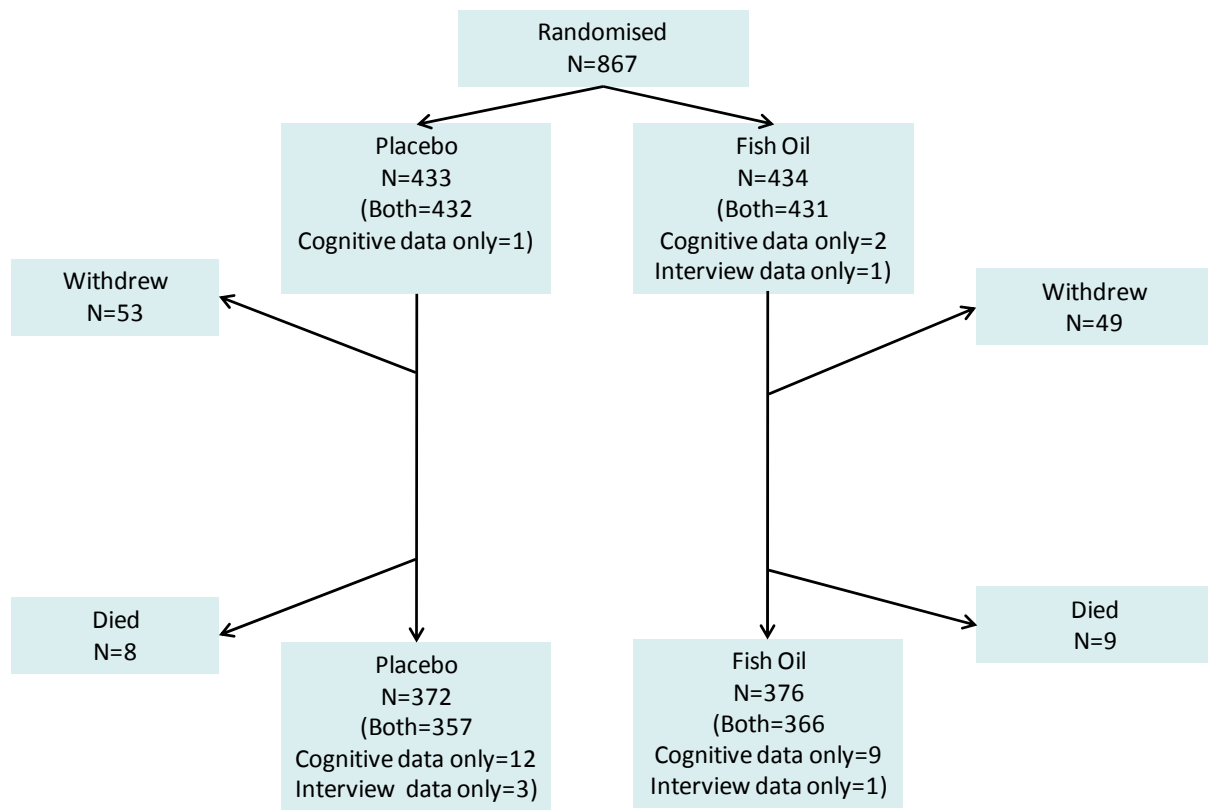


Figure 5: Participant flow in the retinal function sub-study of the OPAL study

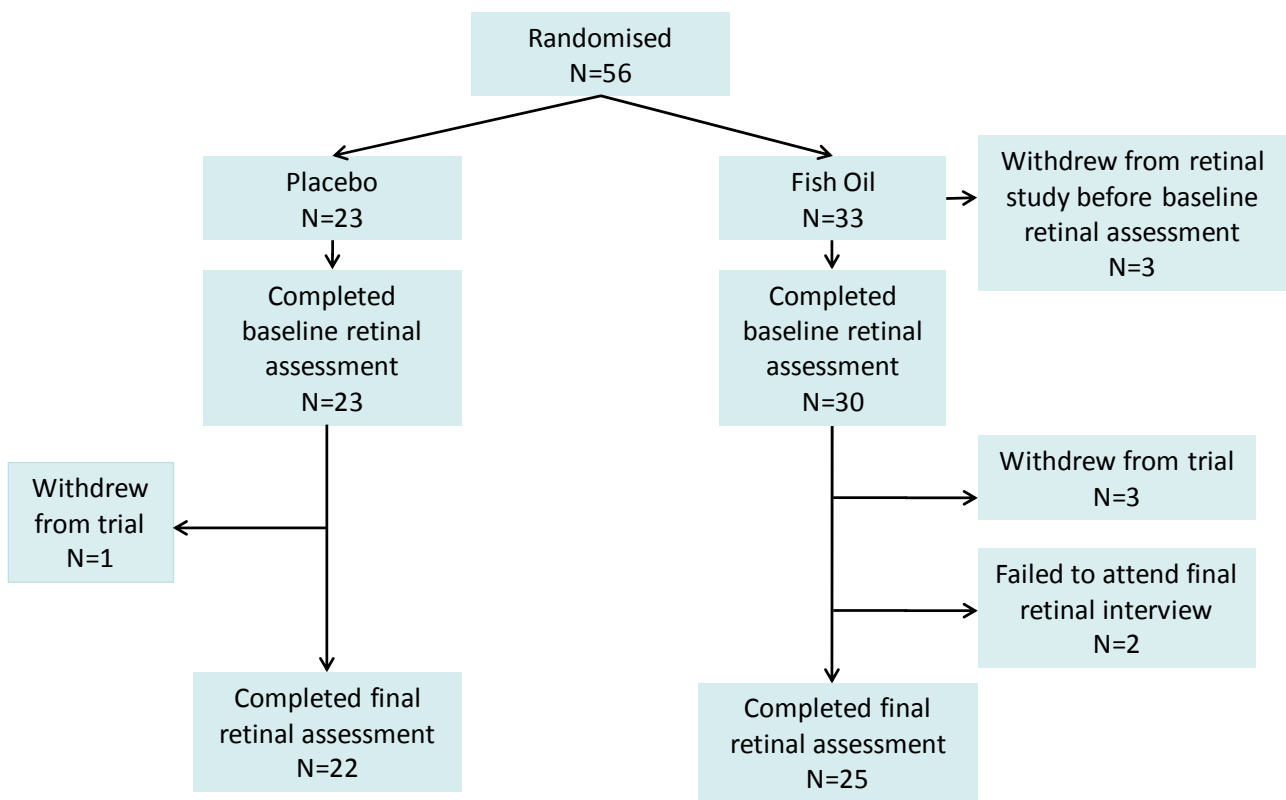


Table 1: Fasting serum fatty acid profiles at 24 months

	Placebo n=119	Fish oil n=116
Palmitic acid	713.2 (16.4)	688.1 (15.5)
Oleic acid (n-9)	700.4 (19.1)	653.9 (15.7)
Linoleic acid (n-6)	814.0 (21.1)	823.0 (19.7)
α -linolenic acid (n-3)	22.0 (0.9)	21.5 (0.8)
Arachidonic acid (n-6)	203.0 (4.8)	178.4 (4.2)
EPA (n-3)	39.1 (3.1)	49.9 (2.7)
DPA (n-6)	31.8 (1.4)	32.0 (1.4)
DPA (n-3)	19.3 (0.6)	18.9 (0.5)
DHA (n-3)	70.7 (2.9)	95.6 (3.1)

Values: mg/l; mean (s.d.)

Table 2: Baseline characteristics of OPAL study participants by trial arm

	Placebo	Fish oil
n	433	434
Sex		
Male (%)	56.6	53.4
Female (%)	43.4	46.6
Age		
mean (years)	74.6	74.7
70-74 years (%)	58.3	56.5
75-79 years (%)	41.7	43.5
Education		
Age at leaving (yrs)	16	16
No qualifications (%)	33.3	32.9
O-level, clerical (%)	25.5	25.5
A-level, univ. (%)	17.8	19.2
Other (%)	23.4	22.5
Vascular health		
MI in 5 yrs (%)	4.4	3.5
Stroke in 5 yrs (%)	2.1	2.3
Blood pressure (mmHg)		
Systolic (mean)	145	144
Diastolic (mean)	77	76
>140/95 (%)	56.9	54.9
BMI (kg/m ²)		
<18.5 (%)	0.9	0.7
>30 (%)	24.3	22.2
MMSE score (median, IQR)	29 (28, 30)	29 (28, 29)
GHQ 30		
Depression (%)	16.7	20.1
Positive life score (mean, s.d.)	15.2 (2.5)	15.1 (2.8)

Table 3: Primary cognitive function outcome at baseline in OPAL study

	Placebo	Fish oil
CVLT		
n (non-missing %)	432 (99.8)	430 (99.1)
Total correct in first 3 trials (incorrect ignored)	23.9 (5.8)	23.9 (6.1)
Words recalled at long delayed recall	7.4 (2.9)	7.4 (2.8)
Values: mean (s.d.)		

Table 4: Primary cognitive function outcome at 24 months in OPAL study

	Placebo	Fish oil	Unadjusted mean diff. (95% CI)	Adjusted ¹ mean diff. (95% CI)
CVLT				
n (non-missing %)	396 (100)	375 (100)		
Total correct in first 3 trials (incorrect ignored)	24.4 (6.4)	24.0 (6.7)		
• Change from baseline	0.5 (5.0)	-0.02 (4.7)	-0.5 (-1.2, 0.2)	-0.5 (-1.2, 0.2)
Words recalled at long delayed recall	7.5 (3.0)	7.6 (3.2)		
• Change from baseline	-0.01 (2.3)	0.1 (2.4)	0.1 (-0.2, 0.4)	0.1 (-0.2, 0.4)

Values: mean (s.d.)

¹Adjusted for age, sex and age at leaving full-time education

Table 5: Secondary cognitive function (z-score) outcomes at 24 months in OPAL study

	Placebo Change from baseline	Fish oil Change from baseline
z-score		
Global	-0.05 (0.6)	-0.05 (0.6)
Memory	-0.03 (0.7)	-0.04 (0.7)
Processing speed	-0.06 (0.9)	-0.02 (0.7)
Executive function	-0.03 (0.8)	-0.04 (0.8)
Global delay	-0.05 (0.7)	-0.01 (0.7)

Values: mean (s.d.)

Table 6: Primary retinal function outcome at baseline in OPAL study

	Placebo	Fish oil
n (non-missing %)	21 (91)	30 (100)
Slope bright flash a-wave ($\mu\text{V}/\text{msec}$)	42.3 (9.4)	43.2 (11.6)
Rod response amplitude b-wave (μV)	147.0 (54.3)	137.8 (55.2)

Values: mean (s.d.)

Table 7: Primary retinal function outcome at 24 months in OPAL study

	Placebo	Fish oil	Unadjusted mean diff. (95% CI)	Adjusted ¹ mean diff. (95% CI)
n (non-missing %)	19 (86)	24 (96)		
Slope bright flash a-wave ($\mu\text{V}/\text{msec}$)	47.5 (14.0)	46.9 (10.3)		
• Change from baseline	3.9 (10.6)	4.4 (8.2)	0.6 (-4.2, 5.4)	0.4 (-4.4, 5.2)
Rod response amplitude b- wave (μV)	141.6 (54.3)	149.1 (58.4)		
• Change from baseline	-4.0 (36.8)	8.8 (40.4)	11.9 (-9.4, 33.2)	11.6 (-11.1, 34.2)

Values: mean (s.d.)

¹Adjusted for age and sex

Table 8: Secondary retinal function outcomes at 24 months in OPAL study

	Placebo	Fish oil
Maximum response (μV)		
Amplitude b-wave	391.6 (103.4)	389.2 (116.6)
• Change from baseline	-3.3 (65.4)	22.1 (71.9)
Amplitude a-wave	275.8 (75.0)	266.7 (64.9)
• Change from baseline	3.3 (51.5)	13.8 (54.3)
Cone flicker (μV)		
Amplitude	77.3 (28.4)	76.5 (25.9)
• Change from baseline	2.3 (20.4)	1.0 (13.4)
Single flash cone (μV)		
Amplitude b-wave	113.1 (38.4)	110.7 (42.5)
• Change from baseline	7.4 (22.0)	4.9 (17.2)
Amplitude a-wave	31.6 (9.3)	33.5 (8.0)
• Change from baseline	2.3 (8.5)	3.4 (7.8)

Values: mean (s.d.)

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