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Alvarez Campano CG, Macleod MJ, Thies F, Aucott L, Macleod MR.
Marine-derived n-3 fatty acids therapy for stroke.
Cochrane Database of Systematic Reviews 2017, Issue 10. Art. No.: CD012815.
DOI: 10.1002/14651858.CD012815.

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[Intervention Protocol]

Marine-derived n-3 fatty acids therapy for stroke

Celia Gabriela Alvarez Campano¹, Mary Joan Macleod², Frank Thies¹, Lorna Aucott³, Malcolm R Macleod⁴

¹The Rowett Institute, University of Aberdeen, Aberdeen, UK. ²Dept of Medicine & Therapeutics, Polwarth Building, Aberdeen, UK. ³Medical Statistics Team, The School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK. ⁴Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK

Contact address: Celia Gabriela Alvarez Campano, The Rowett Institute, University of Aberdeen, Aberdeen, AB25 2ZD, UK. r01cga16@abdn.ac.uk.

Editorial group: Cochrane Stroke Group.

Publication status and date: New, published in Issue 10, 2017.

Citation: Alvarez Campano CG, Macleod MJ, Thies F, Aucott L, Macleod MR. Marine-derived n-3 fatty acids therapy for stroke. *Cochrane Database of Systematic Reviews* 2017, Issue 10. Art. No.: CD012815. DOI: 10.1002/14651858.CD012815.

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ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

To assess the effects of marine-derived n-3 fatty acids administration on functional outcomes and dependence in people with stroke.

BACKGROUND

Description of the condition

Stroke, defined as the acute onset of a persistent focal neurological deficit related to a specific cerebrovascular location (Barret 2013), is a general term that comprises different diseases involving blood vessels supplying the brain (Caplan 2006). Strokes can be broadly classified as haemorrhagic or ischaemic. Haemorrhagic stroke comprises mainly intracerebral haemorrhage (ICH) and subarachnoid haemorrhage (SAH), which account for 5% to 15%, and 5%, respectively, of all acute strokes (Barret 2013). In an ischaemic stroke on the other hand, there is a lack of blood supply for normal functioning of the brain tissue; the three major categories are thrombosis, embolism, and systemic hypoperfusion (Caplan 2006). From the more than 790,000 cases of new or recurrent strokes registered each year in the USA, up to 87% are ischaemic (Mozaffarian 2016). Transient ischaemic attacks (TIAs) are temporary disruptions in circulation to a part of the brain (Caplan 2006). Two regions are found within the infarct in is-

chaemic stroke: the necrotic core, which is irreversibly injured due to rapid cell death; and the ischaemic penumbra, an area that surrounds the core and where neurons remain potentially salvageable (Blondeau 2016; Yao 2013). The penumbra has a life span of only a few hours and, during this period, reperfusion or neuroprotective therapy is needed to prevent irreversible damage (Yao 2013). In response to stroke, physiological and structural changes take place in neuronal circuits surrounding the infarct in order to stimulate neural repair. These include the formation of new connections in cortical areas (termed axonal sprouting, which is also influenced by neurorehabilitation), neural progenitor responses (neurogenesis and gliogenesis), and changes in neuronal excitability in peri-infarct tissue (Carmichael 2016). However, reperfusion after ischaemia can, paradoxically, result in enhanced tissue injury (Eltzschig 2011). Therefore, neuroprotectants that potentially attenuate damage related to reperfusion injury are also needed. In addition, it must be taken into account that theoretically beneficial compounds may have adverse effects during the recovery phase, given that stability and protection is required in the acute phase, whereas neuronal plasticity is needed during the following stages

(Carmichael 2016). The timing of intervention is critical for optimal recovery after stroke: alteplase is effective when administered within 4.5 hours from stroke ictus, while clot retrieval is beneficial up to 7.3 hours (Saver 2016). Due to public awareness campaigns, more people are arriving in emergency departments early after the event, but other than intravenous thrombolysis or thrombectomy, there are no evidence-based neuroprotective agents currently available. In contrast with other cardiovascular events, stroke tends to leave permanent sequelae and generally requires long-term rehabilitation and specialised care (Tanaka 2008). In 2013, there were more than 25 million stroke survivors worldwide and global stroke burden is increasing (Feigin 2015). For these reasons, the need to explore therapeutic options to ameliorate the acute insult remains.

Description of the intervention

High oily fish intake has been related to a reduced mortality from coronary (Hu 2002; Kris-Etherton 2002) and ischaemic heart disease (Zhang 1999) in both sexes. Epidemiological studies have reported mixed results regarding stroke, while some observed an inverse relationship between fish intake and stroke incidence (Gillum 1996; Iso 2001; Keli 1994) and mortality (Hu 2002; Zhang 1999), others have found no significant effect (Morris 1995; Orenca 1996). Given the nature of observational studies, potential confounding factors - such as lifestyle and other dietary compounds - need to be taken into account when assessing the evidence. As oily fish is an important source of omega-3 (n-3) polyunsaturated fatty acids (PUFAs), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Saravanan 2010), potential effects of fish intake on cardiovascular diseases have been attributed to these n-3 PUFAs.

EPA and DHA are synthesised by marine algae and are concentrated in fish and marine oils, in contrast with inland vegetal n-3 fatty acids sources, which contain primarily alpha-linolenic acid (ALA) (Bradbury 2011; Valenzuela 2009). EPA and DHA comprise approximately 30% of the fatty acids present in fish oil; however, their proportion varies according to the type of fish (Calder 2012a). Presently, apart from seafood, these fatty acids can be found in supplements of fish oil, fish liver oil, krill oil, and also as pharmaceutical grade ethyl ester formulations (Calder 2009). Availability of n-3 PUFAs has allowed pharmacological intervention trials using highly purified fatty acids concurrently with food-based trials (Yokoyama 2007). Current marine-derived n-3 PUFAs intake recommendations from different health bodies in Western countries are around 500 mg per day of combined EPA and DHA, the equivalent of two oily fish meals per week (Kris-Etherton 2009).

EPA comprises 20 carbon atoms and five double bonds and it is required for production of eicosanoids (Bradbury 2011). EPA, in its ethyl ester form, has been approved in Japan as a treatment for hyperlipidaemia and peripheral artery disease (Yokoyama 2007). Furthermore, it has been shown to reduce the recurrence of stroke

in a Japanese hypercholesterolaemic population (Tanaka 2008). DHA, with the longest side chain (22 carbon atoms) and six double bonds, is concentrated mainly in the sn-2 position of the phospholipids in cellular membranes (Valenzuela 2013), and it confers particular structural and functional features to them, especially in the retina and in the neuronal synapses of the brain (Bradbury 2011). DHA is essential for normal brain growth and cognitive function (Bazan 2009): dietary supplies are required for membrane replacement, maintaining synaptic integrity and function, gene regulation and synthesis of docosanoids and neuroprotectins (Bazinet 2014). DHA also plays a critical role in neuronal survival and is a potent modulator of brain inflammation (Belayev 2011). DHA therefore has potential roles in prevention and attenuation of ischaemic injury, and also in facilitating repair and neuroplasticity after stroke (Eady 2012).

How the intervention might work

Increasing intakes of n-3 fatty acids from fatty fish could positively influence the prevention of stroke (Ikeya 2013). Furthermore, in people with acute ischaemic stroke, a low serum n-3/n-6 PUFAs ratio on admission has been shown to predict neurological deterioration (Suda 2013). In patients on a low-dose statin therapy, administration of EPA reduced the risk of a recurrent stroke (Tanaka 2008). Marine n-3 fatty acids are suggested to reduce inflammation, improve endothelial activity, and decrease platelet aggregation (Calder 2012a; Calder 2012b). N-3 PUFAs' antithrombotic effects include reducing thrombin formation, decreasing oxidative stress (Gajos 2011), and increasing plaque stability (Thies 2003). Recent findings from animal models showed that unesterified DHA injection or infusion with fish oil within three hours after brain ischaemia reduced total infarct volume by 40% in rats (Belayev 2009), and by 21% in mice (Zhang 2014), compared to placebo; and significantly improved functional outcome after cerebral ischaemia in both models. In a rat model, DHA administration up to five hours after focal ischaemia increased neurobehavioural recovery, reduced brain infarction and oedema, and activated neuroprotectin D1 (NPD1) synthesis in the penumbra (Belayev 2011). NPD1 is part of a family of lipid mediators synthesised from both EPA (E-series resolvins) and DHA (D-series resolvins and protectins) which stimulates cell-protecting and pro-survival signalling (Belayev 2011) and seems to have a role in neuroprotection (Calder 2012a). NPD1 suppresses apoptosis (Bazan 2005) and promotes neurogenesis (Eady 2014). Additionally, DHA exerts an anti-inflammatory effect during ischaemic injury through the attenuation of microglia activation in both young and aged rat models (Belayev 2011; Eady 2014; Hong 2014; Zendedel 2015).

N-3 PUFAs treatment in rats also attenuated the death of both neurons and astrocytes, with the latter being essential for neuronal survival (Belayev 2011; Zendedel 2015). In mice supplemented with n-3 PUFAs-enriched fish oil for three months before and

up to one month after stroke, n-3 PUFAs stimulated the expression of angiopoietin (Ang) 1 and Ang 2, improved the migration and survival of neuroblasts, preserved myelin integrity and promoted oligodendrogenesis after ischaemia, facilitating therefore the recovery of white matter and neurological functions (Zhang 2015). Transgenic *fat-1* mice bred to overproduce n-3 PUFAs have been shown to have long-term behavioural and histological protection against transient focal cerebral ischaemia, exhibiting improvements in revascularization and angiogenesis compared to wild-type littermates (Wang 2014). These results, taken together, suggest that n-3 PUFAs may be beneficial not only in the acute phase but also for long-term functional recovery after cerebral ischaemia.

With respect to the risk of haemorrhagic stroke, evidence assessing the impact of n-3 PUFAs intake shows conflicting results. Early epidemiological studies in Greenlandic Eskimos (or Inuit) observed a relationship between increased fish intake and incidence of haemorrhagic stroke (Dyerberg 1979; Ostergaard Kristensen 1983). This effect is likely due to the EPA component of fish oil, which inhibits platelet aggregation (Jakubowski 1979). However, an augmented incidence of cerebral haemorrhage was not observed in later studies in women with high consumption of fish and n-3 PUFAs (Iso 2001), nor in patients treated with EPA (Nakase 2015).

Why it is important to do this review

Given that stroke is one of the main causes of long-term disability worldwide (Mozaffarian 2016), it is critical to examine the established and potential approaches to protecting the brain during the crucial period of damage and recovery. If n-3 supplements were shown to reduce cerebral ischaemia severity or improve recovery without increasing the risk of cerebral haemorrhage, this would provide a safe, inexpensive, and simple intervention which is easily implementable. Some studies looking at n-3 supplementation and human stroke have been reported. One non-randomised study of stroke patients already taking EPA found no increased risk of haemorrhage but was underpowered for recurrent events (Nakase 2015), while a sub-analysis of the Japan EPA Lipid Intervention Study (JELIS) found reduced risk of recurrent stroke in patients also taking low-dose statin (Tanaka 2008). A study using a guideline-recommended moderate dose of fish oil supplement (0.7 g DHA daily) or placebo on cardiovascular biomarkers, mood- and health-related quality of life in patients with ischaemic stroke found no effect after 12 weeks' supplementation (Poppitt 2009). Heterogeneity in results might be related to variation in doses or intervention time, different pathophysiologies of the clinical outcomes (Mozaffarian 2006), and the potential oxidation of the fish oils (Poppitt 2009). Also, the effect of n-3 fatty acids administration is likely to differ by type of stroke: therefore assessing their impact on total stroke risk might underestimate the strength of the real association with a specific type of stroke (Kris-Etherton

2002; Zhang 1999). No systematic review looking specifically at the effect of n-3 PUFAs as a treatment for stroke has been published. This Cochrane Review will combine and summarise the available outcome evidence from human randomised studies of marine-derived n-3 PUFAs administration after stroke.

OBJECTIVES

To assess the effects of marine-derived n-3 fatty acids administration on functional outcomes and dependence in people with stroke.

METHODS

Criteria for considering studies for this review

Types of studies

We will include randomised controlled trials (RCTs) of marine-derived n-3 fatty acids administration after stroke onset, versus placebo or open control (no placebo), regardless of time lapse between onset and intervention, language, and blinding design. We will exclude quasi-randomised, non-randomised, and observational studies.

Types of participants

We will include all participants with a diagnosis of stroke (including TIA) on the basis of clinical examination findings, diagnostic test results, or according to the definitions used by researchers to enrol participants in their studies, regardless of severity, comorbidities, age, gender, and phase of the disease (from acute to chronic). We will include trials with mixed populations in the meta-analysis when they meet our inclusion criteria and separate data for stroke patients are available.

Types of interventions

We will include trials comparing administration of marine-derived n-3 fatty acids regardless of duration, dosage, route of administration, or type (fatty acids supplements, marine oils or fish intake) with placebo or no intervention. Eligible interventions include but are not limited to: fish oil, krill oil, microalgae oil, cod liver oil, other marine oils, n-3 fatty acids ethyl esters, and consumption of fish (such as salmon, mackerel, tuna, trout, sprat, pilchard, herring, sardine, swordfish).

We will exclude trials in which only one of the groups (treatment or control) received another active therapy. However, we will include trials where the addition of marine-derived n-3 fatty acids

to another treatment is compared to the other treatment alone, therefore assessing the effect of marine-derived n-3 fatty acids.

Types of outcome measures

Primary outcomes

The primary outcome will be efficacy (functional outcome or disability/dependency) for the latest time point of assessment, using a validated scale (e.g. modified Rankin Scale (mRS); Barthel index (BI); or change in National Institutes of Health Stroke Scale (NIHSS)). For studies using multiple scales, we will select the one with the largest amount of data available or, in the case of equal amounts of data, the one reported first in the study.

Secondary outcomes

We will assess the secondary outcomes as follows: after a short follow-up (up to three months), and after a longer follow-up (over three months).

- Vascular-related death.
- Recurrent events: fatal or non-fatal (same type of stroke, i.e. ischaemic or haemorrhagic).
- Incidence of different type of stroke (ischaemic or haemorrhagic): fatal or non-fatal.
- Adverse events: nausea, vomiting, allergic reaction, or other serious adverse events associated with the intervention.
- Quality of life (scales defined by authors).
- Mood (scales defined by authors).

Search methods for identification of studies

See the 'Specialized register' section in the [Cochrane Stroke Group](#) module. We will search for trials in all languages and arrange for the translation of relevant articles where necessary.

Electronic searches

We will search the Cochrane Stroke Group trials register and the following electronic databases.

- Cochrane Central Register of Controlled Trials (CENTRAL; latest issue) in the Cochrane Library.
- MEDLINE Ovid (from 1948) ([Appendix 1](#)).
- Embase Ovid (from 1980).
- CINAHL EBSCO (Cumulative Index to Nursing and Allied Health Literature; from 1982).
- Science Citation Index Expanded - Web of Science (SCI-EXPANDED).
- Conference Proceedings Citation Index- Science - Web of Science (CPCI-S).
- BIOSIS Citation Index.

We developed the MEDLINE search strategy ([Appendix 1](#)) with the help of the Cochrane Stroke Group Information Specialist and will adapt it for the other databases.

We will also search the following ongoing trials registers.

- US National Institutes of Health Ongoing Trials Register ([clinicaltrials.gov](#))
- Stroke Trials Registry ([strokecenter.org/trials/](#))
- ISCRTN Registry ([isrctn.com/](#))
- World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) ([who.int/ictrp/en/](#))

Searching other resources

In order to identify other published, unpublished and ongoing studies we will:

- check the bibliographies of included studies and any relevant systematic reviews identified for further references to relevant trials;
- use the Science Citation Index Reference Search for forward tracking of included trials;
- contact researchers in the field to obtain additional information on relevant trials;
- contact original authors for clarification and further data if trial reports are unclear.

Data collection and analysis

Selection of studies

Two review authors (CGAC, MJM) will independently screen titles and abstracts of the references obtained as a result of our searching activities and will exclude obviously irrelevant reports. We will retrieve the full-text articles for the remaining references and two review authors (CGAC, MJM) will independently screen the full-text articles and identify studies for inclusion, and identify and record reasons for exclusion of the ineligible studies. We will resolve any disagreements through discussion or, if required, we will consult a third author (MRM). We will collate multiple reports of the same study so that each study, not each reference, is the unit of interest in the review. We will record the selection process and complete a PRISMA flow diagram.

Data extraction and management

Two review authors (CGAC, FT) will independently extract data from included studies. Information extracted will include study settings, time frame, type of event, type of intervention, as well as dose and mode of delivery, participants' characteristics, dietary information, outcomes reported, and trials authors' definitions. Discrepancies among authors will be resolved by consultation with a third author (MJM).

Assessment of risk of bias in included studies

Two review authors (CGAC, FT) will independently assess risk of bias for each study using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We will resolve any disagreements by discussion or by involving another author (MRM). We will assess the risk of bias according to the following domains.

- Random sequence generation.
- Allocation concealment.
- Blinding of participants and personnel.
- Blinding of outcome assessment.
- Incomplete outcome data.
- Selective outcome reporting.
- Other bias.

We will grade the risk of bias for each domain as high, low or unclear and provide information from the study report together with a justification for our judgment in the 'Risk of bias' tables.

Measures of treatment effect

For continuous outcomes we will calculate the mean difference (MD) with 95% confidence intervals (CI) for data measured in the same way between trials; and standardized mean difference (SMD) with 95% CI when different scales were used for measurement. For binary outcomes, we will calculate the risk ratios (RR) with 95% CI.

Unit of analysis issues

We anticipate that in most trials the unit of analysis will be individual participants. For studies where there is more than one follow-up period, we will include the longest period recorded to extract outcome data. For non-fatal recurrent events, we will only record the first event of each participant. If we identify studies with non-standard designs (e.g. cross-over trials, cluster randomised studies) we will consider their inclusion, following the guidance in Chapter 16 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

Dealing with missing data

We will contact the authors to request any missing data. If unsuccessful, we will consider the guidelines from the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011) for data imputation.

Assessment of heterogeneity

We will use the I^2 statistic to measure heterogeneity among the trials in each analysis, with a value above 50% indicating substantial heterogeneity. If we identify substantial heterogeneity, we will

explore the reasons and identify if there are clinical or methodological explanations for differences in treatment effects between studies.

Assessment of reporting biases

If possible, we will use funnel plots for all the outcomes to assess reporting bias. If we include fewer than 10 trials in this systematic review, we will assess reporting bias qualitatively on the basis of characteristics of included trials.

Data synthesis

Where we consider that studies can be combined, we will conduct a random-effects model meta-analysis by pooling the appropriate data using Review Manager 5 (Review Manager 2014). If a meta-analysis is not possible, we will summarize the results in a narrative manner, including text, figures and tables.

GRADE and 'Summary of findings' tables

We will create 'Summary of findings' tables (see Table 1 and Table 2 below) to compare the administration of marine-derived n-3 fatty acids versus placebo or no intervention after both a short (up to three months) and a longer (over three months) follow-up, using the following outcomes: efficacy (activities of daily living as measured using mRS or Barthel Index, or neurological impairment measured as change in NIHSS), vascular-related death, recurrent events, incidence of different type of stroke, adverse events, quality of life, and mood. We will use the five GRADE considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) to assess the quality of a body of evidence as it relates to the studies that contribute data to the meta-analyses for the prespecified outcomes (Atkins 2004). We will use methods and recommendations described in Section 8.5 and Chapter 12 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011) using GRADEproGDT software (GRADEproGDT 2015). We will justify all decisions to down- or up-grade the quality of studies using footnotes, and we will make comments to aid the reader's understanding of the review where necessary.

Subgroup analysis and investigation of heterogeneity

If appropriate data are available, we plan to carry out subgroup analyses based on: type of stroke (ischaemic or haemorrhagic), dose, type of intervention (fatty acids supplements, marine oils or fish intake), length of intervention (up to three months and over three months), and initial time of intervention (up to three months and over three months from event onset).

Sensitivity analysis

If we have a sufficient number of studies we will perform sensitivity analyses by: excluding the trials with overall high risk of bias, and excluding studies with substantial missing data.

ACKNOWLEDGEMENTS

We thank the Cochrane Stroke Group, and in particular Hazel Fraser (Managing Editor) and Joshua Cheyne (Trials Search Co-ordinator and Information Specialist), for their support in the development of this protocol.

CG Alvarez Campano is funded by the Mexican Council for Science and Technology (CONACyT) and the Institute of Innovation and Technology Transfer (I²T²) (grant number 457349).

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* Indicates the major publication for the study

ADDITIONAL TABLES

Table 1. Summary of findings template: short follow-up

| Marine-derived n-3 fatty acids therapy compared to no marine-derived n-3 fatty acids therapy for stroke. Follow-up of 3 months or sooner | | | | | |
|--|---|--|--|---------------------------------|----------|
| Patient or population: people with stroke | | | | | |
| Setting: hospital, outpatient, community or home | | | | | |
| Intervention: marine-derived n-3 fatty acids therapy | | | | | |
| Comparison: no marine-derived n-3 fatty acids therapy | | | | | |
| Outcomes | Anticipated absolute effects (95% CI) | Relative effect (95% CI) | N _o of participants (studies) | Quality of the evidence (GRADE) | Comments |
| | Risk with no marine-derived n-3 fatty acids therapy | Risk with marine-derived n-3 fatty acids therapy | | | |

Table 1. Summary of findings template: short follow-up (Continued)

| | | | | | | |
|---------------------------------------|-----|-----|--|--|--|--|
| | apy | apy | | | | |
| Efficacy | | | | | | |
| Vascular-related death | | | | | | |
| Recurrent events | | | | | | |
| Incidence of different type of stroke | | | | | | |
| Adverse events | | | | | | |
| Quality of life | | | | | | |
| Mood | | | | | | |

CI: confidence interval

Table 2. Summary of findings template: longer follow-up

| <p>Marine-derived n-3 fatty acids therapy compared to no marine-derived n-3 fatty acids therapy for stroke. Follow-up longer than 3 months Patient or population: people with stroke Setting: hospital, outpatient, community or home Intervention: marine-derived n-3 fatty acids therapy Comparison: no marine-derived n-3 fatty acids therapy</p> | | | | | | |
|---|---|--|--------------------------|-------------------------------|---------------------------------|----------|
| Outcomes | Anticipated absolute effects (95% CI) | | Relative effect (95% CI) | No. of participants (studies) | Quality of the evidence (GRADE) | Comments |
| | Risk with no marine-derived n-3 fatty acids therapy | Risk with marine-derived n-3 fatty acids therapy | | | | |
| Efficacy | | | | | | |
| Vascular-related death | | | | | | |
| Recurrent events | | | | | | |
| Incidence of different type of stroke | | | | | | |

Table 2. Summary of findings template: longer follow-up (Continued)

| | | | | | | | | | |
|-----------------|--|--|--|--|--|--|--|--|--|
| Adverse events | | | | | | | | | |
| Quality of life | | | | | | | | | |
| Mood | | | | | | | | | |

CI: confidence interval

APPENDICES

Appendix I. MEDLINE (Ovid) search strategy

1. cerebrovascular disorders/ or basal ganglia cerebrovascular disease/ or exp brain ischemia/ or exp carotid artery diseases/ or exp cerebral small vessel diseases/ or exp intracranial arterial diseases/ or exp “intracranial embolism and thrombosis”/ or exp intracranial hemorrhages/ or stroke/ or exp brain infarction/ or stroke, lacunar/ or vasospasm, intracranial/ or vertebral artery dissection/
2. (stroke\$ or poststroke or apoplex\$ or cerebral vasc\$ or brain vasc\$ or cerebrovasc\$ or cva\$ or SAH).tw.
3. ((brain or cerebr\$ or cerebell\$ or vertebrobasil\$ or hemispher\$ or intracran\$ or intracerebral or infratentorial or supratentorial or middle cerebral artery or MCA\$ or anterior circulation or posterior circulation or basilar artery or vertebral artery or space-occupying) adj5 (isch?emi\$ or infarct\$ or thrombo\$ or emboli\$ or occlus\$ or hypoxi\$)).tw.
4. ((brain\$ or cerebr\$ or cerebell\$ or intracerebral or intracran\$ or parenchymal or intraparenchymal or intraventricular or infratentorial or supratentorial or basal gangli\$ or putaminal or putamen or posterior fossa or hemispher\$ or subarachnoid) adj5 (h?emorrhag\$ or h?ematoma\$ or bleed\$)).tw.
5. hemiplegia/ or exp paresis/ or exp Gait Disorders, Neurologic/
6. (hemipleg\$ or hemipar\$ or paresis or paraparesis or parietic).tw.
7. or/1-6
8. exp fish oils/ or fatty acids/ or fatty acids, omega-3/ or docosahexaenoic acids/ or eicosapentaenoic acid/
9. exp Fishes/ or seafood/ or fish products/ or shellfish/ or Dietary Fats/
10. (((fish\$ or cod or mackerel or kipper\$ or pilchards or tuna or trout or sprat\$ or salmon or herring or crab or whitebait or swordfish or sardine\$ or krill or microalgae or marine) adj3 (oil\$ or fat\$ or acid\$ or omega-3 or omega3 or omega 3 or n-3 or polyunsaturat\$)) or PUFA\$).tw.
11. ((Docosahexaenoic or eicosapentaenoic or icosapentaenoic) adj3 acid\$).tw.
12. (EPA or DHA).tw.
13. or/8-12
14. Randomized Controlled Trials as Topic/
15. Random Allocation/
16. Controlled Clinical Trials as Topic/
17. control groups/
18. clinical trials as topic/ or clinical trials, phase i as topic/ or clinical trials, phase ii as topic/ or clinical trials, phase iii as topic/ or clinical trials, phase iv as topic/
19. double-blind method/
20. single-blind method/
21. Placebos/
22. placebo effect/
23. cross-over studies/

24. randomized controlled trial.pt.
25. controlled clinical trial.pt.
26. (clinical trial or clinical trial phase i or clinical trial phase ii or clinical trial phase iii or clinical trial phase iv).pt.
27. (random\$ or RCT or RCTs).tw.
28. (controlled adj5 (trial\$ or stud\$)).tw.
29. (clinical\$ adj5 trial\$).tw.
30. ((control or treatment or experiment\$ or intervention) adj5 (group\$ or subject\$ or patient\$)).tw.
31. (quasi-random\$ or quasi random\$ or pseudo-random\$ or pseudo random\$).tw.
32. ((control or experiment\$ or conservative) adj5 (treatment or therapy or procedure or manage\$)).tw.
33. ((singl\$ or doubl\$ or tripl\$ or trebl\$) adj5 (blind\$ or mask\$)).tw.
34. (cross-over or cross over or crossover).tw.
35. (placebo\$ or sham).tw.
36. trial.ti.
37. (assign\$ or allocat\$).tw.
38. controls.tw.
39. or/14-38
40. 7 and 13 and 39

CONTRIBUTIONS OF AUTHORS

MJM and FT conceived the review.

CGAC drafted the protocol with contributions from MJM, LA, MRM and FT.

All authors approved the protocol prior to publication.

DECLARATIONS OF INTEREST

Celia Gabriela Alvarez Campano: none known.

Mary Joan Macleod: none known.

Frank Thies: none known.

Lorna Aucott: none known.

Malcolm R Macleod: none known.

SOURCES OF SUPPORT

Internal sources

- University of Aberdeen, UK.

External sources

- Mexican Council for Science and Technology (CONACyT) and the Institute of Innovation and Technology Transfer (I²T²) (grant number 457349), Mexico.