PROTOCOL

"A randomised controlled trial to evaluate the effects of omega-3 fatty acid supplementation on peripheral nerve health in type-1 diabetes"

Protocol Number:	nPROOFS-1-v1.2
Phase:	IIb
Sponsor:	The University of Melbourne
Ethics approval:	St Vincent's Hospital, Melbourne

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- Associate investigators

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1. Study overview

1.1. Administrative information

1.1.1. Trial registration

This study has been prospectively registered on the Australia New Zealand Clinical Trials Registry (ANZCTR) http://www.anzctr.org.au/ (ACTRN12618000705280).

1.1.2. Sponsor

This is an investigator-initiated study, sponsored by The University of Melbourne.

1.1.3. Funding

This study will be funded by the Downie research laboratory funds from the Department of Optometry and Vision Science, The University of Melbourne. Support for the project includes a Melbourne Neuroscience Interdisciplinary grant and a Rebecca L Cooper Medical Foundation research grant.

1.1.4. Expected duration of study

This study is expected to run for a total of 2.5 years from recruitment to the completion of the last participant's last visit. The length of treatment for an individual participant, including all follow-up visits, is six months.

1.1.5. Contribution to study

Name	Summary of contribution
Dr Laura Downie Principal Investigator Dept of Optometry & Vision Sciences University of Melbourne, Parkville VIC	Conceived idea for clinical trial; led protocol design and drafting, including development of methodologies for ocular testing; providing funding for the study.
Ms Alexis Ceecee Zhang PhD Student Investigator Dept of Optometry & Vision Sciences University of Melbourne, Parkville VIC	Co-investigator, provided input into methodologies for ocular testing, and design and drafting of trial protocol.
Prof Richard MacIsaac Director, Department of Endocrinology & Diabetes St Vincent's Hospital, Melbourne and	Co-investigator, contributed to protocol, in particular assessments relating to diabetes status.
Dr Leslie Roberts Director, Department of Neurophysiology St Vincent's Hospital, Melbourne	Co-investigator, contributed to protocol, in particular assessments relating to neurophysiology testing.
Dr Jordan Kamel Neurologist St Vincent's Hospital, Melbourne	Co-investigator, contributed to protocol, in particular assessments relating to neurophysiology testing.

1.2. Background and rationale

Diabetic neuropathy is the most common complication of diabetes mellitus, affecting >50% of individuals with the condition (Dyck & Dyck, 1999). The Diabetes Control and Complications Trial demonstrated that intensive blood glucose control could reduce the incidence of neuropathy and potentially slow progressive nerve damage (Albers *et al.*, 2010; Nathan *et al.*, 1993); however, there are currently no clinical therapies for attenuating the onset or progression of neuropathy.

Omega-3 polyunsaturated fatty acids (PUFAs) are essential fatty acids that cannot be synthesised by the human body. Increased consumption of omega-3 PUFAs endogenously generates anti-inflammatory and pre-resolving mediators that have been shown to be beneficial in a range of inflammatory conditions (Simopoulos, 2002). Once ingested, omega-3 PUFAs are incorporated into cell membranes, and play a role in cellular signalling and gene expression (Calder & Grimble, 2002), and maintaining nerve integrity. In animal models of diabetes, omega-3 supplementation can attenuate adverse changes in nerve structure and function (Gerbi *et al.*, 1999; Yee *et al.*, 2010). Derivatives of omega-3 fatty acids have also been shown to restore corneal nerve integrity and restore damage after corneal injury in rabbits (Cortina *et al.*, 2010). However, there has been limited investigation in humans, particularly as related to diabetic neuropathy.

There is some clinical evidence to suggest that omega-3 oral supplements may be useful in treating peripheral neuropathy in diseases other than diabetes. For example, omega-3 supplementation was shown to reduce the severity of symptoms and improve total neuropathy score in paclitaxel-induced peripheral neuropathy (Ghoreishi *et al.*, 2012). Our laboratory has recently shown that omega-3 fatty acids promote corneal nerve regeneration in dry eye disease, which is characterised by a mild degree of corneal neuropathy (Chinnery *et al.*, 2017a). Furthermore, a recent open-label, proof of concept study involving a single-arm of participants with type-1 diabetes, treated for 12 months with omega-3 PUFAs, reported a 20% increase in small nerve fibres using corneal nerves as a primary outcome measure (Lewis *et al.*, 2017). Together, these promising findings provide the rationale for the present proposal, being a randomised, double-masked, placebo-controlled clinical trial to assess the effects of a six-month period of supplementation with omega-3 PUFAs on small nerve fibre structure and function, measured both in the eye and the extremities of the body, in individuals with type-1 diabetes.

JUSTIFICATION OF ASSESSMENT APPROACHES: Ocular manifestations of diabetes in the anterior eye include changes in tear film integrity, impaired wound healing and diabetic cataract, and in the posterior eye, diabetic retinopathy (Negi & Vernon, 2003). Studies suggest that there is an association between peripheral neuropathy and ocular surface complications in diabetes, especially in relation to reduced corneal nerve density and sensitivity, where both reductions in corneal nerve structure and function have been shown to correlate with the severity of diabetic peripheral neuropathy (Malik *et al.*, 2003; Pritchard *et al.*, 2010). Damage to small nerve fibres, including unmyelinated C-fibres and thinly myelinated Aδ-fibres, is recognised to precede the involvement of larger nerve fibres (Breiner *et al.*, 2014; Greene *et al.*, 1999).

Corneal *in vivo* confocal microscopy (IVCM) is the only technique that allows non-invasive imaging of the peripheral nerves in the body. It has been proposed to detect the earliest stages of change that precede the development of large fibre neuropathy in diabetes (Pritchard *et al.*, 2015), and to serve as a marker to monitor changes in neuropathy (Tavakoli *et al.*, 2013). In this study, the structure of the corneal nerves will be visualised using IVCM; we will quantify changes in corneal nerve parameters, as a primary efficacy outcome measure, between baseline and the primary endpoint (six months).

Other ocular parameters to be examined include: tear film integrity, examined using standard clinical techniques (e.g., tear osmolarity, ocular surface staining, inflammatory tear markers) and corneal nerve function (i.e., using corneal aesthesiometry). Routine dilated fundus examination will be performed, being a standard clinical procedure in individuals with diabetes, to assess for any changes of retinal health throughout the study.

In addition to the eye health investigations, we will assess whether there are changes to small nerve fibre function in the peripheral body (ie., feet) using electrophysiology testing methods (i.e., cutaneous silent periods, which assesses thinly myelinated Aδ-fibres) and autonomic testing (i.e., quantitative sudomotor axonal reflex testing, which assesses unmyelinated post-ganglionic sudomotor C-fibres) and quantitative sensory testing, to assess thermal (cold: Aδ-fibres and warm: C-fibres) and vibration (medium myelinated fibres: Aβ) thresholds. Routine nerve conduction studies will also be performed to assess any large nerve fibre damage, and nerve excitability testing, using similar techniques, will detect any changes in these nerve fibres that may precede large nerve fibre damage.

JUSTIFICATION OF COMPARATOR:

Olive oil is chosen as the comparator for omega-3 interventions, as an inert control. The main constituents of olive oil are omega-9 oleic acid (C18:1), which has shown to have no effect in the inflammatory mediators in the body. Olive oils also matches in caloric intake of the active oral supplementation. Participants of double-masked clinical trials have demonstrated a poor ability to differentiate between active fish oil and inactive olive oil as their given intervention, even if they notice a fishy aftertaste (Damico *et al.*, 2002).

2. Study design and participants

2.1. Study objectives

2.1.1. Primary objective

The aim of this randomised, double-masked, placebo-controlled clinical trial is to evaluate the effects of a six month supplementation period with omega-3 polyunsaturated fatty acid (PUFA) supplements on peripheral nerves in individuals with type-1 diabetes.

2.2. Study design

This study is a single-centre, double-masked, randomised, two-arm, parallel-group interventional study comparing the effects of omega-3 EFA oral supplements with olive oil (placebo) supplements over six months. Participants will be randomised according to a 1:1 allocation ratio to omega-3 PUFA supplements (defined below) or an olive oil (placebo) supplement.

The primary outcome measure is the change in central corneal nerve fibre length (CNFL) from baseline (Day 1); the primary analysis endpoint is Day 180 (six months).

2.3. Study setting

This study will be conducted at the Department of Optometry and Vision Sciences at The University of Melbourne, with recruitment and data collection undertaken both at this site and the Endocrinology and Neurology Departments at St Vincent's Hospital, Melbourne.

2.4. Study population and entry criteria

2.4.1. Number of participants

A total of 50 patients will be randomised over the study duration.

Given a two-arm parallel design, with the pre-specified primary outcome of CNFL (taken as a continuous measure), for 80% power at a confidence level of 95%, using an estimated true difference between intervention arms of 2.9 units, mm/mm^2 (Chinnery *et al.*, 2017b), based on an expected standard deviation of 3.2 units, the required sample size is 21 participants per group. To allow for 15% participant attrition, the sample size within each group will be increased by this amount, giving a need to recruit a total of 25 participants in each interventions arm (n=50 for the trial).

2.4.2. Study population characteristics

Participants will be individuals with type-1 diabetes mellitus, for any length of duration, with or without diagnosed neuropathy.

2.4.3. Inclusion criteria

The following are requirements for entry into the study at Visit 1 (Baseline):

1. Male or female, ≥ 18 years of age

2. MNSI >2

3. Written informed consent and documentation, in accordance with privacy requirements, obtained prior to performing any study procedures;

4. Distance best-corrected visual acuity of at least 6/12 Snellen equivalent in **each** eye using a standard visual acuity chart;

5. Intraocular pressure (IOP) ≤ 21 mm Hg in both eyes;

   [*Note:* Participants with primary open-angle glaucoma or ocular hypertension (OHT) may be eligible to participate, provided they are on **stable** monotherapy **bilaterally**, with both eyes IOPs controlled and under 21 mmHg at the baseline visit. Any topical IOP-lowering medications must have a start date of ≥ 3 months prior to Visit 1, with the dosage not expected to change during the study.]

6. Ability to understand and follow study instructions, with the intention of completing all required study visits.

2.4.4. Exclusion criteria

The following are criteria for exclusion from participating in the study at Visit 1 (Baseline):

<u>Systemic</u>

1. Any uncontrolled systemic disease, other than sub-optimally controlled diabetes mellitus;

2. Confirmed neuropathy secondary to causes other than diabetes (e.g., alcohol polyneuropathy. Vitamin B-12 deficiency, folate deficiency, chronic renal failure, hypothyroidism, neurotoxic drug use including chemotherapy);

3. Any of the following general medical conditions: bipolar disorder, atrial fibrillation, implanted defibrillator, familial adenomatous polyposis, systemic immunocompromise;

4. Scheduled or planned systemic surgery over the course of the study;

5. Any known bleeding disorders;

6. Current consumption of a systemic anti-coagulant medication other than aspirin;

7. Females who are currently pregnant or breastfeeding;

8. females of childbearing potential who are planning a pregnancy over the course of the study;

[Definition: For the purposes of this study, females will be considered of childbearing potential unless they are: naturally postmenopausal or permanently sterilised (i.e., a **full** [not partial] hysterectomy). Natural menopause is defined as the permanent cessation of menstrual periods, determined retrospectively after a woman has experienced 12 months of amenorrhea without any other obvious pathological or physiological cause.

If a female participant does become pregnant over the course of the study, the participant will be asked to attend for a 'Study Exit' final visit, and will then be exited from the study with appropriate follow-up **under the direction of the principal investigator**. The **principal investigator** will notify the patient's GP that the patient was being treated with an oral supplement; if necessary, the mask can be broken.]

9. Inability to sit/lie supine comfortably during the examination procedures for any reason.

Ophthalmic

10. Known allergy to or previous reaction to any ocular agents used in the study (i.e., ocular anaesthetics, sodium fluorescein, lissamine green, ocular mydriatics);

11. Scheduled or planned ocular surgery or procedure over the course of the study;

12. Any history of rigid contact lens wear;

13. Grading of diabetic retinopathy worse than moderate retinopathy according to the simplified ETDRS (Wisconsin) classification (ETDRS Research Group, 1991);

14. Presence of any of the following ocular conditions: active ocular infection or inflammation that in the judgment of the investigator may interfere with the interpretation of the study results;

15. Corneal abnormalities or damage that could disrupt normal corneal nerve morphology, including keratoconus, bullous keratopathy, advanced corneal dystrophies, history of neurotrophic keratopathy including herpes keratitis, severe Sjogren's associated dry eye disease;

16. History of refractive surgery or trauma within the past 12 months;

17. Use of autologous serum eye drops within the past three months, or their anticipated use during the course of the study;

18. Participant has a medical or ocular condition, or is in a situation, which in the principal investigator's opinion, may put the participant at significant risk, may confound the study results, or may interfere significantly with participation in the study.

Interventional

19. Current or previous regular consumption of any omega-3 oral supplements (>3 times/week) in the three months preceding Visit 1;

20. Current participation in another interventional drug or device study or anticipated entry into such a within 1 month of enrolment;

21. Known allergy or hypersensitivity to any components of the study supplements;

22. Cultural or religious beliefs that exclude the consumption of certain or all animal products.

2.4.5. Recruitment methods

Initial contact with potential participants will be made in the following ways:

i) After the treating clinician at St Vincent's Hospital or the University of Melbourne eye clinic has identified that a patient who may be eligible to participate in the study, the patient will be asked if they give verbal consent to be contacted by the investigators (either by phone/email) to receive further information. The potential participant will then also be provided with a hard copy of the PICF, and the investigator will contact the potential participant directly to discuss the study.

ii) The co-investigator (Alexis Ceecee Zhang) will have information available about the study for patients who are waiting for clinical appointments at the type-1 diabetes outpatient clinic at St Vincent's Hospital. She will discuss the study with patients whilst they are waiting, and if they are interested in participating, provide them with a hard copy of the PICF and obtain permission to follow-up with them via phone and/or email.

iii) Potential participants who have provided written consent to be contacted for research studies at the University of Melbourne eye care clinic will be identified by chart review (by one of the investigators), with a subsequent email (first preference) or mail-out (if no email details were provided) invitation. Those who respond to the invitation will be provided with the PICF, and then followed up via phone and/or email with regard to arranging a time for a baseline study visit.

iv) Potential participants who respond to study advertisements will be contacted by one of the listed investigators, either via email or phone (depending upon the patient preference), and provided with further information (including the PICF).

3. Study Interventions and Formulations

3.1. Interventions

3.1.1. Omega-3 oral supplements

Treatment arm: triglyceride form omega-3 oral supplement (Caruso's Natural Health Triple Strength fish oil). Each 1500mg capsule contains active ingredients of 540mg Eicosapentaenoic Acid (EPA) and 360mg Docosahexaenoic Acid (DHA).

Participants will be asked to consume two capsules per day; the total active dose of omega-3 triglycerides consumed will be 1080mg EPA +720mg DHA per day for 180 days.

3.1.2. Placebo oral supplement

Control arm: Olive oil (1500mg) oral supplements, two capsules per day, manufactured by BJP Laboratories Pty Ltd, for 180 days.

3.2. Treatment regimen and dosing

Each participant will be instructed to consume two capsules each day, at any time during the day, with or without food, at least two hours before or after medication. Participants will be instructed that they may or may not notice a fishy aftertaste with either treatment options, to alter their expectations, to strengthen the double-masked.

Participants will be provided with a supplement log to document their consumption of capsules each day. Participants will be advised not to 'make up' for missed doses with additional capsules on a given day; a reason for a 'missed dose' should be documented on the supplement log, which will be returned along with any remaining capsules at Visits 3, 4 and 6.

Dose alteration is not permitted. Should participants wish to discontinue dosage for any reason, they must inform the Principal investigator immediately to withdraw from the study.

3.3. Compliance to treatment

Compliance to treatment will be monitored by the analysis of systemic fatty acid profiles at the end of the study (180 days) from baseline.

Assessment of the return of unused capsules at follow-up visits, for counting by an independent research assistant, will also be used to monitor compliance to treatment.

3.4. Concomitant medications and care

Concomitant medications and care, including insulin pumps, with a start date ≥3 months prior to Visit 1 are permitted during the study.

Aspirin use is permitted during the study. Other blood thinning medications (e.g., warfarin, enoxaparin, dipyridamole, clopidogrel) are not permitted; participants taking these medications will not be eligible to participate in the study.

Participants will be asked to maintain their current dietary habits throughout the study and notify the study investigators of any major dietary changes.

3.5. Storage of study product

The study product will be stored in the Downie Laboratory, within the Department of Optometry and Vision Science at the University of Melbourne, in a secure (locked) area, at room temperature (20° to 25° Celsius, in a controlled chamber). Study products will be administered only to participants who have enrolled in the study, at no cost to the participant, in accordance with the conditions specified in this protocol.

3.6. Study product inventory

A detailed inventory will be kept for the study products (e.g., date of dispense, randomisation number, by whom, date of return and accountability of capsules. Study product will only be dispensed by an appropriately-qualified study personnel member. The study product is to be used in accordance with the protocol by participants.

3.7. Assignment of Interventions

3.7.1. Method of masking

Implementation of study allocation protocols will be conducted by an independent data manager. All trial participants and study investigators, including outcome assessors and data analysts, will be masked from the participant allocation.

Oral supplements will be packaged into identical opaque containers by a compounding pharmacist (Dartnell's Pharmacy, Surrey Hills, Victoria), to maintain double-masking. Participants' adherence to treatment will be monitored at follow-up visits by assessment of returned unused capsules by an independent researcher.

The investigators involved in the collection and analysis of data will not be involved in the reconciliation of returned study products, to maintain masking.

3.7.2. Unmasking the study product

During the trial, only when necessary for the safety and proper treatment of a participant, is it permissible for the principal investigator to request the independent data manager to unmask the participant's treatment assignment, to determine which treatment has been assigned, and institute appropriate follow-up care.

Only the principal investigator, following consultation with the independent data manager, is authorised to perform an unmasking procedure. The reason for breaking the code must be recorded in the participant's source documents.

3.7.3. Treatment allocation ratio and method of participant randomisation

A randomised allocation sequence will be generated, in advance, by the independent data manager, using Microsoft Excel, to assign equal numbers of participants in the treatment and placebo groups.

The randomisation schedule will be kept by the independent data manager and will be provided to an independent entity (i.e., compounding pharmacist) for labelling of the investigational product, in sequential order, to a corresponding participant number.

Eligible participants will be enrolled and assigned, by the sequential participant number, to either the treatment group or the placebo group, the treatment allocation for which will not be known to the study personnel who was involved in enrolment (i.e., the study investigator) or the participant.

4. Participant's timeline and schedule of study visits

Test	Repeats	Visit 1 (Day -35)	Visit 2 Day: 1	Visit 3 Day: 30±14	Visit 4 Day: 90±14	Visit 5 Day: 180+/ 24	Visit 6 Day: 180+/ 24	Notes	Time Expected (mins)	Location
Location		UoM	SVH	UoM	UoM	SVH	UoM			
Eligibility Determination										University of Melbourne
PICF	-		■	■	■	■	■		20	
Demographics	-		■	■	■	■	■		10	
Medical History	-		■			■		Medications, concurrent procedures		
Ocular History	-		■			■				
Adverse Events	-		■			■				
Diet Inquiry	-		■			■				
Pregnancy Test	-		■	■	■	■		Females of childbearing potential only	10	
Habitual VA	-	/	■			■	/		5	
Anterior Eye Health										
MNSI questionnaire			■	■	■		■			
OSDI Questionnaire	-		■	■		■			5	
EQ-5D-5L questionnaire			■	■		■				
iMTA questionnaire			■	■		■				
Tear Osmolarity		/	■	■	■	■	/			
Non-contact aesthesiometry	Central		■	■		■		Double-staircase threshold	20	
	Inferior		■	■		■				
Cooled threshold	Central		■	■		■				
	Inferior		■	■		■				
Basal tear collection			■	■	■	■			15	
Phenol red thread test			■	■		■				
Slit lamp										
Slit lamp exam			■			■			5	
Anterior chamber VH grading	-		■	■	■	■		Assessment of anterior chamber angle		
Instill NaFl										
NaFl TBUT			■	■		■				

Test	Repeats	Visit 1 (Day -35)	Visit 2 Day: 1	Visit 3 Day: 30±14	Visit 4 Day: 90±14	Visit 5 Day: 180+/ 24	Visit 6 Day: 180+/ 24	Notes	Time Expected (mins)	Location
Location		UoM	SVH	UoM	UoM	SVH	UoM			
NaFl staining	Cornea		■	■		■		Mag 16X, Max Intensity, Blue Light, Wratten filter Oxford Grading Scale		University of Melbourne
Instill topical anaesthetic										
IOP		╱	■	■	■	■	╱	Goldmann tonometer	5	
Instill mydriatic agent (Visits 1 and 6 only)										
Heidelberg Rostock Tomographer – Corneal module										
Corneal confocal microscopy	Central		■	■		■			10	
	Inferior		■	■		■				
Systemic fatty acid level analysis										
Blood pin-prick test			■	■	■	■			5	
Retinopathy Grading										
Retinal imaging	Right		■	■	■	■			10	
	Left		■	■	■	■				
Recheck eligibility			■	■	■	■				
Randomisation										
Randomisation	-		■	■	■	■	■		5	
Dispense study supplements	-		■	■		■	■			
Collection of unused supplements	-	■	■	■	■	■				
Peripheral Nerve Function										St Vincent's Hospital
MDNS		■		■	■		■		10	
CSP		■		■	■		■		15	
QSART		■		■	■		■		15	
Nerve Excitability		■		■	■		■		15	
Nerve Conduction		■		■	■		■		15	
Systemic Health										
Fasting blood test		■		■	■		■		10	

5. Response measures and summary of data collection methods

5.1. Efficacy measures:

5.1.1. Primary efficacy measures:

Primary Outcome:	Central Corneal Nerve Fibre Length (CNFL) (measured in mm/mm^2 using a Heidelberg Rostock Tomographer III with corneal module, quantified using a fully-automated method (ACCMetrics))
Timepoint:	Change in CNFL from Day 1 at Days 90 +/- 14, 180 +/- 24

5.1.2. Secondary efficacy measures:

Secondary Outcome:	Central Corneal Nerve Fibre Density (CNFD) (measured in mm/mm^2 using a Heidelberg Rostock Tomographer III with corneal module, quantified using a fully-automated method (ACCMetrics))
Timepoint:	Change in CNFD from Day 1 at 90 +/- 14, 180 +/- 24
Secondary Outcome:	Central Corneal Nerve Branch Density (CNBD) (measured in mm/mm^2 using a Heidelberg Rostock Tomographer III with corneal module, quantified using a fully-automated method (ACCMetrics))
Timepoint:	Change in CNBD from Day 1 at Days 90 +/- 14, 180 +/- 24
Secondary Outcome:	Central corneal thresholds to a room-temperature air stimulus (measured in mBar using a non-contact corneal aesthesiometer)
Timepoint:	Change from Day 1 at Days 90 +/- 14, 180 +/- 24
Secondary Outcome:	Central corneal thresholds to a cooled stimulus (measured in mBar using a non-contact corneal aesthesiometer)
Timepoint:	Change from Day 1 at Days 90 +/- 14, 180 +/- 24
Secondary Outcome:	Change in health state (index score and visual analogue score as measured using the EQ-5D-5L questionnaire).
Timepoint:	Change from Day 1 at Day 90 +/- 14, 180 +/- 24

Secondary Outcome:	Michigan Neuropathy Screening Score (MNSI) composite score
Timepoint:	Change from Day 1 at Day 180 +/- 24
Secondary Outcome:	Michigan Diabetic Neuropathy Score (MDNS) (measured through clinical examination)
Timepoint:	Change from Day 1 at Day 180 +/- 24
Secondary Outcome:	Routine nerve conduction studies (NCS) –sural sensory amplitude (measured using Dantec Keypoint G4 Workstation)
Timepoint:	Change from Day 1 at Day 180 +/- 24
Secondary Outcome:	Routine nerve conduction studies (NCS) – peroneal motor velocity (measured using Dantec Keypoint G4 Workstation)
Timepoint:	Change from Day 1 at Day 180 +/- 24

Secondary Outcome:	Routine nerve conduction studies (NCS) – tibial motor minimum F-wave latency (measured using Dantec Keypoint G4 Workstation)
Timepoint:	Change from Day 1 at Day 180 +/- 24
Secondary Outcome:	Nerve excitability profile: Depolarising threshold electrotonus (TEd) (peak), TEd (90- 100 ms), hyperpolarising threshold electrotonus (Teh) (90-100 ms), TEd (10- 20 ms), superexcitability, subexcitability, resting current–voltage (I/V) slope, strength duration time constant (measured with QTRAC software using TROND protocol)
Timepoint:	Change from Day 1 at Day 180 +/- 24
Secondary Outcome:	Quantitative sudomotor axonal reflex testing (QSART), measured at the foot in sweat volume in nL using the QSWEAT system.
Timepoint:	Change from Day 1 at Day 180 +/- 24
Secondary Outcome:	Cutaneous silent period testing: upper and lower limb (measuring silent period onset latency and duration)
Timepoint:	Change from Day 1 at Day 180 +/- 24

5.2. Safety measures:

Safety Outcome:	Adverse events (AEs) reported
Timepoint:	Change from Day 1 at Days 30 +/- 14, 90 +/- 14, 180 +/- 24
Safety Outcome:	Change in habitual visual acuity measured using a standard eye chart
Timepoint:	Change from Day 1 at Days 30 +/- 14, 90 +/- 14, 180 +/- 24
Safety Outcome:	Change in intraocular pressure (IOP) measured using a Goldmann tonometer
Timepoint:	Change from Day 1 at Day 180 +/- 24
Safety Outcome:	Diabetic retinopathy graded using the Wisconsin Scale grading system
Timepoint:	Change from Day 1 at Day 180 +/- 24
Secondary Outcome:	Blood samples (measuring fasting glucose, fasting lipid profile [LDL, HDL, total cholesterol, triglycerides], FBC, ESR, CRP, Vitamin B-12, Folate, TSH, UEC, HbA1c, liver function, electrolytes)
Timepoint:	Change from Day 1 at Day 180 +/- 24

5.3. Compliance measures:

Compliance Outcome:	Total omega-3 status obtained from blood finger spot test (Waite Lipid Analytical Services)
Timepoint:	Change from Day 1 at Day 180 +/- 24
Compliance Outcome:	Total omega-9 status obtained from blood finger spot test (Waite Lipid Analytical Services)
Timepoint:	Change from Day 1 at Day 180 +/- 24

Compliance Outcome:	Total omega-6 status obtained from blood finger spot test (Waite Lipid Analytical Services)
Timepoint:	Change from Day 1 at Day 180 +/- 24
Compliance Outcome:	Omega-6:Omega-3 ratio obtained from blood finger spot test (Waite Lipid Analytical Services)
Timepoint:	Change from Day 1 at Day 180 +/- 24

Exploratory outcome measures:

1. Compliance outcome: supplement count at Day 180 +/- 24

2. Efficacy of masking: Guessing of treatment at Day 180 +/- 24
   - Guessing by participant
   - Guessing by examiner

3. Change in central corneal dendritic cell count (CDC) (measured in cells/mm2 using a Heidelberg Rostock Tomographer III with corneal module) from Day 1 at Days 90 +/- 14, 180 +/- 24

4. Change in peripheral corneal nerve fibre length (CNFL) (measured in mm/mm2 using a Heidelberg Rostock Tomographer III with corneal module) from Day 1 at Days 90 +/- 14, 180 +/- 24

5. Change in peripheral corneal dendritic cell count (CDC) (measured in cells/mm2 using a Heidelberg Rostock Tomographer III with corneal module) from Day 1 at Days 90 +/- 14, 180 +/- 24

6. Change in peripheral corneal thresholds to a room air stimulus (measured in mBar using a non-contact corneal aesthesiometer) from Day 1 at Days 90 +/- 14, 180 +/- 24

7. Change in peripheral corneal thresholds to a cooled stimulus (measured in mBar using a non-contact corneal aesthesiometer) from Day 1 at Days 90 +/- 14, 180 +/- 24

8. Routine nerve conduction studies (NCS) – ulnar sensory amplitude, velocity and latency (measured using Dantec Keypoint G4 Workstation) from Day 1 at Day 180 +/- 24

9. Routine nerve conduction studies (NCS) – median sensory velocity, amplitude and latency (measured using Dantec Keypoint G4 Workstation) from Day 1 at Day 180 +/- 24

10. Routine nerve conduction studies (NCS) – sural sensory velocity and latency (measured using Dantec Keypoint G4 Workstation) from Day 1 at Day 180 +/- 24

11. Routine nerve conduction studies (NCS) – median motor velocity, amplitude and latency (measured using Dantec Keypoint G4 Workstation) from Day 1 at Day 180 +/- 24

12. Routine nerve conduction studies (NCS) – tibial motor amplitude and latency (measured using Dantec Keypoint G4 Workstation) from Day 1 at Day 180 +/- 24

13. Routine nerve conduction studies (NCS) – peroneal motor amplitude and latency (measured using Dantec Keypoint G4 Workstation) from Day 1 at Day 180 +/- 24

14. Change in sweat volume in the forearm measured using quantitative sudomotor axonal reflex testing (QSART) from Day 1 at Day 180 ± 14

15. Change in sweat volume in the proximal leg measured using quantitative sudomotor axonal reflex testing (QSART) from Day 1 at Day 180 ± 14

16. Change in sweat volume in the distal leg measured using quantitative sudomotor axonal reflex testing (QSART) from Day 1 at Day 180 ± 14

17. Change in nerve excitability profile (additional parameters) from Day 1 at Day 180 ± 14
    - Stimulus (mA) for 50% max response
    - Rheobase (mA)
    - Stimulus-response\slope
    - Peak response\(mV)
    - Minimum I/V slope
    - Temperature (°C)
    - RRP (ms)
    - Polarizing current\(% threshold)
    - Polarizing current\(mA)
    - Latency (ms)
    - TEd(40-60ms)
    - TEh(10-20ms)
    - TEd(undershoot)
    - TEh(overshoot)
    - S2 accommodation
    - Accommodation half-time (ms)
    - Hyperpol. I/V slope
    - Refractoriness at 2.5ms (%)
    - TEh(20-40ms)
    - TEh(slope 101-140ms)
    - Refractoriness at 2 ms (%)
    - Superexcitability at 7 ms (%)
    - Superexcitability at 5 ms (%)
    - TEd20 (peak)

18. Change in dry eye symptoms (measured using the validated Ocular Surface Disease Index (OSDI) questionnaire) from Day 1 at Days 90 +/- 14, 180 +/- 24

19. Change in tear stability measured using corneal sodium fluorescein from Day 1 at Days 90 +/- 14, 180 +/- 24

20. Change in tear osmolarity: electrolyte concentration of the tears (measured in mOsm/L using the TearLab system (TearLab Corporation Pty Ltd) from Day 1 at Day 180 +/- 24

21. Change in tear production measured using the phenol red thread test (R+L) from Day 1 at Days 90 +/- 14, 180 +/- 24

22. Additional slit lamp microscopy grading
    - Blepharitis grading from 0 - 4
    - Meibomian gland dysfunction grading (MGD) from 0 - 4
    - Conjunctival redness grading from 0 - 4
    - Limbal redness grading from 0 – 4

23. Change in corneal sodium fluorescein (NaFl) staining score, as the average of two eyes, from Day 1 at Day 90 +/- 14, Day 180 +/- 24
    - Corneal staining graded from 0 – 4

24. Change in tear composition markers from Day 1 at Day 180 +/- 24

25. Change in indirect healthcare costs measured using the iMTA-iPCQ health economics questionnaire at Day 1 and Day 180

26. Change in ONH structure retinal neuro-retinal fibre layer (RNFL) thickness in the right eye measured using Optic Coherence Tomography (OCT) from Day 1 at Day 180 +/- 24

27. Change in macular thickness in the right eye measured using Optical Coherence Tomography (OCT) from Day 1 at Day 180 +/- 24

28. Change in foveal avascular zone area in the right eye, measured using Optical Coherence Tomography Angiography (OCT-A) from Day 1 at Day 180 +/- 24

29. Change in retinal vascular calibre in the right eye, measured using Optical Coherence Tomography Angiography (OCT-A) from Day 1 at Day 180 +/- 24

6. Methods of data collection and study reports

6.1. Summary

All study data will be uploaded or manually entered (as appropriate) onto REDCap, a secure web-based application for research data capture, management and analysis (hosted on the University of Melbourne data centre infrastructure, provided via the National eResearch Collaboration tools and Resources project).

The co-investigator, Alexis Ceecee Zhang, will be responsible for ensuring that data are properly recorded and that all hard copy documents are securely stored.

Any protocol deviations will be documented and must also be reported to the Principal Investigator. Any actions arising from the protocol deviation will also be noted. Deviations identifying issues for protocol review will be amended as per Section 9.4.

6.2. Data Collection

Source documents in this study will include participants' ophthalmic records, relevant medical records, completed questionnaires, the patient study files (case report forms, CRFs), as well as the results of any diagnostic tests, such as blood tests and systemic fatty acid profiles.

Identifiable information will be collected and stored in a separate password-protected file that is only accessible to study investigators. The following data and information will be recorded:

- Identifiable participant information at baseline: name; contact details; study enrolment date
- A statement that written informed consent (including the date) was obtained prior to any study procedures being performed
- Participant ID number
- Dates of all study visits
- Demographic information at baseline (age, sex, date of birth, ethnicity)
- Height and weight at baseline
- Medical and ophthalmic histories at baseline
- Pre-study and concomitant medications, including insulin type, administration method and dosage
- Study procedures performed at the University of Melbourne (UoM)
    - Habitual distance refractive correction in each eye at baseline.
    - Habitual distance visual acuity in each eye at baseline, day 30 ± 14, day 90 ± 14 and day 180 ± 24.
    - EQ-5D-5L questionnaire score at baseline, day 30 ± 14, day 90 ± 14 and day 180 ± 24.
    - Ocular Surface Disease Index (OSDI) questionnaire score at baseline, day 90 ± 14 and day 180 ± 24.
    - Tear osmolarity in each eye at baseline and day 180 ± 24.
    - Corneal sensitivity thresholds in the right eye at baseline, day 90 ± 14 and day 180 ± 24.
    - Phenol red thread test score in each eye at baseline, day 90 ± 14 and day 180 ± 24.

  - Tear collection time and volume collected at baseline and day 180 ± 24.
  - Slit lamp examination gradings in each eye at baseline, day 90 ± 14 and day 180 ± 24.
  - Intraocular pressure (IOP) in each eye at baseline and day 180 ± 24.
  - Diabetic retinopathy (DR) and diabetic macular oedema grading (Wisconsin classification) in each eye at baseline and day 180 ± 24.
  - Optical coherence tomography angiography (OCT-A) imaging at baseline and day 180 ± 24.
  - Corneal sub-basal nerve plexus and dendritic cell parameters, measured using *in vivo* confocal microscopy (IVCM) in the right eye at baseline, day 90 ± 14 and day 180 ± 24.
  - Systemic fatty acid concentrations at baseline and day 180 ± 24.
- Study procedures performed at St Vincent's Hospital Melbourne (SVHM)
  - Michigan Neuropathy Screening Instrument (MNSI) and Michigan Diabetic Neuropathy Score (MDNS) examination score at baseline and day 180 ± 24.
  - Nerve conduction study (NCS) parameters for the median motor, peroneal motor, tibial motor, median sensory, sural sensory, ulnar sensory nerves at baseline and day 180 ± 24.
  - Cutaneous silent periods (CSP) measurements of the upper and lower limbs at baseline and day 180 ± 24.
  - Quantitative Sudomotor Axonal Reflex Test (QSART) measurements of the forearm, proximal leg, distal leg and foot at baseline and day 180 ± 24.
- Blood test results
  - Blood pathology test results at baseline and day 180 ± 24.
  - Systemic fatty acid profiles at baseline and day 180 ± 24.
- Occurrence, grading and status of any adverse events, including their potential association with the investigational product or a study procedure
- Number and proportion of supplements returned for each participant
- Participant's and examiner's forced-choice guesses of the treatment allocation (omega-3 PUFAs or placebo) on day 180 ± 24.
- The date the participant exited the study, and a notation as to whether participant completed the study,

The co-investigator (Alexis Ceecee Zhang) and Principal Investigator will be jointly responsible for ensuring that data are properly transferred from CRFs and correctly recorded.

6.3. Data Management

6.3.1. Data coding

All data collected directly by the investigators for this study will be de-identified using a participant ID code, assigned to the participant when they sign the informed consent form. This ID code will be used as an identifier on all study documentation, and be used for the labelling of body tissue samples (i.e., blood, tears), which are processed off-site. The only individuals who will have the capacity to identify the participant's study results (by having access to the code linking the participant ID code to the participant's identity) will be the investigators listed on this application, and the participant themselves. The data will not be identifiable to any other persons.

6.3.2. Security and storage of data

Study data will be collected in both paper (hard copy) files and electronic formats.

For paper source documents, de-identified data, recorded during study examinations, will be stored in a binder (marked with the participant's ID code), and subsequently entered into the REDCap database (using each participant's ID code). The paper study source documents will be kept in a locked research room – The Downie Laboratory within the Department of Optometry and Vision Sciences, accessible only by research staff and students of the Downie Laboratory.

Data captured during study examinations in an electronic format will be labelled with the participant's ID code, and subsequently uploaded into the REDCap database. Only researchers name on the project application will have direct access to the raw study data.

6.4. Individual Data Reporting

Should a participant ask for details about the results of any of their study tests, immediate verbal feedback can be given by one of the study investigators. A report, summarising key findings relating to a participant's eye and/or peripheral nerve health, can also be made available, further to the participant's request, to potentially assist in guiding the participant's ongoing clinical care.

6.5. Study summary

At the conclusion of the study, a study summary report will be made available to participants who indicate wishing to receive this report during the written informed consent process.

6.6. Retention of study documentation

All study related data, participant records, consent forms, patient privacy documentation, records of the distribution and use of all study products, and copies of case report forms will be maintained on file. Electronic data and paper files will be kept for a minimum of 15 years, but possibly longer if the data is judged of continuing value at that time.

7. Safety measures

7.1. Summary

Safety measures will include:

- Adverse events query (Visits 1, 3, 4 and 6)
- Habitual distance visual acuity, BCVA (Visits 1, 3, 4 and 6)
- Intraocular pressure, IOP (Visits 1 and 6)
- Retinal imaging and ocular fundus examination (Visits 1 and 6)
- Basic blood tests (Visits 2 and 5)

7.2. Data Monitoring

This study is a single-site, investigator-initiated clinical trial (with procedures being undertaken at two Melbourne locations – St Vincent's Hospital and the University of Melbourne). Professor Richard MacIsaac, Director - Director, Department of Endocrinology and Diabetes at St Vincent's Hospital, will act as the medical safety monitor for the trial. He possesses the requisite expertise to assist and advise the HREC about reports of serious adverse events. All adverse events will be discussed by the Principal Investigator (Dr Laura Downie) and the medical monitor before reporting to the relevant HREC or other regulatory agencies, as required.

7.3. Adverse events (AEs)

7.3.1. Assessments and documentations of adverse events

All AEs and SAEs will be immediately (within 24 hours) reported to the Principal Investigator by the research team member.

At Visit 1, after informed consent and before the initial study products are administered, all pre-treatment adverse events will be documented (e.g., allergic response to topical anaesthetic). Conditions that are present at screening and do not deteriorate will not be considered adverse events.

After the study product supply, all treatment-emergent post-treatment adverse events will be documented. Abnormal laboratory values will not be considered adverse events unless deemed clinically significant by the medical monitor, and documented as such.

If adverse events occur, the first concern will always be the safety of the study participants. All adverse events, including severity, action taken and likely relationship to the study product will be documented throughout the study.

At each visit, the co-investigator or principal investigator will begin the examination by querying for adverse events by asking each participant a general, non-directed question such as "How have you been feeling since the last visit?" Directed questioning and examination will then be carried out as appropriate.

The description of each AE recorded will include:

- A description of the AE;
- The onset date, duration, date of resolution;
- Severity (assessed as per section 7.4;
- Seriousness (i.e., is it an SAE?);

- Actions taken;
- The outcome;
- The likelihood of the relationship of the AE to the study treatment (Unrelated, Possible, Probable, Definite).

For each adverse event reported, the number and percent of participants affected will be tabulated. Separate adverse event tables will be generated for pre-treatment AEs and post-treatment AEs. All adverse events will be presented in the listings. Other safety variables, including biomicroscopy findings, BCVA, IOP, retinal fundus photograph and blood test results will be analysed.

DEFINITIONS:

- An adverse event is **any** untoward medical occurrence in a participant or clinical investigation of participant administered a pharmaceutical product. It does not necessarily have a causal relationship with treatment.

  An adverse event can therefore be **any** unfavourable or unintended sign, symptom, or disease temporally associated with the use of a study (investigational) product, whether or not it is thought to be related to the study (investigational) product. In addition, during the screening examination (Visit 1), pre-treatment adverse events will be assessed regardless of the administration of a pharmaceutical product.

  All adverse events judged as having a reasonable possibility of a causal relationship to an investigational medicinal product will qualify as adverse reactions. The expression 'reasonable causal relationship' means to convey, in general, that there is evidence or argument to suggest a causal relationship.

Note: Details relating to adverse events must be collected once informed consent has been obtained, regardless of whether or not the patient has been administered study product. Progression of treatment indication including new or worsening of anticipated clinical signs or symptoms, which are collected as clinical efficacy variables and assessed as unequivocally associated with the disease progression and /or lack of efficacy, should NOT be reported as adverse events unless the disease progression is greater than anticipated in the natural course of the disease.

7.3.2. Serious adverse events (SAEs)

- A serious adverse event is any adverse event that results in any of the following outcomes:
  - death
  - a life-threatening adverse event
  - inpatient hospitalisation or prolongation of existing hospitalisation (for any reason)
  - a persistent or significant disability/incapacity
  - a congenital anomaly/birth defect
  - a diagnosis of cancer
  - any abortion (spontaneous or non-spontaneous)

- A Suspected Unexpected Serious Adverse Reaction (SUSAR) is any SAE that is both suspected to be related to the study treatment and is unexpected (i.e., not consistent with applicable product information)

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardise the participant or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (See below for procedures for reporting a serious adverse event.)

Pre-planned surgeries or procedures for pre-existing, known medical conditions, for which a participant requires hospitalisation are not reportable as a serious adverse event. Any pre-planned surgery or procedure should be clearly documented in the site source documents by the medically qualified investigator at the time of the participant's entry into the study. If it has not been documented at the time of the patient's entry into the study, then it should be documented as a serious adverse event and reported as such.

7.4. Severity of AEs

A clinical determination will be made of the intensity of **every** adverse event. The severity assessment for a clinical adverse event must be completed using the following definitions as guidelines:

- Mild - awareness of sign or symptom, but easily tolerated.
- Moderate - discomfort enough to cause interference with usual activity.
- Severe - incapacitating with inability to work or do usual activity.
- Not applicable - in some cases, an adverse event may be an 'all or nothing' finding which cannot be graded.

7.5. Relationship to study product or study procedure

A determination will be made in relation to the likely relationship (if any) between an adverse event and the study product or study procedure, as applicable. A causal relationship is present if a determination is made that there is a reasonable possibility that the adverse event may have been caused by the study product or study procedure.

Adverse events will subsequently be classified according to the safety reporting flowchart in the NHMRC safety monitoring and reporting guidelines. Any serious adverse event occurring during the study period (beginning with informed consent) and for at least 28 days after the last dose of study product must be immediately reported according to HREC guidelines, no later than 24 hours after learning of a serious adverse event. All SAEs will be immediately (within 24 hours) reported to the medical monitor and the principal investigator. The medical monitor will make a judgment in relation to whether the SAE is likely related to the study product, and whether this outcome affects the potential safety of other participants in the study.

7.6. Procedures for reporting adverse events

All adverse events will be recorded on the appropriate sections in REDCap and the principal investigator will be informed within 24 hours**.** Any adverse event that is marked as 'ongoing' at the exit visit will be followed-up, as appropriate.

All SUSARs will be reported to the Institution (University of Melbourne Human Research Ethics Committee) and the Therapeutic Goods Administration (TGA) as soon as possible, and no later than seven calendar days for fatal or life threatening SUSARs, and 15 calendar days for all other SUSARs.

An annual safety report will be generated by the Principal Investigator and provided to the governing institutional human research ethics committee (HREC), as required by the HREC, local regulations, and the governing health authorities to provide a clear summary of the evolving safety profile of the trial. The annual safety report will include

- a brief description and analysis of new and relevant findings (as permissible);
- a brief discussion of the implications of the safety data to the trial's risk-benefit ratio;
- a description of any measures taken or proposed to minimise risk

The Principal Investigator will complete the annual review and provide the HREC and investigators with updates/addenda to the Investigator's Brochure, should new or relevant information become available.

The Principal Investigator will notify the Institution, the TGA and investigators (co-investigators and associate investigators) of all significant safety issues, amendments, temporary halt or early termination of a trial. Significant safety issues that meet the definition of an urgent safety measure should be notified within 72 hours, and all other significant safety issues should be notified within 15 calendar days

Definitions:

Significant safety issues are issues that adversely affect the safety of participants or materially impact on the continued ethical acceptability or conduct of the trial. These may require an urgent safety measure, an amendment, a temporary halt or an early termination of a trial.

Urgent Safety Measures (USMs) are significant safety issues where sponsors or trial investigators act immediately to protect participants from an immediate hazard to their health and safety.

7.7. Administrative items

This study protocol is to be conducted in accordance with the applicable Good Clinical Practice (GCP) regulations and guidelines (e.g., the International Conference on Harmonisation (ICH) Guideline on GCP).

All safety monitoring and reporting will be conducted according to the NHMRC 'Safety monitoring and reporting in clinical trials involving therapeutic goods" handbook.

8. Statistical Analysis

8.1. Statistical methods

A separate statistical analysis plan will be developed and finalised prior to study unmasking.

Data relating to participant demographics and baseline clinical characteristics will be analysed with a repeated measures model or ordered logistic regression model, as appropriate. For the primary outcome, analyses will be performed on data from right eyes. For slit lamp examination, analyses will be performed on the average of data from both eyes. For neurophysiological data, analyses will be performed on data from one limb only.

8.1.1. Populations to be analysed

The statistical analysis will follow an intention-to-treat approach (i.e., will include data from all participants randomised into the study, regardless of whether they received the study intervention). This approach preserves the prognostic balance in the study arms achieved by randomisation.

8.1.2. Handling of missing data

The proposed statistical approach provides valid inference in the presence of missing data if the missing data mechanism is ignorable (missing completely at random or missing at random) and includes participants who have a baseline and at least one post-baseline measurement of the primary outcome measure (CNFL). Missing values will be checked and reported across treatment arms and follow-up time points.

8.1.3. Methods of analysis

The primary outcome measure is the change in central corneal nerve fibre length (CNFL) from baseline (Day 1); the primary analysis endpoint is Day 180 (six months).

A range of secondary outcome measures, including safety outcomes, will be investigated (as detailed in Section 5.1).

For continuous outcome measures, a repeated measures model will be used to assess for differences in the post-treatment means between groups (i.e., the main effect of group) at each time point, with the baseline (Day 1) values used as a covariate. For binary and categorical outcome measures, an ordered logistical regression model will be used. For all analyses, an alpha level of 0.05 will be adopted for statistical significance. Unless otherwise specified, data will be expressed as mean plus/minus standard deviation.

8.2. Interim analysis

We do not intend to undertake interim data analyses, unless there is a concern in relation to safety. In this case, an independent statistician will perform the analyses, and consult with the medical monitor, who will make a judgment with regard to the termination of the trial.

9. Ethics and dissemination

9.1. Compliance with informed consent regulations

Written informed consent must be obtained from each potential participant prior to any study-related activities or procedures in the study. Consent will be obtained by a named study investigator on this project.

9.2. Compliance with human research ethics committee (HREC) regulations

This study is to be conducted in accordance with HREC regulations or applicable IEC regulations, in full conformance with principles of the "Declaration of Helsinki". The investigator must obtain approval from a properly constituted HREC prior to initiating the study and re-approval or review at least annually.

9.3. Compliance with Good Clinical Practice (GCP)

This protocol is to be conducted in accordance with the applicable GCP regulations and guidelines.

9.4. Protocol amendments

This study will be conducted in compliance with the current version of the protocol. Any modifications made to the protocol that may affect the scientific intent, study design, participant safety or participant's willingness to continue participation will be written as an amendment to the current protocol, and will be submitted to the HREC for approval prior to becoming effective.

9.5. Participant confidentiality

Participant confidentiality will be strictly held in trust by the study investigators and all associated research staff. This confidentiality extends to all biological samples and all clinical information collected relating to participants. The study protocol, documentation and all data generated as part of this study will be held in strict confidence. No information concerning the study or the data collected will be released to any unauthorised third party.

All laboratory specimens, evaluation forms or any other reports that leave the study sites will be identified only by the participant identification number to maintain participant confidentiality. Clinical information will not be released without the permission of the participant, except as necessary for monitoring by HREC or other regulatory agencies.

Any publications of the study results will not include identifiable information about any participants. Appropriate precautions will be taken to maintain confidentiality of medical records and personal information.

9.6. Declaration of Interests

No declarations of interests are reported by any of the participating investigators or study personnel.

9.7. Dissemination policy

A study summary to participants will be made available at the conclusion of the study. Participants will also be advised that they are welcome to phone or email the researchers if they are interested in receiving more information about any aspect of the research program.

Participants can request a copy of their individual study findings from the investigators; this information can either be provided verbally on the day, or in the form of a written report.

The findings from this study will be published as part of Alexis Ceecee Zhang's PhD thesis and are also expected to be presented at relevant scientific meetings and/or submitted for publication to refereed journals. For the purpose of dissemination of the research results, all participants will be non-identifiable, as data are collated and averaged with the data of other participants in each group.

The full protocol, participant level dataset and statistical codes formed as part of this study will not be available for public access.

10. Study visit schedule and procedures

10.1. Overview of entry procedures

Prospective participants, as defined by the inclusion and exclusion criteria, will be considered for enrollment into this study.

10.2. Informed consent and privacy

After the study is discussed with the potential participant, a person wishing to participate must provide written informed consent prior to any study-related procedure being undertaken. Each potential participant who provides informed consent will be assigned a participant ID code that will be used on all documentation and labelling related to this study. Eligible participants will also be assigned a randomisation number that will be used to assign treatment throughout the study.

After informed consent is obtained at Visit 1 and before randomisation into the study occurs, all pre-treatment adverse events will be documented.

10.3. Treatment period

Participants entering the study product treatment period must continue to meet all inclusion and exclusion criteria.

10.4. Visits and associated procedures

Clinical evaluations are intended to be performed by the same clinician throughout the study, whenever possible. If it is not possible to use the same evaluator to follow the patient, then evaluations should ideally overlap (i.e., examine the patient together and discuss findings) for at least one visit.

<u>Visit 1 (including Screening), The University of Melbourne, 2 hours</u>

The following procedures will be performed at Visit 1

- Written informed consent
- Demographic information
- Medical and ophthalmic histories
- Inclusion/exclusion criteria assessment
- Pre-treatment adverse event assessment
- Diet questioning
- Urine pregnancy test (for females of childbearing potential only)
- Habitual/ best corrected visual acuity
- MNSI questionnaire
- OSDI questionnaire
- EQ-5D-5L questionnaire
- iMTA questionnaire
- Tear osmolarity
- Basal (non-stimulated) tear collection
- Non-contact aesthesiometry

- Slit lamp assessment
- Anterior chamber angle assessment
- TBUT
- Ocular surface staining (Oxford scale)
- IOP (with anaesthesia and fluorescein using a Goldmann tonometer)
- Corneal confocal microscopy (with anaesthesia)
- Dilated fundus examination (with mydriatic eye drops) and non-invasive retinal imaging
- Pin-prick blood sample for omega-3 fatty acid analysis
- Re-check all eligibility requirements
- Dispense masked study products (if the participant meets the required qualifications)

Randomised participants will be provided with a supplement intake log (to mark off when they consume their supplements each day), given instructions with regard to how to take their supplements and be advised about the dose regimen to be followed. The supplement label (which specifies details with regard to: optimal storage conditions, clinical trial use only, keep out of reach of children, etc.) should also be read through and explained with the participant.

Participants will be asked to commence taking oral supplements (Day 1) on the day that their baseline nerve functions tests are conducted at Visit 2.

Visit 2 (Day 1; St Vincent's Hospital, 2 hours)

This visit will assess peripheral nerve fibre function, as per routine nerve conduction examinations, for diabetic neuropathy. The following procedures will be performed at this visit:

- Brief medical history (including related neuropathy symptoms)
- Michigan Diabetic Neuropathy Score
- Cutaneous silent period testing
- Quantitative sudomotor axonal reflex testing
- Nerve conduction studies
- Nerve excitability tests
- Blood samples

Visit 3 (23 to 37 days, The University of Melbourne, 1 hour)

The following procedures will be performed at Visit 3:

- Adverse event assessment
- Diet questioning
- Medical and ophthalmic histories
- Habitual/best corrected visual acuity
- Slit lamp examination

Visit 4 (76 to 104 days, The University of Melbourne, 1 hour)

The following procedures will be performed at Visit 4:

- Adverse event assessment
- Diet questioning
- Medical and ophthalmic histories

- OSDI questionnaire
- Habitual/best corrected visual acuity
- Non-contact aesthesiometry
- Slit-lamp examination
- TBUT
- Ocular surface staining (Oxford scale)
- Corneal confocal microscopy (with anaesthesia)
- Dispense of masked study product

Visit 5 (156 to 204 days, St Vincent's Hospital Melbourne, 2 hours)

The following procedures will be performed at Visit 5:

- Brief medical history (including related neuropathy symptoms)
- Michigan Diabetic Neuropathy Score
- Cutaneous silent period testing
- Quantitative sudomotor axonal reflex testing
- Nerve conduction studies
- Nerve excitability tests
- Blood samples

Visit 6 (156 to 204 days, The University of Melbourne, 2 hours) or Early exit

- Adverse event assessment
- Medical and ophthalmic histories
- Diet questioning
- Urine pregnancy test (for females of childbearing potential only)
- Habitual/ best corrected visual acuity
- MNSI questionnaire
- OSDI questionnaire
- EQ-5D-5L questionnaire
- iMTA questionnaire
- Tear osmolarity
- Basal (non-stimulated) tear collection
- Non-contact aesthesiometry
- Slit lamp examination
- Anterior chamber angle assessment
- TBUT
- Ocular surface staining (Oxford scale)
- IOP (with anaesthesia and fluorescein using a Goldmann tonometer)
- Corneal confocal microscopy (with anaesthesia)
- Dilated fundus examination (with mydriatic eye drops) and non-invasive retinal imaging
- Pin-prick blood sample
- Collection of unused study supplements

Unscheduled visits

Unscheduled visits may be arranged, as necessary, to ensure the safety and well-being of participants during the study. Source documentation should be completed for all parameters

measured for each unscheduled visit. If the unscheduled visit is an exit visit, then all exit visit procedure data should be captured and the Visit 6 CRF should be completed.

At a minimum, the following procedures should be performed at an unscheduled visit:

- Adverse event assessment
- Concomitant medication assessment
- Concurrent procedures assessment
- BCVA
- Slit lamp biomicroscopy

All other procedures should be performed on indication, as indicated by the participant's presenting symptoms and signs.

10.5. Instructions to eligible participants

Eligible participants will be asked to attend a total of 6 study visits; four visits will be at the University of Melbourne, and two will be performed at St Vincent's Hospital (at the start and end of the supplementation period), within four weeks of the initial and final visits.

At Visit 1, eligible participants will be dispensed study products consisting of their randomised treatment allocation. Study investigators will counsel the participant to consume 2 capsules each day, with or without food, at least two hours before or after medications. Participants will be provided with a supplement log to document their consumption of capsules each day. Participants will be advised not to 'make up' for missed doses with additional capsules on a given day; a reason for a 'missed dose' should be documented on the supplement log, which will be returned along with any remaining capsules at Visits 3, 4 and 6.

An appointment will be arranged for participants within four weeks of Visit 1, to attend Visit 2 at St Vincent's Hospital, Melbourne. Participants will be asked to start their first dose on the day of Visit 2 (Day 1), following their assessments at St Vincent's Hospital. Participants will be advised that they should maintain their regular dietary habits and medications (including ocular and systemic) and notify us of any change in medications of dosage throughout the study.

Appointments for Visits 2 and 5 at St Vincent's Hospital will be arranged for the participants by the study investigators, at Visits 1 and 4 respectively.

Participants will be encouraged to contact the Principal Investigator at any time should they have any questions about the study or the instructions they have been given.

10.6. Compliance with the protocol

If a participant is inadvertently enrolled with significant deviation(s) from the specified inclusion and exclusion criteria, he/she may be discontinued from the study at the discretion of the Principal Investigator.

Participants will be asked at Visits 3, 4 and 6 whether they have had any changes to their medications (including dosage) since their last study visit. To ensure compliance with usage of study product after randomisation, participants will be queried about their study product usage. The examiner will ensure that, based upon how the participant answers such questions, that the participant has been compliant with the protocol study product usage instructions.

Participants will be scheduled for follow-up visits and these will be timed to occur as close as possible to the day specified in the visit schedule. If a patient is unable to come to their next scheduled follow-up visit within the visit tolerance window for that visit, every effort will be made to reschedule the visit as soon as possible. If a patient is absolutely unable to come in for that visit, then that visit will be considered a missed visit.

10.7. Early discontinuation of participants

Participants may voluntarily withdraw from the study at any time. If a participant decides to voluntarily withdraw from the study and he/she alerts a member of the study personnel by phone, the participant will be asked to fill in a form of withdrawal and return to the site for an early exit (equivalent to Visit 6) assessment.

Upon early discontinuation, participants will be asked to return all unused study product. If it is determined during a study visit that a patient should be discontinued from the study early for safety or other reasons, the study investigators must immediately consult the principal investigator to discuss this matter. Whenever possible, all early exit procedures and assessments will be conducted.

10.8. Withdrawal criteria

A participant can be withdrawn from the study if he/she is not able or willing to use the study product in compliance with the protocol; a female of child-bearing potential becomes pregnant; or, if he/she is not willing to comply with the required study procedures at each visit.

Withdrawal of a participant **must** be discussed with the principal investigator, who will directly advise the participant of their need to withdraw. A 'Form of Withdrawal" must be completed by the participant indicating their intent to withdraw from the study.

11. Examination procedures, tests, equipment and techniques

11.1. Ophthalmic Examinations and fatty acid analysis (University of Melbourne)

11.1.1. Examination room

All study visits will be undertaken in a quiet, ophthalmic examination room that is maintained at an average temperature of 20 ± 5^0C, and humidity of 55 ± 5%. Full room lighting is to be maintained for all procedures except for the examination of ocular surface staining, tear-break-up-time (TBUT) with sodium fluorescein and retinal fundus photographs.

11.1.2. Medical and ophthalmic histories and adverse events

At Visit 1, the study investigator will take a thorough medical and ophthalmic history. All current medications (including prescription and over-the-counter products) will be recorded on the appropriate CRF. Ophthalmic and general surgical histories will be documented. General medical conditions and current medications will be carefully checked against the study exclusion criteria (see Section 2.4.4). All known allergies will be documented, with an indication regarding the severity of the allergic reaction (if known).

At Visits 3, 4 and 6, the study investigator will ask the participant with regard to whether their general health or medications have changed, in any way, since the previous visit (this includes dosages/form of medications). Any procedures that the participant has undergone (e.g., an X-ray) will be documented on the appropriate CRF. Adverse events and serious adverse events will be documented as detailed in Section 7.3.1).

11.1.3. Diet questioning

At Visits 1, 3, 4 and 6, the study investigator will ask participants about their prior month intake of foods that are known to be naturally high in omega-3 essential fatty acids, EFAs (i.e., specific fish, oils and nuts). This information will be used to approximately assay the natural dietary intake of omega-3 EFAs.

11.1.4. Pregnancy test

At Visits 1 and 6, females of childbearing potential (see Section 2.4.4 for definition of 'childbearing potential' as applicable to this study) will undergo a urine pregnancy test, which will be performed by the examiner.

This procedure involves the (potential) participant providing a urine sample (approximately 20mL volume) into a collection cup, in the privacy of the clinic toilet. The participant will be advised to leave their urine sample on the bathroom bench and to return to the clinical examination room. The examiner will then immediately test the urine sample by following the directions provided in a commercially available urine pregnancy test. Medical examination gloves should be worn by the examiner when handling the sample and performing the pregnancy test with the participant's urine sample. Upon completion of the pregnancy test, the urine sample should be immediately disposed of via toilet flushing. The collection cup should also be disposed of.

A potential participant must have a negative pregnancy test result in order to be eligible to participate in this study. In the event that a pregnancy test result is returned as 'positive', the researcher will recommend that the potential participant consult their GP (or another medical

doctor if they do not have a GP). A letter will be provided to the participant to take to their doctor. The investigator will advise the patient that the doctor will be able to perform additional testing to confirm or refute whether they are pregnant and to facilitate appropriate medical care. The study optometrist will offer the patient the option of helping them facilitate this appointment with their chosen medical practitioner if required.

11.1.5. Habitual/best corrected visual acuity

At each visit, the habitual visual acuity will be measured using a Snellen visual acuity chart for testing at 3 metres. If the habitual visual acuity in each eye at Visit 1 is equal to or better than 6/12, this correction will be used for ALL study visits. If the habitual visual acuity in either eye is worse than 6/12, subjective refraction will be performed at Visit 1 to obtain the best spectacle corrected visual acuity (BSCVA) in each eye. If this is equal to or better than 6/12, provided that the participant meets all other requirements, participant will be enrolled into the study. If the BSCVA in either eye is worse than 6/12, the participant will not be able to participate in the study. The BSCVA will be recorded on the participant's CRF.

To provide standardised assessments of visual acuity during the study, all assessments should be consistently carried out using the same lighting conditions during the entire study (i.e., under normal consulting room lighting conditions such that the chart is evenly lit without glare).

The right eye will be tested first, with the left eye occluded by the examiner. The patient will be asked to start reading from the 6/24 line and to read the letters from left to right on each line; the patient should be informed that the chart contains letters only and no numbers. The patient should be asked to read slowly to achieve best identification of each letter and not to proceed to the next letter until he/she has given a definite answer.

If the patient changes a response (e.g., "that was a C not an O") before he/she has read aloud the next letter, then the change must be accepted. If the patient changes a response after having read the next letter, then the change will not be accepted. The examiner must not point to specific letters during the test.

When the letters become difficult to read, or if the patient identifies a letter as one of 2 letters, he/she should be asked to choose one letter and, if necessary, to guess. Patients should be encouraged to continue trying even when letters become difficult to read. Visual acuity testing should stop when 3 or more errors are made on the same line.

The left eye will then be tested using the same procedures outlined above.

11.1.6. EQ-5D-5L questionnaire

At Visits 1, 3, 4, and 6, participants will complete the EQ-5D-5L questionnaire (see below). The two-part questionnaire is a standardised measure of health status that provides a measure of health for clinical and economic appraisal (Herdman *et al.*, 2011).

The first part is a descriptive system comprises 5 domains: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each domain will be rated by the participant as one of five levels, from 1 (no problem) to 5 (extreme problems), for quantifying their general health state. Only one response will be permitted per domain. A five-digit code, generated from the ratings (e.g., 11111), will be converted into an index value using the 'EQ-5D-5L Crosswalk Index Value Calculator' using the value set developed for the United Kingdom (available from

https://euroqol.org/eq-5d-instruments/eq-5d-5l-about/valuation-standard-value-sets/crosswalk-index-value-calculator/).

The second part of the questionnaire is a visual analogue scale ranging from 0 (the worst health you can imagine) to 100 (the best health you can imagine). Participants are asked to rate their health at the present moment (i.e., on the day of testing) by marking an 'X' on the scale and writing the number they have marked in the corresponding box.

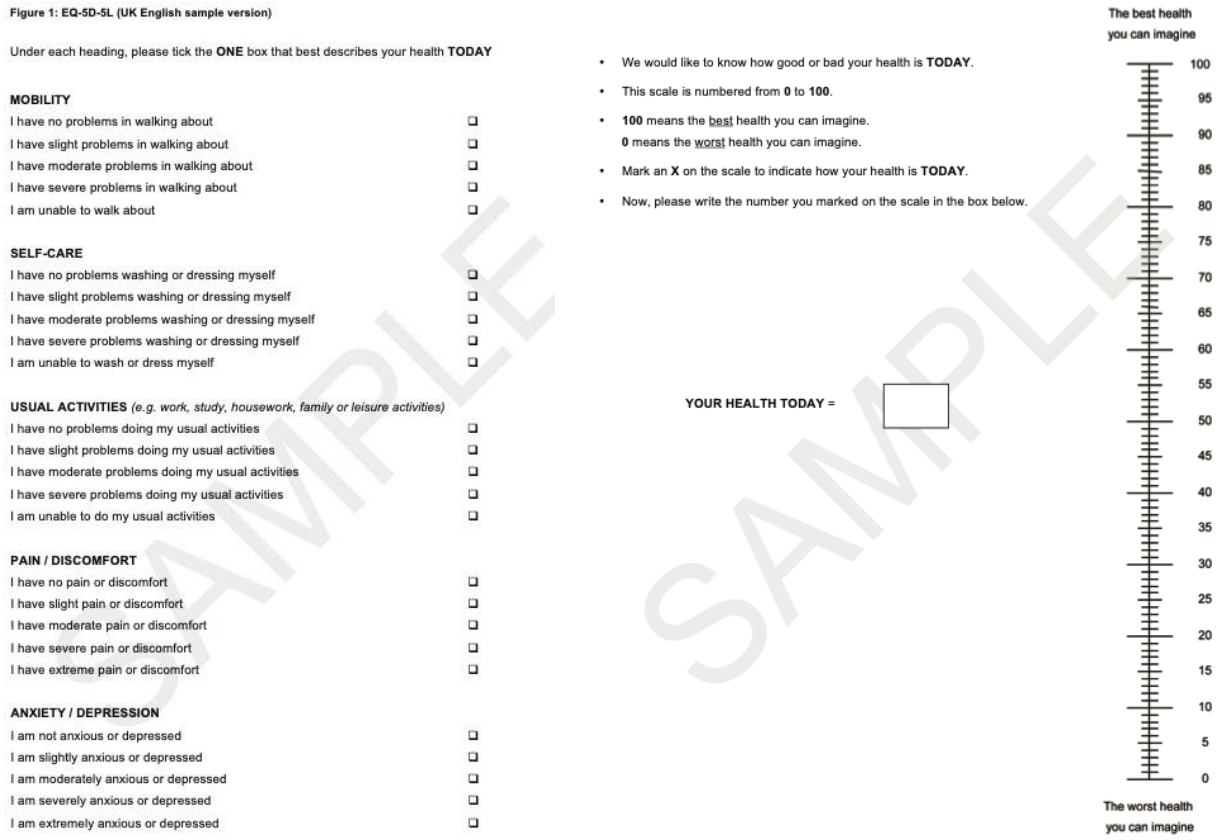


Figure 1: EQ-5D-5L (UK English sample version)

Under each heading, please tick the **ONE** box that best describes your health **TODAY**

MOBILITY
- I have no problems in walking about ❑
- I have slight problems in walking about ❑
- I have moderate problems in walking about ❑
- I have severe problems in walking about ❑
- I am unable to walk about ❑

SELF-CARE
- I have no problems washing or dressing myself ❑
- I have slight problems washing or dressing myself ❑
- I have moderate problems washing or dressing myself ❑
- I have severe problems washing or dressing myself ❑
- I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*
- I have no problems doing my usual activities ❑
- I have slight problems doing my usual activities ❑
- I have moderate problems doing my usual activities ❑
- I have severe problems doing my usual activities ❑
- I am unable to do my usual activities ❑

PAIN / DISCOMFORT
- I have no pain or discomfort ❑
- I have slight pain or discomfort ❑
- I have moderate pain or discomfort ❑
- I have severe pain or discomfort ❑
- I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION
- I am not anxious or depressed ❑
- I am slightly anxious or depressed ❑
- I am moderately anxious or depressed ❑
- I am severely anxious or depressed ❑
- I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is **TODAY**.
- This scale is numbered from **0** to **100**.
- **100** means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an **X** on the scale to indicate how your health is **TODAY**.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐

The best health you can imagine
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
The worst health you can imagine

11.1.7. Productivity costs questionnaire

At Visits 1 and 6, participants will complete the productivity costs questionnaire developed by the Institute for Medical Technology Assessment (iMTA) (Erasmus Universiteit Rotterdam, Rotterdam, 2013). This questionnaire estimates the indirect healthcare costs outside of the healthcare system that is associated with productivity losses (Bouwmans *et al.*, 2015). The questionnaire will be administered in writing by the participant. The questionnaire includes general questions about the participant's education attainment and current work status and questions about productivity loss.

Productivity losses will be valuated according to the iProductivity Cost Questionnaire (iPCQ) manual (https://www.imta.nl/questionnaires/). Scoring will be performed for the following modules: i) absenteeism; ii) presenteeism; iii) productivity losses due to unpaid work. Absenteeism will be scored based on the number of days a participant has missed work in the last four weeks as a result of being sick. If long-term absences were noted, defined as absences from work of longer than four weeks, absenteeism will be scored using the friction-cost method as described in the questionnaire scoring manual. Presenteeism will be calculated as (Number

of workdays impaired) times by (1 – (efficiency score/10)]) times by the number of hours per workday for that participant. Lost productivity due to unpaid work will be calculated as (the number if days missed) multiplied by (the number of house help was needed per day to make up the work).

11.1.8. Ocular surface disease index (OSDI) questionnaire

At Visits 1, 4 and 6, participants will complete an OSDI questionnaire (see below). The purpose of this assessment is to capture a range of ocular surface symptoms, including symptoms related to dry eye, their severity, and their impact on the patient's ability to function, scaled into a 0 (no disease) to 100 (maximum severity of disease) score. The participant's OSDI score will be calculated as (the sum of scores for all questions answered × 100) divided by (total number of questions answered × 4).

Have you experienced any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light? . .	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Have problems with your eyes limited you in performing any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Have your eyes felt uncomfortable in any of the following situations *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned? . . .	4	3	2	1	0	N/A

OSDI questionnaire

11.1.9. Tear osmolarity

At Visits 1 and 6 tear osmolarity will be assessed. Following appropriate monthly (hyperosmolar control solutions) and daily (electronic test cards) calibration (as recommended by the manufacturer) of the measurement pen labelled '2', tear osmolarity will be measured at each visit in each eye. A 50 nanolitre tear sample will be taken from the inferior temporal tear meniscus using the TearLab™ Osmolarity System (TearLab™ Corp., San Diego, CA). Measurements will be taken once in each eye, beginning with the right eye and followed by the left eye. To avoid contaminating factors, participants will be instructed to not have instilled any eye drops at least two hours to their appointment.

If the testing card touches any eye lashes, the testing card will be discarded, and the measurement will be repeated in that eye. Tear osmolarity for both eyes will be recorded. For analysis, the tear osmolarity value used will be the higher value of the two eyes, taken from one eye per participant.

11.1.10. Non-contact corneal aesthesiometry

At Visits 1, 4 and 6, corneal sensitivity will be measured using a non-contact corneal aesthesiometer (NCCA). Both room temperature and cooled air will be used to stimulate nerves with different sensory receptors in different locations of the cornea. The instrument will be positioned to fire a small stream of air, with a predetermined pressure (measured in mBar) as set by the examiner, from 1 cm away from the cornea. Both central cornea (i.e., half way between the temporal and nasal limbus) and inferior peripheral cornea (i.e., at 1mm above the most inferior limbus point) will be measured.

The participant will be seated comfortably on the slit lamp biomicroscope, where the instrument will be mounted, and asked to wear over-the-ear headphones, which will transmit a series of auditory cues to run each trial. The participant will also be given a small number pad with tactile pads to register their response to detecting a stimulus (i.e., yes and no).

The auditory cue consists of a short beep (0.5s) followed by a brief break (2s) and a long beep (4 seconds). For each individual trial, participants will be instructed that they should blink as frequently as they wish when they hear the first beep, but to stop blinking as soon as they hear the start of the second (i.e., long) beep as they may or may not feel a small stream of air during that time. The audio cue will be terminated by a double beep to indicate the end of the trial; after hearing this cue, the participant should register their response (i.e., yes or no) on the keypad.

The sensory threshold will be determined using a double-staircase threshold technique designed using a customised MATLab software. Trials will be repeated at discrete increments of 0.1 mbar for three reversals, until a final threshold can be calculated.

If participants respond 'yes' to two stimuli at 0.1 mbar, false positives will be checked by the examiner by initiating the testing sequence, but not releasing a stimuli. If participants respond 'yes' to two false positive checks, testing will stop, and the participants will be reinstructed. After instructions are given to the participant, testing will re-start from the beginning of the double staircase algorithm for that measurement.

Corneal sensitivity thresholds will be measured for the central and peripheral cornea to both room and cooled stimuli (4 measurements). For the cooled air setting, the instrument cooling will be turned on for three minutes prior to re-initiation of testing, as per manufacturer's instructions.

11.1.11. Basal tear sample collection

At Visits 1 and 6, tear samples will be collected using disposable 20µL glass microcapillary tubes (Drummond Scientific Co, USA). The participant will lie in a lateral decubitus position on a medical examination table. A 10µL basal (non-stimulated) tear sample will be collected via capillary flow from each eye, from the lateral tear meniscus, with the lateral canthus gently opposed. Tear samples will be collected from the right eye first, followed by the left eye. To minimise any potential reflex tearing, utmost care will be taken not to directly contact the conjunctiva, cornea or eyelid margins with the micro-capillary tube during tear collection. Tear collection flow rate will be monitored for each sample collected. Any samples with a flow rate greater than 10µL/min will be discarded to exclude dilution effects caused by reflex tearing. The collected tear samples will be immediately transferred to appropriately-labelled 50µL Eppendorf vials (Eppendorf, Westbury, NY) and stored at 4^0C. Within four hours of collection,

the vials will be securely stored, in locked facilities, at -80^{0}C until required for multiplex-CBA analysis.

Note: All Eppendorf vials will be double-labelled (with permanent marker on the cap of the vial and with a paper/sticky-tape label on the vial, according to the pre-defined labelling schema).

11.1.12. Phenol red thread test

At Visits 1 and 6, phenol red thread test will be performed as a measure of resting tear volume. The phenol red thread (Entol Research Cell, London, UK) will be gently folded and removed by the examiner from the sterile packaging without touching the thread. The folded end will be hooked in the participant's inferior lid by gently pulling down the inferior lid margin, approximately a third of the way from the temporal canthus. After exactly 15 seconds, as timed by a stopwatch, the thread will be removed, and the wetted length will be measured immediately using the ruler supplied on the packaging and recorded. Examination will be performed in both eyes; the right eye will be assessed first.

11.1.13. Assessment of anterior chamber angle

At Visits 1 and 6, the anterior chamber angle will be assessed using the Van Herick technique to estimate the depth of the angle, before instillation of the mydriatic (0.5% tropicamide) eye drops. This assessment will be performed at the slit lamp using a single optic section projected onto the peripheral cornea at 16X magnification, using the brightest illumination.

The peripheral anterior chamber depth will be estimated against the peripheral corneal depth to obtain a subjective estimation of the depth of the peripheral anterior chamber angle.

11.1.14. Slit lamp biomicroscopy examination

At each visit, slit lamp biomicroscopy (without pupil dilation) will be performed on both eyes, with the room lights off. Observations for the slit lamp biomicroscopy examination (using white light and 10-16x magnification) will be graded using the standardised Efron grading scale for:

- Conjunctiva: nasal and temporal redness
- Limbal redness
- MG dysfunction: extent of capping
- Blepharitis (anterior)

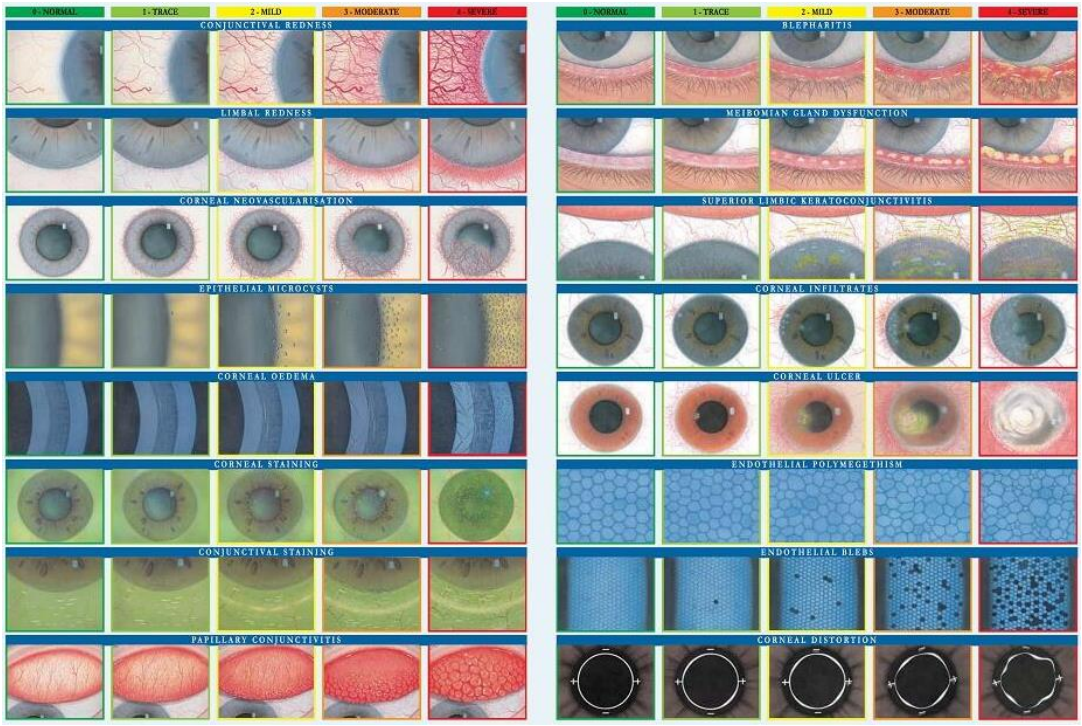
Grades range from 0 to 4, and will be recorded in 0.1 step increments. Observations with regard to clinically notable findings will be recorded for the conjunctiva, cornea, and tear film.

The presence of anterior chamber inflammation will be evaluated using a maximum intensity white light, under 25X magnification and a 1x1mm slit beam. The presence of cells and flare will be graded using the Standardization of Uveitis Nomenclature (SUN) grading scales (as per the grading scheme below).

SUN Grading Scheme for Anterior Chamber Cells	
Grade	**Cells in 1mm x 1 mm slit lamp beam**
0	<1
0.5+	1-5
1+	6-15
2+	16-25
3+	26-50
4+	>50

SUN Grading Scheme for Anterior Chamber Flare	
Grade	**Description**
0	None
1+	Faint
2+	Moderate (Iris and lens details clear)
3+	Marked (Iris and lens details hazy)
4+	Intense (Fibrin or plastic aqueous)

SUN Grading Schemes for Anterior Chamber Cells and Flare (Jabs *et al.*, 2005)



Efron anterior eye grading scale

11.1.15. Tear break up time (TBUT) with fluorescein

At Visits 1, 4 and 6, TBUT with fluorescein will be measured in both eyes. The sodium fluorescein strips (Amcon dry eye tests, DET Nomax Inc., St Louis MO) will be moistened with non-preserved saline and applied onto the superior bulbar conjunctiva with the upper eyelid retracted and the patient in downgaze to deliver an approximate fluid volume of 1µL to each eye. The NaFL strip will not be shaken after wetting with saline. A single instillation of sodium fluorescein should be used for both TBUT and corneal staining.

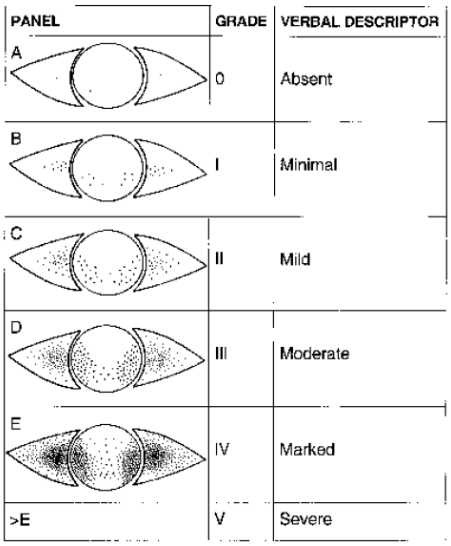
TBUT will be performed with a slit lamp at 10X magnification using a cobalt blue illumination and a yellow barrier filter (Wratten filter) to enhance contrast, set at the highest illumination intensity. Participants will be asked to first blink normally 2 to 3 times to evenly spread the

fluorescein then to not blink for as long as they can. The timing of the stopwatch will begin as soon as they start holding the blink. The stopwatch is stopped on the first occurrence of true tear break-up, not just local thinning or tear film irregularity. Three consecutive TBUTs will be performed per eye and the time in seconds for each of the three measurements will be recorded (to two decimal places).

11.1.16. Sodium fluorescein (NaFl) staining – Oxford scale

At each visit, NaFl staining of the cornea will be evaluated in each eye. Sodium fluorescein staining will be evaluated using a slit lamp with full slit width and 16X magnification with cobalt blue illumination and a yellow barrier filter (Wratten filter) to enhance contrast. The yellow barrier filter must be placed directly in front of the objective lens of the slit lamp. The slit lamp's light source must also be set to high intensity (increased voltage) when the cobalt blue and enhancement filters are in place.

NaFl staining should be graded two minutes after instillation of the sodium fluorescein, during which time TBUT is measured. Corneal staining will be assessed using the 5-point Oxford scale (see below), ranging from 0 to 5, in 0.1 grading steps.



Oxford grading scale

11.1.17. Intra-ocular pressure (IOP)

At Visits 1 and 6, intraocular pressure (with anaesthesia and fluorescein) will be measured for each eye. One drop of 0.5% proxymetacaine hydrochloride (Alcon Laboratories, French Forest, NSW, Australia) will be instilled into the lower conjunctival sac. After exactly four minutes, the participant should gently close their eyes and any excess fluid should be gently dabbed away from the eyelids using a tissue. Direct, active blotting of the *cul de sac* is not permitted.

Measurements should be taken using a calibrated Goldmann applanation tonometer. Additional sodium fluorescein can be applied if needed. One measurement should be recorded for each eye (in mmHg); the time of measurement should also be noted.

11.1.18. Corneal confocal microscopy

At Visits 1, 4 and 6, corneal confocal microscopy will be performed to capture *in-vivo* images of the corneal sub-basal nerve plexus, using a Heidelberg Retinal Tomograph III with the Rostock Corneal Module (Heidelberg Engineering GmbH, Heidelberg, Germany). Topical anaesthesia will be instilled using the methods described above. A high-viscous eye gel (GenTealEyes. Novartis, North Ryde. NSW, Australia) will couple a single-use, sterile polymethylmethacrylate 'Tomocap' to the microscope objective lens.

Participants will be asked to fixate on a central target with the non-dominant eye while the right eye (i.e., same eye tested as NCCA) is examined. A sufficient number of images will be captures using ''sequence" mode setting on the microscope of the central corneal region as a representation of that area. Participants will then be asked to fixate on a superior target while the inferior cornea (1mm above the inferior most limbus) will be imaged using the same methods as above.

Raw images will be saved on a secure server that is only accessible to the investigators. All captured images that are blurred, where the imaging depth was inconsistent, or where excessive vignette obstructs nerve visibility will be removed from the analysis sample.
For each participant, 12 images from the central cornea and eight images from the peripheral cornea will be randomly selected for analysis. Images will be checked by the investigator to ensure that any overlap is kept minimal (i.e., less than 20%).

Central corneal sub-basal nerve parameters will be quantified using an automatic image analysis software (ACCMetrics software v2, The University of Machester) (Dabbah *et al.*, 2011). Sub-basal nerve parameters for each participant will be taken as the average value of the twelve images analysed for the following corneal nerve parameters: corneal nerve fibre length (CNFL; mm/mm^2), corneal nerve fibre density (CNFD; $fibres/mm^2$), corneal nerve branch density (CNBD; branches on main $fibre/mm^2$) and corneal nerve total branch density (CTBD; total $branches/mm^2$).

For the peripheral cornea, images will be analysed using ImageJ software (ImageJ; U. S. National Institutes of Health, Bethesda, Maryland, USA; https://imagej.nih.gov/ij/). Each image will be manually traced using NeuronJ software (Meijering *et al.*, 2004). For each image, CNFL (mm/mm^2) will be derived as (The Sum of total nerve length) divided by 0.16. For each participant, the CNFL will be taken as the average value of the eight images analysed.

Central and peripheral dendritic cell count will be performed for each participant using the same set of randomly selected images as for corneal sub-basal nerve parameters. Dendritic cell count will be performed using the cell count plugin on ImageJ. For the central cornea, cell count will be stratified into three morphological phenotypes according to Lagali *et al*., (2018), as the following: i) type-1, putative mature dendritic cells (mDCs), as bright, reflective cells with extensions of long-dendrites; ii) type-2; putative immature dendritic cells (imDCs), as small, reflective bodies either without dendrites or with short dendrites, and iii) type-3; putative globular cells, as round or oval cells bodies, that are round and larger than DCs and without any visible dendrites (Lagali *et al.*, 2018). For the peripheral cornea, the total dendritic cell count per image will be quantified (without any putative subtype classification). The number of cells per meters squared ($cells/mm^2$) will be calculated as (the number of cells per image) divided by 0.16.

11.1.19. Systemic fatty acid pin-prick test

At Visits 1 and 6, a finger pin-prick test will be taken to obtain a small amount of blood samples for fatty acid analysis. Gloves will be worn by the examiner while performing the procedure. The side of the participant's finger will be lightly rubbed to stimulate blood flow and thoroughly rubbed with isopropyl alcohol. A trigger of the pin-prick device will be released to release a small, shallow lancet against the testing area to create a pin-prick puncture. 2-3 drops of blood will be released after gentle squeezing and mounted directly onto PUFAcoat paper supplied in the testing kit.

All test kits will be labelled with the participant's corresponding code, sealed and couriered to Waite Lipid Analytic Service (The University of Adelaide) for analysis within four weeks from collection date, as per manufacturer's instructions.

11.1.20. Diabetic retinopathy grading

At Visits 1 and 6, diabetic retinopathy will be assessed through a dilated pupil, and retinopathy stages will be graded using the simplified ETDRS (Wisconsin) classification below.

Retinopathy stage	**Definition**
Minimal NPDR (level 20)	Ma only
Mild NPDR (level 35)	Ma and one or more of: retinal haem, HEx, CWS, but not meeting Moderate NPDR definition
Moderate NPDR (levels 43, 47)	H/Ma ≥ std photo 2A in at least one quadrant and one or more of: CWS, VB, IRMA, but not meeting Severe NPDR definition
Severe NPDR *preproliferative* (level 50+)	Any of : H/Ma >std photo 2A in all four quadrants, IRMA >std photo 8A in one or more quadrants, VB in two or more quadrants
PDR (level 60+)	Any of: NVE or NVD <std photo 10A, vitreous/ preretinal haem and NVE <½ disc area (DA) without NVD
High-risk PDR (level 70+)	Any of: NVD>¼ to ⅓ disc area, or with vitreous/ preretinal haem, or NVE>½ DA with vitreous/ preretinal haem
Advanced PDR	High-risk PDR with tractional detachment involving macula or vitreous haem obscuring ability to grade NVD and NVE
Macular Oedema	Retinal thickening within 2 disc diameters of macular centre
Clinically Significant Macular Oedema (CSME)	Retinal thickening within 500µm of macular centre or hard exudates within 500µm of macular centre with adjacent thickening

Classification of diabetic retinopathy into retinopathy stages derived from NHMRC "Clinical Practice Guidelines for the Management of Diabetic Retinopathy"

In addition, non-invasive images will be taken of the retina, including retinal fundus photographs and optical coherence tomography (OCT) images, as a permanent record of posterior segment health. For retinal fundus photography, two 30-degree retinal fundus

photographs will be taken from each eye at macula and disc fixations. The following OCT images will be captured: 1) Two OCT-angiography scans of the central macula (3.0x3.0 mm) in the right eye only; 2) Two OCT-angiography scans of the optic nerve head (6.0x6.0 mm) in the right eye only; 3) One standard OCT scan of the optic nerve head in the right eye only; 4) One standard OCT scan of the central macula (horizontal) in both eyes.

11.2. Nerve fibre function testing (St Vincent's Hospital, Melbourne)

At Visits 2 and 5, detailed neurophysiological assessment of small and large peripheral nerve fibres will be performed. These testing procedures will involve:

11.2.1. Michigan Neuropathy Screening Index

The Michigan Neuropathy Screening Index (MNSI) will be administered by the investigator (see below). Participants must score >3 in the two-part screening instrument, across both part A and part B, to be eligible for inclusion in the study. The physical assessment component (Part B) will be performed by a trained examiner.

Patient Version

MICHIGAN NEUROPATHY SCREENING INSTRUMENT

A. History (To be completed by the person with diabetes)

Please take a few minutes to answer the following questions about the feeling in your legs and feet. Check yes or no based on how you usually feel. Thank you.

1.	Are you legs and/or feet numb?	☐ Yes	☐ No
2.	Do you ever have any burning pain in your legs and/or feet?	☐ Yes	☐ No
3.	Are your feet too sensitive to touch?	☐ Yes	☐ No
4.	Do you get muscle cramps in your legs and/or feet?	☐ Yes	☐ No
5.	Do you ever have any prickling feelings in your legs or feet?	☐ Yes	☐ No
6.	Does it hurt when the bed covers touch your skin?	☐ Yes	☐ No
7.	When you get into the tub or shower, are you able to tell the hot water from the cold water?	☐ Yes	☐ No
8.	Have you ever had an open sore on your foot?	☐ Yes	☐ No
9.	Has your doctor ever told you that you have diabetic neuropathy?	☐ Yes	☐ No
10.	Do you feel weak all over most of the time?	☐ Yes	☐ No
11.	Are your symptoms worse at night?	☐ Yes	☐ No
12.	Do your legs hurt when you walk?	☐ Yes	☐ No
13.	Are you able to sense your feet when you walk?	☐ Yes	☐ No
14.	Is the skin on your feet so dry that it cracks open?	☐ Yes	☐ No
15.	Have you ever had an amputation?	☐ Yes	☐ No

Total: _____

MICHIGAN NEUROPATHY SCREENING INSTRUMENT

B. Physical Assessment (To be completed by health professional)

1. Appearance of Feet

	Right	**Left**
a. Normal	☐ 0 Yes ☐ 1 No	Normal ☐ 0 Yes ☐ 1 No
b. If no, check all that apply:		If no, check all that apply:
Deformities	☐	Deformities ☐
Dry skin, callus	☐	Dry skin, callus ☐
Infection	☐	Infection ☐
Fissure	☐	Fissure ☐
Other	☐	Other ☐
specify: _____		specify: _____

	Right		**Left**	
	Absent	Present	Absent	Present
2. Ulceration	☐ 0	☐ 1	☐ 0	☐ 1

	Right			**Left**		
	Present	Present/ Reinforcement	Absent	Present	Present/ Reinforcement	Absent
3. Ankle Reflexes	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1
	Present	Decreased	Absent	Present	Decreased	Absent
4. Vibration perception at great toe	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1
5. Monofilament	Normal	Reduced	Absent	Normal	Reduced	Absent
	☐ 0	☐ 0.5	☐ 1	☐ 0	☐ 0.5	☐ 1

Signature:_____ Total Score _____ /10 Points

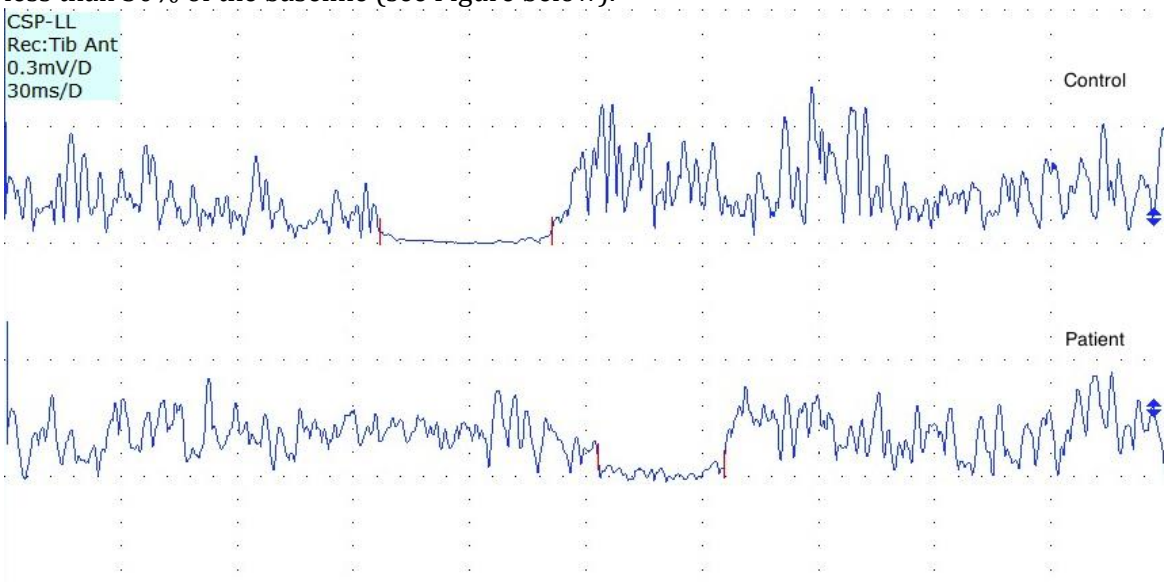
11.2.1 Michigan Diabetic Neuropathy Score (MDNS) and routine nerve conduction studies (NCS)

The first part of the MDNS is a clinical examination, which is then followed by routine nerve conduction studies. Vibratory threshold perception is assessed with a 128 Hz tuning-fork. Light touch is assessed with a 10g monofilament applied to the dorsum of the great toe. Deep tendon reflexes and muscle strength testing formed the remainder of the clinical examination score. Nerve conduction studies are then performed using an electromyographic (EMG) device (Dantec Keypoint G4 Workstation), examining the right upper and lower extremities, and included median, peroneal and tibial motor studies, and sural, median and ulnar sensory studies. Abnormal parameters are defined as amplitudes, conduction velocities or latencies that exceeded two standard deviations from the normal values of our laboratory. Testing is performed with a target temperature of 32 degrees Celsius in the upper extremity and 30 degrees Celsius in the lower extremity. Participants are given a composite score (from class 0 to 3) based on their clinical examination and nerve conduction study results.

11.2.2 Cutaneous Silent Periods (CSPs)

The CSP is recorded from both upper and both lower extremities using the same EMG device above. Filters are set at 20 Hz to 10 kHz, sweep speed is 300 ms, and sensitivity is 300 μV. In the upper extremities, electrical stimulations are delivered to the second digit using ring electrodes (median nerve). The sensory threshold is first determined by gradually increasing electrical current stimulations of 0.5 ms duration, and the participant indicated the lowest current intensity that is felt. Standard recording electrodes are placed with the recording electrode over the contracting abductor pollicis brevis (APB) muscle, and the reference electrode at the base of

the thumb. The patient abducts the thumb at steady sub-maximal contraction. The technician provides downwards resistance, using EMG sound to provide auditory feedback and semi-quantitatively ensure that contraction is approximately 50% of maximal. While the participant is maintaining this contraction, 10 consecutive electrical stimuli of 15 times the sensory threshold are delivered to the index finger. If the CSPs obtained are poorly formed or absent, and the patient does not perceive the stimuli as painful, then stimulus intensity is increased in 10 mA increments up to 100 mA. Traces are then averaged and rectified. The CSP onset latency is then quantitatively defined by the time after stimulation when the EMG signal drops below 50% of baseline, and the CSP duration is defined by the time period when the EMG signal was less than 50% of the baseline (see Figure below).



Top – Control: normal onset latency and duration
Bottom – Patient: abnormal prolonged onset latency and shortened duration

The process is repeated in the lower extremities in a similar manner, using a stimulating probe behind the lateral malleolus (sural nerve), with the recording electrode over the belly of tibialis anterior whilst the patient maintains steady ankle dorsiflexion.

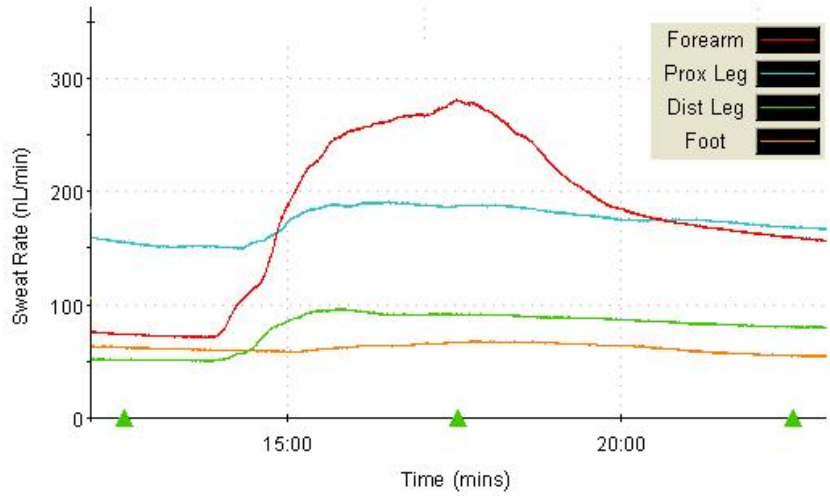
Reference values for CSP are taken from St Vincent's Hospital Melbourne reference values for small fibre testing, as below

	Onset (ms)	SD (ms)	**ULN (ms)**	Duration (ms)	SD (ms)	**LLN (ms)**
Upper extremity	72.9	7.9	**86.4**	51.1	9.6	**33.8**
Lower extremity	100.7	9.2	**118.7**	54.4	10.7	**38.3**

*n=50 (26 M, 24F). Reference limits are in **bold**.*

11.2.3 Quantitative Sudomotor Axonal Reflex Testing (QSART)

QSART is performed using the Q-Sweat unit (WR Medical Electronics Co., Stillwater, Minnesota), which uses a desiccant pack as its dry air source. Four multi-compartment sweat capsules are placed over standard recording sites on the right side at the forearm, proximal leg, distal leg and foot. A 10% acetylcholine gel is iontophoresed into the skin at a constant current of 2 mA for five minutes to stimulate postganglionic sudomotor unmyelinated nerves, and the sweat responses are assessed during this time plus an additional five minutes (see Figure below).



Example of abnormal reduced sweat volume from foot site.

Reference values for QSART are taken from St Vincent's Hospital Melbourne reference values for small fibre testing, as below

	Total vol (uL)	**LLN (uL)**	ULN (uL)	Latency	LLN (s)	ULN (s)
Forearm						
Men	2.0	**0.5**	3.7	102	28	191
Women	1.1	**0.3**	2.9	82	35	148
Prox Leg						
Men	1.6	**0.4**	3.9	107	47	197
Women	1.4	**0.4**	3.9	84	37	153
Distal Leg						
Men	1.9	**0.3**	3.3	95	24	190
Women	1.2	**0.4**	3.2	79	38	143
Foot						
Men	1.1	**0.2**	2.6	128	45	190
Women	0.8	**0.2**	1.5	116	42	353

*n=40 (20 M, 20F). Reference limits are in **bold**.*

11.2.4 Nerve Excitability Test

Sensory and motor axonal excitability studies are undertaken on the median nerve. Axonal excitability studies are performed using an automated computerized system (Qtrac© Institute of Neurology, Queens Square, UK). Stimulation is computer controlled and converted to current using an isolated linear bipolar constant current simulator (maximal output ± 50 mA; DS5, Digitimer, Welwyn Garden City, UK). The median nerve is stimulated at the wrist with an anode electrode placed 10 cm proximal over bone. Compound motor action potentials (CMAPs) are recorded from the abductor pollicis brevis muscle with the reference electrode placed 4 cm distal. Compound sensory action potentials (CSAPs) are recorded from the second digit, utilizing ring electrodes placed at the proximal and distal interphalangeal joints for recording and reference, respectively. Electronic noise is removed (via Hum Bug 50/60 Hz Noise Eliminator, Quest Scientific Instruments, North Vancouver, Canada). Temperature is monitored to ensure this is maintained above 32 degrees Celsius.

Multiple excitability parameters are recorded via the TROND protocol including: (i) threshold electrotonus (TE), a measure of inter-nodal conductances and membrane potential; (ii) recovery cycle (RC) of excitability, an assessment of the recovery of excitability following an action potential marking the function of nodal Na^+ channels and (iii) current–threshold

relationship (I/V), a measure of the rectifying properties of the axon. Target amplitude for threshold tracking is automatically set to approximately 40% of supramaximal response amplitude, utilizing the area of steepest slope of the stimulus response curve. Changes in the threshold current required achieving the target amplitude are tracked on-line, with the tracking steps proportional to the error between target amplitude and actual response.

11.3 Basic blood samples

Blood tests to assess diabetes control will be performed, including fasting blood sugar level and HbA1c. Patients that have any disorder other than diabetes that could cause symptoms or signs of peripheral neuropathy will be excluded from participating. Thus, participants will be tested for B12 levels, folate levels, lipid profile, inflammatory markers (CRP, ESR), liver function and electrolytes.

12 APPENDICES

12.2 Informed consent materials

Participant information sheet/consent form

12.3 Biological Specimens

The following biological specimen will be collected for this study

- Tear samples (as per section 11.1.10)
- Blood capillary samples (as per section 11.1.18)
- Basic blood samples (as per section 11.3)
- Urine samples (as per section 11.1.4)

At the time of collection, tubes containing the tear samples will be labelled with a participant ID number (assigned by the researchers) and the laterality (R or L eye). Similarly, PUFA-coated test cards with the blood spot assays will be labelled with a participant ID number. This method allows the privacy of the donor to be protected. This system also keeps the samples identity unknown to the people involved in the analyses of the samples

Females of childbearing potential will undergo a urine pregnancy test, to confirm their eligibility to participate, which will be performed by the study investigator. Upon completion of the pregnancy test, the urine sample and collection will be immediately disposed of.

All other samples (including tear samples and blood spot assay samples) will be destroyed once the research is completed. The samples will be disposed of according to standard laboratory practices in a manner that does not risk the confidentiality of the participants.

Blood samples will be collected for this study and can form part of the participant's routine care by St Vincent's Pathology. Testing will be performed by experienced personnel at St Vincent's Pathology, upon request with a pathology slip as provided by Prof Richard MacIsaac. Results collected as a part of this study will then be entered into the participants CRF in coded forms as required.

12.4 Abbreviation

UoM	The University of Melbourne
SVHM	St Vincents Hospital (Melbourne)
PUFA	Polyunsaturated Fatty Acids
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
CNFL	Corneal Nerve Fibre Length
CNFD	Corneal Nerve Fibre Density
CNBD	Corneal Nerve Branch Density
CDC	Corneal Dendritic Cell
IVCM	In-Vivo Confocal Microscopy
NCCA	Non-Contact Corneal Aesthesiometry
BCVA	Best Corrected Visual Acuity
NaFl	Sodium Fluorescein
TBUT	Tear Break-Up Time
IOP	Intraocular Pressure
OSDI	Ocular Surface Disease Index
OPAS	Ocular Pain Assessment Score
DFE	Dilated Fundus Examination
AACG	Acute Angle Closure Glaucoma
HbA1C	Glycated Hemoglobin
FBC	Full Blood Count
CRP	C-Reactive Protein
ESR	Erythrocyte Sedimentation Rate
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
MDNS	Michigan Diabetic Neuropathy Score
QSART	Quantitative Sudomotor Axonal Reflex Test
CSP	cutaneous silent period
NCS	Nerve Conduction Studies
QST	Quantitative Sensory Test
CPI	Coordinating Principal Investigator
AEs	Adverse Events
SAEs	Serious Adverse Events
SUSAR	Suspected Unexpected Serious Adverse Reaction
TGA	Therapeutics Goods Administration
WLAS	Waite Lipid Analytical Services
REDCap	Research Data Capture
CRF	Case Report Form

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Statistical Analysis Plan

"A randomised controlled trial to evaluate the effects of omega-3 fatty acid supplementation on peripheral nerve health in type-1 diabetes"

Version: 1.4

Authors:

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Date: <30-06-2020>

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1. Introduction

Primary objective

The aim of this randomised, double-masked, placebo-controlled superiority clinical trial is to evaluate the effects of a six-month supplementation period with long-chain omega-3 polyunsaturated fatty acid (PUFA) supplements on peripheral nerve parameters in individuals with type-1 diabetes.

Study design

This study is a single-centre, double-masked, randomised, two-arm, parallel-group interventional trial comparing the effects of oral omega-3 PUFA supplements relative to olive oil (placebo) supplements over six months. Participants are randomised according to a 1:1 allocation ratio to either long-chain omega-3 PUFA supplements (defined below) or an olive oil (placebo) supplement.

Study setting

This study is being conducted at the Department of Optometry and Vision Sciences at The University of Melbourne, with recruitment and data collection undertaken both at this site and the Departments of Endocrinology and Neurology at St Vincent's Hospital, Melbourne.

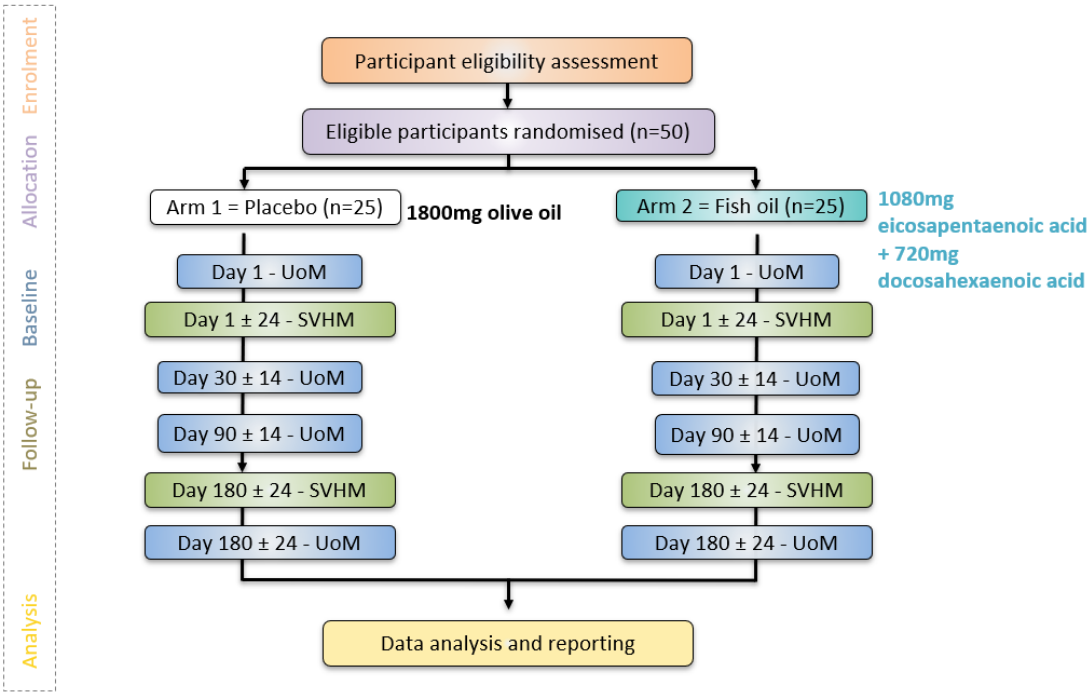
Participants

A total of 50 participants with type-1 diabetes mellitus were planned to be recruited. The first participant was enrolled on July 6, 2018; the last participant's last visit took place on March 10, 2020.

Given a two-arm parallel design, with a pre-specified primary outcome of corneal nerve fibre length (CNFL), taken as a continuous measure, for 80% power at a

confidence level of 95%, using an estimated true difference between intervention arms of 2.9 units, mm/mm^2, (Chinnery et al., 2017), based on an expected standard deviation of 3.2 units, the required sample size was determined to be 21 participants per group. To allow for 15% participant attrition, the sample size within each group was increased by this amount, giving a need to recruit a total of 25 participants in each intervention arm (n=50 for the trial; Figure 1). During the recruitment phase, the target sample size was reduced to 43 in total, in view of the timeline feasible for completing the study and having had fewer dropouts than anticipated.

Figure 1. Planned study flow diagram



Abbreviations: SVHM, St Vincent's Hospital Melbourne; UoM, University of Melbourne.

Data source

Following direct recording of data onto hard-copy source worksheets, the study data was entered into REDCap, a secure web-based application for research data capture, management and analysis (hosted on the University of Melbourne data centre infrastructure, provided via the National eResearch Collaboration tools and Resources project).

All data collected directly by the investigators for this study was de-identified using a participant ID code, and the participant ID code was used as an identifier on all study documentation, and for the labelling of body fluid samples (i.e., blood, tears), which are processed off-site.

The following data and information was to be recorded:

- Identifiable participant information at baseline: name; contact details; study enrolment date
- A statement that written informed consent (including the date) was obtained prior to any study procedures being performed
- Participant ID number
- Dates of all study visits
- Demographic information at baseline (age, sex, date of birth, ethnicity)
- Height and weight at baseline
- Medical and ophthalmic histories at baseline
- Pre-study and concomitant medications, including insulin type, administration method and dosage
- Study procedures performed at the University of Melbourne (UoM)
    - Habitual distance refractive correction in each eye at baseline.
    - Habitual distance visual acuity in each eye at baseline, day 30 $\pm$ 14, day 90 $\pm$ 14 and day 180 $\pm$ 24.
    - EQ-5D-5L questionnaire score at baseline, day 30 $\pm$ 14, day 90 $\pm$ 14 and day 180 $\pm$ 24.
    - Ocular Surface Disease Index (OSDI) questionnaire score at baseline, day 90 $\pm$ 14 and day 180 $\pm$ 24.
    - Tear osmolarity in each eye at baseline and day 180 $\pm$ 24.
    - Corneal sensitivity thresholds in the right eye at baseline, day 90 $\pm$ 14 and day 180 $\pm$ 24.
    - Phenol red thread test score in each eye at baseline, day 90 $\pm$ 14 and day 180 $\pm$ 24.

- Tear collection time and volume collected at baseline and day 180 ± 24.
- Slit lamp examination gradings in each eye at baseline, day 90 ± 14 and day 180 ± 24.
- Intraocular pressure (IOP) in each eye at baseline and day 180 ± 24.
- Diabetic retinopathy (DR) and diabetic macular oedema grading (Wisconsin classification) (ETDRS Research Group, 1991) in each eye at baseline and day 180 ± 24.
- Optical coherence tomography angiography (OCT-A) imaging at baseline and day 180 ± 24.
- Corneal sub-basal nerve plexus and dendritic cell parameters, measured using *in vivo* confocal microscopy (IVCM) in the right eye at baseline, day 90 ± 14 and day 180 ± 24.
- Systemic fatty acid concentrations at baseline and day 180 ± 24.

- Study procedures performed at St Vincent's Hospital Melbourne (SVHM)
  - Michigan Neuropathy Screening Instrument (MNSI) and Michigan Diabetic Neuropathy Score (MDNS) examination score at baseline and day 180 ± 24.
  - Nerve conduction study (NCS) parameters for the median motor, peroneal motor, tibial motor, median sensory, sural sensory, ulnar sensory nerves at baseline and day 180 ± 24.
  - Cutaneous silent periods (CSP) measurements of the upper and lower limbs at baseline and day 180 ± 24.
  - Quantitative Sudomotor Axonal Reflex Test (QSART) measurements of the forearm, proximal leg, distal leg and foot at baseline and day 180 ± 24.
- Blood test results
  - Blood pathology test results at baseline and day 180 ± 24.
  - Systemic fatty acid profiles at baseline and day 180 ± 24.

- Occurrence, grading and status of any adverse events, including their potential association with the investigational product or a study procedure
- Number and proportion of supplements returned for each participant
- Participant's and examiner's forced-choice guesses of the treatment allocation (omega-3 PUFAs or placebo) on day 180 ± 24.
- The date the participant exited the study, and a notation as to whether participant completed the study

2. Analysis Objectives

Aim 1

To evaluate the effects of a six-month supplementation period with oral long-chain omega-3 PUFA supplements on peripheral nerve health in individuals with type-1 diabetes, as measured by the change from baseline in central corneal nerve fibre length (CNFL) at day 180.

Aim 2

To evaluate the effects of a six-month supplementation period with oral long-chain omega-3 PUFA supplements in individuals with type-1 diabetes on the change from baseline at day 180 for:

Key secondary outcomes:

Other corneal nerve structure and function parameters

[2 main variables for corneal nerve structure: central corneal nerve branch density (CNBD) and central corneal nerve fibre density (CNFD)

2 main variables for corneal nerve function: central corneal sensitivity thresholds to room and cooled temperatures]

Secondary outcomes:

- Patient-reported quality of life

[2 main variables: EQ-5D-5L VAS and Index]

- Small nerve fibre function

[6 main variables: CSP onset latency and duration in the upper limb, CSP onset latency and duration in the lower limb, QSART sweat volume and latency in the foot]

- Large nerve fibre function

[2 symptom/clinical sign composite score variables: MNSI and MDNS (clinical examination) scores]

[3 main neurophysiology variables: sural SNAP amplitude, peroneal motor conduction velocity and tibial minimum F-wave]

- Median nerve excitability

[8 main variables: Depolarising threshold electrotonus (TEd) (peak), TEd (90-100 ms), hyperpolarising threshold electrotonus (Teh) (90-100 ms), TEd (10-20 ms), superexcitability, subexcitability, resting current–voltage (I/V) slope, strength duration time constant]

Aim 3

To evaluate the safety of a six-month supplementation period with oral long-chain omega-3 PUFAs in individuals with type-1 diabetes, by evaluating the incidence of adverse events, change in habitual distance visual acuity, change in intraocular pressure, change in diabetic retinopathy grading, and change in blood pathology parameters from baseline at day 180.

3. Analysis sets/Populations

The statistical analysis aims to include all available data for all participants fulfilling all of the eligibility criteria. For the analyses of efficacy variables, participants will be included in the group to which they were randomised (i.e., as-randomised), referred to as the Intention-To-Treat sample. For the analyses of safety variables, participants will be included in the group they were treated (as-treated, if different from the randomised group assignment), referred to as the Safety sample.

Inclusion criteria

The following are the inclusion criteria for participant eligibility at Visit 1 (Baseline):

1. Male or female, ≥ 18 years of age;
2. MNSI > 2;
3. Written informed consent and documentation, in accordance with ethics requirements and ICH Good Clinical Practice (GCP), obtained prior to performing any study procedures;
4. Distance best corrected visual acuity of at least 6/12 Snellen equivalent in each eye using a standard logMAR visual acuity chart, measured at 3 metres;
5. Intraocular pressure (IOP) ≤ 21 mmHg in both eyes, measured using applanation tonometry;
6. Ability to understand and follow study instructions, with the intention of completing all required study visits.

Exclusion criteria

The following are the exclusion criteria for participant eligibility at Visit 1 (Baseline):

<u>Systemic</u>

1. Any uncontrolled systemic disease, other than sub-optimally controlled type-1 diabetes mellitus;

2. Confirmed neuropathy secondary to causes other than diabetes (e.g., alcohol polyneuropathy, Vitamin B-12 deficiency, folate deficiency, chronic renal failure, hypothyroidism, neurotoxic drug use including chemotherapy);
3. Any of the following general medical conditions: bipolar disorder, atrial fibrillation, implanted defibrillator, familial adenomatous polyposis, systemic immunocompromise;
4. A scheduled or planned systemic surgery over the course of the study;
5. Any known bleeding disorders;
6. Current consumption of a systemic anti-coagulant medication other than aspirin;
7. Females who are currently pregnant or breastfeeding;
8. Females of childbearing potential who are planning a pregnancy over the course of the study;
9. Inability to sit/lie supine comfortably during the examination procedures for any reason.

Ophthalmic

10. Known allergy to, or previous reaction to, any ocular agents used in the study (i.e., ocular anaesthetics, sodium fluorescein, lissamine green, ocular mydriatics);
11. Scheduled or planned ocular surgery over the course of the study;
12. Any history of rigid contact lens wear;
13. Grading of diabetic retinopathy worse than ‘moderate’ in either eye according to the simplified ETDRS (Wisconsin) classification (ETDRS Research Group 1991);
14. Presence of any of the following ocular conditions: active ocular infection or inflammation that in the judgment of the investigator may interfere with the interpretation of the study results;
15. Corneal abnormalities or damage that could disrupt normal corneal nerve morphology, including keratoconus, bullous keratopathy, advanced corneal dystrophies, history of neurotrophic keratopathy including herpes keratitis and severe Sjogren’s associated dry eye disease;

16. History of refractive surgery or trauma within the past 12 months;
17. Use of autologous serum eye drops within the past three months, or their anticipated use during the course of the study;
18. Participant has a medical or ocular condition, or is in a situation, which in the principal investigator's opinion, may put the participant at significant risk, may confound the study results, or may interfere significantly with participation in the study.

Interventional

19. Current or previous regular consumption of any omega-3 oral supplements (>3 times/week) in the past three months;
20. Current participation in another interventional drug or device study or anticipated entry into such a study within one month of enrolment;
21. Known allergy or hypersensitivity to any components of the study supplements;
22. Cultural or religious beliefs that exclude the consumption of certain or all animal products.

4. Endpoints and Covariates

All variables are listed in Appendix 1 and the definition of the derived variables can be found in Appendix 2.

5. Handling of Missing Values and Other Data Conventions

During data collection, regular verification of the source documents was undertaken to minimise the potential for missing data. Data collected outside the specified time windows for each study visit will be set to missing (see Table 1 in Section 10). The number and percentage of missing data will be calculated for each of the outcomes listed in Appendix 1.

For outcomes analysed using the constrained longitudinal data analysis technique outlined in Section 6.2, we will use a likelihood-based approach that relies on the underlying assumption that the probability of missing outcome data is not related to the missing data, but to some of the observed measured data in the model (Missing At Random [MAR]). For outcomes analysed using the (ordered) logistic regression model, we will exclude those with missing baseline or missing post-baseline data (i.e., incomplete cases) relying on the underlying assumption that the missing outcome data is missing completely at random (MCAR).

For corneal nerve sensitivity thresholds, the instrument has a lowest measurable threshold (i.e., floor) of 0.1 mbar. All measured values that were recorded to be below this lower limit will be replaced by a value of the lower limit divided by two (i.e., 0.05 mbar) (Beal 2001).

6. Statistical Methodology

Baseline characteristics

See Table 1 in Appendix 4.

Participant demographic and baseline variables will be summarised and presented in a table (see Table 1 in Appendix 4) by treatment group using: frequencies and percentages (based on the non-missing sample size at baseline) for discrete variables, mean and standard deviation for continuous variables, or median and quartiles (25th to 75th percentile) for non-symmetrical continuous variables. If variables have missing data, we will report the non-missing sample size either in the table or in the footnote of the table.

Statistical procedures

See Tables 2 – 5 in Appendix 4.

All outcomes will be summarised by visit and treatment group using descriptive statistics. Depending on the distribution, one of the following models will be fitted:

- Continuous repeated measures data collected at more than one visit will be analysed using a constrained longitudinal data analysis technique (Liang 2000). The outcome (dependent variable) will consist of the baseline and post-baseline values. The model will assume a common baseline mean across the two treatment arms due to random allocation. It will incorporate time point (study visit) as a categorical variable, thus it will not assume a specific trajectory over time. In addition to study visit, the model will include treatment as the main factor and the treatment by study visit interaction. The variance–covariance among the repeated measurements will be defined as unstructured. In the case of non-convergence, we will consider alternative structures (first-order autoregressive, Toeplitz, compound symmetry). Using this model, study participants with a missing baseline or a missing post-baseline value will be included in the analysis but study participants with no outcome values will be excluded. This model will yield unbiased results when the outcome data are Missing At Random. We will report the estimate and two-sided confidence interval of the absolute difference between two treatment groups in the change from baseline to 3 months and 6 months post-baseline (if applicable). Before fitting this model, the distribution of the outcome will be explored. In addition, we will examine if there are potential violations of underlying model assumptions (e.g. residuals). As a result, we may apply an appropriate transformation (e.g. log-transformation) before fitting the final model.
- Binary data will be analysed using a logistic regression model. We will report the estimate and two-sided confidence interval of the odds ratio between the two treatment groups.
- Categorical data will be analysed using an ordinal logistic regression model. We will report the estimate and two-sided confidence interval of the odds ratio between the treatment groups.

Details of which model will be used for each of the primary, key secondary, and secondary outcomes can be found in Appendix 3.

Compliance

See Table 4 in **Appendix 4**.

The compliance outcomes (such as the percentage of omega-3 and omega-6 fatty acids present in erythrocytes (determined from dried blood spot tests [analysed by Waite Lipid Analysis Services, Australia]) at the study endpoint (day 180) will be analysed using a linear regression model, adjusted for baseline. Before fitting this model, the distribution of the outcome will be explored. In addition, we will examine if there are potential violations of underlying model assumptions (e.g., residuals). As a result, we may apply an appropriate transformation (e.g., log-transformation) before fitting the final model. The proportion of returned capsules (counted by an independent, masked reconciler) will be summarised by treatment group.

Compliance (%) will be derived as 100 times [(actual number of capsules taken over the study period) divided by (total number of capsules that should have been taken over the study period)], with study period based on actual trial participation duration. The actual number of capsules taken over the study period is derived as the number of capsules dispensed minus the number of capsules returned. Participants with compliance less than 75% or above 125% will be considered as non-compliant to study drug intake (AusPAR2011; NCT01797185). A sensitivity analysis will be performed for the primary outcome to examine the treatment effect when non-compliant cases are excluded from the analysis.

Concomitant medications

Concomitant medications will be categorised by their therapeutic class, according to the United States Pharmacopeia Drug Classification system. The number and

percentage of participants taking concomitant medications in each class, for each participant group, will be tabulated and reported in the supplementary information.

Adverse events

Information about adverse events will be collated, summarised and provided in the supplementary information. The incidence of participants with adverse events at each visit, type of event, event severity, and a judgement regarding its association with the investigational product or a study procedure, will be tabulated and reported in the supplementary information.

7. Measures to Adjust for Multiplicity, Confounders, Heterogeneity

Adjustments for confounders

In addition to the models specified above that only include treatment group, we will fit two adjusted models to the outcomes linked to the primary, key secondary, and secondary aims that adjusts for:

Adjusted model 1:

- Age (years)
- Diabetes duration (years)
- HbA1c (mmol/mol)

Adjusted model 2:

- Age (years)
- Diabetes duration (years)
- HbA1c (mmol/mol)
- Baseline CNFL (mm/mm^2) (continuous) if it is imbalanced between groups at baseline

- Baseline omega-3 index (%) (continuous) if it is imbalanced between groups at baseline.

Results will be compared with the unadjusted analyses and discussed in the main study report.

Subgroup analyses

Exploratory subgroup analyses will be performed for the primary outcome for the following subgroups:

- CNFL at baseline (above 12.5mm/mm^2 and below or equal to 12.5 mm/mm^2), as the threshold considered diagnostic for diabetic sensorimotor polyneuropathy in type-1 diabetes (Perkins et al., 2018).
- Presence of small fibre neuropathy (yes/no) at baseline (physician-diagnosed, using results from abnormal QSART or CSP) (Kamel et al., 2015).

Subgroup (main effect) and the subgroup-by-treatment-by-visit interaction (as well as subgroup-by-treatment and subgroup-by-visit interaction) terms will be added to the unadjusted model to evaluate whether the treatment effect differs between subgroup categories at the different time points. Results of the subgroup analyses will be summarised using Forest plots, presenting the estimate and two-sided 95% confidence interval of the treatment effect within each subgroup level along with corresponding p-value and including the p-value for heterogeneity of the treatment effect between the subgroups.
Analyses of any other subgroups will be considered as *post hoc* exploratory analyses.

7.1.1 Multiple testing

The primary outcome at day 180 will be evaluated using the two-sided 5% level of significance.

Multiple testing for the key secondary outcomes related to corneal nerve parameters at day 180 will be assessed using the Benjamini-Hochberg stepwise method with a false discovery rate of 5% for: corneal nerve fibre density (CNFD), corneal nerve branch density (CNBD), central corneal sensitivity thresholds to room temperature, and central corneal sensitivity thresholds to cooled temperature. For these key secondary outcomes at day 180, we will report the multiplicity adjusted p-values.

For all other post-baseline time points and outcomes, we will report multiplicity unadjusted p-values. Given the large number of statistical tests, the probability of finding at least one false positive finding will be close to 100%. For this reason, we will exercise caution and in addition to p-values, associations will be interpreted based on the magnitude and direction of the association and width of the 95% confidence intervals.

All tests will be performed two-sided, and we will report two-sided 95% confidence intervals.

8. Sensitivity Analyses

Additional sensitivity analyses will be performed for the outcomes linked to the primary and key secondary aims for the following dataset:

- Dataset excluding participants deemed non-compliant (see definition in Section 6.3).

9. QC Plans

Source data verification of the data entered on REDCap was performed by the Principal Investigator (PI). Data was manually checked for accuracy from the source worksheets/original digital data record, for all participants, for the primary outcome (CNFL) and key secondary outcomes (CNFD, CNBD, central corneal sensitivity thresholds to room temperature, and central corneal sensitivity thresholds to cooled temperature). In addition, a subset of data comprising at least 20% of the full dataset,

was randomly selected by the PI for verification, to ensure that the observations and findings are recorded correctly and completely. Data found as incorrectly entered were verified against the original source documents, corrected on REDCap and recorded accordingly.

10. Programming Plans

A list of all planned tables and figures and their templates can be found in Appendix 4.

Tolerance windows will be used for converting visit dates into visit numbers according to the study visit schedule before summarising or analysing the data. The relative day of testing will be derived as the visit date minus the supplementation start date. The visit windows in Table 1 will be applied to the testing dates.

Table 1. Visit windows

Visit	Target day	Lower limit (incl.)	Upper limit (incl.)
Day 1	Supplement start date (Day 0)	Day -35	1
Day 30	Day 30	Day 14	Day 44
Day 90	Day 90	Day 76	Day 104
Day 180	Day 180	Day 156	Day 204

Assessments performed outside of these visit windows will not be included in the analyses. If a participant has more than one assessment performed within the same visit window, then the assessment closest to the target date will be used. The visit windows will be applied to the study variables described in Appendix 1.

11. REFERENCES

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12. Appendix 1

VARIABLES IN THE DATA SET

	Variable label	Unit	Variable in data set
	Baseline		
R	Sex	0 = Female 1 = Male	<sex>
R	Age	Years	<age>
R	Diabetes duration (year(s) since diagnosis)	Years	<hx_dm_duration>
D	Body mass index	Kg/m^2	<bmi>
R	Insulin intake method	0 = pump 1 = multiple daily injections	< hx_insulin_type>
R	Concomitant medications	0 = no 1 = yes	<hx_concomitant_medications>
R	Regular soft contact lens wear (at least once per week)	0 = No 1 = Yes	<ohx_scl>
R	History of ophthalmic surgery	0 = No 1 = Yes	<ohx_surgeries>
D	Diagnosed as having an abnormal small fibre function using either QSART or CSP	0 = Normal 1 = Abnormal	<sfn_qsart_csp>
R	Completed study	0 = No 1 = Yes	<completed_study>
	Primary efficacy		
R	Central Corneal nerve fibre length [right eye]	mm/mm^2	<ivcm_cnfl_c>
	Secondary and exploratory efficacy outcomes		
	Corneal nerve parameters: main secondary		
R	Central corneal nerve fibre density [right eye]	$nerves/mm^2$	<ivcm_cnfd_c>
R	Central corneal nerve branch density [right eye]	$branches/mm^2$	<ivcm_cnbd_c>
R	Central corneal sensitivity threshold to room temperature [right eye]	mBar	<ncca_rm_c>
R	Central corneal sensitivity threshold to cooled temperature [right eye]	mBar	<ncca_cd_c>
	Corneal nerve parameters: exploratory		
R	Central corneal dendritic cell density [right eye]	cells/mm2	<ivcm_dcc_c_total>
R	Peripheral corneal nerve fibre length [right eye]	mm/mm2	<ivcm_cnfl_p>

	Variable label	**Unit**	**Variable in data set**
R	Peripheral corneal dendritic cell density [right eye]	cells/mm^2	<ivcm_dcc_p_total>
R	Peripheral corneal sensitivity threshold to room temperature [right eye]	mBar	<ncca_rm_p>
R	Peripheral corneal sensitivity threshold to cooled temperature [right eye]	mBar	<ncca_cd_p>
	Quality of life: secondary		
D	Change in health state: EQ-5D-5L index score	Range: -0.569 to 1	<eq5d5l_index>
R	Change in health state: EQ-5D-5L VAS score	Range: 0 to 100	<eq5d5l_vas>
	Small fibre function: secondary		
R	Cutaneous silent period - upper limb latency	ms	<csp_upper_lat>
R	Cutaneous silent period - upper limb duration	ms	<csp_upper_duration>
R	Cutaneous silent period - lower limb latency	ms	<csp_lower_lat>
R	Cutaneous silent period - lower limb duration	ms	<csp_lower_duration>
R	QSART sweat volume - measured at the foot	µL	<qsart_foot_vol>
R	QSART sweat latency - measured at the foot	s	<qsart_foot_lat>
	Small fibre function: exploratory		
D	QSART neuropathy grading (neuropathy/ length-dependent)	0 = None 1 = Length-dependent SFN 2 = Non-length dependent SFN	<qsart_neuropathy>
R	QSART: forearm volume	uL	<qsart_forearm_vol>
R	QSART: forearm latency	s	<qsart_forearm_lat>
R	QSART: proximal leg volume	uL	<qsart_proxleg_vol>
R	QSART: proximal leg latency	s	<qsart_proxleg_lat>
R	QSART: distal leg volume	uL	<qsart_distleg_vol>
R	QSART: distal leg latency	s	<qsart_distleg_lat>
	Large fibre function: secondary		
R	Michigan Neuropathy Screening Instrument (MNSI) score	Range: 0 to 23	<mnsi_total>
R	Michigan Diabetic Neuropathy Score (MDNS) composite score: clinical examination	4 Levels: 0 = Class 0 1 = Class 1 2 = Class 2	<mdns_clin_class>

	Variable label	**Unit**	**Variable in data set**
		3 = Class 3	
R	Nerve conduction studies: Sural SNAP amplitude (Mid. lower leg-Lat. malleolus)	uV	<sensory_sural_amp>
R	Nerve conduction studies: Peroneal CMAP velocity (Bl. knee – ankle)	m/s	<motor_peroneal_vel>
R	Nerve conduction studies: Tibial minimum F-wave latency (Ankle – Abd hal)	ms	<motor_tibial_fminlat>
	Large fibre function: exploratory		
R	Michigan Diabetic Neuropathy Score (MDNS) composite score: neurophysiology composite score (NCS)	4 Levels: 0 = Class 0 1 = Class 1 2 = Class 2 3 = Class 3	<mdns_neurophys_class>
R	NCS: median motor latency (wrist-APB)	ms	<motor_median_lat1>
R	NCS: median motor amplitude (wrist-APB)	mV	<motor_median_amp1>
R	NCS: median motor latency (elbow-wrist)	ms	<motor_median_lat2>
R	NCS: median motor amplitude (elbow-wrist)	mV	<motor_median_amp2>
R	NCS: median motor velocity (elbow-wrist)	m/s	<motor_median_vel>
R	NCS: peroneal motor latency (ankle-EDB)	ms	<motor_peroneal_lat1>
R	NCS: peroneal motor amplitude (ankle-EDB)	mV	<motor_peroneal_amp1>
R	NCS: peroneal motor latency (Bl. knee-Ankle)	ms	<motor_peroneal_lat2>
R	NCS: peroneal motor amplitude (Bl. knee-Ankle)	mV	<motor_peroneal_amp2>
R	NCS: tibial motor latency (Ankle–Abd hal)	ms	<motor_tibial_lat>
R	NCS: tibial motor amplitude (Ankle–Abd hal)	mV	<motor_tibial_amp>
R	NCS: tibial motor velocity (Ankle–Abd hal)	m/s	<motor_tibial_vel>
R	NCS: Median sensory latency (Dig II–Wrist; orthodromic)	ms	<sensory_median_lat1>
R	NCS: Median sensory amplitude (Dig II–Wrist; orthodromic)	uV	<sensory_median_amp1>
R	NCS: Median sensory velocity (Dig II–Wrist; orthodromic)	m/s	<sensory_median_vel1>

	Variable label	**Unit**	**Variable in data set**
R	NCS: Median sensory latency (Wrist-Dig II; antidromic)	ms	<sensory_median_lat2>
R	NCS: Median sensory amplitude (Wrist-Dig II; antidromic)	uV	<sensory_median_amp2>
R	NCS: Median sensory velocity (Wrist-Dig II; antidromic)	m/s	<sensory_median_vel2>
R	NCS: Sural sensory latency (Mid. lower leg-Lat. malleolus)	ms	<sensory_sural_lat>
R	NCS: Sural sensory velocity (Mid. lower leg-Lat. malleolus)	m/s	<sensory_sural_vel>
R	NCS: ulnar sensory latency (Dig V-Wrist; orthodromic)	ms	<sensory_ulnar_lat1>
R	NCS: ulnar sensory amplitude (Dig V-Wrist; orthodromic)	uV	<sensory_ulnar_amp1>
R	NCS: ulnar sensory velocity (Dig V-Wrist; orthodromic)	m/s	<sensory_ulnar_vel1>
R	NCS: ulnar sensory latency (Wrist – Dig V; antidromic)	ms	<sensory_ulnar_lat2>
R	NCS: ulnar sensory amplitude (Wrist – Dig V; antidromic)	uV	<sensory_ulnar_amp2>
R	NCS: ulnar sensory velocity (Wrist – Dig V; antidromic)	m/s	<sensory_ulnar_vel2>
	Nerve excitability: secondary		
R	Median motor nerve excitability profile: TEd (peak)	-	<nes_22_ted_peak>
R	Median motor nerve excitability profile: TEd (90-100 ms)	-	<nes_18_ted_90_100>
R	Median motor nerve excitability profile: TEh (90-100 ms)	-	<nes_10_teh_90_100>
R	Median motor nerve excitability profile: TEd (10-20 ms)	-	<nes_11_ted_10_20>
R	Median motor nerve excitability profile: Superexcitability	%	<nes_12_superexcitability>
R	Median motor nerve excitability profile: Subexcitability	%	<nes_13_subexcitability>
R	Median motor nerve excitability profile: Resting current-voltage (I/V) slope	-	<nes_6_resting_iv>
R	Median motor nerve excitability profile: strength duration time constant	ms	<nes_2_sdtc>
	Nerve excitability: exploratory		
R	Nerve excitability: Stimulus (mA) for 50% max response	mA	<nes_1_stimulus_50_max>
R	Nerve excitability: Rheobase (mA)	mA	<nes_3_rheobase>
R	Nerve excitability: Stimulus-	-	<nes_4_stimulus_respo

	Variable label	**Unit**	**Variable in data set**
	response\slope		nse_slope>
R	Nerve excitability: Peak response (mV)	mV	<nes_5_peak_response>
R	Nerve excitability: Minimum I/V slope	-	<nes_7_min_iv>
R	Nerve excitability: Temperature (ºC)	C	<nes_8_temp>
R	Nerve excitability: RRP (ms)	ms	<nes_9_rrp>
R	Nerve excitability: Nerve excitability: Polarizing current	% threshold	<nes_14_pol_current_threshold>
R	Nerve excitability: Polarizing current\(mA)	mA	<nes_15_pol_current_ma>
R	Nerve excitability: Latency (ms)	ms	<nes_16_latency>
R	Nerve excitability: TEd(40-60ms)	-	<nes_17_ted_40_60>
R	Nerve excitability: TEh(10-20ms)	-	<nes_19_teh_10_20>
R	Nerve excitability: TEd(undershoot)	-	<nes_20_ted_undershoot>
R	Nerve excitability: TEh(overshoot)	-	<nes_21_teh_overshoot>
R	Nerve excitability: S2 accommodation	-	<nes_23_s2_accom>
R	Nerve excitability: Accommodation half-time (ms)	ms	<nes_24_accom_half_time>
R	Nerve excitability: Hyperpol. I/V slope	-	<nes_25_hyperbol_iv_slope>
R	Nerve excitability: Refractoriness at 2.5ms (%)	%	<nes_26_refractoriness_2.5ms>
R	Nerve excitability: TEh(20-40ms)	-	<nes_27_teh_20_40>
R	Nerve excitability: TEh(slope 101-140ms)	-	<nes_27_teh_20_40>
R	Nerve excitability: Refractoriness at 2 ms (%)	%	<nes_29_refractoriness_2ms>
R	Nerve excitability: Superexcitability at 7 ms (%)	%	<nes_30_refractoriness_7ms>
R	Nerve excitability: Superexcitability at 5 ms (%)	%	<nes_31_refractoriness_5>
R	Nerve excitability: TEd20(peak)	-	<nes_32_ted20_peak>
	Signs of dry eye disease: exploratory		
D	Ocular Surface Disease Index score	score 0 - 100	<ocul_osdi>
R	Tear osmolarity (highest of the two eyes)	mOsmol/L	<ocul_osmol_highest>
R	Sodium fluorescein tear break-up time in the right eye (average of three repeat measurements)	s	<ocul_nafl_tbut_r>
R	Phenol red thread test (average of	mm	<ocul_prt_ave>

	Variable label	**Unit**	**Variable in data set**
	both eyes)		
R	Anterior blepharitis grading (average of both eyes)	Efron scale score 0-4 (continuous)	<ocul_bleph_ave>
R	Meibomian gland dysfunction grading (average of both eyes)	Efron scale score 0-4 (continuous)	<ocul_mgd_ave>
R	Conjunctival redness grading (average of both eyes)	Efron scale score 0-4 (continuous)	<ocul_conj_red_ave>
R	Limbal redness grading (average of both eyes)	Efron scale score 0-4 (continuous)	<ocul_limbal_red_ave>
R	Corneal sodium fluorescein staining score (average of both eyes)	Oxford Score 0-4 (continuous)	<ocul_nafl_stain_ave>
	Indirect healthcare costs: exploratory		
D	iMTA iPCQ - loss of productivity due to absences from paid work	hours	<imta_absenteeism>
D	iMTA iPCQ - loss of productivity due to presenteeism	hours	<imta_presenteeism>
D	iMTA iPCQ - loss of productivity of unpaid work	hours	<imta_unpaid>
	Safety outcomes		
R	Habitual distance visual acuity measured using a standard eye chart - Right eye	LogMAR	<ocul_va_r_logmar>
R	Habitual distance visual acuity measured using a standard eye chart - Left eye	LogMAR	<ocul_va_l_logmar>
R	Intraocular pressure - Right eye	mmHg	<ocul_iop_r>
R	Intraocular pressure - Left eye	mmHg	<ocul_iop_l>
R	Diabetic retinopathy grading - Worst eye retinopathy grading	6 Grades: 0 = None 1 = Minimal 2 = Mild 3 = Moderate 4 = Severe 5 = Proliferative	<dr_retinopathy>
R	Diabetic macular oedema grading - Worst eye macular oedema grading	3 Grades: 0 = None 1 = Macular oedema 2 = Clinically significant macular oedema	<dr_dme>
R	Bloods: HbA1C, mmol/mol	mmol/mol	<bt_hba1c_mmol>
R	Bloods: cholesterol	mg/dL	<bt_cholesterol>
R	Bloods: HDL	mmol/L	<bt_hdl>
R	Bloods: LDL	mmol/L	<bt_ldl>
R	Bloods: folate	nmol/L	<bt_folate>
R	Bloods: vitamin B-12	pmol/L	<bt_b12>

	Variable label	Unit	Variable in data set
R	Bloods: creatinine	umol/L	<bt_creatinine>
R	Adverse events	0 = None 1 = AE 2 = SAE	<adverse_events>
R	Bloods: haemoglobin (Hb)	g/L	<bt_hb>
R	Bloods: platelets	$x10^9$/L	<bt_platelets>
R	Bloods: white blood cells (WBC)	$x10^9$/L	<bt_wbc>
R	Bloods: HbA1C, Percentage (%)	%	<bt_hba1c_percent>
R	Bloods: triglycerides	mmol/L	<bt_triglycerides>
R	Bloods: ALT	U/L	<bt_alt>
R	Bloods: T-Bil	umol/L	<bt_tbil>
R	Bloods: ALP	U/L	<bt_alp>
R	Bloods: GGT	U/L	<bt_ggt>
R	Bloods: Na+	mmol/L	<bt_sodium>
R	Bloods: K+	mmol/L	<bt_potassium>
R	Bloods: CO2	mmol/L	<bt_bicarb>
R	Bloods: TSH	mU/L	<bt_tsh>
R	Bloods: eGFR	mL/min/1.73m^2 Categorical: 0 = >90 1 = <90	<bt_egfr>
	Compliance outcomes		
R	Omega-3 index	%	<pufa_n3_index>
R	Omega-3: EPA concentration	%	<pufa_n3_epa>
R	Omega-3: DHA concentration	%	<pufa_n3_dha>
R	Omega-6 concentration	%	<pufa_n6_total>
R	Omega-9 concentration	%	<pufa_n9_total>
R	Omega-6:Omega-3 ratio	-	<pufa_n6_n3_ratio>
R	Compliance: capsule count	%	<capsules_taken>
	Efficacy of masking		
R	Intervention guess: participant	0 = Placebo 1 = Omega-3	<guess_participant>
R	Intervention guess: outcome assessor	0 = Placebo 1 = Omega-3	<guess_examiner>

D = derived outcome; R = raw outcome. SFN = small fibre neuropathy
Colour codes: orange = primary outcome; green = main secondary outcomes; white = exploratory outcomes.

13. Appendix 2

DEFINITIONS OF DERIVED VARIABLES IN THE DATA SET

Derived variable	Range	Derived from

Body mass index		Weight/(height)^2
OSDI score derived as (sum of scores for all questions answered) × 100]/[(total number of questions answered) × 4	0 to 100	Derived using the OSDI scoring sheet
Change in health state: EQ-5D-5L index score	-0.569 to 1	Derived using the UK index value set
MDNS – class of neuropathy (clinical examination)	Classes 0 to 3	Derived using MDNS scoring sheet
MDNS – class of neuropathy (electrophysiology)	Classes 0 to 3	Derived using MDNS scoring sheet
Evidence of neuropathy using QSART	0 = None 1 = Length dependent SFN 2 = Non-length dependent SFN	Clinically derived using QSART scores at forearm, foot, proximal leg and distal leg
Evidence of small fibre neuropathy using abnormal QSART or CSP	0 = None 1 = SFN	Clinically derived using an abnormal QSART or CSP score
iMTA iPCQ - loss of productivity due to absence from paid work		Method derived from iPCQ Manual
iMTA iPCQ - loss of productivity due to presenteeism		Method derived from iPCQ Manual
iMTA iPCQ - loss of productivity of unpaid work		Method derived from iPCQ Manual

SFN = small fibre neuropathy

14. Appendix 3

LIST OF ANALYSES FOR MAIN OUTCOMES

The distribution column of this table will be completed after unmasking, based on an examination of the distribution along with potential violation of the model assumptions to inform the need for a transformation and which transformation is most appropriate.

Outcome	Distribution/Transformation	Days	Analysis model
Primary efficacy			
Central Corneal nerve fibre length [right eye]		1, 90, 180	Repeated measures model.
Key secondary efficacy			
Corneal nerve parameters			
Central corneal nerve fibre density [right eye]		1, 90, 180	Repeated measures model.
Central corneal nerve branch density [right eye]			
Central corneal sensitivity threshold to room temperature [right eye]		1, 90, 180	Repeated measures model.
Central corneal sensitivity threshold to cooled temperature [right eye]			
Secondary efficacy			
Quality of life			
Change in health state: EQ-5D-5L index score		1, 90, 180	Repeated measures model.
Change in health state: EQ-5D-5L VAS score			
Small nerve fibre function			
Cutaneous silent period - upper limb onset		1, 180	Repeated measures model.
Cutaneous silent period - upper limb duration			
Cutaneous silent period - lower limb onset			
Cutaneous silent period - lower limb duration			
QSART sweat volume -measured at the foot		1, 180	Repeated measures model.
QSART sweat latency -measured at the foot			

Outcome	Distribution/Transformation	Days	Analysis model
Large nerve fibre function			
Michigan Neuropathy Screening Instrument (MNSI) score		1, 180	Repeated measures model.
Michigan Diabetic Neuropathy Score (MDNS) composite score: clinical examination class	Categorical variables with 4 ordinal levels	1, 180	Ordered logistic regression model
Nerve conduction studies: Sural SNAP amplitude (Mid. lower leg-Lat. malleolus)		1, 180	Repeated measures model.
Nerve conduction studies: Peroneal CMAP velocity (Bl. knee – ankle)			
Nerve conduction studies: Tibial minimum F-wave latency (Ankle – Abd hal)			
Nerve excitability			
Median motor nerve excitability profile: TEd (10-20 ms)		1, 180	Repeated measures model.
Median motor nerve excitability profile: TEd (peak)			
Median motor nerve excitability profile: TEd (90-100 ms)			
Median motor nerve excitability profile: TEh (90-100 ms)			
Median motor nerve excitability profile: Superexcitability			
Median motor nerve excitability profile: Subexcitability			
Median motor nerve excitability profile: Resting I/V slope			
Median motor nerve excitability profile: strength duration time constant			
Safety			
Habitual visual acuity measured using a standard eye chart - Right eye		1, 30, 90, 180	Repeated measures model.
Habitual visual measured using a standard eye chart - Left eye		1, 30, 90, 180	
Intraocular pressure - Right eye		1, 180	Repeated measures model.
Intraocular pressure - Right eye		1, 180	

Outcome	Distribution/Transformation	Days	Analysis model
Diabetic retinopathy grading - Worst eye retinopathy grading	Categorical variable with 4 ordinal levels	1, 180	Ordered logistic regression model
Diabetic retinopathy grading - Worst eye macular oedema grading	Categorical variable with 3 ordinal levels.		
Bloods: HbA1C (mmol/mol)		1, 180	Repeated measures model.
Bloods: cholesterol			
Bloods: HDL			
Bloods: LDL			
Bloods: folate			
Bloods: vitamin B-12			
Bloods: creatinine			
Compliance			
Omega-3 index		1, 180	Repeated measures model.
Omega-3: EPA concentration			
Omega-3: DHA concentration			
Omega-6 concentration			
Omega-9 concentration			
Omega-6:Omega-3 ratio			

15. Appendix 4
LIST OF TABLES/FIGURES

Number	Title – analysis set
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Table 2	Central corneal nerve efficacy outcomes (Intention-To-Treat sample)
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Figure 1	CONSORT flow diagram
Figure 2	Efficacy plots for primary outcome and key secondary corneal parameters (Intention-To-Treat sample)
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Table S3*	Corneal nerve parameters (Intention-To-Treat sample)
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* These tables will consist of summary statistics for all visits (thus not limited to baseline and day 180) along with the analysis for all post-baseline time-points.

Example of Table 1: Baseline Participant Characteristics (Intention-To-Treat sample)

Characteristic	**Omega-3 PUFAs (N=XX)**	**Placebo (N=XX)**
Male sex, n (%)	XX (XX.X)	XX (XX.X)
Age (years), median (IQR)	X.X (X.X)	X.X (X.X)
Duration of type-1 diabetes (years), median (IQR)	X.X (X.X)	X.X (X.X)
Body mass index (kg/m²), median (IQR)	X.X (X.X)	X.X (X.X)
Insulin pump, n (%)	XX (XX.X)	XX (XX.X)
Contact lens wear, n (%)	XX (XX.X)	XX (XX.X)
History of (any) ophthalmic surgery, n (%)	XX (XX.X)	XX (XX.X)
Presence of small fibre neuropathy,* n (%)	XX (XX.X)	XX (XX.X)

*Abnormal small fibre function defined as having abnormal QSART or abnormal CSP at baseline (Kamel, 2015)

Example of Table 2: Central corneal nerve efficacy outcomes (Intention-To-Treat sample)

	Omega-3 PUFAs			**Placebo**			**Omega-3 PUFAs vs Placebo**	
	Baseline* (N=XX)	Day 180* (N=XX)	Change from baseline* (N=XX)	Baseline (N=XX)	Day 180* (N=XX)	Change from baseline* (N=XX)	Estimate (95% CI)	p-value†
Primary outcome								
CNFL (mm/mm²)								
Key secondary outcomes								
CNFD (nerves/mm²)								
CNBD (branches/mm²)								
Central corneal sensitivity thresholds (mbar) - Room temperature - Cooled temperature								

* Mean and standard deviation, unless stated otherwise.

† Multiplicity-adjusted p-value for the key secondary outcomes.

Abbreviations: CNBD; corneal nerve branch density CNFD, corneal nerve fibre density; CNFL, corneal nerve fibre length

Example of Table 3: Clinical neurophysiology outcomes (Intention-To-Treat sample)

	Omega-3 PUFAs			**Placebo**			**Omega-3 PUFAs vs Placebo**	
	Baseline* (N=XX)	Day 180* (N=XX)	Change from baseline* (N=XX)	Baseline* (N=XX)	Day 180* (N=XX)	Change from baseline* (N=XX)	Estimate (95% CI)	p-value†
Quality of life - EQ 5D-5L Index - EQ 5D-5L VAS								
Small nerve fibre function								
Cutaneous silent periods - Upper limb latency onset (ms) - Upper limb duration (ms) - Lower limb latency onset (ms) - Lower limb duration (ms)								
Quantitative Sudomotor Axonal Reflex Test at the foot - Sweat Volume (µL) - Response Latency (s)								
Large nerve fibre function								
Neuropathy score - MNSI (out of 13) - MDNS clinical examination (out of 46)								
Nerve conduction studies - Sural sensory amplitude (µV) - Peroneal motor velocity (ms^{-1}) - Tibial minimum F-wave latency (s)								
Nerve excitability								
- TEd (10–20 ms) - TEd (peak) - TEd (90–100 ms) - TEh (90–100 ms) - Superexcitability (%) - Subexcitability (%) - Resting I/V slope - SDTC, ms								

* Mean and standard deviation, unless stated otherwise.
† p-values are not adjusted for multiple testing.

Example of Table 4: Compliance (Safety sample)

	Omega-3 PUFAs			**Placebo**			**Omega-3 PUFAs vs Placebo**	
	Baseline* (N=XX)	Day 180* (N=XX)	Change from baseline* (N=XX)	Baseline* (N=XX)	Day 180* (N=XX)	Change from baseline* (N=XX)	Estimate (95% CI) (N=XX)	p-value†
Omega-3 PUFAs, % - Omega-3 index - EPA - DHA								
Total omega-3, %								
Total omega-6, %								
Total omega-9, %								
Omega-6:Omega-3 ratio								

* Mean and standard deviation, unless stated otherwise.
† p-values are not adjusted for multiple testing.

Abbreviations: MDNS, Michigan Diabetic Neuropathy score; MNSI, Michigan Neuropathy Screening Index; TSH, thyroid stimulating hormone;

Example of Table 5: Safety outcomes and blood biochemistry (Safety sample)

	Omega-3 PUFAs			**Placebo**			**Omega-3 PUFAs vs Placebo**	
	Baseline * (N=XX)	Day 180* (N=XX)	Change from baseline* (N=XX)	Baseline* (N=XX)	Day 180* (N=XX)	Change from baseline* (N=XX)	Estimate (95% CI) (N=XX)	p-value†
Adverse events - At least one AE or SAE, n (%) - At least one AE, n (%) - At least one SAE, n (%)								
Habitual distance visual acuity (LogMAR) - Right eye - Left eye								
Intraocular pressure (mmHg) - Right eye - Left eye								
Diabetic retinopathy in the worse eye, n (%) - None - Mild - Moderate - Severe - Proliferative								
Diabetic macular oedema in the worse eye, n (%) - None - Macular oedema - Clinically significant macular oedema								
Blood biochemistry								
HbA1C (mmol/mol)								
Cholesterol (mmol/L)								
HDL (mmol/L)								
LDL (mmol/L)								
Creatinine (µmol/L)								
Vitamin B12 (pmol/L)								
Folate (nmol/L)								

* Mean and standard deviation unless stated otherwise.
† p-values are not adjusted for multiple testing.
Abbreviations: HbA1C, haemoglobin A1c; HDL, high-density lipoproteins; LDL, low density lipoproteins.

Changes from the planned analysis in the Statistical Analysis Plan

1. A sensitivity analysis was performed for outcomes with substantial imbalance at baseline either in the mean (continuous outcomes) or geometric mean (log base e transformed outcomes) between groups. The imposed constraint of balance at baseline of the constrained longitudinal data analysis model was removed by adjusting the primary model for the interaction between baseline and treatment in addition to the factors already part of the longitudinal data analysis model.
2. We had intended to analyse categorical and binary variables using (ordinal) logistic regression analysis. However, this model could not be fitted due to the distribution of the data within and between groups. Instead, for categorical and binary variables, change from baseline at the study endpoint for each participant was categorised as negative change, positive change, or no change. Fisher's exact test was used to compare the proportions of participants between treatment groups. For MDNS scores, in changing from analysing this parameter as a continuous variable to a categorical variable, the class of neuropathy (class 0–3 as defined by the MDNS) was derived as the highest score from the clinical scoring or neurophysiology component.
3. Between-group comparisons of dry eye symptom scores using the exploratory outcome of Ocular Surface Disease Index (OSDI) (appendix 3) could not be performed using the prespecified repeated measures model due to the number of scores with the value of zero. For the OSDI outcome, comparison of change between the omega-3 PUFA and placebo groups was tested using the non-parametric Wilcoxon rank sum test. The estimate and 95% confidence intervals between-groups were obtained using the Hodges-Lehman non-parametric estimator.
4. Total omega-3 concentrations (%) are not additionally analysed as EPA and DHA concentrations have been reported.
5. Exploratory outcomes for indirect health care were not analysed.

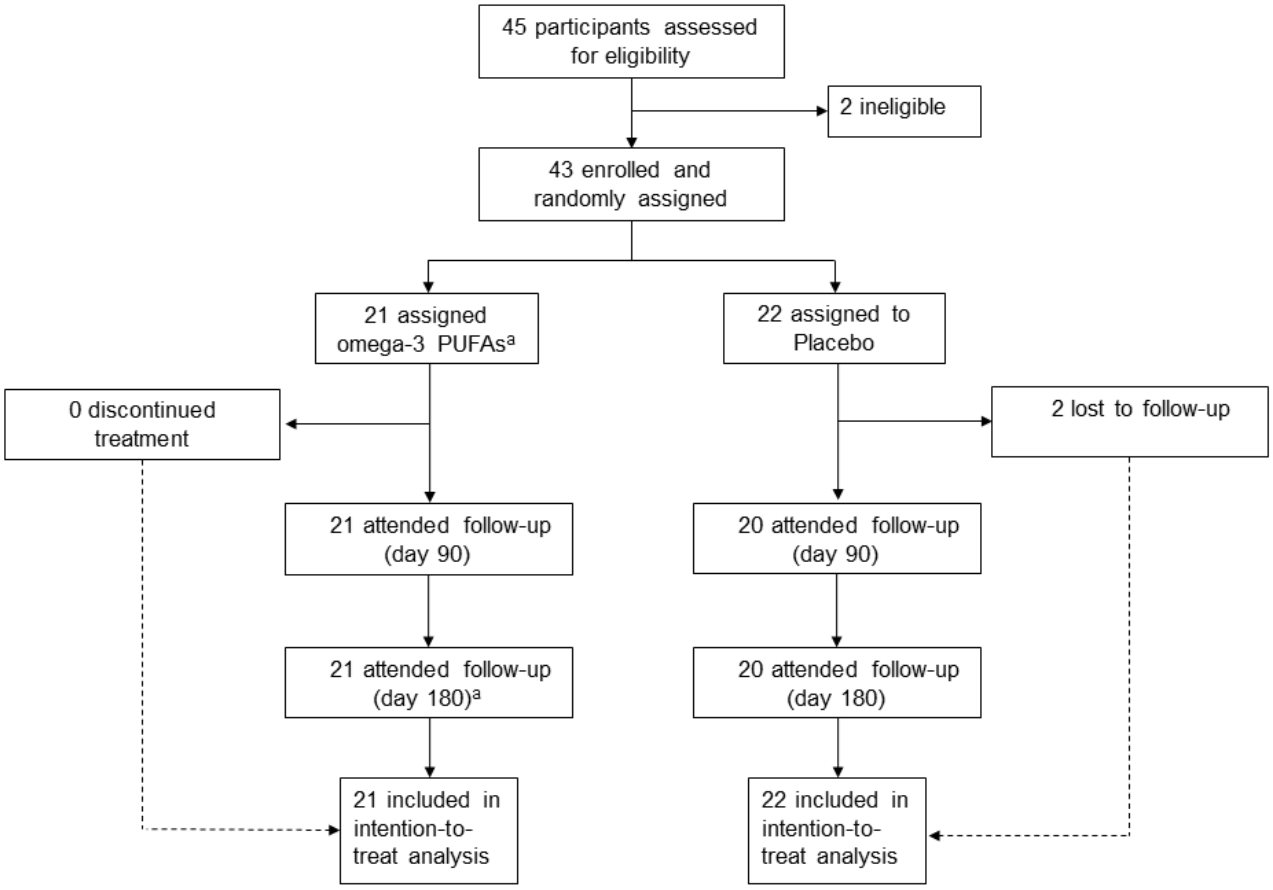

Figure S1. CONSORT diagram. PUFA=polyunsaturated fatty acid. [a]One participant in the omega-3 PUFA group completed the study but did not undergo procedures relating to small or large nerve fibre function in the limbs.

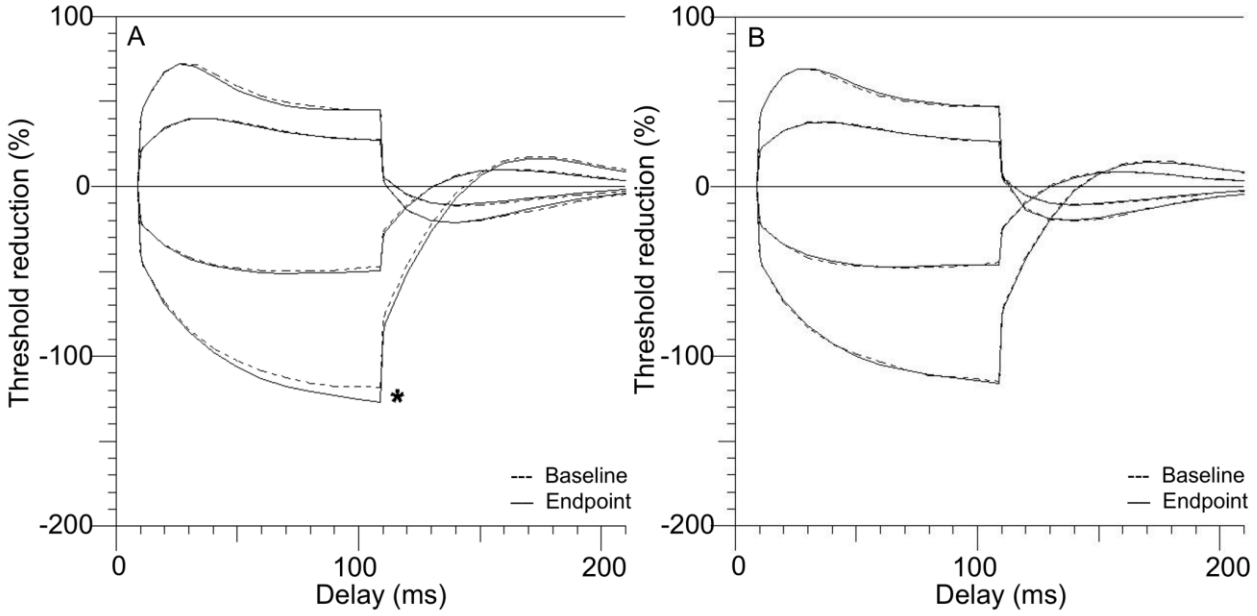

Figure S2. Axonal nerve excitability studies at baseline and endpoint (Intention-To-Treat sample). Threshold electrotonus at day 0 (dashed line) and day 180 (solid line) in (A) the omega-3 PUFA group and (B) the placebo group. *Asterisk indicates significant between-group difference for change in hyperpolarising threshold electrotonus at the 90-100ms interval (TEh 90-100 ms) during a -40% control threshold stimulus.

Table S1. Summary of concomitant systemic medications (Intention-To-Treat sample)

Medication category	**Omega-3 PUFAs** *(n=21)*	**Placebo** *(n=22)*
None	5 [24%]	7 [32%]
At least one concomitant medication	16 [76%]	15 [68%]
Blood Products and Modifiers	1 [5%]	1 [5%]
Analgesics	2 [10%]	3 [14%]
Antibacterials	1 [5%]	1 [5%]
Anticonvulsants	0 [0%]	2 [9%]
Antidepressants	1 [5%]	3 [14%]
Antiparasitics	0 [0%]	1 [5%]
Blood Glucose Regulators	1 [5%]	0 [0%]
Cardiovascular agents	15 [71%]	13 [59%]
Contraceptives	2 [10%]	1 [5%]
Dermatological agents	1 [5%]	3 [59%]
Electrolytes/Minerals/Metals/ Vitamins	12 [57%]	6 [27%]
Vitamins	7 [33%]	5 [23%]
Electrolyte/Mineral Replacement	5 [24%]	1 [5%]
Gastrointestinal Agents	3 [14%]	0 [0%]
Genetic or Enzyme Disorder: Replacement, Modifiers, Treatment	1 [5%]	1 [5%]
Hormonal Agents, Stimulant/Replacement/ Modifying (Thyroid)	1 [5%]	2 [9%]
Immunological Agents	1 [5%]	1 [5%]
Inflammatory Bowel Disease Agents	0 [0%]	1 [5%]
Respiratory Tract/ Pulmonary Agents	2 [10%]	1 [5%]
Sleep Disorder Agents	1 [5%]	0 [0%]

Data reported as the number of participants with the medications. Concomitant medications are categorised using the United States Pharmacopeia (USP; United States Pharmacopeial Convention) Drug Classifications 2019, published on https://www.usp.org/

Table S2. Compliance outcomes using systemic fatty acid profiles (Intention-To-Treat sample)

	Omega-3 PUFAs			Placebo			Omega-3 PUFAs vs Placebo	
	Baseline *(n=21)*	Day 180 *(n=21)*	Change from baseline *(n=21)*	Baseline *(n=22)*	Day 180 *(n=19)*	Change from baseline *(n=19)*	Estimate (95% CI) *(n=43)*	p-value[a]
Omega-3 Index	4.9 (0.8)	8.2 (1.7)	3.3 (1.8)	4.6 (1.0)	4.8 (1.3)	0.12 (1.0)	3.3 (2.4,4.2)	<0.001
EPA, %	0.6 (0.2)	2.11 (0.7)	1.5 (0.8)	0.6 (0.2)	0.7 (0.4)	0.08 (0.4)	1.4 (1.1,1.8)	<0.001
DHA, %	1.9 (0.5)	3.0 (0.7)	1.1 (0.7)	1.8 (0.7)	1.8 (0.7)	0.01 (0.5)	1.1 (0.7,1.5)	<0.001
Total omega-6, %	25.0 (4.7)	25.8 (3.4)	0.8 (4.1)	26.9 (2.8)	28.3 (2.6)	1.52 (4.0)	-2.1 (-3.9,-0.3) [b]	0.023 [b]
Total omega-9, %	20.9 (5.0)	21.1 (2.5)	0.3 (5.6)	21.0 (3.8)	20.5 (1.9)	-1.26 (3.5)	0.7 (-0.7,2.0)	0.34
Omega-6:omega-3 ratio	6.1 (1.7)	3.7 (0.9)	-2.4 (2.0)	7.2 (1.8)	7.3 (1.8)	0.2 (1.9)	-3.5 (-4.3,-2.6)[c]	<0.001[c]

Data are reported as mean and standard deviation. DHA=docosahexaenoic acid. EPA=eicosapentaenoic acid. PUFA=polyunsaturated fatty acid.

[a] p-values are not adjusted for multiple testing.

[b] In a sensitivity analysis adjusting for imbalance at baseline, the treatment effect for total omega-6 is -0.59 (-3.09,1.92) %; unadjusted p-value = 0.65.

[c] In a sensitivity analysis adjusting for imbalance at baseline, the treatment effect for omega-6:omega-3 ratio is -2.54 (-3.77,1.32) %; unadjusted p-value <0.001.

Table S3. Safety outcome: Summary of adverse events (Safety sample)

	Omega-3 PUFAs *(n=21)*	**Placebo** *(n=22)*	**Omega-3 PUFAs vs Placebo p-value**
Summary of all adverse events[a]			
At least one AE or SAE, n [%]	9 [43%]	7 [32%]	0·54
At least one AE, n [%]	9 [43%]	6 [27%]	0·35
At least one SAE, n [%]	0 [0%]	1 [5%]	1·0
Unrelated adverse events[b]			
Haematologic	0	1	··
Ocular			··
Posterior segment/retinal condition (not associated with diabetes)	0	2	
Light sensitivity/headache	1	2	··
Respiratory symptoms	4	2	··
Gastrointestinal (unrelated SAE)	0	1	··
Musculoskeletal pain/cramping	1	1	··
Change in blood glucose control	0	1	··
Dermatological: rash or skin changes	2	0	··
Cardiovascular: heart palpitations	1	0	··
Immunologic: unrelated allergic reaction	1	0	··
Potentially-related adverse events[b]			
Gastrointestinal: nausea[c]	2	1	··

AE=adverse event. SAE=serious adverse event. PUFA=polyunsaturated fatty acid.

[a]Data are reported as number of individuals with at least one AEs.

[b]Data are reported as the number of adverse events.

[c]Gastrointestinal effects were deemed to be possibly related to the treatment due to biological plausibility.

Table S4. Corneal structure (*in vivo* confocal microscopy) and function (non-contact aesthesiometry) outcomes baseline, day 90, and day 180 (Intention-To-Treat sample)

	Omega-3 PUFAs					Placebo					Omega-3 PUFAs vs Placebo			
	Baseline *(n=21)*	**Day 90** *(n=19)*	**Change from baseline at day 90** *(n=19)*	**Day 180** *(n=21)*	**Change from baseline at day 180** *(n=21)*	**Baseline** *(n=22)*	**Day 90** *(n=18)*	**Change from baseline at day 90** *(n=18)*	**Day 180** *(n=19)*	**Change from baseline at day 180** *(n=19)*	**Estimate (95% CI) Day 90** *(n=43)*	**p-value**	**Estimate (95% CI) Day 180** *(n=43)*	**p-value** [a]
Central CNFL (mm/mm^2)	11·49 (3·34)	13·09 (2·45)	1·62 (1·92)	13·55 (3·58)	2·06 (1·73)	12·38 (3·21)	12·30 (3·79)	-0·15 (1·12)	11·41 (3·66)	-0·72 (1·68)	1·80 (0·83,2·78)	<0·001	2·70 (1·64,3·75)	<0·001
Central CNFD ($nerves/mm^2$)	19·93 (7·72)	22·45 (5·84)	2·78 (3·99)	23·41 (8·08)	3·48 (4·15)	20·27 (7·36)	20·92 (8·32)	0·49 (3·19)	18·45 (7·57)	-1·57 (4·02)	2·41 (0·26,4·56)	0·028	4·98 (2·51,7·44)	<0·001
Central CNBD ($beanches/mm^2$)	19·69 (10·76)	24·92 (7·46)	5·65 (8·23)	27·06 (13·59)	7·37 (6·34)	23·48 (10·27)	21·58 (11·98)	-2·84 (6·39)	19·54 (12·64)	-3·68 (7·35)	7·31 (3·01,11·61)	0·001	11·23 (7·01,15·45)	<0·001
Peripheral CNFL (mm/mm^2)	5·09 (2·82)	4·40 (2·34)	-0·92 (2·19)	4·83 (2·23)	-0·26 (2·58)	5·20 (2·56)	4·47 (2·42)	-0·52 (2·09)	3·39 (2·49)	-1·90 (2·65)	-0·20 (-1·35,0·94)	0·73	1·51 (0·24,2·77)	0·020
Central corneal sensitivity threshold to room temperature (mbar), median (IQR) [b]	0·47 (0·38 - 0·75)	0·47 (0·25 - 0·80)	-0·15 (-0·20 - 0·12)	0·38 (0·25 - 0·60)	-0·12 (-0·20 - - 0·00)	0·30 (0·28 - 0·45)	0·36 (0·25 - 0·57)	0·01 (-0·00 - 0·20)	0·30 (0·22 - 0·62)	0·12 (-0·03 - 0·20)	0·73 (0·50,1·04) [b,c]	0·084 [c]	0·68 (0·45,1·02) [b,c]	0·061 [c]
Central corneal sensitivity threshold to cooled temperature (mbar), median (IQR) [b]	0·43 (0·30 - 0·70)	0·40 (0·22 - 0·68)	-0·07 (-0·22 - 0·05)	0·35 (0·22 - 0·60)	-0·07 (-0·20 - 0·03)	0·32 (0·15 - 0·47)	0·36 (0·20 - 0·60)	0·09 (0·00 - 0·20)	0·40 (0·20 - 0·52)	0·05 (-0·00 - 0·20)	0·73 (0·48,1·12) [b,c]	0·15c	0·68 (0·45,1·04) [b,c]	0·077 [c]
Central corneal dendritic cell density ($cells/mm^2$), median (IQR) [b]	19·79 (10·42 - 34·38)	31·25 (17·19 - 45·83)	6·77 (-6·77 - 15·63)	30·21 (16·15 - 44·79)	7·81 (-3·65 - 19·79)	37·76 (18·23 - 88·02)	34·64 (15·10 - 71·35)	-8·07 (-20·31 - - 0·52)	21·88 (16·67 - 55·73)	-2·08 (-11·46 - 15·10)	2·22 (1·32,3·75) [b,c]	0·0030 [c]	2·65 (0·90,7·79) [b,c]	0·077 [c]

	Omega-3 PUFAs (continued)					Placebo (continued)					Omega-3 PUFAs vs Placebo (continued)			
	Baseline *(n=21)*	**Day 90** *(n=19)*	**Change from baseline at day 90** *(n=19)*	**Day 180** *(n=21)*	**Change from baseline at day 180** *(n=21)*	**Baseline** *(n=22)*	**Day 90** *(n=18)*	**Change from baseline at day 90** *(n=18)*	**Day 180** *(n=19)*	**Change from baseline at day 180** *(n=19)*	**Estimate (95% CI) Day 90** *(n=43)*	**p-value**	**Estimate (95% CI) Day 180** *(n=43)*	**p-value** [a]
Peripheral corneal dendritic cell density (cells/mm^2), median (IQR)[b]	69·53 (43·75 - 124·22)	64·84 (46·09 - 119·53)	5·47 (-8·59 - 18·75)	66·41 (42·19 - 82·03)	-2·34 (-46·88 - 6·25)	105·08 (76·56 - 187·50)	94·14 (45·31 - 151·56)	-11·33 (-41·41 - 17·97)	75·00 (60·94 - 135·16)	-10·16 (-40·62 - 10·94)	2·18 (0·78,6·06) [b,c]	0·14[c]	2·03 (0·73,5·62) [b,c]	0·18[c]
Peripheral corneal sensitivity threshold to room temperature (mbar), median (IQR)[b]	0·62 (0·35 - 0·95)	0·55 (0·43 - 0·80)	-0·05 (-0·20 - 0·10)	0·50 (0·25 - 0·88)	-0·08 (-0·38 - 0·20)	0·44 (0·28 - 0·52)	0·50 (0·30 - 0·65)	0·07 (-0·03 - 0·18)	0·45 (0·28 - 0·75)	0·23 (-0·17 - 0·28)	0·73 (0·46,1·18) [b,c]	0·20[c]	0·75 (0·46,1·21) [b,c]	0·23[c]
Peripheral corneal sensitivity threshold to cooled temperature (mbar), median (IQR)[b]	0·52 (0·28 - 0·70)	0·45 (0·40 - 0·73)	-0·05 (-0·15 - -0·02)	0·32 (0·25 - 0·65)	-0·10 (-0·27 - 0·13)	0·36 (0·28 - 0·45)	0·43 (0·25 - 0·65)	0·03 (-0·12 - 0·33)	0·35 (0·30 - 0·55)	0·02 (-0·08 - 0·12)	0·96 (0·56,1·63) [b,c]	0·87[c]	0·87 (0·58,1·32) [b,c]	0·51[c]

Mean and standard deviation, unless stated otherwise. All values are derived from examination of the right eye of all participants. CNBD=corneal nerve branch density. CNFD=corneal nerve fibre density. CNFL=corneal nerve fibre length. PUFA=polyunsaturated fatty acid.

[a] p-values are not adjusted for multiple testing.

[b] Descriptive data are presented as median and interquartile range; estimates are presented as geometric mean ratio of the change from baseline in Omega-3 PUFAs vs Placebo at day 180

[c] Estimate and 95% CI after accounting for imbalance at baseline in the statistical analysis model.

Table S5. Exploratory outcome: large nerve fibre function (motor nerve conduction studies) (Intention-To-Treat sample)

	Omega-3 PUFAs			Placebo			Omega-3 PUFAs vs Placebo	
	Baseline *(n=20)*[d]	**Day 180** *(n=20)*[d]	**Change from baseline at day 180** *(n=20)*	**Baseline** *(n=22)*	**Day 180** *(n=19)*	**Change from baseline at day 180** *(n=19)*	**Estimate (95% CI) Day 180** *(n=42)*	**p-value**[a]
Median motor latency, Wrist-APB (ms)	3·55 (0·52)	3·45 (0·44)	-0·10 (0·16)	3·83 (0·80)	3·88 (0·77)	0·01 (0·35)	-0·16 (-0·32,-0·01)	0·043
Median motor latency, Elbow-APB (ms)	8·03 (0·73)	7·83 (0·68)	-0·20 (0·28)	8·52 (1·32)	8·50 (1·33)	-0·06 (0·49)	-0·19 (-0·43,0·06)	0·13
Median motor amplitude, Wrist stimulation (mV)	7·93 (1·96)	8·59 (1·91)	0·66 (0·77)	7·61 (2·65)	7·88 (2·84)	0·49 (1·08)	0·20 (-0·38,0·78)	0·50
Median motor amplitude, Elbow stimulation (mV)	7·54 (1·96)	8·20 (1·83)	0·66 (0·69)	7·33 (2·59)	7·56 (2·79)	0·46 (1·06)	0·22 (-0·34,0·77)	0·44
Median motor velocity, Elbow–wrist (ms^{-1})	54·04 (3·50)	54·68 (4·52)	0·63 (3·01)	51·16 (4·89)	51·43 (5·76)	0·47 (2·69)	0·21 (-1·58,2·00)	0·82
Peroneal motor latency, Ankle–EDB (ms), median (IQR) [b]	3·83 (3·38 - 4·53)	3·72 (3·31 - 4·34)	-0·10 (-0·56 - 0·26)	3·72 (3·42 - 4·56)	3·80 (3·55 - 4·12)	0·09 (-0·50 - 0·25)	0·98 (0·92,1·03)[b]	0·40
Peroneal motor latency, Below knee–EDB (ms), median (IQR)[†]	11·90 (11·15 - 12·75)	11·40 (11·05 - 12·60)	-0·10 (-1·25 - 0·30)	12·15 (11·10 - 13·40)	11·90 (11·20 - 13·00)	-0·10 (-0·30 - 0·00)	0·99 (0·96,1·03)	0·68
Peroneal motor amplitude, Ankle stimulation (mV)	4·19 (2·62)	4·63 (2·53)	0·44 (1·11)	3·89 (1·97)	4·27 (2·16)	0·29 (0·64)	0·17 (-0·40,0·73)	0·56
Peroneal motor amplitude, Below knee stimulation (mV)	3·51 (2·44)	3·78 (2·24)	0·27 (0·98)	3·26 (1·79)	3·59 (1·91)	0·27 (0·64)	0·02 (-0·48,0·52)	0·94
Peroneal motor velocity, Below· knee–ankle (ms^{-1})	42·32 (3·86)	42·04 (5·16)	-0·28 (4·46)	41·04 (5·16)	41·14 (4·92)	0·08 (3·73)	0·01 (-2·43,2·46)	0·99
Tibial motor latency, Ankle–Abd hal (ms), median (IQR) [b,c]	3·38 (3·04 - 4·15)	3·58 (3·10 - 4·39)	0·20 (-0·34 - 0·51)	3·46 (3·15 - 4·00)	3·96 (3·34 - 4·32)	0·08 (-0·30 - 0·63)	0·96 (0·87,1·06) [b]	0·3
Tibial motor amplitude, Ankle–abductor hallucis (mV)[c]	11·59 (5·25)	10·94 (4·21)	-0·61 (1·98)	10·33 (5·26)	10·28 (5·55)	-0·06 (1·73)	-0·39 (-1·51,0·73)	0·50
Tibial motor velocity, Ankle–Abd hal (ms^{-1})[c]	56·07 (5·97)	55·49 (5·54)	-0·25 (2·13)	54·24 (6·10)	54·10 (5·48)	-0·09 (1·82)	0·04 (-1·19,1·27)	0·95

Mean and standard deviation, unless stated otherwise. Abd hal= abductor hallucis. APB= Abductor Pollicis Brevis. EDB= extensor digitorum brevis

[a] p-values are not adjusted for multiple testing.

[b] Descriptive data are presented as median and interquartile range; estimates are presented as geometric mean ratio of the change from baseline in Omega-3 PUFAs vs Placebo at day 180

[c] For tibial motor nerve conduction, number of participants analysed in the treatment group was n=19 at baseline and n=20 at day 180; number analysed in the placebo group was n=22 at baseline and n=18 at day 180.

[d] One participant in the omega-3 PUFA group completed the study but did not undergo procedures relating to small or large nerve fibre function in the limbs.

Table S6. Exploratory outcome: large nerve fibre function (sensory nerve conduction studies) (Intention-To-Treat sample)

	Omega-3 PUFAs			Placebo			Omega-3 PUFAs vs Placebo	
	Baseline *(n=20)*[b]	**Day 180** *(n=20)*[b]	**Change from baseline at day 180** *(n=20)*	**Baseline** *(n=22)*	**Day 180** *(n=19)*	**Change from baseline at day 180** *(n=19)*	**Estimate (95% CI) Day 180** *(n=42)*	**p-value**[a]
Median sensory latency, orthodromic (ms)	3·03 (0·43) *(n=20)*	3·01 (0·41) *(n=20)*	-0·02 (0·16) *(n=20)*	3·09 (0·47) *(n=20)*	3·10 (0·48) *(n=17)*	-0·05 (0·31) *(n=17)*	0·01 (-0·14,0·16) *(n=40)*	0·89
Median sensory amplitude, orthodromic (µV)	13·53 (6·62) *(n=20)*	13·00 (5·63) *(n=20)*	-0·53 (3·87) *(n=20)*	12·94 (7·83) *(n=22)*	12·53 (7·42) *(n=19)*	-0·37 (4·08) *(n=19*	0·01 (-2·16,2·18) *(n=42)*	1·0
Median sensory velocity, orthodromic (ms^{-1})	54·93 (8·87) *(n=20)*	54·60 (7·88) *(n=20)*	-0·33 (3·17) *(n=20)*	53·44 (7·72) *(n=20)*	53·33 (8·24) *(n=16)*	-0·03 (3·99) *(n=16)*	-0·14 (-2·40,2·13) *(n=40)*	0·91
Median sensory latency, antidromic (ms)	3·27 (0·48) *(n=19)*	3·20 (0·48) *(n=20)*	-0·06 (0·17) *(n=19)*	3·33 (0·67) *(n=21)*	3·35 (0·60) *(n=18)*	-0·04 (0·41) *(n=18)*	-0·05 (-0·23,0·13) *(n=40)*	0·60
Median sensory amplitude, antidromic (µV)	35·72 (18·82) *(n=19)*	33·61 (18·20) *(n=20)*	-1·12 (4·02) *(n=19)*	34·45 (21·36) *(n=22)*	31·08 (21·82) *(n=19)*	-3·35 (7·27) *(n=19*	2·25 (-1·36,5·85) *(n=41)*	0·22
Median sensory velocity, antidromic (ms^{-1})	53·28 (8·71) *(n=19)*	53·75 (8·27) *(n=20)*	0·30 (3·61) *(n=19)*	52·15 (8·87) *(n=21)*	51·94 (8·87) *(n=17)*	-0·29 (4·69) *(n=17)*	0·75 (-1·89,3·39) *(n=40)*	0·58
Ulnar sensory latency, orthodromic (ms)	2·49 (0·21) *(n=20)*	2·44 (0·22) *(n=20)*	-0·05 (0·15) *(n=20)*	2·51 (0·37) *(n=21)*	2·56 (0·35) *(n=19)*	-0·00 (0·19) *(n=18)*	-0·07 (-0·16,0·03) *(n=41)*	0·15
Ulnar sensory amplitude, orthodromic (µV)	9·18 (4·67) *(n=20)*	8·61 (4·18) *(n=20)*	-0·57 (2·30) *(n=20)*	7·15 (4·83) *(n=22)*	7·58 (4·78) *(n=19)*	0·53 (1·37) *(n=19)*	-0·81 (-1·93,0·31) *(n=42)*	0·16
Ulnar sensory velocity, orthodromic (ms^{-1})	57·98 (5·45) *(n=20)*	57·94 (5·21) *(n=20)*	-0·04 (3·31) *(n=20)*	57·40 (7·98) *(n=21)*	56·29 (7·01) *(n=18)*	-0·69 (4·59) *(n=17)*	0·95 (-1·40,3·29) *(n=41)*	0·43
Ulnar sensory latency, antidromic (ms)	31·66 (16·04) *(n=19)*	29·87 (14·66) *(n=20)*	-0·82 (5·51) *(n=19)*	28·80 (17·45) *(n=22)*	29·02 (19·19) *(n=19)*	0·47 (9·55) *(n=19)*	-1·19 (-6·04,3·66) *(n=41)*	0·63
Ulnar sensory amplitude, antidromic (µV)	55·75 (6·15) *(n=19)*	56·13 (4·83) *(n=20)*	0·34 (5·12) *(n=19)*	54·15 (7·51) *(n=22)*	55·62 (8·67) *(n=18)*	1·10 (6·09) *(n=18)*	-0·35 (-3·73,3·02) *(n=41)*	0·84

Ulnar sensory velocity, antidromic (ms^{-1})	2·74 (0·28) *(n=19)*	2·64 (0·25) *(n=20)*	-0·09 (0·24) *(n=19)*	2·85 (0·62) *(n=22)*	2·71 (0·30) *(n=19)*	-0·16 (0·50) *(n=19)*	-0·02 (-0·16,0·11) *(n=41)*	0·73

	Omega-3 PUFAs (continued)			**Placebo (continued)**			**Omega-3 PUFAs vs Placebo (continued)**	
	Baseline *(n=20)*[b]	**Day 180** *(n=20)*[b]	**Change from baseline at day 180** *(n=20)*	**Baseline** *(n=22)*	**Day 180** *(n=19)*	**Change from baseline at day 180** *(n=19)*	**Estimate (95% CI) Day 180** *(n=42)*	**p-value**[a]
Sural sensory latency, orthodromic (ms)	3·72 (0·27) *(n=20)*	3·70 (0·37) *(n=20)*	-0·02 (0·32) *(n=20)*	3·67 (0·48) *(n=21)*	3·72 (0·55) *(n=19)*	-0·01 (0·42) *(n=18)*	-0·02 (-0·25,0·21) *(n=41)*	0·87
Sural sensory amplitude, orthodromic (µV)	11·93 (7·11) *(n=20)*	11·44 (6·70) *(n=20)*	-0·49 (3·88) *(n=20)*	12·26 (7·80) *(n=22)*	12·27 (8·25) *(n=19)*	-0·37 (2·84) *(n=19)*	-0·20 (-2·26,1·86) *(n=42)*	0·85
Sural sensory velocity, orthodromic (ms^{-1})	47·79 (3·90) *(n=20)*	48·24 (5·06) *(n=20)*	0·45 (3·54) *(n=20)*	48·25 (7·50) *(n=21)*	70·91 (99·31) *(n=18)*	24·29 (102·69) *(n=17)*	-22·98 (-66·48,20·51) *(n=41)*	0·30

Mean and standard deviation, unless stated otherwise. IQR=interquartile range. PUFAs=polyunsaturated fatty acids.

[a]p-values are not adjusted for multiple testing.

[b]One participant in the omega-3 PUFA group completed the study but did not undergo procedures relating to small or large nerve fibre function in the limbs.

Table S7. Exploratory outcome: small nerve fibre function (quantitative sudomotor axonal reflex testing) (Intention-To-Treat sample)

	Omega-3 PUFAs			Placebo			Omega-3 PUFAs vs Placebo	
	Baseline *(n=20)*[e]	**Day 180** *(n=20)*[e]	**Change from baseline at day 180** *(n=20)*	**Baseline** *(n=22)*	**Day 180** *(n=17)*	**Change from baseline at day 180** *(n=17)*	**Estimate (95% CI) Day 180** *(n=42)*	**p-value**[a]
Forearm: sweat volume (µL)[b]	1·74 (1·35)	1·50 (1·52)	-0·24 (0·97)	1·49 (0·79)	2·02 (1·36)	0·54 (1·07)	-0·76 (-1·42,-0·11)	0·022
Forearm: response latency (s)[b]	104·35 (44·98)	121·65 (38·71)	17·30 (63·91)	113·14 (37·11)	101·24 (43·56)	-10·76 (38·61)	21·66 (-4·47,47·79)	0·10
Proximal leg: sweat volume (µL)[c]	1·04 (0·49)	1·05 (0·46)	0·01 (0·42)	1·19 (0·51)	0·93 (0·56)	-0·16 (0·47)	0·16 (-0·16,0·47)	0·33
Proximal leg: response latency (s)[c]	118·71 (41·65)	120·79 (42·23)	1·00 (55·71)	127·36 (46·45)	129·69 (43·89)	10·56 (59·44)	-7·67 (-39·21,23·86)	0·63
Distal leg: sweat volume (µL)[d]	1·52 (1·06)	1·44 (1·17)	-0·06 (0·88)	1·72 (1·03)	1·81 (1·32)	0·22 (1·31)	-0·31 (-1·00,0·38)	0·38
Distal leg: response latency (s)[d]	105·95 (52·50)	119·11 (34·47)	15·61 (56·51)	117·27 (49·42)	124·33 (64·15)	4·83 (87·00)	-5·38 (-39·02,28·26)	0·75

Mean and standard deviation, unless stated otherwise. PUFAs=polyunsaturated fatty acids.

[a] p-values are not adjusted for multiple testing.

[b] For QSART in the forearm, number of participants analysed in the treatment group was n=20 at baseline and at day 180; number analysed in the placebo group was n=22 at baseline and n=17 at day 180.

[c] For QSART in the proximal leg, number of participants analysed in the treatment group was n=17 at baseline and n=14 at day 180; number analysed in the placebo group was n=14 at baseline and n=12 at day 180. Estimates for between-group change derived from n=31.

[d] For QSART in the distal leg, number of participants analysed in the treatment group was n=20 at baseline and n=18 at day 180; number analysed in the placebo group was n=22 at baseline and n=18 at day 180.

[e] One participant in the omega-3 PUFA group completed the study but did not undergo procedures relating to small or large nerve fibre function in the limbs.

Table S8. Exploratory outcome: nerve excitability (Intention-To-Treat sample)

	Omega-3 PUFAs			Placebo			Omega-3 PUFAs vs Placebo	
	Baseline *(n=18)* [d]	**Day 180** *(n=16)*[d]	**Change from baseline at day 180** *(n=16)*	**Baseline** *(n=22)*	**Day 180** *(n=18)*	**Change from baseline at day 180** *(n=18)*	**Estimate (95% CI) Day 180** *(n=40)*	**p-value**[a]
TEd peak (%)	70·24 (4·24)	69·45 (4·69)	-0·80 (3·42)	67·58 (4·48)	69·39 (9·02)	1·37 (9·81)	-2·43 (-7·24,2·39)[‡]	0·32
TEd 90–100 (%)	45·08 (3·09)	44·94 (3·92)	-0·18 (4·21)	44·12 (4·89)	47·49 (13·85)	4·34 (15·21)	-2·90 (-9·87,4·07)	0·42
TEh 90–100 (%)	-117·02 (17·34)	-126·35 (21·27)	-6·56 (12·56)	-117·77 (19·66)	-115·24 (19·16)	1·61 (14·66)	-9·33 (-18·41,-0·25)	0·044
TEd 10–20 (%)	70·52 (4·60)	70·27 (4·71)	-0·24 (3·78)	67·99 (4·30)	68·00 (4·71)	-0·74 (4·25)	1·09 (-1·42,3·60)	0·40
Superexcitability (%)	-23·45 (5·11)	-23·48 (5·35)	0·15 (3·14)	-21·14 (6·79)	-21·60 (6·73)	-0·10 (4·70)	-0·19 (-2·73,2·34)	0·88
Subexcitability (%)	13·61 (2·78)	13·78 (3·04)	0·08 (2·60)	12·75 (4·10)	12·51 (3·29)	-0·43 (4·07)	0·95 (-0·88,2·78)	0·31
SDTC (ms)	0·49 (0·10)	0·48 (0·09)	-0·02 (0·08)	0·50 (0·11)	0·49 (0·09)	-0·01 (0·13)	-0·01 (-0·07,0·05)	0·74
Resting I/V slope, median (IQR) [b]	0·61 (0·54 - 0·65)	0·58 (0·56 - 0·63)	0·01 (-0·03 - 0·04)	0·62 (0·56 - 0·71)	0·63 (0·56 - 0·76)	0·08 (-0·07 - 0·14)	0·96 (0·77,1·19)[b]	0·73
Stimulus (mA) for 50% max response, median (IQR)[b]	4·00 (3·63 - 4·41)	4·86 (4·20 - 5·82)	0·72 (0·31 - 1·58)	4·20 (3·34 - 5·92)	4·44 (4·28 - 5·41)	0·41 (-0·21 - 1·10)	1·11 (0·96,1·28)[b]	0·16
Rheobase (mA), median (IQR)[b]	2·68 (2·03 - 2·82)	3·02 (2·73 - 3·88)	0·69 (0·17 - 1·21)	2·60 (2·10 - 3·40)	3·08 (2·50 - 3·64)	0·29 (-0·12 - 0·83)	1·10 (0·92,1·32)[b]	0·29
Stimulus-response slope	4·68 (1·53)	4·64 (1·51)	-0·00 (0·65)	4·73 (1·12)	4·90 (1·06)	0·18 (0·92)	-0·20 (-0·70,0·30)	0·44
Peak response (mV)	73·22 (91·26)	98·92 (94·33)	25·30 (81·73)	48·79 (62·38)	62·60 (87·98)	12·77 (106·27)	25·82 (-29·82,81·45)	0·36
Minimum I/V slope	0·24 (0·05)	0·23 (0·04)	-0·01 (0·04)	0·24 (0·04)	0·24 (0·04)	-0·00 (0·03)	-0·01 (-0·03,0·01)	0·41
RRP (ms)	3·26 (0·32)	3·07 (0·31)	-0·20 (0·33)	3·42 (0·51)	3·24 (0·53)	-0·20 (0·39)	-0·06 (-0·27,0·16)	0·59
Latency (ms)	7·14 (0·64)	6·97 (0·60)	-0·15 (0·29)	7·33 (0·81)	7·38 (0·86)	0·00 (0·53)	-0·19 (-0·47,0·09)	0·18
TEd 40-60 (%)	53·63 (2·86)	51·76 (3·60)	-1·96 (3·28)	53·25 (7·51)	55·32 (11·98)	1·80 (14·30)	-3·59 (-9·65,2·47)	0·25
TEh 10-20 (%)	-75·53 (6·75)	-77·25 (5·52)	-0·69 (3·63)	-76·58 (6·72)	-74·57 (4·83)	2·41 (5·62)	-2·90 (-5·33,-0·47)	0·019
TEd undershoot (%)	-20·89 (3·54)	-20·59 (3·89)	0·34 (2·79)	-20·58 (3·95)	-19·37 (5·09)	1·78 (4·93)	-1·30 (-3·86,1·25)	0·32

TEh overshoot (%)	17·11 (4·88)	17·16 (3·92)	-0·33 (3·14)	16·88 (3·89)	15·99 (4·29)	-1·16 (3·03)	0·95 (-0·93,2·83)	0·32
	Omega-3 PUFAs (continued)			**Placebo (continued)**			**Omega-3 PUFAs vs Placebo (continued)**	
	Baseline *(n=18)* [d]	**Day 180** *(n=16)*[d]	**Change from baseline at day 180** *(n=16)*	**Baseline** *(n=22)*	**Day 180** *(n=18)*	**Change from baseline at day 180** *(n=18)*	**Estimate (95% CI) Day 180** *(n=40)*	**p-value**[a]
S2 accommodation	23·85 (6·70)	24·50 (2·70)	-0·62 (2·61)	21·62 (6·28)	21·90 (7·03)	0·24 (10·19)	2·48 (-1·19,6·14)	0·19
Accommodation half-time (ms)	43·57 (7·21)	40·12 (4·05)	-1·96 (3·92)	44·84 (9·80)	43·97 (6·16)	-1·28 (10·44)	-3·21 (-6·52,0·10)	0·058
Hyperpolarizing I/V slope	0·32 (0·07)	0·33 (0·06)	0·01 (0·10)	0·32 (0·05)	0·33 (0·05)	0·02 (0·05)	-0·01 (-0·05,0·03)	0·68
Refractoriness at 2·5 ms (%)	37·35 (20·08)	24·67 (14·49)	-13·93 (18·51)	37·41 (20·44)	28·03 (20·14)	-9·92 (19·20)	-3·65 (-13·55,6·25)	0·47
TEh 20-40 (%)	-93·61 (9·98)	-96·72 (9·91)	-1·37 (5·24)	-93·57 (10·15)	-91·53 (7·74)	2·42 (6·80)	-4·24 (-7·78,-0·70)	0·019
TEh slope 101-140 (%)	2·07 (0·39)	2·22 (0·44)	0·08 (0·26)	2·05 (0·36)	2·00 (0·44)	-0·06 (0·22)	0·15 (-0·01,0·31)	0·071
Refractoriness at 2 ms (%), median (IQR) [b]	84·75 (68·47 - 126·47)	70·52 (51·73 - 104·75)	-27·64 (-45·77 - 6·64)	84·82 (69·84 - 114·53)	73·74 (57·64 - 86·94)	-18·74 (-34·35 - -3·02)	0·97 (0·74,1·29) [b]	0·86
Superexcitability at 7 ms (%)	-23·38 (5·28)	-22·50 (5·40)	1·04 (2·74)	-20·92 (6·66)	-21·21 (6·08)	0·08 (5·47)	0·28 (-2·36,2·93)	0·83
Superexcitability at 5 ms (%)	-22·90 (4·97)	-24·73 (5·52)	-1·71 (4·46)	-20·51 (7·69)	-21·34 (7·91)	-0·64 (4·26)	-1·48 (-4·27,1·30)	0·30
TEd 20 peak (%)	39·52 (3·31)	39·58 (3·80)	0·04 (2·48)	37·73 (3·00)	37·63 (3·38)	-0·89 (3·04)	0·62 (-1·31,2·56)[c]	0·24

Mean and standard deviation, unless stated otherwise. IQR=interquartile range. I/V=current/voltage. RRP= relative refractory period. PUFAs=polyunsaturated fatty acids. SDTC=strength duration time constant, TEd=depolarising threshold electrotonus. TEh= hyperpolarising threshold electrotonus.

[a] p-values are not adjusted for multiple testing.

[b] Descriptive data are presented as median and interquartile range; estimates are presented as geometric mean ratio of the change from baseline in Omega-3 PUFAs vs Placebo at day 180

[c] Estimate and 95% CI after accounting for imbalance at baseline in the statistical analysis model

[d] In the omega-3 PUFA group, one person completed the study but did not undergo procedures relating to small or large nerve fibre function in the limbs; two people could not tolerate the procedure.

Table S9. Exploratory outcomes: anterior ocular examination at baseline, day 90, and day 180 (Intention-To-Treat sample)

	Omega-3 PUFAs					Placebo					Omega-3 PUFAs vs Placebo			
	Baseline *(n=21)*	**Day 90** *(n=19)*	**Change from baseline at day 90** *(n=19)*	**Day 180** *(n=21)*	**Change from baseline at day 180** *(n=21)*	**Baseline** *(n=22)*	**Day 90** *(n=18)*	**Change from baseline at day 90** *(n=18)*	**Day 180** (n=19)	**Change from baseline at day 180** *(n=19)*	**Estimate (95% CI) Day 90** *(n=43)*	**p-value** [a]	**Estimate (95% CI) Day 180** *(n=43)*	**p-value** [a]
Ocular Surface Disease Index score (0-100), median (IQR)[b,c]	6·3 (2·1 - 12·5)	6·3 (2·1 - 13·9)	0·0 (-4·1 - 2·1)	6·8 (2·1 - 10·4)	0·0 (-2·1 - 2·1)	5·3 (2·1 - 8·3)	4·2 (0·0 - 6·3)	-0·0 (-2·8 - 0·0)	4·2 (0·0 - 12·5)	0·0 (-4·2 - 4·2)	0 (-2·8,2·2)[c]	0·59	0 (-3,4·2)[c]	0·74
Phenol red thread test (mm) [d]	14·1 (4·3)	14·8 (2·8)	1·0 (4·0)	15·0 (4·4)	0·6 (5·3)	13·9 (4·7)	13·1 (4·1)	-1·0 (4·7)	13·2 (4·7)	-0·2 (4·0)	1·8 (-0·3,3·8)	0·087	1·3 (-1·4,4·0)	0·33
Anterior blepharitis grading [e]	1·48 (0·81)	1·24 (0·56)	-0·35 (0·71)	0·98 (0·61)	-0·50 (0·70)	1·08 (0·82)	1·26 (0·85)	0·21 (0·61)	1·36 (0·81)	0·21 (0·71)	-0·33 (-0·69,0·02)	0·066	-0·54 (-0·90,-0·18)	0·0030
Meibomian gland dysfunction grading [e]	1·63 (0·84)	1·35 (0·57)	-0·35 (0·78)	1·23 (0·68)	-0·39 (0·72)	1·00 (0·76)	1·26 (0·85)	0·24 (0·59)	1·31 (0·77)	0·26 (0·72)	-0·56 (-0·98,-0·13)[f]	0·011 [f]	-0·67 (-1·11,-0·23)[f]	0·0030[f]
Conjunctival redness grading[e]	0·88 (0·36)	0·85 (0·30)	-0·07 (0·29)	0·82 (0·40)	-0·06 (0·26)	0·79 (0·32)	0·88 (0·33)	0·14 (0·29)	0·82 (0·30)	0·05 (0·35)	-0·13 (-0·29,0·03)	0·099	-0·07 (-0·24,0·11)	0·47
Limbal redness grading [e]	0·69 (0·55)	0·65 (0·50)	-0·07 (0·36)	0·61 (0·53)	-0·07 (0·37)	0·52 (0·37)	0·71 (0·50)	0·23 (0·40)	0·52 (0·30)	0·03 (0·29)	-0·24 (-0·47,-0·01)	0·040	-0·04 (-0·23,0·15)	0·67
Sodium fluorescein tear break-up time (s) [right eye], median (IQR) [b]	7·18 (5·49 - 9·61)	10·23 (7·09 - 13·88)	2·52 (0·45 - 5·31)	10·77 (7·91 - 12·93)	4·79 (-0·49 - 7·16)	9·99 (7·40 - 18·83)	11·30 (7·54 - 16·05)	-0·44 (-5·95 - 4·55)	10·44 (7·71 - 14·22)	-1·26 (-4·43 - 1·75)	1·39 (1·04,1·86)[f]	0·028 [f]	1·64 (1·20,2·23)[f]	0·0020[f]
Osmolarity (mOsm) [highest of two eyes]	307·0 (12·4)	..	..	309·1 (10·4)	2·1 (14·3)	301·2 (13·6)	..	..	305·21 (11·0)	3·5 (16·9)	..	..	3·2 (-3·4,9·7)	0·34
Ocular surface staining, n [%]														
<0·5	14 [67%]	14 [74%]	..	12 [57%]	..	16 [73%]	11 [61%]	..	16 [84%]	..	..	0·057	..	0·16
0·5 - <1·0	3 [14%]	4 [21%]	..	5 [24%]	..	3 [14%]	4 [22%]	..	2 [11%]	..				
1·0-<1·5	1 [5%]	0 [0%]	..	3 [14%]	..	1 [5%]	3 [17%]	..	0 [0%]	..				
1·5-<2·0	1 [5%]	1 [5%]	..	1 [5%]	..	1 [5%]	0 [0%]	..	1 [5%]	..				
2·0 – 2·5	1 [5%]	0 [0%]	..	0 [0%]	..	1 [5%]	0 [0%]	..	0 [0%]	..				

2·5-<3·0	1 [5%]	0 [0%]	··	0 [0%]	··	0 [0%]	0 [0%]	··	0 [0%]	··
>3·0	0 [0%]	0 [0%]	··	0 [0%]	··	0 [0%]	0 [0%]	··	0 [0%]	··

Mean and standard deviation, unless stated otherwise. All values are derived from the average value of two eyes, unless otherwise indicated. IQR=interquartile range. PUFAs=polyunsaturated fatty acids.

[a] p-values are not adjusted for multiple testing.

[b] Estimates are presented as geometric mean ratio of the change from baseline in Omega-3 PUFAs vs Placebo at day 180

[c] p-value for treatment comparison in change from baseline values was derived using the Wilcoxon rank sum test. Estimates and 95% confidence intervals at days 90 and 180 were derived using Hodges-Lehman non-parametric estimator.

[d] Number of participants analysed for phenol red thread test in the treatment group was n=21 at baseline, n=19 at day 90 and n=19 at day 180; number analysed in the placebo group was n=22 at baseline, n=18 at day 90 and n=17 at day 180. Estimates for between-group change derived from n=43

[e] Using the Efron grading scale

[f] Estimate and 95% CI are presented as geometric mean ratio of the change from baseline in Omega-3 PUFAs vs Placebo at day 180, after accounting for imbalance at baseline in the statistical analysis model.

Table S10. Safety outcome: blood pathology parameters at baseline and day 180 (Safety sample)

	Omega-3 PUFAs			Placebo			Omega-3 PUFAs vs Placebo	
	Baseline *(n=21)*	**Day 180** *(n=20)*	**Change from baseline at day 180** *(n=20)*	**Baseline** *(n=19)*	**Day 180** *(n=16)*	**Change from baseline at day 180** *(n=16)*	**Estimate (95% CI) Day 180** *(n=40)*	**p-value**[a]
Haemoglobin (HB) (g/L)	140·5 (9·3)	139·8 (11·4)	-0·7 (5·9)	141·7 (10·8)	139·7 (15·3)	-1·3 (7·9)	1·0 (-3·5,5·4)	0·67
Platelets (x10^9/L)	259·2 (40·3)	257·0 (47·8)	-2·3 (20·1)	244·5 (57·3)	251·6 (64·5)	9·6 (30·5)	-10·2 (-26·6,6·3)	0·23
HbA1c (%)	7·34 (0·81)	7·44 (0·79)	0·10 (0·51)	7·82 (1·11)	7·81 (0·94)	0·13 (0·54)	-0·11 (-0·42,0·21)	0·50
Alkaline phosphatase (ALP) (U/L)	75·3 (18·0)	76·6 (19·6)	1·2 (7·4)	79·1 (26·0)	83·4 (33·3)	6·5 (11·3)	-4·9 (-10·9,1·1)	0·11
Sodium (mmol/L)	140·2 (2·0)	140·1 (2·2)	-0·1 (2·1)	139·4 (3·0)	140·7 (3·2)	0·9 (2·9)	-0·9 (-2·4,0·6)	0·22
Potassium (mmol/L)	4·46 (0·28)	4·35 (0·29)	-0·11 (0·31)	4·32 (0·36)	4·52 (0·36)	0·15 (0·40)	-0·20 (-0·39,-0·00)	0·045
Carbon dioxide (mmol/L)	27·3 (2·7)	27·2 (2·0)	-0·10 (2·5)	26·7 (2·2)	27·1 (3·1)	0·3 (2·2)	-0·1 (-1·5,1·2)	0·85
Thyroid stimulating hormone (TSH)(mU/L)	1·88 (0·86)	1·70 (0·65)	-0·23 (0·56)	1·65 (1·34)	1·96 (1·90)	0·39 (0·73)	-0·64 (-1·06,-0·23)	0·0020
White blood cells (x10^9/L), median (IQR)[b]	5·60 (4·60 - 6·50)	5·60 (4·50 - 6·60)	-0·20 (-0·60 - 0·60)	5·55 (4·85 - 7·65)	5·90 (5·05 - 7·45)	0·20 (-0·90 - 1·00)	0·99 (0·84,1·17)[b]	0·94
Triglycerides (mmol/L), median (IQR)[b]	0·80 (0·60 - 0·80)	0·60 (0·50 - 0·90)	-0·15 (-0·25 - - 0·05)	0·70 (0·60 - 1·00)	0·65 (0·55 - 0·80)	-0·10 (-0·30 - 0·10)	0·99 (0·84,1·16)[b]	0·87
Alanine transaminase (ALT) (U/L), median (IQR)[b]	19·0 (14·0 - 24·0)	20·0 (16·0 - 26·0)	1·0 (-2·0 - 4·0)	17·0 (12·0 - 25·0)	18·0 (15·0 - 27·0)	1·0 (-1·0 - 5·0)	1·1 (0·9,1·3)[b]	0·62
Total bilirubin (T-Bil) (umol/L), median (IQR)[b]	13·0 (10·0 - 17·0)	14·0 (10·5 - 16·5)	0·0 (-2·0 - 3·5)	13·0 (8·0 - 16·0)	9·50 (8·5 - 14·5)	-2·0 (-5·0 - 0·0)	1·2 (1·0,1·6)[b]	0·097
Gamma-glutamyl transferase (GGT) (U/L), median (IQR)[b]	15·0 (11·0 - 27·0)	15·0 (13·0 - 21·0)	1·0 (0·0 - 5·0)	15·0 (10·0 - 17·0)	16·0 (12·5 - 19·5)	2·0 (-2·0 - 3·0)	1·1 (0·9,1·3)[b]	0·32
Estimated glomerular filtration rate (eGFR), n [%]								
>90	13 [62%]	13 [62%]	··	11 [73%]	11 [73%]	··	··	1·0
<90	8 [38%]	8 [38%]	··	4 [7%]	4 [7%]	··		

Mean and standard deviation, unless stated otherwise. IQR=interquartile range. PUFAs=polyunsaturated fatty acids.

[a]p-values are not adjusted for multiple testing.

[b]Descriptive data are presented as median and interquartile range; estimates are presented as geometric mean ratio of the change from baseline Omega-3 PUFAs vs Placebo at day 180