Effect of omega-3 supplementation on neuropathy in type 1 diabetes

A 12-month pilot trial

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ABSTRACT

Objective: To test the hypothesis that 12 months of seal oil omega-3 polyunsaturated fatty acids (ω-3 PUFA) supplementation will stop the known progression of diabetic sensorimotor polyneuropathy (DSP) in type 1 diabetes mellitus (T1DM).

Methods: Individuals with T1DM and evidence of DSP as determined by a Toronto Clinical Neuropathy Score ≥1 were recruited to participate in a single-arm, open-label trial of seal oil ω-3 PUFA supplementation (10 mL·d^{-1}; 750 mg eicosapentaenoic acid, 560 mg docosapentaenoic acid, and 1,020 mg docosahexaenoic acid) for 1 year. The primary outcome was the 1-year change in corneal nerve fiber length (CNFL) measured by in vivo corneal confocal microscopy, with sensory and nerve conduction measures as secondary outcomes.

Results: Forty participants (53% female), aged 48 ± 14 years, body mass index 28.1 ± 5.8 with diabetes duration of 27 ± 18 years, were enrolled. At baseline, 23 participants had clinical DSP and 17 did not. Baseline CNFL was 8.3 ± 2.9 mm/mm^2 and increased 29% to 10.1 ± 3.7 mm/mm^2 ($p = 0.002$) after 12 months of supplementation. There was no change in nerve conduction or sensory function.

Conclusions: Twelve months of ω-3 supplementation was associated with increase in CNFL in T1DM.

ClinicalTrials.govidentifier: NCT02034266.

Classification of evidence: This study provides Class IV evidence that for patients with T1DM and evidence of DSP, 12 months of seal oil omega-3 supplementation increases CNFL. *Neurology®* 2017;88:2294–2301

GLOSSARY

BMI = body mass index; **CNBD** = corneal nerve branch density; **CNFA** = corneal nerve fiber area; **CNFD** = corneal nerve fiber density; **CNFL** = corneal nerve fiber length; **DHA** = docosahexaenoic acid; **DM** = diabetes mellitus; **DSP** = diabetic sensorimotor polyneuropathy; **HRV** = heart rate variability; **IVCCM** = in vivo corneal confocal microscopy; **LDI$_{FLARE}$** = laser Doppler imaging flare technique; **mTCNS** = modified Toronto Clinical Neuropathy Score; **NPD1** = neuroprotectin D1; **T1DM** = type 1 diabetes mellitus; **TCNS** = Toronto Clinical Neuropathy Score; **ω-3 PUFA** = omega-3 polyunsaturated fatty acid.

Diabetic sensorimotor polyneuropathy (DSP) is the most prevalent complication of diabetes mellitus (DM), affecting over 50% of individuals diagnosed with type 1 (T1) or type 2 DM.[1] There are currently no treatments to stop the onset or progression of this disease.[2] The Diabetes Control and Complications Trial showed that intensive blood glucose control in T1DM could decrease the incidence of DSP and slow the progression of nerve damage[3,4]; however, there are no disease-modifying therapies to stop or reverse nerve injury.[5]

Recent DSP intervention trials have focused on large fiber function and have generally not been effective.[6–8] Examination of DSP disease progression suggests that small fiber damage precedes large fiber dysfunction.[9] In vivo corneal confocal microscopy (IVCCM) measurement of corneal nerve fiber length (CNFL) is being validated as a small fiber test to identify future DSP risk.[10–12] Longitudinal analysis of a T1DM cohort showed a mean 1-year change in CNFL was −1.6%, while those with stable T1DM and healthy volunteers showed a 5% increase per year.[13]

Supplemental data at Neurology.org

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Table 1 Baseline characteristics of trial participants

Characteristics	Mean ± SD or n (%)
Clinical characteristics	
Age, y	48 ± 14
Female	21 (53)
Diabetes duration, y	27 ± 14
BMI	28.1 ± 5.8
SBP/DBP, mm Hg	126/70 ± 13/9
Smoking	7 (18)
Alcohol use	32 (80)
Family history of diabetes	22 (55)
Family history of neuropathy	5 (13)
Toronto Clinical Neuropathy score	
No neuropathy (1–5)	16 (40)
Mild neuropathy (6–8)	14 (35)
Moderate neuropathy (9–11)	10 (25)
Modified Toronto Clinical Neuropathy score	
1–10	24 (60)
11–20	13 (32.5)
>20	3 (7.5)
Diabetic sensorimotor polyneuropathy (n = 39)	
Absent neuropathy	16 (41)
Diagnosed neuropathy	23 (59)
IVCCM CNFL 300 μm lens, mm/mm^2	8.3 ± 2.3
No DSP, low risk, CNFL >15 mm/mm^2 (n)	19.0 ± 4.6 (6)
No DSP, high risk, CNFL <15 mm/mm^2 (n)	11.0 ± 2.1 (10)
DSP present, CNFL <15 mm/mm^2 (n)	10.5 ± 4.5 (23)
Sural nerve amplitude, μV	2.7 ± 9.1
Sural nerve conduction velocity, m/s	43.8 ± 11.0
Peroneal nerve amplitude, ankle, mV	3.2 ± 4.6
Peroneal nerve conduction velocity, fibular head, m/s	38.8 ± 6.6
Biochemical characteristics	
A1C, %	7.6 ± 1.0
Total cholesterol, mmol/L	4.23 ± 0.95
LDL cholesterol, mmol/L	2.20 ± 0.70
HDL cholesterol, mmol/L	1.61 ± 0.44
Non-HDL cholesterol, mmol/L	2.61 ± 0.91
Triglycerides, mmol/L	1.25 ± 2.20
TSH, mIU/L	2.28 ± 1.41
Creatinine, μmol/L	72 ± 17
eGFR, mL/min/1.73 m^2	87 ± 16
CRP, mg/L	3.8 ± 7.5

Abbreviations: BMI = body mass index; CNFL = corneal nerve fiber length; CRP = C-reactive protein; DBP = diastolic blood pressure; DSP = diabetic sensorimotor polyneuropathy; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; IVCCM = in vivo corneal confocal microscopy; LDL = low-density lipoprotein; SBP = systolic blood pressure; TSH = thyroid-stimulating hormone.

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs) are essential for the development and maintenance of nerve structure and function.[14] In animal models of diabetes, ω-3 PUFAs supplementation was shown to attenuate changes in nerve structure and function[15–18]; however, there has been limited investigation in humans.[19,20]

This open-label proof-of-concept trial investigated if 12 months of ω-3 PUFA supplementation could stop the known progression of DSP in T1DM by measuring changes in IVCCM CNFL and gold standard clinical measures of small and large fiber function.

METHODS

Study design. This single-arm, open-label pilot trial was conducted and reported according to the CONSORT statement. The study was performed at the Prosserman Family Neuromuscular Clinic at the University Health Network, Toronto, Canada.

Standard protocol approvals, registrations, and patient consents. The protocol and consent procedures were conducted in accordance with Health Canada good clinical practice and were approved by the University Health Network Research Ethics Board. All study patients were at least 18 years of age and provided written informed consent. This trial is registered at ClinicalTrials.gov as NCT02034266.

Participants. Individuals aged 18 years or above with previously diagnosed T1DM as defined by the 2008 Canadian Diabetes Association guidelines of any duration and a Toronto Clinical Neuropathy Score (TCNS) ≥1 were included in the study (table 1). The TCNS was used to ensure patients had a sign or symptom of neuropathy and to classify DSP severity (no [0–5], mild [6–8], moderate [9–11], or severe [>12] neuropathy).[21] To ensure a broad spectrum of DSP, at least 7 patients were included in each of the no, mild, and moderate neuropathy groups. Patients were also evaluated using the modified TCNS (mTCNS) as this scale is more sensitive to change in sensory function[22] and established consensus criteria for DSP diagnosis.[23]

Individuals were excluded from the trial if they presented with neuropathy due to nondiabetic causes (e.g., familial, alcoholic, nutritional, uremic); current eye infection, corneal damage, or severe movement disorder to preclude safe IVCCM examination; regular use of ω-3 PUFA supplements or consuming fish >2 times per week in the 30 days prior to enrollment in the study determined by investigator interview; or had an allergy to proparacaine (the ocular topical anesthetic used for the IVCCM examination).

The baseline characteristics of the study population have been described previously.[12]

DSP risk profile. DSP risk was classified using baseline IVCCM CNFL and established consensus criteria for DSP diagnosis.[23] Participants' future DSP risk was classified using a proposed CNFL threshold of >/<15 mm/mm^2 [11,12] and current clinical DSP +/− criteria[23] (table 1). Patients with no DSP and CNFL <15 mm/mm^2 were considered at high risk for future DSP. Patients with no DSP and CNFL >15 mm/mm^2 were considered at low risk for future DSP.

Study intervention. Participants supplemented their diet with an oral 10 mL dose of seal oil ω-3 PUFAs with 2,330 mg of essential fatty acids (750 mg eicosapentaenoic acid, 560 mg docosapentaenoic acid, and 1,020 mg docosahexaenoic acid [DHA]) (Auum Inc., Timmons, Ontario, Canada) divided into 2 daily 5 mL doses with breakfast and dinner. Our study used a high dose of ω-3 PUFAs compared to previous studies examining ω-3 PUFA supplementation on nerve function in diabetes[19,20] or risk of cardiovascular events in diabetes[24] to test potential therapeutic effect. Chemical analysis of ω-3 PUFA derived from seals has determined that fatty acid esterification occurs primarily at the *sn*-1,3 position of the triacyl-*sn*-glycerol molecule, compared to the *sn*-2 position of fish ω-3 PUFAs.[25] This difference in stereochemistry is thought to alter ω-3 PUFA metabolism leading to higher absorption and greater bioavailability,[26] especially from sublingual digestion of the *sn*-3 ω-3 PUFA.[27]

Classification of evidence. The primary research question was the following: Can 12 months of seal oil ω-3 PUFA supplementation stop the known progression of DSP in T1DM as measured by CNFL? This study provides Class IV evidence that for patients with T1DM and evidence of DSP, 12 months of seal oil omega-3 supplementation increases CNFL.

Primary outcome. The primary outcome of this trial was the 12-month change in IVCCM CNFL. Patients had bilateral examinations of the nerve plexus adjacent to the Bowman layer of the cornea using the Rostock Cornea Module of the Heidelberg Tomograph III using a 300 lens (Heidelberg Engineering, Smithfield, RI) to determine CNFL according to our validated procedure.[28,29] Images from each trial visit were coded to conceal order. A trained technician, blinded to the image code, selected the most technically sound image from each eye for automated analysis of CNFL (mm/mm^2) (ACCMetrics Image Analysis software v2.0).[30]

Secondary outcomes. ***In vivo corneal confocal microscopy.*** Secondary IVCCM measures included corneal nerve fiber density (CNFD) (fibers/mm^2), corneal nerve branch density (CNBD) (branches/mm^2), and corneal nerve fiber area (CNFA) (mm^2/mm^2). The measurement of baseline CNFL for DSP risk factor analysis was performed using semiautomated analytical software (CCMetrics Image Analysis software v1.1) as previously described.[11] CNFL in mm/mm^{-2} was calculated semiautomatically by having the examiner digitally trace over the nerve fibers and branches of the selected images.

Nerve conduction studies. For nerve conduction studies, a total of 10 sensory and motor parameters from the dominant and nondominant leg (peroneal and sural nerves) and the nondominant limb (median and ulnar nerves) were obtained using the Counterpoint device (Alpine Biomed Corporation, Fountain Valley, CA) according to the standards of the American Association for Neuromuscular and Electrodiagnostic Medicine.[23] These include distal motor and sensory latencies, sensory nerve action potential amplitudes, compound muscle action potential amplitudes, F-wave latencies, and motor and sensory nerve conduction velocities.

Small and large nerve fiber function. Three tests of nerve fiber function were assessed. Two tests of small fibers included cooling detection thresholds and axon reflex-mediated neurogenic vasodilation in response to cutaneous heating by the laser Doppler imaging flare technique (LDI_{FLARE}). A single test of large fiber function, vibration perception thresholds, was performed. Vibration perception threshold testing (Neurothesiometer, Bailey Instruments Limited, Trafford Park, UK) and cooling detection thresholds testing (Medoc TSA-II NeuroSensory Analyzer, Ramat-Yishai, Israel) were done using method of limits algorithms.[31] LDI_{FLARE} was measured using a MoorLDI2 laser Doppler blood perfusion imager (Moor Instruments, Axminster, UK).

Autonomic function. Heart rate variability (HRV) function was measured for 1 minute at rest and during deep breathing. Changes in heart rate (adaptation of the Diabetes Control and Complications Trial protocol)[32] and R-R variation (on the CounterPoint [Medtronic, Langhorne, PA] EMG device) were measured using the beat-to-beat heart rate variability as endorsed by the American Diabetes Association[33] and implemented by our research group.[34]

Clinical assessment. All patients underwent a comprehensive medical and neurologic evaluation, including detailed assessments of symptoms and signs of neuropathy, comorbidities, and biochemical tests including fasting plasma glucose, glycated hemoglobin A_{1c}, serum lipids, thyroid-stimulating hormone, creatinine, urinary albumin excretion, and serum C-reactive protein concentration.

Follow-up assessment. IVCCM and a fasting blood sample collected for omega-3 analysis were performed at 4 and 8 months, while all trial procedures performed at baseline were repeated at 12 months.

Sample size. Sample size was estimated from longitudinal IVCCM data from a cohort of participants with T1DM.[13] We hypothesized that ω-3 PUFA supplementation would be associated with a group annual change in CNFL that approximates those with stable CNFL (mean annual change +5%) rather than the cohort behavior, which showed an annual change −1.6%. Based on these data, we anticipated a baseline CNFL reading of 14.6 mm/mm^{-2}, a Pearson correlation coefficient of 0.75 between baseline and follow-up CNFL readings, and an SD in the difference between baseline and follow-up readings of 2 mm/mm^2. Using these parameters, we calculated that a sample size of 31 participants had 80% power to detect an annual increase in CNFL of 5% using a paired Student *t* test. Conservatively estimating attrition rates exceeding 15%, we aimed to accrue 40 study participants.

Statistical analysis. Statistical analyses were performed using SAS v9.2 (SAS Institute, Cary, NC). Analysis of the primary outcome, the 12-month change in IVCCM CNFL, was performed using a paired Student *t* test. For participants who did not complete the full trial but completed at least the 4-month follow-up, their last IVCCM observation was carried forward to the 12-month measure (n = 4).

Changes in secondary outcomes were compared at baseline and 12 months using paired Student *t* tests (normally distributed variables) or the Wilcoxon signed-rank test (nonparametric variables). The Benjamini-Hochberg procedure with a false discovery rate of 0.10 was used to adjust for multiple comparisons; otherwise statistical significance for all tests was set at $p < 0.05$.

The CNFL change from baseline at 4, 8, and 12 months in different subgroups was compared. Participants were divided into subgroups in 2 ways: (1) baseline DSP status and (2) baseline DSP risk: no DSP with low future risk (CNFL >15 mm/mm^2), no DSP with high future risk (CNFL <15 mm/mm^2), and DSP present. A responder/nonresponder analysis was performed. Participants were defined as responders if the 12-month change in CNFL was ≥5%.

RESULTS

A total of 40 participants with T1DM were enrolled in this trial between March 2014 and June 2015 and follow-up was finished in June

2016. There are primary outcome data for 36 participants who completed at least the first 4 months of the trial. Thirty-two participants completed the full 12-month protocol (figure 1).

Primary outcome. ***Corneal nerve fiber length.*** Per protocol analysis of the 4 participants who completed at least 4 months of supplementation combined with the 32 participants who completed the full 12-month protocol showed CNFL increased 29.2 ± 45.9% from 8.3 ± 2.9 mm/mm^2 to 10.1 ± 3.7 mm/mm^2 ($p = 0.002$) (figure 2, A and B). Analysis of only the 32 participants who completed the full 12-month protocol showed CNFL increased 30.4 ± 47.7% from 8.3 ± 3.0 mm/mm^2 to 10.2 ± 3.8 mm/mm^2 ($p = 0.004$). CNFL change from baseline at 4 months was 17.1 ± 46.5% from 8.3 ± 2.9 mm/mm^2 to 9.1 ± 3.2 mm/mm^2 ($p = 0.3$) and at 8 months was 17.7 ± 37.6% 8.3 ± 2.9 mm/mm^2 to 9.6 ± 3.1 mm/mm^2 ($p = 0.04$).

Subgroup analysis. Twenty-five participants responded to the ω-3 PUFA intervention and 11 did not. CNFL change in the responders was 50.5 ± 37.7% and −19.4 ± 15.2% in the nonresponders ($p < 0.001$). Within the nonresponders were 8 participants who lost ≥−14% CNFL. At baseline, nonresponders had higher CNFL compared to responders ($p = 0.01$).

At baseline, participants with diagnosed DSP (n = 20) showed lower CNFL (7.1 ± 2.6 mm/mm^2) compared to those without baseline DSP (n = 15) (9.5 ± 2.8 mm/mm^2) ($p = 0.01$). At 12 months, CNFL was higher compared to baseline in both groups ($p < 0.05$) and the groups were not different from each other ($p = 0.5$) (figure 2C).

When participants were grouped by DSP status and future disease risk, those with high DSP risk (n = 10) and diagnosed DSP (n = 23) increased 59% and 27%, respectively ($p < 0.05$ for both comparisons to baseline levels) (figure 2D). Participants with low disease risk increased 7%, which was not different from baseline.

Secondary outcomes. ***IVCCM parameters.*** Change in other IVCCM parameters are shown in table 2. CNBD was higher at 12 months ($p = 0.03$), while other morphologic measures, CNFD and CNFA, were not different ($p = 0.07$, $p = 0.07$).

Nerve conduction study. Large fiber nerve function measured by nerve conduction study showed no

Figure 1 Consort diagram of participants

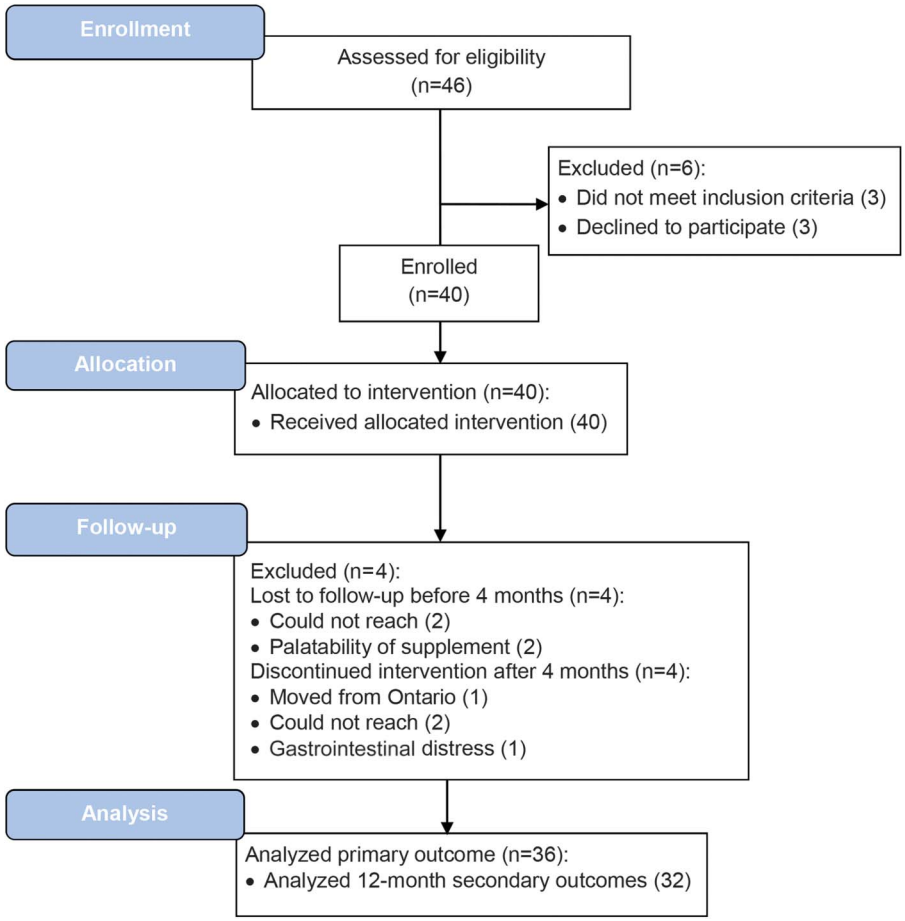
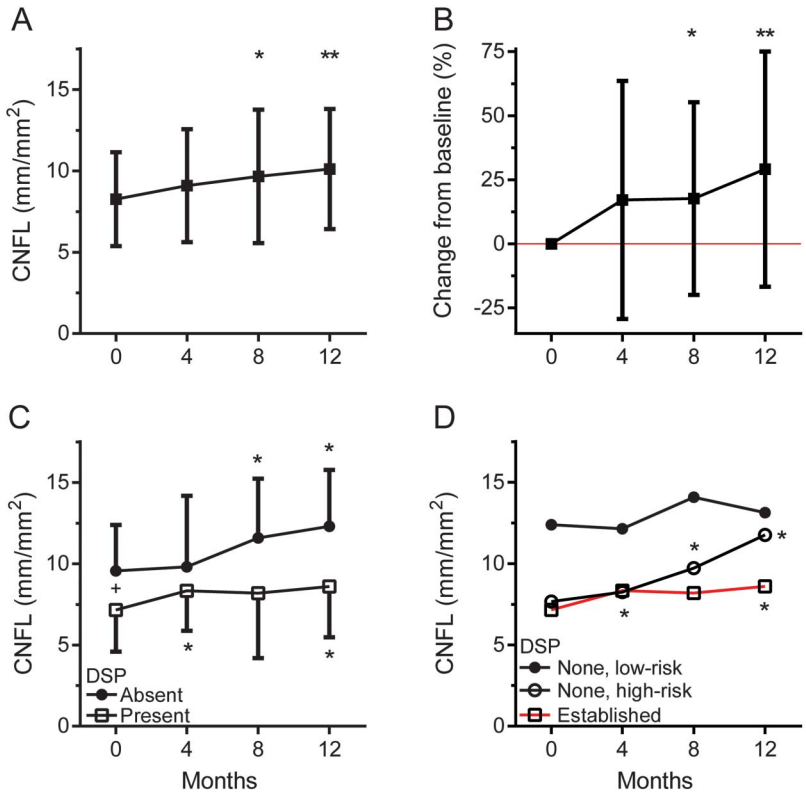


Figure 2 Change in corneal nerve fiber length (CNFL) during 12 months of omega-3 supplementation



(A) Primary outcome analysis shows change from baseline ($p = 0.002$). (B) CNFL percent change from baseline. (C) Change in CNFL in participants with and without baseline diabetic sensorimotor polyneuropathy (DSP). Baseline CNFL was higher in the DSP absent subgroup (+, $p = 0.01$). (D) CNFL change by DSP diagnosis and future DSP risk. The low-risk group was stable at +7%. The high-risk and DSP present groups increased by 56% and 27%, respectively. Analyses were performed using paired Student *t* tests. All analyses are compared to baseline. Data are shown as mean ± SD (*$p < 0.05$, **$p < 0.01$).

change from baseline after the 12-month supplementation period ($p > 0.05$) (table 3 and table e-1 at Neurology.org). In those without baseline DSP, the dominant and nondominant peroneal motor nerve amplitudes increased 0.7 ± 1 mA and 0.6 ± 0.6 mA compared to −0.2 ± 0.7 mA and −0.1 ± 0.8 mA in those with DSP ($p = 0.01$).

Sensory nerve function. Change in small and large fiber sensory function is shown in table 3.

The change in cooling detection threshold, vibration perception threshold, or heart rate variability was not different based on baseline DSP status ($p > 0.05$). Participants without baseline DSP showed an increase in LDI_{FLARE} area of 0.2 ± 0.29 cm^2 compared to those with baseline DSP −0.08 ± 0.31 cm^2 ($p = 0.03$).

Clinical assessment. DSP symptom score measured by TCNS was 6 ± 3 at baseline and 5 ± 3 at 12 months ($p = 0.15$), while the mTCNS was 9 ± 6 at baseline and 8 ± 6 at 12 months ($p = 0.09$). Participants' body mass increased from 79.6 ± 16.7 kg to 82.6 ± 16.5 kg ($p < 0.001$). Body mass index (BMI) was also increased from 26.8 ± 4.0 at baseline to 27.9 ± 4.0 at 12 months ($p < 0.001$). Systolic and diastolic blood pressures were not different from baseline at 12 months ($p > 0.3$).

DISCUSSION The present trial showed that 12 months of seal oil ω-3 PUFA supplementation increased CNFL 29% in comparison to our hypothesized margin of stability, corresponding to +5%. Though it does not prove causality, this trial is the first to provide proof-of-principle data that a targeted nutritional intervention can stop and reverse small fiber damage in a cohort of 40 participants with T1DM, regardless of baseline DSP diagnosis or disease severity. Furthermore, 12 months of seal oil ω-3 PUFA prevented progression of clinical disease symptoms and prevented declines in small and large fiber sensory and functional measures.

Owing to the broad spectrum of nerve damage within our trial population, we were able to explore the response across the disease spectrum. Participants at high risk for future DSP and those with diagnosed DSP showed the best response to the ω-3 PUFA intervention (figure 2, C and D). This supports the potential of ω-3 PUFA supplementation to act as a disease-modifying therapy for those at risk of DSP. Furthermore, participants with advanced disease were highly responsive to the ω-3 PUFA supplementation. This finding is surprising as more advanced nerve injury can attenuate the response to therapy.[35]

We proposed that 12 months of ω-3 PUFA supplementation acted to improve essential fatty acid

Table 2 Change in in vivo corneal confocal microscopy (IVCCM) measures

IVCCM measure	0 months	4 months	8 months	12 months	*p* Value
CNFL, mm/mm²	8.1 ± 3.0	9.1 ± 3.5	9.7 ± 4.1	10.1 ± 3.7	0.002
CNBD, br/mm²	10.6 ± 12.5	14.8 ± 17.2	16.7 ± 19.1	19.6 ± 19.7	0.03
CNFD, fib/mm²	16.2 ± 10.7	18.8 ± 10.7	21.0 ± 12.8	23.1 ± 13.8	0.07
CNFA, mm²/mm²	0.0033 ± 0.0011	0.0037 ± 0.0016	0.0037 ± 0.0015	0.0039 ± 0.0015	0.07

Data are shown as mean ± SD. *p* Values are for change from baseline to 12 months.
Abbreviations: CNBD = corneal nerve branch density; CNFA = corneal nerve fiber area; CNFD = corneal nerve fiber density; CNFL = corneal nerve fiber length.

Table 3 Baseline and 12-month nerve conduction study, quantitative sensory testing, and autonomic function

	0 months	12 months	*p* Value
Nerve conduction study			
Sural nerve amplitude, μv	2.7 ± 9.1	3.0 ± 6.7	0.4
Sural nerve conduction velocity, m/s	43.8 ± 11.0	44.0 ± 17.4	0.6
Peroneal nerve amplitude, ankle, mV	3.2 ± 4.6	3.4 ± 2.9	0.2
Peroneal nerve conduction velocity, fibular head, m/s+	38.8 ± 6.6	39.2 ± 6.2	0.2
Peroneal nerve F-wave, ms	55.5 ± 18.3	56.8 ± 9.9	0.06
Small and large nerve fiber function			
Cooling detection threshold, °C	25.2 ± 4.7	25.4 ± 5.2	0.8
Vibration perception threshold-hand, V	4.5 ± 2.7	4.9 ± 2.2	0.06
Vibration perception threshold-toe, V	15.2 ± 8.9	16.1 ± 9.5	0.2
LDI_{FLARE} area, cm^2	1.9 ± 0.7	1.9 ± 0.6	0.6
Autonomic function			
Resting heart rate, beats/min	62 ± 7	64 ± 8	0.1
Resting heart rate, deep breathing, beats/min	62 ± 8	64 ± 9	0.4
Heart rate variability, %	16 ± 11	12 ± 8	0.01[a]
Heart rate variability, deep breathing, %	24 ± 14	24 ± 13	0.9

Data are shown as mean ± SD.
[a] Represents a significant *p* value when adjusted for multiple comparisons.
Abbreviation: LDI_{FLARE} = laser Doppler imaging flare technique.

dysmetabolism that occurs in T1DM.[20] As this dysmetabolism is associated with low DHA status, it is likely that the high concentration of DHA in the trial supplement (1,020 mg/d) supported nerve growth. Mechanistically, DHA is the precursor to neuroprotectin D1 (NPD1), a lipid mediator with potent anti-inflammatory and neurogenic properties.[14] As such, DHA and NPD1 have been shown to support nerve regeneration in spinal cord[35] and corneal nerves.[36]

The current trial had several strengths. A high dose of ω-3 PUFA was used to test the therapeutic effect of supplementation compared to other recent trials of ω-3 PUFA supplementation in DM.[37] This trial recruited participants with a broad spectrum of nerve injury measured by the TCNS and a balanced number of patients with and without diagnosed DSP.

The findings of this trial are limited by the single-arm, open-label design; however, this approach is justified as this is the first trial in humans to investigate the effect of ω-3 PUFA supplementation using gold standard clinical outcomes along with novel in vivo measures of small fiber structure. To strengthen the validity of IVCCM endpoints within our study design and limit selection bias, images were selected by technicians who were blinded to from what visit the images were obtained. Within the single-arm design of this trial, we acknowledge that some participants with low baseline CNFL could have naturally regressed towards the mean. It is important to note that our subgroup analyses are hypothesis-generating given the small group size.

The participants in this trial tolerated the oil supplementation well and there were no previously unrecognized adverse effects of supplementation (figure 1). Body mass and BMI increased over the 12-month period, which is in contrast to previous ω-3 PUFA supplementation studies[38] and warrants further investigation. While inherent impaired metabolism in diabetes might account for some of the change, there were 5 participants who showed >6 kg increase in body mass and accounted for half of the 3 kg increase. This study did not include advanced measures of metabolic control or body composition so the effect of ω-3 PUFA supplementation on these outcomes should be evaluated in future trials.

Examination of HRV data showed 5 participants with >10% decrease during normal breathing only, which accounted for the significant change. As our measurement of HRV deep breathing at 6 breaths per minute is a more sensitive measurement of cardiovagal[34] function, the interpretation of the normal breathing results should be made with caution given the small sample size.

This trial provides proof of principle that 12 months of seal oil ω-3 PUFA supplementation supported early nerve regeneration in a T1DM cohort with a broad spectrum of nerve injury. Participants showed a 29% increase in CNFL over 12 months,

while our hypothesized change was 5%. Moreover, supplementation appears to have prevented progression of clinical symptoms and sensory and motor impairments observed in natural history of DSP. Findings from this trial support the development of a future randomized clinical trial.

AUTHOR CONTRIBUTIONS

E.J.H.L., B.A.P., R.P.B., T.M.S.W., and V.B. designed the study. E.J.H.L. and L.E.L. performed the statistical analysis. All authors contributed to the discussion and reviewed and edited the manuscript. E.J.H.L. and V.B. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

STUDY FUNDING

This trial was supported by an operating grant to V.B. from the Canadian Diabetes Association (operating grant #OG-2-13-4093-VB). E.J.H.L. was supported by a Banting and Best Diabetes Centre Studentship, an Ontario Graduate Scholarship, a James F. Crothers Family Fellowship in Peripheral Nerve Damage, and a Peterborough K.M. Hunter Graduate Studentship. All supplements were donated by Auum Inc.

DISCLOSURE

E. Lewis has received omega-3 supplements for research purposes from Auum Inc. B. Perkins and L. Lovblom report no disclosures relevant to the manuscript. R. Bazinet has received research grants from Bunge Ltd., Arctic Nutrition, the Dairy Farmers of Canada, and Nestle Inc., as well as travel support from Mead Johnson and mass spectrometry equipment and support from Sciex; is on the executive board of the International Society for the Study of Fatty Acids and Lipids and held a meeting on behalf of Fatty Acids and Cell Signaling, both of which rely on corporate sponsorship; has given expert testimony in relation to supplements and the brain; and provides complementary fatty acid analysis for farmers, food producers, and others involved in the food industry, some of whom provide free food samples. T. Wolever is a part owner, President, and Medical Director of Glycemic Index Laboratories, Toronto, Canada, and has authored several popular diet books on the glycemic index, for which he has received royalties from Phillipa Sandall Publishing Services and CABI Publishers. He has received consultant fees, honoraria, travel funding, or served on the scientific advisory board for McCain Foods, Temasek Polytechnic, Singapore, Glycemic Index Symbol Program, CreaNutrition AG, and the National Sports and Conditioning Association. His wife is an employee and part owner of Glycemic Index Laboratories. V. Bril reports no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received October 14, 2016. Accepted in final form March 21, 2017.

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Neurology 2017;88;2294-2301 Published Online before print May 17, 2017
DOI 10.1212/WNL.0000000000004033