



# Association between the systemic Omega-3 Index and corneal nerve architecture

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## Background

- Long-chain omega-3 polyunsaturated fatty acids (PUFAs) have neuroprotective properties, including in diabetes.<sup>1</sup>
- The Omega-3 Index is a measure of the relative concentration of the long-chain omega-3 fatty acids, EPA and DHA, in erythrocyte membranes.
- Low Omega-3 Index (<4%) has been associated with poorer systemic health outcomes; an Omega-3 index of over 8% is considered optimal.<sup>2</sup>
- A relationship between the Omega-3 Index and corneal nerve health could indicate a potential role for omega-3 PUFAs in modulating peripheral nerve health in diabetes.

## Aim

To investigate the association between systemic omega-3 fatty acid levels and corneal nerve parameters in healthy controls and those with diabetes.

## Methods

### Prospective, cross-sectional clinical study

- Diabetes (n=26) and healthy control (n=21) participants with no neuropathy symptoms, defined as a score of <16 using the Norfolk Quality of Life Questionnaire–Diabetic Neuropathy (Norfolk QOL-DN).<sup>3</sup>

### Ocular surface assessments

Dry eye disease diagnosed according to TFOS DEWS II criteria,<sup>4</sup> using:

- Dry eye symptoms: Ocular Surface Disease Index (OSDI)
- Tear osmolarity (Tearlab Corp; mOsm/L)
- Clinical examination: slit lamp examination and grading, tear break-up time with sodium fluorescein, ocular surface staining

### Central corneal sub-basal nerve plexus structure

- Measured using *in-vivo* confocal microscopy (IVCM) images, from the average of 12 non-overlapping regions in the central cornea
- Parameters quantified using automated software (ACCMetrics)
  - Corneal nerve fibre length (CNFL; mm/mm<sup>2</sup>)
  - Corneal nerve fibre density (CNFD; fibres/mm<sup>2</sup>)
  - Corneal nerve branch density (CNBD; branches on main fibre/mm<sup>2</sup>)
  - Corneal nerve total branch density (CTBD; total branches/mm<sup>2</sup>)

### Central corneal sensitivity

- Measured using non-contact corneal esthesiometry with both room temperature stimuli (~25°C) and cooled stimuli (~20°C)

### Systemic omega-3 fatty acid levels

- Erythrocyte analysis using capillary dried blood spot (DBS) and analysed by an independent laboratory (Waite Lipid Analysis Service (WLAS), University of Adelaide, Australia)<sup>5</sup>

### Statistical Analyses

- Inter-group comparisons:
  - Independent sample t-test (normally distributed variables)
  - Mann-Whitney-U test (non-normally distributed variables)
- The relationship between systemic fatty acid levels and corneal nerve parameters:
  - Multiple linear regression, adjusted for for age, sex, neuropathy symptom score, and the presence of both diabetes and dry eye disease

## Results

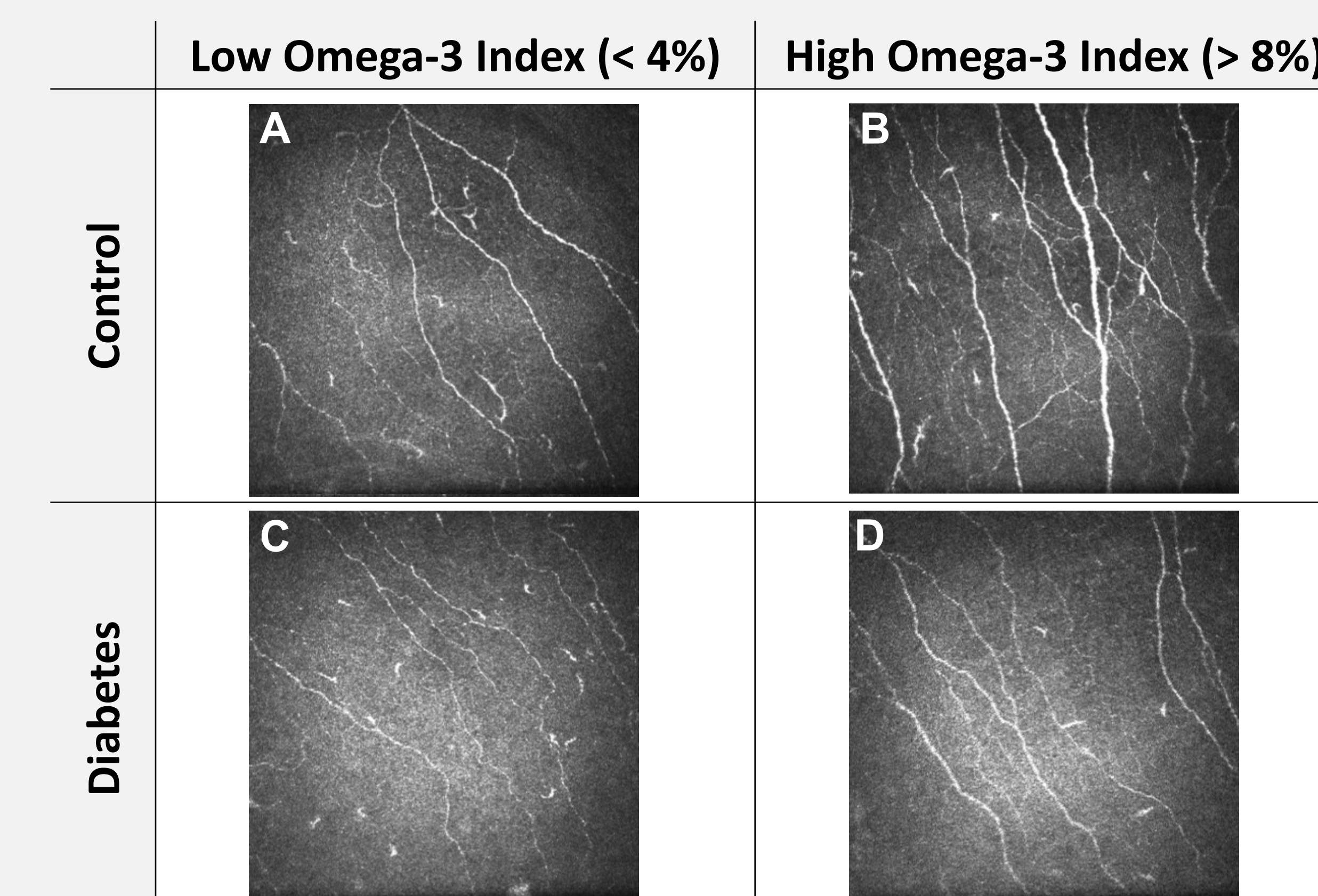
### Tables 1 & 2: Participant characteristics

Baseline clinical parameter	Control (n=21)	Diabetes (n=26)	P-value
Age, years	48 (26–64)	49.0 (30–63)	0.86
Sex, % female	67	58	0.56
Diabetes type, % type 1	N/A	58	-
Diabetes duration, years	N/A	13 (6–22)	-
HbA1c, %	N/A	7.0 (6.6–8.0)	-
Presence of dry eye disease <sup>†</sup> , %	9.5	15.4	0.68
CNFL, mm/mm <sup>2</sup>	<b>14.71 ± 2.99</b>	<b>12.60 ± 3.41</b>	<b>0.030*</b>
CNFD, fibres/mm <sup>2</sup>	24.67 ± 6.05	20.99 ± 6.67	0.054
CNBD, branches on main fibre/mm <sup>2</sup>	<b>35.00 ± 12.57</b>	<b>23.60 ± 11.45</b>	<b>0.002*</b>
CTBD, total branches/mm <sup>2</sup>	<b>50.88 ± 18.72</b>	<b>35.48 ± 16.49</b>	<b>0.005*</b>
Corneal sensitivity threshold, mbar			
- Room temperature	<b>0.28 (0.18–0.35)</b>	<b>0.59 (0.42–0.75)</b>	<b>&lt;0.001*</b>
- Cooled temperature	<b>0.15 (0.05–0.23)</b>	<b>0.48 (0.35–0.69)</b>	<b>&lt;0.001*</b>

Blood fatty acid parameter	Control (n=21)	Diabetes (n=26)	P-value
Total AA (22:4 omega-6), %	<b>6.77 ± 1.50</b>	<b>5.61 ± 1.63</b>	<b>0.016*</b>
Total EPA (20:5 omega-3), %	0.60 (0.45–0.73)	0.62 (0.51–0.82)	0.52
Total DHA (22:6 omega-3), %	2.05 (1.88–2.60)	1.96 (1.49–2.31)	0.26
Omega-3 Index, %	5.54 (4.54–6.06)	5.00 (4.25–5.68)	0.32

Data are shown as mean ± SEM or median (IQR). AA=Arachidonic acid. \*indicates a statistically significant difference (p < 0.05). <sup>†</sup>Dry eye disease was diagnosed according to TFOS DEWS II criteria.

### Omega-3 Index and corneal nerve structure



**Figure 1:** Representative IVCM images from control participants with Omega-3 Indices of 3.89% (A) and 8.50% (B), and diabetes participants with Omega-3 Indices of 3.85% (C) and 9.61% (D)

### Table 3: Multiple linear regression models for dependent variable: central CNFL

Predictor variables:	β	p-value	β	p-value	β	p-value	β	p-value
	Omega-3 index		EPA		DHA		Total omega-6	
Fatty acid variable	<b>0.33</b>	<b>0.02*</b>	0.25	0.064	<b>0.32</b>	<b>0.027*</b>	-0.03	0.86
Age	<b>-0.46</b>	<b>0.001*</b>	<b>-0.48</b>	<b>0.001*</b>	<b>-0.41</b>	<b>0.003*</b>	<b>-0.44</b>	<b>0.004*</b>
Diabetes (Present)	<b>-0.30</b>	<b>0.03*</b>	<b>-0.33</b>	<b>0.02*</b>	-0.27	0.051	<b>-0.33</b>	<b>0.045*</b>
Sex (Male)	0.13	0.35	0.07	0.61	0.13	0.348	-0.02	0.90
Dry eye disease (Present)	0.10	0.42	0.08	0.56	0.12	0.367	-0.07	0.61
Norfolk score	-0.03	0.82	-0.02	0.90	-0.05	0.739	-0.04	0.81
Model statistics	R=0.62. R <sup>2</sup> =0.39. F=4.22. <b>p=0.002.</b>		R=0.59. R <sup>2</sup> =0.35. F=3.60. <b>p=0.006.</b>		R=0.61. R <sup>2</sup> =0.37. F=3.99. <b>p=0.003.</b>		R=0.54. R <sup>2</sup> =0.30. F=2.75. <b>p=0.025.</b>	

β, standardised regression coefficient. \* indicates a statistically significant difference (p < 0.05).

### Table 4: Multiple linear regression models for dependent variable: central CNFD

Predictor variables:	β	p-value	β	p-value	β	p-value	β	p-value
	Omega-3 index		EPA		DHA		Total omega-6	
Fatty acid variable	<b>0.35</b>	<b>0.01*</b>	0.24	0.09	<b>0.37</b>	<b>0.013*</b>	-0.16	0.33
Age	<b>-0.44</b>	<b>0.001*</b>	<b>-0.47</b>	<b>0.002*</b>	<b>-0.39</b>	<b>0.005*</b>	<b>-0.45</b>	<b>0.003*</b>
Diabetes (Present)	-0.27	0.054	<b>-0.30</b>	<b>0.039*</b>	<b>-0.24</b>	<b>0.09*</b>	<b>-0.36</b>	<b>0.031*</b>
Sex (Male)	0.12	0.38	0.054	0.70	0.13	0.34	0.01	0.94
Dry eye disease (Present)	0.13	0.32	0.10	0.45	0.15	0.26	0.10	0.48
Norfolk score	-0.004	0.98	0.006	0.97	-0.024	0.86	-0.04	0.81
Model statistics	R=0.60. R <sup>2</sup> =0.36. F=3.79. <b>p=0.004.</b>		R=0.56. R <sup>2</sup> =0.31. F=2.97. <b>p=0.017.</b>		R=0.60. R <sup>2</sup> =0.36. F=3.82. <b>p=0.004.</b>		R=0.52. R <sup>2</sup> =0.27. F=2.51. <b>p=0.037.</b>	

β, standardised regression coefficient. \* indicates a statistically significant difference (p < 0.05).

## Other findings

- A positive association exists between both the systemic Omega-3 Index and erythrocyte DHA levels, with each of CNFL and CNFD, independent of age or diabetes status.
- Participant age and diabetes status were the only factors associated with corneal sensitivity thresholds. Neither systemic omega-3 nor omega-6 fatty acid levels were related to corneal sensitivity thresholds with room-temperature or cooled stimuli.

## Conclusion

This study newly describes a relationship between the systemic Omega-3 Index and CNFL and CNFD, the major anatomical features of the corneal sub-basal nerve plexus. These findings suggest that omega-3 fatty acid intake may influence corneal nerve health.

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