Corneal confocal microscopy for the diagnosis of diabetic peripheral neuropathy: A systematic review and meta-analysis

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Keywords

CCM, Diabetic peripheral neuropathy, Diagnosis

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ABSTRACT

Introduction: Corneal confocal microscopy (CCM) is a rapid non-invasive ophthalmic imaging technique that identifies corneal nerve fiber damage. Small studies suggest that CCM could be used to assess patients with diabetic peripheral neuropathy (DPN).

Aim: To undertake a systematic review and meta-analysis assessing the diagnostic utility of CCM for sub-clinical DPN (DPN⁻) and established DPN (DPN⁺).

Data sources: Databases (PubMed, Embase, Central, ProQuest) were searched for studies using CCM in patients with diabetes up to April 2020.

Study selection: Studies were included if they reported on at least one CCM parameter in patients with diabetes.

Data extraction: Corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL), and inferior whorl length (IWL) were compared between patients with diabetes with and without DPN and controls. Meta-analysis was undertaken using RevMan V.5.3.

Data synthesis: Thirty-eight studies including ~4,000 participants were included in this meta-analysis. There were significant reductions in CNFD, CNBD, CNFL, and IWL in DPN⁻ vs controls (P < 0.00001), DPN⁺ vs controls (P < 0.00001), and DPN⁺ vs DPN⁻ (P < 0.00001).

Conclusion: This systematic review and meta-analysis shows that CCM detects small nerve fiber loss in subclinical and clinical DPN and concludes that CCM has good diagnostic utility in DPN.

INTRODUCTION

Diabetic peripheral neuropathy (DPN) affects ~50% of patients with diabetes and leads to significant morbidity including neuropathic pain, erectile dysfunction, and foot ulceration¹. Currently, the diagnosis of DPN in clinic relies on symptoms, loss of sensation to the 10 g monofilament, neurological examination, and occasionally electrophysiology². However, these methods do not reliably detect small nerve fiber damage which occurs in early DPN³.

In 2003, we showed that the ophthalmic technique of corneal confocal microscopy (CCM) can identify corneal small nerve

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fiber loss in patients with early and established DPN⁴. Subsequently we and others demonstrated good diagnostic utility for DPN⁵⁻⁷, comparable to intra-epidermal nerve fiber density (IENFD)^{8,9}. CCM also predicts incident DPN^{8,10} and identifies individuals at higher risk of developing DPN¹¹. However, some studies have failed to demonstrate corneal nerve fiber loss in patients with and without DPN^{12,13}, which has been attributed to a small sample size¹³ and variances in image acquisition and analysis protocols¹⁴.

We have undertaken a systematic review and meta-analysis to generate a definitive single estimate for the diagnostic utility of CCM in sub-clinical and clinical DPN.

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METHODS

Data sources and searches

This systematic review and meta-analysis is reported in accordance with MOOSE guidelines¹⁵. The protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on November 2020 (CRD42018093498). Four databases were chosen to search for this systematic review: PubMed, EMBASE (Ovid), CENTRAL, and web of science (WoS)- (1900-present). In the PubMed and CENTRAL database both Mesh subject headings and keywords were searched; in Embase-(1988-present) Emtree subject headings and keywords were utilized. Numerous terms were tested for relevancy and the final search strings for the three databases can be found in Table S1 in the supplement. Article language was limited to English and no date restrictions were set. A segment of the grey literature was searched through the use of dissertation and theses (ProQuest) and Clinicaltrials.gov. The databases were searched from inception to April 2020.

We included observational studies that reported on at least one of the following CCM parameters: corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL), or inferior whorl length (IWL) in any of the following three groups: patients with type 1 and/or type 2 diabetes with diabetic peripheral neuropathy (DPN⁺), without diabetic peripheral neuropathy (DPN⁻), and controls. Cross-sectional and longitudinal observational studies were included in this systematic review and meta-analysis. Narrative reviews, systematic reviews, correspondence, and case reports were excluded. Study country, age, diagnosis (DPN⁺, DPN⁻, control), duration of diabetes, HbA1c, software used for image analysis, CNFD, CNBD, CNFL, and IWL were extracted when available. Studies using CCMetrics, ACCMetrics, ImageJ, and other morphometric software to quantify CNFD, CNBD, and CNFL were included. IWL was quantified using CCMetrics and ACCmetrics only. Data presented as median (IQR) were converted into mean ± SD using an online calculator and data presented as mean ± SEM were converted into mean \pm SD using the RevMan calculator¹⁶. HbA1c presented in (%) was also converted into (mmol/mol) using the NGSP calculator, where NGSP % must be between 3 and 20¹⁷. Original studies that staged DPN as per the diabetic neuropathy study group in Japan (DNSGJ) were classified as: DPN⁻ for stage I, DPN⁺ for stages II-V, for metaanalysis reporting purpose^{18,19}. Stage I was reported as DPN⁻ and stages II-III were reported as DPN⁺ in this study²⁰. Patients classified according to the modified neuropathy disability score (NDS) were grouped as: scores between 0-2 (DPN⁻) and 3-10 (DPN⁺)^{21,22}. No neuropathy was classified as DPN⁻ and mild-severe neuropathy was classified as DPN⁺²³⁻²⁶. No differentiation was made for either painful or painless DPN and both were classified as DPN^{+27,28}. Where the vibration perception threshold (VPT) was used, <15V was classified as DPN⁻ and $\geq 15V$ as DPN⁺⁴.

Study selection

After the removal of duplicates, all citations were screened for relevance using the full citation, abstract, and indexing terms, before excluding studies deemed as irrelevant. Where there was a lack of consensus a third (senior) author was consulted. Duplicates were removed and the most recent and complete versions of the studies were reviewed for eligibility. Relevant studies were assessed by two reviewers (HG and INP) to assess eligibility according to the pre-specified inclusion and exclusion criteria. Full manuscripts of these potentially eligible citations were obtained. Two reviewers made the final inclusion and exclusion decisions independently and in the case of disagreement, a third reviewer was consulted to resolve any conflicts. A flow chart of search results was produced (Figure S1). A data collection tool was developed to extract the data from each study. Data verification was undertaken by two reviewers (HG and INP). In the event of missing data, the authors were emailed to obtain unpublished data.

Data extraction and quality assessment

The included studies were assessed using the Cochrane Collaborations tool for assessing the risk of bias (section 8.5)²⁹. The tool categorizes the risk of bias into high, moderate, low, or unclear risk. This tool assessed six domains: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias, where applicable. Quality assessment was undertaken by two reviewers (AK and GP). If the risk of bias of a study was unclear, the effect of removing the study was checked and relevant outcomes were reported (Table S2).

Data synthesis and analysis

Meta-analysis was performed in RevMan (version 5.3)³⁰. Random effects meta-analysis was used in anticipation of heterogeneity due to differences in study population and type and duration of diabetes. The mean difference (MD) with a 95% confidence interval (CI) was calculated for CNFD, CNBD, CNFL, and IWL. The Chi-squared (χ^2) test was used to test for difference between subgroups. The I² statistic was calculated, which is derived from Cochrane's chi-squared test Q and is used to describe the percentage of between-study variations attributed to variability in the true exposure effect²⁹. An I² value of 0–40% was classified as not important, 30–60% moderate, 50–90% substantial, and 75–100% considerable²⁹.

RESULTS

The search strategy identified 1,310 records (Figure S1). In total, 557 papers were screened on the basis of titles and abstracts, of which 508 were excluded, leaving 49 full text papers of which 38 were included in the meta-analysis.

Study characteristics

The studies were conducted in Canada^{10,26,31–33}, United Kingdom^{4,8,9,21,24,25,28,34–42}, Germany²⁷, Denmark¹², Australia^{43–49}, Japan^{18,19,22,50}, and China^{23,51} (Table 1).

Table 1 Characteristics	s of the included studie	S										I
Study	Country	Group	и	Age (years)	Duration of	HbA1c% –	CCM Type	Software for	Assessr	nent with	CCM	
					diabetes (years)	mmol/mol		image analysis	CNFD	CNBD	CNFL IWL	\neg
Ahmed <i>et al.</i> ³¹	Canada	DPN+	33 56	50 ± 14.3 349 + 148	31.4 ± 13.5 176 + 14	8.7 ± 2.1–72 74 + 13–57	HRT-II	CCMetrics	Ļ	5	Ļ	I
		Control	32	38.9 ± 17.6	NA – SV	NS						
Ostrovski <i>et al.</i> ³²	Canada	DPN+	13	56.2 ± 8.7	34.8 土 13	8.5 ± 2.2–69	HRT-III	CCMetrics	Ļ	Ļ	Ļ	
		DPN-	13	30.3 ± 13.7	10.7 ± 6.2	7.5 ± 1.3–58		ACCMetrics				
		Control	20	41.3 ± 17.3	N/A	5.5 ± 0.4–37						
Lovblom <i>et al.</i> ¹⁰	Canada	DPN+	11	38 ± 16	21 ± 9	8.1 ± 1.6-65	HRT-III	CCMetrics	5	Ļ	5	
		DPN-	57	34 土 15	17 ± 12	7.6 ± 1.3-60						
Sivaskandarajah <i>et al.</i> ³³	Canada	DPN+	33	48.5 土 13.7	32.3 ± 13.1	8.4 ± 1.6–68	HRT-III	CCMetrics	Ļ	Ļ	Ļ	
		DPN-	63	32.7 ± 13.6	17.3 ± 12.2	7.5 ± 1.2–58						
		Control	2	38.3 ± 16.4	N/A	5.6 ± 0.4-38						
Hertz <i>et al.²⁶</i>	Canada	DPN+	14	NS	NS	NS	HRT-III	CCMetrics	Ļ	Ļ	5	
		DPN-	12	NS	NS	NS						
		Control	20	41.4 土 17.3	N/A	5.5 ± 0.4–37						
Alam <i>et al.</i> 9	UK	DPN+	31	53.3 ± 11.9	37.2 ± 13.1	8.5 ± 1.5–69	HRT-III	CCMetrics	Ļ	Ļ	Ļ	
		DPN-	30	38.8 ± 12.5	17.2 ± 12	8 ± 1.3–64						
		Control	27	41 ± 14.9	N/A	5.5 ± 0.3						
Azmi et al. ³⁴	UK	DPN+	29	61.9 土 12.3	46 土 13.9	8.3 ± 1.3	HRT-III	ACCMetrics	Ļ	Ļ	5	
		Control	32	47.7 土 1.6	N/A	5.7 ± 0.6						
Chen <i>et al.</i> ³⁵	UK	DPN+	29	63 ± 12	19.9 ± 11.7	8.6 ± 3.6-70.4 ± 16	HRT-III	CCMetrics	Ļ	Ļ	Ļ	
		DPN-	63	44 ± 15	20 ± 11.1	8 土 4.1-63.9 土 21.2		ACCMetrics				
		Control	8	46 土 15	N/A	5.6-37.4 ± 3.5						
Brines et al. ²¹	UK	DPN+	60	35.3 土 14.3	35.3 ± 14.3	8.2 ± 1.3-66	HRT-III	ACCMetrics	Ļ	Ļ	Ļ	
		DPN-	21	37.1 ± 16.5	17.9 ± 15.1	7.9 ± 1.3–63						
		Control	8	46.2 土 16.9	N/A	5.7 ± 0.3–39						
Petropoulos <i>et al.³⁶</i>	UK	DPN+	25	60.1 ± 10.2	24.8±19.5	7.6 ± 1.5-60	HRT-III	CCMetrics	5		∽ ∽	
		DPN-	28	42.4 ± 14.7	16.2 ± 9.3	NS						
		Control	15	NS	N/A	5.4±0.5–36						
Chen <i>et al.</i> ⁸	UK	DPN+	17	59 ± 11	39 土 14	8.5 ± 1.3-69	HRT-III	CCMetrics	Ļ	Ļ	5	
		DPN-	46	44 ± 13	23 ± 15	8.2 ± 1.4–66		ACCMetrics				
:		Control	26	44 ± 15	N/A	5.5 ± 0.3–37						
Petropoulos <i>et al.³⁷</i>	UK	DPN+	61	56.5 土 13.2	35.33 ± 14.3	8.4 ± 1.8–68	HRT-III	CCMetrics	Ļ	Ļ	Ļ	
		DPN-	50	44.2 ± 15.6	23 ± 14	7.9 ± 1.7–63						
:		Control	47	52 ± 13.2	N/A	5.6 ± 0.3–38						
Petropoulos <i>et al.³⁸</i>	LK	DPN+	100	NS	34.4 ± 17.3	7.9 ± 1.6-63	HRT-III	CCMetrics	Ļ	Ļ	Ļ	
		DPN-	86	NS	24.2 ± 21.2	7.7 ± 1.6–61		ACCMetrics				
		Control	55	51.7 土 11.4	N/A	5.5 ± 0.3-37						
Ponirakis <i>et al.</i> ³⁹	UK	DPN+	46	60.75 ± 8.9	36.5 土 14.4	8.6 ± 0.4–70	HRT-III	CCMetrics	Ļ	Ļ	4	
		DPN-	2	45.5 ± 14.4	22.25 ± 13	7.62 ± 0.48-60						

Table 1 (Continued)												
Study	Country	Group	и	Age (years)	Duration of	HbA1c% -	CCM Type	Software for	Assessm	nent with	CCM	
					diabetes (years)	mmol/mol		image analysis	CNFD	CNBD	CNFL	
Quattrini <i>et al.</i> ²⁴	Я	DPN+ DPN-	4 0 i	59.3 ± 17.25 43.5 ± 10.2 55 ± 10.5	NS NS	8.01 ± 2.32-64 7.16 ± 1.26-55	Confoscan-P4	Morphometric software	Ļ	Ļ	Ļ	
Tavakoli <i>et al.</i> ⁴⁰	Я	DPN+	0 <u>7</u> 6 į	59 土 10.5 59 土 18.2 55 土 11.1 57 - 10.0	17.8 ± 29.55 10.7 ± 10.6	NJ 8.2 ± 2.70-66 8.1 ± 1.57-65	Confoscan-P4	Morphometric software	5	Ļ	Ļ	
Tavakoli <i>et al.</i> ²⁵	N	DPN+ DPN- DPN-	- 6 7 ×	55 ± 19.8 59 ± 20 57 ± 13	NVA 59 ± 20 57 ± 13	 <0.5 < 48 8.30 ± 30.14-67 7.88 ± 10.23-63 5 < 40 	Confoscan-P4	CCMetrics	Ļ	Ļ	Ļ	
Kalteniece <i>et al.</i> 41	Ч	DPN+ DPN- DPN-	69 69 60	стс 62.08 ± 11.6 46.9 ± 13.2 5037 ± 13.7	10.04 ± 17.8 16.04 ± 12.2 NZA	~3.0-40 7.19 ± 10.16-55 7.72 ± 2.06-61 5.48 + 00.42 36	HRT-III	CCMetrics	Ļ	Ļ	Ļ	Ļ
Kalteniece <i>et al.</i> ²⁸	ЛК	DPN+	140 30	65.09 ± 1.13 61.0 ± 1.13	21.8 ± 2.05	7.5±0.17-58 5.63+0006-38	HRT-III	CCMetrics	Ļ	Ļ	Ļ	Ļ
Malik <i>et al.</i> ⁴	Я	DPN+ DPN- Control	0 ⁴ ⁴ ⁶	59.2 ± 9.9 53 ± 18.5 578 + 11.5	23.4 ± 6.25 21.3 ± 3.6 N/A	2.00 ± 0.0000 ± 0.00000 ± 0.0000 ± 0.00000000	Confoscan-P4	Morphometric software	Ļ	Ļ	Ļ	
Ponirakis <i>et al.</i> ⁴²	Я	DPN+ DPN- Control	33 141 23	64.1 ± 1.79 44.3 ± 2.19 41.8 ± 1.63	37.6 ± 3.2 23.3 ± 2.03 N/A	7.9 ± 0.26-63 7.5 ± 0.18-58 5.20 ± 0.12-34	HRT-III	ACCMetrics	Ļ			
Puttgen <i>et al.²⁷</i>	Germany	DPN+ Control) 116 46	67.3 ± 9 66 ± 5.2	17.6 土 13 N/A	2.27 ± 0.12 − 51 7.41 ± 1.3−57 5.44 ± 0.23−36	HRT-III	CCMetrics ACCMetrics	ĻI	Ļ	ĻI	
Andersen <i>et al.</i> ¹²	Denmark	DPN+ DPN- Control	27 117 25	71.4 ± 3.1 69.7 ± 2.7 71.2 ± 0.69	12.2 ± 1.23 11.67 ± 1.12 N/A	6.95 ± 0.48–52 6.6 ± 0.33–49 5.5 ± 0.22–37	HRT-III	ACCMetrics	Ļ	Ļ	Ļ	
Tummanapalli <i>et al.</i> ⁴⁴	Australia	DPN+ DPN- Control	28 35 34	NS	NS	8.45 ± 0.569 7.59 ± 0.659	HRT-III	ACCMetrics	Ļ	Ļ	Ļ	Ļ
Dehghani <i>et al.⁴⁷</i>	Australia	DPN+ DPN- Control	17 20 13	NS	NS	NS	HRT-III	CCMetrics ACCMetrics			Ļ	
Tummanapalli <i>et al.</i> ⁴⁹	Australia	DPN+ DPN- Control	23 27	47 ± 15 32 ± 10 37 + 11	22 ± 13 15 ± 9 N/A	8.89 ± 1.9–74 7.83 ± 1.02–62 NS	HRT-III	ACCMetrics	Ļ	Ļ	Ļ	Ļ
Tummanapalli <i>et al.</i> ⁴³	Australia	DPN+	35 35	51 ± 9.5 44.5 ± 11	NS	8 ± 1.4-64 8 ± 2-64	HRT-III	ACCMetrics	Ļ	Ļ	Ļ	Ļ
Pritchard <i>et al.</i> ⁴⁸	Australia	DPN+ DPN- Control	25 82 80	NS NS 37.0 ± 17.8	NS	SN	HRT-III	CCMetrics			Ļ	ς Ι

Study	Country	Group	Ч	Age (years)	Duration of	HbA1c% –	CCM Type	Software for	Assessn	nent with	CCM	
					diabetes (years)	mmol/mol		image analysis	CNFD	CNBD	CNFL	ML
Edwards <i>et al.</i> ⁴⁶	Australia	DPN+	88	58±9	23 ± 14	8.2 ± 1.7-66	HRT-III	CCMetrics		 	 	Ļ
		DPN-	143	48 土 16	14 土 12	7.8 ± 1.2–62						
		Control	61	52 ± 14	N/A	5.4 土 0.3-36						
Dehghani <i>et al.</i> ⁴⁵	Australia	DPN+	39	NS	NS	NS	HRT-III	ACCMetrics	Ļ	Ļ	Ļ	
		DPN-	108	NS	NS							
		Control	8	NS	N/A							
Ishibashi <i>et al.</i> ¹⁸	Japan	DPN+	55	56.4 土 14.1	9.6 土 16.3	8.03 ± 3.0-64	HRT-III	ImageJ	Ļ	Ļ	Ļ	
		DPN-	23	48.1 土 10.6	5.8 ± 5.8	7.7 ± 2.11–61		1				
		Control	28	50.2 土 7.41	N/A	5.6 ± 0.26-38						
Ishibashi <i>et al.</i> ¹⁹	Japan	DPN+	153	56.03 ± 10.3	12.4 土 8.2	8.3 ± 3.5–67	HRT-III	ImageJ	Ļ	Ļ	Ļ	
		DPN-	47	53.4 土 7.54	10.5 土 14.8	7.3 ± 1.4–56		I				
		Control	6	53.6 ± 12.65	N/A	5.7 ± 0.32-39						
Ishibashi <i>et al.</i> ²²	Japan	DPN+	115	54.4 ± 19.1	7.9 土 11.4	9.06 ± 4.4–76	HRT-III	ImageJ	Ļ	Ļ	Ļ	
		DPN-	47	52.4 土 9.6	5 土 4.5	8.5 ± 1.4–69						
		Control	45	52.8 土 4.7	N/A	5.5 ± 0.03-37						
Ishibashi <i>et al.</i> ⁵⁰	Japan	DPN+	18	59.4 ± 8.1	13.6 土 10.61	9 土 1.74-75	HRT-III	ImageJ	Ļ	Ļ	Ļ	
		DPN-	57	54.4 土 12.1	6.7 土 6.34	9.1 ± 2.4–76						
		Control	42	53.1 ± 11.7	N/A	5.7 ± 0.4-39						
Li <i>et al.</i> ⁵¹	China	DPN+	79	70.15 ± 7.34	12.58 ± 7.28	7.94 ± 1.86-63	HRT-II	COMetrics	Ļ	Ļ	Ļ	
		DPN-	49	67.12 ± 6.01	9.79 ± 7.09	7.07 ± 0.96-54		ACCMetrics				
		Control	24	68.3 ± 5.19	N/A	5.88 ± 0.82-41						
Xiong <i>et al.</i> ²³	China	DPN+	79	70.3 ± 10	12.57 ± 10.2	7.95 ± 3.4-63	HRT-II	ImageJ	Ļ	Ļ	Ļ	
		DPN-	49	67.12 ± 6.13	9.79 ± 7.14	7.07 ± 1.68–54						
		Control	24	68.63 ± 5.2	N/A	5.88 ± 0.83-41						
Pritchard <i>et al.⁷⁰</i>	Australia, Canada, UK	DPN+	16	51 土 14	29 土 16	8 土 1.1-64	HRT-III	CCMetrics			Ļ	
		DPN-	74	42 ± 16	15 土 12	7.9 ± 1.2–63						
Pritchard <i>et al.⁵²</i>	Australia, UK	DPN+	48	57 土 11	34 土 16	8.6±1.8-70	HRT-III	CCMetrics		Ļ	Ļ	
		DPN-	100	43 ± 16	20 ± 15	8 土 1.264						
		Control	99	46 ± 15	N/A	5.5 ± 0.3–37						

Figure 1 | (a) Forest plots of corneal nerve fiber density (CNFD) in patients with diabetic peripheral neuropathy (DPN⁺) and without diabetic peripheral neuropathy (DPN⁻). (b) Forest plots of corneal nerve fiber density (CNFD) in patients with diabetic peripheral neuropathy (DPN⁺) and healthy control. (c) Forest plots of corneal nerve fiber density (CNFD) in patients without diabetic peripheral neuropathy (DNP⁻) and healthy control.

Corneal nerve fiber density

DPN⁺ vs DPN⁻

Twenty-nine studies^{4,8–10,12,18,19,21–26,31–33,35,37–44,50,51} with 3,214 (1,677 DPN⁺ and 1,537 DPN⁻) participants were included in the meta-analysis. The CNFD (fiber/mm²) was significantly lower in the DPN⁺ group compared with the DPN⁻ group (MD = -7.01, 95% CI -7.45 to 6.57, P < 0.00001) (CCMetrics (MD = -6.83, 95% CI -7.82 to -5.84, P < 0.00001), ACCMetrics (MD = -7.77, 95% CI -7.82 to -7.22, P < 0.00001), ImageJ (MD = -3.48, 95% CI -4.64 to -2.33, P < 0.00001), and morphometric software (MD = -11.40, 95% CI -15.42 to -7.38, P < 0.00001)). There was a significant difference in the magnitude of the CNFD reduction in the DPN⁺ group between studies ($\chi^2 = 19.32$, P = 0.0002) (Figure 1a).

DPN^+ vs control

Twenty-nine studies^{4,8,9,12,18,19,21–28,31–35,37,38,40,41,43–45,50,51} with 3377 (1994 DPN⁺ and 1383 control) participants were included in the meta-analysis. The CNFD (fiber/mm²) was significantly lower in the DPN⁺ group compared with the controls (MD = – 11.94, 95% CI –12.25 to –11.62, P < 0.00001) (CCMetrics (MD = –10.83, 95% CI –11.26 to –10.40, P < 0.00001), ACCMetrics (MD = –13.75, 95% CI –14.26 to –13.25, P < 0.00001), ImageJ (MD = –8.98, 95% CI –10.40 to –7.55, P < 0.00001), and morphometric software (MD = –22.26, 95% CI –27.67 to –16.85, P < 0.00001). There was a significant difference in the magnitude of the CNFD reduction in the DPN⁺ group between studies ($\chi^2 = 15.50$, P = 0.001) (Figure 1b).

DPN⁻ vs control

Twenty-seven studies^{4,8,9,12,18,19,21–26,31–33,35,37,38,40–45,50,51} with 3,035 (1,620 DPN⁻ and 1,415 control) participants were included in the meta-analysis. The CNFD (fiber/mm²) was significantly lower in the DPN⁻ group compared with the controls (MD = -5.85, 95% CI -6.12 to -5.57, P < 0.00001) (CCMetrics (MD = -5.76, 95% CI -6.15 to -5.37, P < 0.00001), ACCMetrics (MD = -5.91, 95% CI -6.32 to -5.50], P < 0.00001), ImageJ (MD = -5.89, 95% CI -7.13 to -4.65, P < 0.00001), and morphometric software (MD = -11.07, 95% CI -16.34 to -5.80, P < 0.0001). There was no significant difference in the magnitude of the CNFD reduction in the DPN⁻ group between studies ($\chi^2 = 4.01$, P = 0.26) (Figure 1c).

Corneal nerve branch density

DPN⁺ vs DPN⁻

Thirty studies^{4,8–10,12,18,19,21–26,31–33,35,37–41,43–46,50–52} with 3,552 $(1,763 \text{ DPN}^+ \text{ and } 1,789 \text{ DPN}^-)$ participants were included in

the meta-analysis. The CNBD (branch/mm²) was significantly lower in the DPN⁺ group compared with the DPN⁻ group (MD = -3.36, 95% CI -4.11 to -2.61, P < 0.00001) (CCMetrics (MD = -10.37, 95% CI -12.56 to -8.18, P < 0.00001), and ACCMetrics (MD = -8.20, 95% CI -10.20 to -6.20, P < 0.00001). There was a significant difference in the extent of the CNBD reduction in the DPN⁺ group between studies ($\chi^2 = 30.97$, P < 0.00001), (Figure 2a).

DPN⁺ vs control

Thirty studies^{4,8,9,12,18,19,21–28,31–35,37,38,40,41,43–46,50,51} with 3,460 (2,072 DPN⁺ and 1,388 control) participants were included in the meta-analysis. The CNBD (branch/mm²) was significantly lower in the DPN⁺ group compared with the controls (MD = -11.00, 95% CI -11.65 to -10.35, P < 0.00001) (CCMetrics (MD = -20.87, 95% CI -22.05 to -19.68, P < 0.00001), ACCMetrics (MD = -7.34, 95% CI -8.35 to -6.32, P < 0.00001), ImageJ (MD = -4.79, 95% CI -6.05 to -3.53, P < 0.0001), and morphometric software (MD = -21.81, 95% CI -26.61 to -17.01, P = 0.0003)). There was a significant difference in the magnitude of the CNBD reduction in the DPN⁺ group between studies ($\chi^2 = 30.98$, P < 0.00001) (Figure 2b).

DPN⁻ vs control

Twenty-six studies^{4,8,12,18,19,21–24,26,31–33,35,37,38,40,41,43–46,50–52} with 2,813 (1,606 DPN⁻ and 1,207 control) participants were included in the meta-analysis. The CNBD (branch/mm²) was significantly lower in the DPN⁻ group compared with the controls (MD = -6.37, 95% CI –7.31 to –5.44, P < 0.00001) (CCMetrics (MD = –11.08, 95% CI –13.40 to –8.75, P < 0.00001), ACCMetrics (MD = –11.17, 95% CI –13.46 to – 8.88, P < 0.00001), ImageJ (MD = –3.34, 95% CI –4.52 to – 2.17, P < 0.0001), and morphometric software (MD = –16.26, 95% CI –21.14 to –11.37, P = 0.007)). There was a significant difference in the magnitude of the CNBD reduction in the DPN⁻ group between studies ($\chi^2 = 33.32$, P < 0.00001) (Figure 2c).

Corneal nerve fiber length

DPN⁺ vs DPN⁻

Thirty-four studies^{4,8–10,12,18,19,21–26,31–33,35,37–41,43–48,50–53} with 3,868 (1,855 DPN⁺ and 2,013 DPN⁻) participants were included in the meta-analysis. The CNFL (mm/mm²) was significantly lower in the DPN⁺ group compared with the DPN⁻ group (MD = -3.08, 95% CI -3.58 to -2.58, P < 0.00001) (CCMetrics (MD = -3.74, 95% CI -4.49 to -2.99,

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(a)	OPN+		DPN-		Mean Difference	Mean Difference
Study or Subgroup Mean [2.1.1 CCMetrics Study and additional study of the study of	no./mm2] SD (no	Jan 22 Total Mean (no./mm2] SD [no	p./mm2] Total Weight IV.	Random, 95% CI (no./mm2)	IV, Random, 95% CI (no./mm2)
Ahmed 2012 Alam 2017	28 19.8	9 33 9.2 31	39 30.1	10 56 2.8N 6.7 30 2.8N	-11.00 [-15.04, -6.96] -10.30 [-14.33, -6.27]	
Chen 2015 Petropoulos 2015	13.5 22.4	9.1 17 9.6 25	22.6 31.2	7.3 46 2.5N 8.2 28 2.5N	-9.10 [-13.91, -4.29] -8.80 [-13.64, -3.96]	
Chen 2015 Petroposios 2013 Tavaitoli 2011	13.3 18.4 23.5	8.7 29 9.9 61 21.9 96	21.9 26.9 31.86	7.6 63 3.0N 9.5 50 3.0N 14 42 2.0N	-8.60 [-12.28, -4.92] -8.50 [-12.12, -4.88] -8.36 [-14.45, -2.27]	
Ponizakis 2015 Petropoulos 2014 Loviblom 2015	21.5 14.4 39.4	8.7 46 8.9 100 12.3 11	29.85 20.1 44.7	8.14 64 3.2N 8.7 86 3.5N 10.8 54 1.5N	-8.35 [-11.56, -5.14] -5.70 [-8.23, -3.17] -5.30 [-13.12, 2.52]	
Hertz 2011	31.2 16.88	10.1 14 7.39 79	36.27 18.98	5.7 12 1.9N 7.21 49 3.5N	-5.07 [-11.27, 1.13] -2.10 [-4.69, 0.49]	
Kalteriece 2018 Subtotal (95% CI) Heterogenetty: Tau ² = 9.03; CM ² = 46	24.5 .32, df = 14 (* < 6	7.48 69 637 0.0001); f ² = 70%	26.31	8.16 47 3.48 703 40.3N	-1.61 [-4.74, 1.12] -7.61 [-9.50, -5.72]	•
Test for overall effect: Z = 7.89 (P < 0 2.1.2 ACCMetrics	(0001)					
Chen 2015 Alam 2017 Chen 2018	16.9 19.8	10.1 17 9.2 31	28.3 30.1	7.2 46 2.3N 6.7 30 2.8N	-11.40 [-16.63, -6.17] -10.30 [-14.33, -6.27] -10.30 [-14.43, -6.27]	
Ostrovski 2015 Tummanapali 2020	30.3 17.55	10.54 13 4.86 23	40.6 26.86	9.46 13 1.5N 4.41 27 3.5N	-10.30 [-18.00, -2.60] -9.31 [-11.90, -6.72]	
Ponirakis 2016 Brines 2018 Petropoulos 2014	14.2 15.3 20.5	1.6 33 8.9 60 9.5 100	22.9 21.5 26.7	1.3 41 4.2N 7.3 21 2.9N 8.5 86 3.5N	-6.20 [-9.38, -6.02] -6.20 [-10.05, -2.35] -6.20 [-8.79, -3.61]	· _
Tumnanapali, Issar 2020 Tumnanapali, Wilcox 2019 11 2019	19.5 19.55	4.48 35 6.48 28 7.39 79	25.47 25.2	4.53 35 3.7N 5.19 35 3.4N 7.21 49 3.5N	-5.67 [-8.08, -3.86] -5.65 [-8.60, -2.70] -2.10 [-4.69, 0.49]	-
Andersen 2018 Subtotal (95% CI)	19.21	7.09 27 475	19.47	5.74 117 3.4N 563 37.6N	-0.26 [-3.13, 2.61] -6.86 [-8.70, -5.02]	• +
Test for overall effect: Z = 7.30 (P < 0.	.46001)	0.00001); F = 85%				
2.1.3 imagej tshtashi 2013 tshthashi 2014	21.9	5-22 18 14.09 55	28.42	5.3 57 3.4N 3.74 23 2.8N	-6.52 [-9.30, -3.74] -4.90 [-8.93, -0.87]	-
Ishibashi 2016 Ishibashi 2015 Viseo 2018	22.3 21.63	9.33 115 10 153	25.3 24.1	4.9 47 3.7N 4.8 47 3.7N	-3.00 [-5.21, -0.79] -2.47 [-4.57, -0.37]	=
Subtotal (95% CI) Heterogeneity: Tau ⁴ = 1.29; CH ⁴ = 6.8	5, df = 4 (f = 0.1	420 43; f = 425	****	223 17.1%	-3.62 [-5.18, -2.06]	•
Test for overall effect: Z = 4.55 (P < 0 2.1.5 Morphometric software	.66001)					
Malik 2003 Tavakoli 2010 Deamini 2007	22.8 21.59 21.9	7.45 14 22.02 67 21.2 44	37.2 31.63 29.05	4.6 4 2.0N 13.6 34 1.7N 9.71 30 1.7N	-14.40 [-20.36, -8.44] -10.04 [-17.02, -3.06] =7.16 -15.84	
Subtotal (95% CI) Heterogenetty: Tau ² = 0.25; Ch ² = 2.0	21.9 24, cf = 2 c7 = 0.3	125 (6); f ² = 25	49.43	48 5.0%	-7.12 [-13.54, 1.34] -11.39 [-15.45, -7.32]	•
Test for overall effect: Z = 5.49 (P < 0 Total (95% C0	.00001)	1677		1537 100.0N	-6.84 [-8.01, -5.67]	•
Heterogeneity: Tau ² = 8.38; Ch ² = 16 Test for overall effect: Z = 11.45 (P < 1 Test for subscenare of forecasts (P < 1)	8.49, df = 34 (P < 0.00001) 19.32, df = 3.4P	0.00001); P = 80%				-20 -10 0 10 20 Note DPN Less DPN
(b)						
(D) Study or Subgroup M	DPN Nean (no./mm2) Si	i+ D [no./mm2] Total Me	Contr an [no./mm2]_SD	ol [no./mm2] Total Weight	Mean Difference IV, Random, 95% CI [no./mm2]	Mean Difference IV, Random, 93% CI [no./mm2]
2.2.1 CCMetrics Chen 2015 Chen 2018	16.9	10.1 17 9.9 29	36.8 36.17	5.3 26 2.4N 6.2 84 2.9S	-19.50 [-25.12, -14.68] -18.77 [-22.61, -14.93]	
Perropoulos 2013 Alam 2017 Tavakoli 2011	18.4 19.8 21.5	9.9 61 9.2 31 21.9 94	37.2	7.2 47 3.18 5.1 27 2.98 13.5 26 1 0	-18.60 (-21.83, -15.37) -17.40 (-21.17, -13.63) -17.28 (-24.07, -10.40)	
Petroposka 2014 Sivaskandarajah 2013	20.5	9.5 100 10.4 33	37.2	6.7 55 3.3N 12 64 2.6N	-16.70 [-19.27, -14.13] -16.20 [-20.81, -11.50]	
Anned 2012 Petropoulos 2015 Kabeniece 2018	28 22.4 24.5	9 33 9.6 25 7.48 69	43 37.2 35.42	11 64 2.88 5.9 15 2.58 7.5 22 2.88	-15.00 [-19.09, -10.91] -14.80 [-19.60, -10.00] -10.92 [-14.52, -7.32]	<u> </u>
Kalteniece 2020 Ostrovski 2015 Bernen 2019	22.11 30.3 22.6	0.89 140 10.54 13	32.58 40.3	1.26 30 3.7% 9.34 20 1.5%	-10.47 [-10.94, -10.00] -10.00 [-17.04, -2.96] -640 [-6.67, -4.13]	<u> </u>
Delighani, Pitchard, Edwards 2014 U 2019	16.3 33.51	8.3 39 8.96 79	22.3	8 60 3.0% 7.64 24 2.5%	-6.00 [-9.30, -2.70] -2.17 [-5.81, 1.47]	
Subtotal (95% Cl) Heterogenety: Tau ² = 18.70; Ch ² = 15	31.2 i2.96, df = 15 (P <	0.00001); P = 90%	\$1.9	5.4 20 2.08 630 44.2%	-0.40 [-7.41, 6.01] -12.59 [-14.96, -10.21]	•
Test for overall effect: Z = 10.43 (P < 0 2.2.2 ACCMetrcis	.00001)					
Azmi 2019 Chen 2015 Petroposées 2014	10.3 13.5 14.4	5.7 29 9.1 17 8.9 100	30.8 31.3 30	7.3 32 3.1X 6.5 26 2.5X 6.9 55 3.3X	-20.50 [-23.77, -17.23] -17.60 [-22.60, -12.60] -15.60 [-18.12, -13.08]	
Chen 2018 Pontrakis 2016	13.3 14.2	8.7 29 1.6 33	28.9	6.9 84 3.08 0.92 70 3.78	-15.60 [-19.09, -12.11] -14.60 [-15.19, -14.01]	· .
Brines 2018 Tummanapalii 2020 Ostrovski 2015	17.55 17.52	4.56 23 8.13 13	28.8 30.03 28.06	4.8 48 3.38 3.95 29 3.38 8.55 20 2.28	-13.30 [-16.13, -10.87] -12.48 [-14.93, -10.03] -10.54 [-16.33, -4.75]	-
Tummanapalli, Willox 2019 Puttgen 2019 Li 2019	19.55 19.05 16.88	6.48 28 7.9 116 7.39 79	29.25 25.9 23.18	4.32 34 3.28 6.5 46 3.38 5.77 24 3.28	-9.70 [-12.51, -6.89] -6.85 [-9.22, -4.48] -6.30 [-9.13, -3.47]	-
Andersen 2018 Subtotal (95% CI)	19.21	7.09 27 554	23.16	5.15 25 3.08 493 37.0%	-3.95 [-7.30, -0.60] -12.24 [-14.64, -9.83]	•
Test for overal effect: Z = 9.99 (P < 0.0	00001)					
ishibashi 2013 Ishibashi 2014	21.9	5.22 18 14.09 55	35.5 33.1	14.3 42 2.5N 5 28 2.8N	-13.60 [-18.55, -8.65] -10.70 [-14.86, -6.54]	
ishibashi 2016 Ishibashi 2015 Xiong 2018	22.3 21.43 33.41	9.33 115 10 153 12.8 79	33 31.7 35.32	8.05 45 3.2N 5.7 40 3.3N 5.78 55 3.1N	-10.70 [-13.61, -7.79] -10.07 [-12.44, -7.70] -1.91 [-5.12, 1.30]	=
Subtotal (95% CI) Heterogenety: Tau ² = 14.64; Ch ² = 24 Test for overall effect: Z = 4.88 (P < 0.0	1.80, df = 4 (P < 0) 00001)	420 0001); ² = 84N		210 14.8%	-9.24 [-12.95, -5.52]	•
2.2.4 Morphometric software						
Malk 2003 Quality 2007	22.8 21.9	7.45 14 21.2 44	44.5	14.1 18 1.78 19.56 15 1.08	-21.70 [-29.29, -14.11] -21.30 [-33.01, -9.59]	
Heterogenety: Tau ² = 0.00; Ch ² = 0.1/ Test for overall effect: Z = 0.07 (P < 0.1	6, df = 2 (P = 0.92 00001)	125); i ² = 0%		50 4.0%	-44-40 [-27-87, -16.85]	-
Total (95% CI) Heterogenety: Tau ² = 13.46; Ch ² = 41	16.58, df = 35 (P <	1994 0.00001); F = 925		1383 100.0%	-12.34 [-13.72, -10.96]	
Test for overall effect: $Z = 17.52$ ($P < 0$ Test for subgroup differences: $Ch^{\mu} = 1$	5.50, df = 3 (P = 0	.001), # = 80.6%				More DPN Less DPN
(C) Study or Subgroup M	DPN lean (no./mm2) 55	l- D [no./mm2] Total Me	Contri an [no./mm2] SD	ol [no./mm2] Total Weight	Mean Difference IV, Random, 95% CI (no./mm2)	Mean Difference IV, Random, 93% CI [no./mm2]
2.3.1 CCMetrics Petropules 2014 Petropules 2013	26.7	8.5 M	37.2	6.7 55 3.6N	-10.50 [-13.02, -7.98]	
Kalteniece 2018 Tasakoli 2011	26.9 26.31 31.86	8.16 47 14 42	35.42 40.78	7.5 22 2.4N 13.5 26 1.1N	-9.11 (-13.02, -5.20) -8.92 (-15.62, -2.22)	
Chen 2015 Chen 2018 Alam 2017	28.3 27.7 30.1	7.2 46 7.9 63 6.7 30	36.8 36.17 37.2	5.3 26 3.2% 6.2 84 3.8% 5.1 27 3.1%	-6.50 [-11.41, -5.55] -6.47 [-10.83, -6.11] -7.10 [-10.17, -4.03]	=
Petropoulos 2015 Pontrakts 2016 Debrani, Pitchard, Educatio 2014	31.2	8.2 28 1.3 41 2.1 108	37.2 28.8 22.1	5.9 15 2.2N 0.92 70 5.3N	-6.00 [-10.26, -1.74] -3.90 [-6.33, -3.43] -4.00 [-6.43]	· .
Ahmed 2012 Andersen 2018 Standarden 2018	39 19.47	10 56 5.74 117	43 23.16	11 64 2.5N 5.15 25 3.9N	-4.00 [-7.76, -0.24] -3.69 [-5.96, -1.42]	=
zweskandarajan 2013 Ostrovski 2015 Li 2019	42.3 40.6 35.68	9.4 63 9.46 13 7.64 49	45.3 40.3 35.32	12 64 2.5N 9.34 20 1.2N 5.55 79 3.7N	-5.00 [-6.75, 0.75] 0.30 [-6.27, 6.87] 0.36 [-2.10, 2.82]	-
Hertz 2011 Subtotal (95% CI) Heterogenety: Tau ² = 6.64: Chi ² = 82 A	36.27 63, cf = 15 (r < 0.1	5.7 12 851 00001); r ² = 62N	31.9	9.4 20 1.6N 704 46.7%	4.37 [-0.86, 9.60] -5.53 [-7.05, -4.00]	•
Test for overall effect: Z = 7.10 (P < 0.0 2.3.2 ACCMetrics	00001)					
Petropoulos 2014 Chen 2015 Britan 2018	26.7	8.5 86 7.3 46 7.3 21	37.2 31.3	6.7 55 3.6N 6.5 26 2.9N	-10.50 [-13.02, -7.98] -8.70 [-11.97, -5.43] -7.10 [-10.65, -1.64]	=
Chen 2018 Pontrakis 2016	21.5 21.9 22.9	7.6 63 1.3 41	28.9 28.8	6.9 84 3.7N 6.92 70 5.5N	-7.00 (-0.35, -3.95) -7.00 (-0.35, -4.61) -5.90 (-6.35, -5.45)	-
LI 2019 Tummanapall, Wilcox 2019 Andersen 2018	18.98 25.2 19.47	7.21 49 5.19 35 5.74 117	23.18 29.25 23.16	5.77 79 3.7% 4.32 34 3.9% 5.15 25 3.9%	-4.20 [-6.59, -1.81] -4.05 [-6.30, -1.80] -3.69 [-5.96, -1.42]	=
Tummanapalii 2020 Ostrovski 2015 Subtotal (95% CI)	26.86 27.27	4.41 27 8.49 13 498	30.03 28.06	3.95 29 3.9N 8.55 20 1.4N 482 35.4%	-3.17 [-5.37, -0.97] -0.79 [-0.73, 5.15] -5.70 [-7.02, -4.341	
Heterogenetity: Tau ² = 2.79; Ch ² = 33.1 Test for overall effect: $Z = 8.48 \text{ (P} < 6.0$	\$7, cff = \$ (P < 0.0 90001)	001); F = 735				•
2.3.3 Imagej Ishibashi 2016	25.3	4.9 47	33	8.05 45 3.4N	-7.70 [-10.44, -4.96]	-
təhtbashi 2015 İshibashi 2013 təhtbashi 2014	24.1 28.42 27.3	4.8 47 5.3 57 5.74 23	\$1.7 35.5 33.1	5.7 40 3.9% 14.3 42 2.0% 5 28 5.7%	-7.60 (-9.84, -5.36) -7.08 (-11.62, -2.54) -5.80 (-8.20, -3.46)	
Xiong 2018 Subtotal (95% CI) Heleroperativ: Tax ² = 7.88 · Ch ² = 10.1	35.48	7.7 49 223 007): F = 794	35.32	5.78 24 3.0N 179 16.0N	0.36 [-2.80, 3.52] -5.59 [-8.40, -2.79]	•†
Test for overall effect: Z = 3.91 (P < 0.0	0001)	www.da = rdB				
Quatrini 2007 Tavakoli 2010	29.05 31.43	3.07 10 13.6 34	43.2 45.6	19.56 15 0.6N 18.43 17 0.6N	-14.15 [-24.23, -4.07] -13.97 [-23.85, -4.09]	
Mark 2003 Subtotal (95% Cl) Heterogenety: Tau ² = 0.00; Chi ² = 1.54	37.2 6, df = 2 (? = 0.46)	4.6 4 48); i² = 0%	44.5	14.1 18 0.9N 50 2.0%	-7.30 [-15.22, 0.62] -11.07 [-16.34, -5.80]	+
Test for overall effect: Z = 4.12 (P < 0.0 Total (95% CI)	9001)	1620		1415 100.0%	-5.76 [-6.56, -4.97]	•
Heterogeneity: $Tas^4 = 2.91$; $Ch^4 = 141$ Test for overall effect: $Z = 14.22$ ($P < 0$ Test for subgroup differences: $Ch^4 = 4$.)	.06, df = 33 @ < 6 .00001} 01, df = 3 @ = 0.2	1.00001); F = 77%				-20 -10 0 10 20 More DPN Less DPN

Test for overall effect: Z = 14.22 (P < 0.00001) Test for subgroup differences: $Os^{\mu} = 4.01$, df = 3 (P = 0.26), P = 25.1N

(a) Study or Substrain	DPN+ Mean (no./mm2) SD	no./mm21 Total Mea	DPN-	ino./mm21 Total Weight IV	Mean Difference Random, 95% Cl Ino./mm21	Mean Difference IV. Random. 95% CI (no./mm2)
3.1.1 CCMetrics Perceptulos 2013 Principal 2014	33.4 40.1	28.13 61 32.1 48	\$5.5 61.7	26.1 50 2.1N 37.2 160 1.8N	-22.10 [-32.21, -11.93] -21.60 [-33.25, -9.95]	
Pontrakts 2015 Sheakandarajah 2013 Alam 2012	87.1 18.2	62.7 46 13.3 33	104.03	55.46 64 0.6N 19.9 63 3.2N	-16.53 [-39.58, 5.72] -16.40 [-23.09, -9.71] -15.20 [-23.09, -9.71]	
Loubion 2015 Ahmed 2012	24.7	13.5 11 12 33	39.2 29	19.5 54 2.3N 16 56 3.5N	-14.50 [-24.02, -4.98] -12.00 [-17.86, -6.14]	
Chen 2018 Ildwards 2012 Tavakoil 2011	45.6 57.8 11.24	51.3 29 49.7 88 22.9 96	69.5 19.4	31.3 63 1.4% 51.4 143 1.3% 12.51 42 3.4%	-11.80 (-25.57, 1.97) -11.70 (-25.07, 1.67) -8.16 (-14.10, -2.22)	
LI 2019 Chen 2015 Kalteniece 2018	25.03 48.2 56.94	15.95 79 32.9 17 34.14 69	33.02 56.1 64.29	17.6 49 3.4N 30.3 46 1.0N 35.1 47 1.6N	-7.99 [-14.04, -1.94] -7.90 [-25.82, 10.02] -7.35 [-20.22, 5.52]	
Petropoulos 2014 Ostrovski 2015 Harter 2011	48.7	33.2 100 12.21 13	54.9 28.21 20	35.7 86 2.2N 11.67 13 2.4N	-6.20 -16.16, 3.76 -5.99 -15.17, 3.19 -6.00 -16.06, 8.00	
Dehghani, Pitchard, Edwards 2014 Subtotal (95% CI)	23.7	20.9 39 807	24.2	17.4 108 2.9N 1026 36.3%	-0.50 [-7.83, 6.83] -10.61 [-13.50, -7.71]	•
Heterogenety: Tas" = 11.65; Ch" = 2 Test for overall effect: Z = 7.18 (P < 0	(4.69, df = 16 0 ^p = 0) (.00001)	(d); r = 35 %				
3.1.2 ACCMetrics Tummanapall, Willow 2019 Petropoulos 2014	22.25 20.1	6.38 28 18.7 100	\$3.75 31.4	15.2 35 3.4N 25.6 86 3.2N	-11.30 [-17.09, -5.91] -11.30 [-17.84, -4.76]	-
Chen 2015 Tummanapall, Issar 2020 Tummanapall 2020	15.4 24.5 22.08	12.1 17 9.29 35 8.58 23	26.2 34.7 32.04	15.1 46 3.0N 12.86 35 3.7N 11.98 27 3.5N	-10.80 [-18.02, -3.58] -10.20 [-15.46, -4.94] -9.96 [-15.68, -4.24]	=
Chen 2018 Li 2019 Orizzaki 2015	16 23.66	15.5 29 15.6 79	25.9 32.96	17.5 63 3.0N 19.3 49 3.3N	-9.30 (-17.01, -2.75) -9.30 (-15.71, -2.85)	
Brines 2018 Andersen 2018	18.73 24.56	13.73 60 13.79 27	21.6	11.7 21 3.4N 13.98 117 3.5N	-2.87 [-8.96, 3.22] 0.31 [-5.48, 6.10]	+
Heterogenety: Tas ² = 7.43; Ch ² = 15 Test for overall effect: Z = 6.06 (P < 6	5.35, df = 9 (* = 0.08 0.00001)	(² - 41N		492 32.0%	-6.20 [-10.86, -5.55]	•
3.1.3 imagej Xiong 2018	24.5	2.33 79	33.02	17.5 49 3.6N	-8.52 (-13.45, -3.59)	
shibashi 2014 Ishibashi 2015 Ishibashi 2015	9.9 8.53	10.38 55 6.6 153	13.1 9.9 9.46	6 23 4.3N 3.43 47 4.5N 5.1 57 4.6N	-3.20 [-6.88, 0.48] -1.37 [-2.80, 0.06] -0.61 [-3.31, 2.08]	1
lahibashi 2016 Subtotal (95% CI)	9.5	6.4 115 420	9.5	2.7 47 4.9N 223 22.5%	0.00 [-1.40, 1.40] -1.82 [-3.68, 0.04]	•
Heterogenetity: Tas* = 2.70; Ch* = 12 Test for overall effect: Z = 1.91 (P = 0	2.55, cf = 4 (F = 0.01) 0.06)	(P = 688				
3.1.4 Morphometric software Taxakoli 2010 Multi: 2003	7.35	13.42 67	17.42	11.6 34 3.6N	-9.47 [-14.57, -4.37]	
Quatrini 2007 Subtotal (95% CI)	7.06	13.5 44	8.1	5.7 10 3.7N 48 9.2N	-1.04 [-6.37, 4.29] -5.74 [-11.98, 0.49]	•
Test for overall effect: Z = 1.61 (P = 0				1944 144.4	-3101	
Heterogenetity: $Tau^2 = 19.67$; $Ch^2 = 1$ Test for overall effect: $Z = 7.62$ ($P < 0$	(46.41, df = 34 (P < 0 .00001)	1763 0.00001); i ^z = 77%		1789 100.0%	=7.39 [+9.33, =5.64] 	-20 -10 0 10 20 More DPN Leve DPN
Test for subgroup differences: Chi ⁴ =	30.97, df = 3 (P < 0.0	0001), i* = 90.3N				and an a set of the set
(b) Study or Subgroup	DPN: Mean [no./mm21_Sn	[to.(mm2] Total Max	Contro (no./mm21_50	l (no./mm2) Total Weight IV	Mean Difference Random, 95% Cl Inc./mm ⁻²¹	Mean Difference IV, Random, 95% CI (no./mm2)
3.2.1 CCMetrics Peroposes 2013 Chrop 2015	33.4	28.13 61	96.6	42.1 47 2.48	-63.20 [-77.15, -49.25]	
Petropoulos 2014 Pritchard 2014	48.7	33.2 100 32.1 48	92.7 83.5	38.6 55 2.6N 45.8 60 2.4N	-44.00 [-56.10, -31.50] -43.40 [-58.12, -28.68]	
Chen 2018 Kalteniece 2020 Kalteniece 2018	45.6 51.07 56.94	31.3 29 3.6 140 34.14 69	90.98 89.64	5.4 30 3.18 34 22 2.28	-41.50 [-55.33, -27.67] -39.91 [-41.93, -37.89] -32.70 [-49.03, -16.37]	-
Edwards 2012 Shuskardarajah 2013 Ahmed 2012	57.8 18.2 17	49.7 88 13.3 35 12 33	79.7 39.7 35	55.4 61 2.2N 16.9 64 2.9N 14 64 3.0N	-21.90 [-39.25, -4.55] -21.50 [-27.64, -15.36] -18.00 [-23.34, -12.66]	-
Li 2019 Alam 2017 Tanakai 2011	25.03	15.95 79 32 31	41.48 60.7	16.5 24 2.98 27.9 27 2.38	-16.45 [-23.93, -8.97] -15.30 [-30.72, 0.12]	_
Hertz 2011 Dehghani, Pitchard, Edwards 2014	25 23.7	18.3 14 20.9 39	37.2 35.1	17.7 20 2.5% 23.8 60 2.8%	-12.20 [-24.61, 0.21] -11.40 [-20.30, -2.50]	
Puttgen 2019 Subtotal (95% CI)	17.8	6.5 116 1006	22.8	4.9 46 3.18 736 44.5%	-9.17 [-17.18, -1.16] -5.00 [-6.85, -3.15] -26.23 [-35.73, -16.73]	
Heterogenety: Tau ² = 363.90; Ch ² = Test for overall effect: Z = 5.41 (P < 0	719.07, df = 16 0 < 0.00001)	0.00001); F = 58%				
3.2.2 ACCMetrics Petropoulos 2014 Azmi 2019	20.1	18.7 100 8.1 29	50.4	24.7 55 2.98 17.3 32 2.98	-30.30 [-37.79, -22.81] -29.80 [-36.48, -23.12]	-
Chen 2015 Tummanapalli 2020 Chen 2018	15.4 22.08	12.1 17 8.58 23	44.6	17.2 26 2.8% 14.25 29 2.9%	-29.20 -37.96, -20.44] -25.24 -31.50, -18.98]	-
Tummanapall, Wilcox 2019 Brines 2018	22.25 18.73	6.58 28 13.73 60	42.3	12.4 34 3.08 14.4 48 3.08	-20.05 [-24.88, -15.22] -17.77 [-23.12, -12.42]	<u> </u>
Ostrovski 2015 Andersen 2018	23.66 13.25 24.56	15.6 79 13.13 13 13.79 27	36.2 24.99 29.8	12.87 24 2.98 12.16 20 2.88 11.96 25 2.98	-12.54 [-18.73, -6.35] -11.74 [-20.65, -2.83] -5.24 [-12.24, 1.76]	-
Puttgen 2019 Subtotal (95% Cl) Heterogenety: Tau ⁴ = 151.14; Ch ⁴ =	11.9 248.14, df = 10 (P <	4.1 116 521 0.00001); # = 96%	15.1	3.1 46 3.15 423 32.2%	-3.20 [-4.37, -2.03] -18.76 [-26.29, -11.23]	◆ ¹
Test for overall effect: Z = 4.88 (P < 0 3.2.3 Image)	.00001)					
Xiong 2018 Ishibashi 2016 Ishibashi 2013	24.5 9.5	2.33 79 6.4 115	41.48	16.41 24 2.98 8.05 45 3.18 2.13 42 3.18	-16.98 [-23.57, -10.39] -5.00 [-7.63, -2.37] -4.95 [-8.14, -1.76]	
ishibashi 2014 ishibashi 2015	9.9 8.53	10.38 55 6.4 153	14.2 12.3	5.24 28 3.18 5.1 40 3.18	-4.30 [-7.66, -0.94] -3.77 [-5.67, -1.87]	-
Heterogenety: Tas ² = 6.03; Ch ² = 14 Test for overall effect Z = 4.31 (P < 0	1.39, df = 4 (7 = 0.00 0.0001)	420 6); 1 ⁴ = 72 N		179 15.25	-214 (-0140) -2123	•
3.2.4 Morphometric software Malk 2003	24.6	15.5 14	78.9	30.4 18 2.3%	-54.30 [-70.52, -38.08]	
Quattrini 2007 Tavakoli 2010 Subtotal (95% C0	7.06	13.5 44 13.42 67 125	27.39 25.38	12.82 15 2.98 12.33 17 2.98 50 8.0%	-20.33 [-27.95, -12.71] -17.43 [-24.11, -10.75] -28.61 [-44.16, -13.07]	
Heterogenety: Tau ² = 160.18; Ch ² = Test for overall effect: Z = 3.61 (P = 0	17.20, df = 2 (P = 0) 0.0003)	0002); # = 88%				-
Total (95% CI) Heterogenety: Tau ⁴ = 179.84; Ch ⁴ =	1428.71, df = 35 (P	2072 < 0.00001); I ⁴ = 98N		1388 100.0%	-20.89 [-25.53, -16.26]	+ 100 -50 0 50 100
Test for subgroup differences: Ch ⁴ =	30.98, df = 3 (P < 0.0	0001), F = 90.3%				More DPN Less DPN
(c)	DPN-		Contro		Mean Difference	Mean Difference
3.3.1 CCMetrics Petropoulos 2013		36.1 50	96.6	42.1 47 1.8%	-41.10 [-56.75, -25.45]	
Petropoulos 2014 Chen 2015 Chen 2018	54.9 56.1 57.4	35.7 86 30.3 46 31.3 63	92.7 92.8 87.1	38.6 55 2.3N 36.4 26 1.7N 36.7 84 2.6N	-37.80 [-50.49, -25.11] -36.70 [-53.21, -20.19] -29.70 [-40.72, -18.68]	<u> </u>
Kalteniese 2018 Pritchard 2014 Delighani, Pitchard, Edwards 2014	64.29 61.7 24.2	35.1 47 37.2 100 17.4 108	89.64 83.5 35.1	34 22 1.68 45.8 60 2.18 23.8 60 1.68	-25.35 [-42.74, -7.56] -21.80 [-35.49, -8.11] -10.90 [-17.76, -4.67]	
Edwards 2012 Li 2019 Hertz 2011	69.5 33.02	51.4 143 17.6 49	79.7	55.4 61 1.78 16.5 24 3.38 17.7 20 2.26	-10.20 -26.46, 6.66] -8.46 [-18.70, -0.22]	=
Taxahol 2011 Ahmed 2012	19.4 29	12.51 42 16 56	26.47	11.32 26 3.9N 14 64 3.9N	-7.07 [-12.84, -1.30] -6.00 [-11.42, -0.58]	-
Swiskandarajan 2013 Ostrovski 2015 Subtotal (95% Cl)	34.6 28.21	19.9 63 11.47 13 878	39.7 31.39	10.3 64 3.7N 10.24 20 3.4N 633 38.5N	-5.10 [-11.53, 1.33] -3.18 [-10.95, 4.59] -16.20 [-21.97, -10.42]	•
Heterogeneity: $Tau^2 = 90.02$; $Ch^2 = 6$ Test for overall effect: $Z = 5.50$ ($P < 0$	19.36, df = 13 (* < 0.) 1.00001)	00001); P = 81N				
s.3.2 ACCMetrics Petropoulos 2014 Chen 2015	31.4 26.2	25.6 86 15.1 46	50.4 44.6	24.7 55 3.28 17.2 26 3.38	-19.00 -27.48, -10.52] -18.40 -26.32, -10.481	-
Turninanapalii 2020 Brines 2018 Chen 2018	32.04	11.98 27 11.7 21 17.5 55	47.32 36.5	14.25 29 3.6N 14.4 48 3.7N 17.7 84 1.08	-15.28 [-22.16, -8.40] -14.90 [-21.35, -8.45] -12.80 [-18.54 -2.65]	÷
Turrmanapall, Wilkox 2019 Ostrovski 2015	33.75 19.23	15.2 35 16.76 13	42.3 24.99	12.4 34 3.7N 12.16 20 2.7N	-8.55 [-15.09, -2.01] -5.76 [-16.31, 4.79]	-
Li 2019 Subtotal (95% CI)	24.25 32.96	19.3 49 457	36.2	11.20 25 4.08 12.87 24 3.58 345 31.6%	-3.24 [-10.85, -0.22] -3.24 [-10.70, 4.22] -11.45 [-15.12, -7.78]	•
Test for overall effect: Z = 6.12 (P < 6	(9.65, df = 5 (f = 0.0 (.00001)	1); r' = 50%				
				16.41 34 1.98	-8.46 [-16.65, -0.27]	-
3.3.3 imagej Xiong 2018 bhibashi 2016	33.02 9.5	17.5 49 2.7 47	41.48	8.05 45 4.55	-5.00 [-7.48, -2.52]	-
3.3.3 Imagej Xkorg 2018 bihbash 2016 bihbash 2013 bihbash 2013 bihbash 2015	33.02 9.5 9.46 9.9	17.5 49 2.7 47 5.1 57 3.43 47 6 21	41.48 14.5 13.8 12.3 14.2	8.05 45 4.58 7.13 42 4.58 5.1 40 4.68 5.24 28 147	-5.00 [-7.48, -2.52] -4.34 [-6.87, -1.81] -2.40 [-4.26, -0.54] -1.10 [-4.26, -0.54]	-
3.3.3 Imagej Xiog 2018 Uvltaski 2016 Uvltaski 2013 Uvltaski 2015 Uvltaski 2015 Uvltaski 2014 Subtotal (95% CO) Heteroperety, Tap ⁴ = 1.38; Ch ⁴ = 6.	33.02 9.3 9.46 9.9 13.1 78, df = 4 (F = 0.15);	17.5 49 2.7 47 5.1 57 3.43 47 6 23 223 f = 41N	41.48 14.5 13.8 12.3 14.2	8.65 45 458 7.13 42 4.58 5.1 40 4.68 5.24 28 4.48 179 21.35	-5.00 [-7.48, -2.52] -4.34 [-6.87, -1.81] -2.40 [-4.26, -0.54] -1.10 [-4.23, 2.63] -3.46 [-5.11, -1.81]	-
3.3.3 Imagej Xlog 2018 Julijska 2016 Julijska 2016 Julijska 2015 Julijska 2015 Subitota (0555 CO Heterogenety: Tas ² = 1.38; Ch ² = 6. Test for overall effect Z = 4.12 of < 6 3.3.4 Morphometric software	33.02 9.5 9.46 9.9 13.1 78, df = 4 (P = 0.15); 0001)	17.5 49 2.7 47 5.1 57 3.43 47 6 23 223 f = 41%	41.48 14.5 13.8 12.3 14.2	1041 44 545 7.13 42 4.58 5.1 40 4.68 5.24 28 4.48 179 21.35	-5.00 [-7.48, -2.52] -4.34 [-6.87, -1.81] -2.40 [-4.26, -0.54] -1.10 [-4.23, 2.03] -3.46 [-5.13, -1.81]	-
3.3.3 Integral solution of the solution of th	33.02 9.5 9.46 9.9 13.1 78, df = 4 (P = 0.15); 0.0001) 32.3 8.1 17.42	17.5 49 2.7 47 5.1 57 3.43 47 6 23 223 f=418 9.04 4 5.7 10 11.8 34	41.48 14.5 13.8 12.3 14.2 78.9 27.38 25.38	30,4 16 1.2% 5,12 40 4.6% 5,24 28 4.4% 5,24 28 4.4% 179 21.3% 30,4 16 1.2% 12,82 15 3.5%	-5.08 [-7.48, -2.52] -4.14 [-6.87, -1.81] -2.48 [-4.26, -0.54] -1.10 [-4.23, 2.63] -3.46 [-5.11, -1.81] -46.60 [-63.20, -30.40] -19.29 [-26.68, -11.50] -7.96 [-5.44, -0.48]	
1.3.3 Investig Nakap 2018 Nakap 2018 Nakap 2018 Nakap 2018 Nakap 2018 Nakap 2018 Nakap 2018 Nakap 2018 Nakap 2018 Naka 2	33.02 9.5 9.46 9.9 13.1 76, df = 4 (P = 0.15); 10001) 32.3 8.1 17.42 18.76, df = 2 (P < 0.4	17.5 49 2.7 47 5.1 57 3.43 47 6 23 223 r = 418 9.04 4 5.7 10 11.8 34 46 0001); r = 856	41,48 14.5 13.8 12.3 14.2 78.9 27,39 25,38	30.5 45 4.58 7.13 42 4.58 5.1 40 4.68 5.24 2.8 4.48 179 21.35 30.4 18 1.79 12.82 13 15.8 12.33 50 6.75	-5.08 [-7.48, -2.52] -8.14 [-6.57, -1.81] -2.48 [-6.57, -1.81] -3.46 [-5.11, -1.81] -4.66 [-5.11, -1.81] -4.66 [-5.12, -3.0.40] -15.29 [-5.40, -0.481] -2.2.78 [-15.40, -0.481] -2.2.78 [-15.41, -6.85]	
1.3.1 marging Yung 2016 white with 2016 white with 2016 white with 2016 white with 2016 white with 2018 white with 20	33.02 9.5 9.46 9.9 13.1 78. df = 4 (P = 0.13); 0.0001) 32.3 8.5 17.42 18.76, df = 2 (P < 0.) 1007)	17.5 49 2.7 47 5.1 57 3.43 47 6 23 7 - 41% 9.04 4 5.7 10 11.4 34 46 0001); r = 850 1606 1606	41.48 14.5 13.8 12.3 14.2 78.9 27.39 25.38	160 +5 +53 7.13 +2 +53 5.1 +0 +48 5.24 +83 +179 21.35 179 21.35 30.4 18 1.79 12.82 15 3.58 12.33 17 3.58 12.07 100.05	-5.00 [-7.48, -2.21 -4.34 [-6.57, -1.81] -2.40 [-4.56, -0.54] -1.10 [-4.21, 2.431] -3.46 [-5.11, -1.43] -4.66 [-5.11, -1.43] -19.29 [-26.68, -11.30] -7.96 [-15.64, -0.48] -22.79 [-39.21, -6.35] -12.01 [-14.73, -9.30]	

Figure 2 | (a) Forest plots of corneal nerve branch density (CNBD) in patients with diabetic peripheral neuropathy (DPN⁺) and without diabetic peripheral neuropathy (DPN⁻). (b) Forest plots of corneal nerve branch density (CNBD) in patients with diabetic peripheral neuropathy (DPN⁺) and healthy control. (c) Forest plots of corneal nerve branch density (CNBD) in patients without diabetic peripheral neuropathy (DNP⁻) and healthy control.

P < 0.00001), ACCMetrics (MD = -2.80, 95% CI -3.57 to -2.04, P < 0.00001), ImageJ (MD = -1.57, 95% CI -2.06 to -1.09, P < 0.00001), and morphometric software (MD = -3.49, 95% CI -5.63 to -1.35, P = 0.001). There was a significant difference in the magnitude of the CNFL reduction in the DPN⁺ group between studies ($\chi^2 = 25.42$, P < 0.00001) (Figure 3a).

DPN⁺ vs control

Thirty-two studies^{4,8,9,12,18,19,21–26,28,31–35,37,38,40,43–47,50–52} with 3,459 (2,036 DPN⁺ and 1,423 control) participants were included in the meta-analysis. The CNFL (mm/mm²) was significantly lower in the DPN⁺ group compared with the controls (MD = -6.05, 95% CI -6.77 to -5.34, P < 0.00001) (CCMetrics (MD = -6.91, 95% CI -8.06 to -5.76, P < 0.00001), ACCMetrics (MD = -5.49, 95% CI -7.03 to -3.95, P < 0.00001), ImageJ (MD = -4.14, 95% CI -4.72 to -3.56, P < 0.00001), and morphometric software (MD = -6.07, 95% CI -8.64 to -3.50, P < 0.00001). There was a significant difference in the magnitude of CNFL reduction between studies ($\chi^2 = 19.59$, P = 0.0002) (Figure 3b).

DPN⁻ vs control

Thirty studies^{4,8,9,12,18,19,21–26,31–33,35,37,38,40,41,43–48,50–52} with 3,149 (1,786 DPN⁻ and 1,363 control) participants were included in the meta-analysis. The CNFL (mm/mm²) was significantly lower in the DPN⁻ group compared with the controls (MD = -2.87, 95% CI -3.34, -2.40, P < 0.00001) (CCMetrics (MD = -3.12, 95% CI -4.06 to -2.19, P < 0.00001), ACCMetrics (MD = -2.63, 95% CI -3.43 to -1.83, P < 0.00001), ImageJ (MD = -2.78, 95% CI -3.35 to -2.22, P < 0.00001), and morphometric software (MD = -2.68, 95% CI -3.48 to -1.88, P < 0.00001). There was no difference in the magnitude of the CNFL reduction in the DPN⁻ group between studies ($\chi^2 = 0.72$, P = 0.87), (Figure 3c).

Inferior whorl length

DPN⁺ vs DPN⁻

Six studies^{8,41,43,44,48} with 459 (205 DPN⁺ and 254 DPN⁻) participants were included in the meta-analysis. The IWL (mm/ mm²) was significantly lower in the DPN⁺ group compared with the DPN⁻ group (MD = -4.11, 95% CI -5.10 to -3.12, P < 0.00001) (CCMetrics (MD = -3.42, 95% CI -5.47 to -1.36, P = 0.001), and ACCMetrics (MD = -4.40, 95% CI -5.53 to -3.28, P < 0.00001). There was no significant difference in the magnitude of the CNFL reduction in the DPN⁺ group between studies ($\chi^2 = 0.68$, P = 0.41), (Figure 4a).

DPN⁺ vs control

Six studies^{8,28,41,43,44,48} with 520 (310 DPN⁺ and 210 control) participants were included in the meta-analysis. The IWL (mm/mm²) was significantly lower in the DPN⁺ group compared with the controls (MD = -10.36, 95% CI -13.30 to -7.42, P < 0.00001) (CCMetrics (MD = -11.62, 95% CI -15.97 to -7.28, P < 0.00001), and ACCMetrics (MD = -8.32, 95% CI -9.40 to -7.24, P < 0.00001)). There was no significant difference in the extent of the IWL reduction in the DPN⁺ group between studies ($\chi^2 = 2.08$, P = 0.15), (Figure 4b).

DPN⁻ vs control

Five studies^{8,41,43,44,48} with 399 (219 DPN⁻ and 180 control) participants were included in the meta-analysis. The IWL (mm/mm²) was significantly lower in the DPN⁻ group compared with the controls (MD = -3.81, 95% CI -4.56 to -3.06, P < 0.00001) (CCMetrics (MD = -4.43, 95% CI -5.56 t0 -3.29, P = 0.003), and ACCMetrics (MD = -3.34, 95% CI -4.33 to -2.34, P < 0.00001). There was no significant difference in the extent of IWL reduction in the DPN⁻ group between studies ($\chi^2 = 2.11$, P = 0.15), (Figure 4c).

DISCUSSION

In this large systematic review and meta-analysis of over 3,000 participants, CCM demonstrated a consistent reduction in four major corneal nerve parameters in patients with DPN compared with healthy controls and those without DPN. Furthermore, we demonstrate a lesser but significant reduction in all corneal nerve parameters in patients without DPN compared with controls, suggesting that CCM detects early sub-clinical DPN. This is consistent with the demonstration of corneal nerve loss in subjects with impaired glucose tolerance⁵⁴, recently diagnosed type 2 diabetes⁵⁵ and children with type 1 diabetes⁵⁶. The greater corneal nerve loss in patients with DPN compared with those without DPN is consistent with studies showing that corneal nerve loss is associated with the severity of DPN^{4,24,51,57,58} and has good sensitivity and specificity for diagnosing DPN⁵⁻⁷. Both CNFD and IENFD have a comparable diagnostic performance for DPN^{8,9,59}, although in a study of patients with recently diagnosed type 2 diabetes there were differences in the extent of small nerve fiber damage between CCM and skin biopsy⁵⁷. Additionally, a reduction in corneal nerve parameters is associated with incident DPN^{10,53,60} and greater corneal nerve loss⁴¹, and augmented nerve branching²⁷ occurs in patients with painful diabetic neuropathy. CCM could act as a biomarker as defined by the NIH Biomarkers

(a) Study or Subgroup	DPN+ Hean [mm/mm2] SD [n	en/mm2] Total Mean	DPN- (mm/mm2) SD (mm	n/mm2] Total Weight N.:	Mean Difference Random, 95% Cl [mm/mm2]	Mean Difference IV, Randoer, 95% CI (mm/mm2)
LL1 CCMetrics Petroposks 2015	17	7.5 25	23.4	7 28 1.15	-6.40[-10.32, -2.48]	_
Alam 2017 Ahmed 2012 Semiconduction 2013	15.8	7 31 34 33	21.5	4.8 30 1.6% 4.3 54 2.7% 4.3 61 2.7%	-5.70 -8.70, -2.70 -5.40 -7.27, -3.83 -5.50 -7.21, -3.70	-
Chen 2018 Chen 2015	14.6	8.2 29 8.3 17	20.1 20.2	5.3 63 1.5N 5.1 46 1.0N	-5.50 -8.76, -2.24 -5.40 -9.61, -1.19	=
Antronald 2014 Ontronald 2015 Hentr 2011	14 12 12.45	4.23 13 3.3 14	19.1 16.9 17.12	5.6 100 2.38 3.73 13 1.68 3.83 12 1.88	-5.10 -7.24, -2.36 -4.30 -7.93, -1.81 -4.54 -7.44, -1.84	-
Ponizakis 2015 Loubium 2015 Petrosenia 2014	19.45	8.48 46 3.8 11 7.6 100	24.08	5.097 64 1.8N 3.7 54 2.0N	-4.43 (-7.38, -1.88) -3.80 (-6.25, -1.35) -3.60 (-5.66, -1.54)	-
Tavakoli 2011 Pritchard, Delighani 2015	4,41	45 16 35 25	7.6 16.7	3.8 42 3.0N 3.5 82 2.8N	-3.19 -4.65, -1.73 -2.50 -4.07, -0.33	-
Edwards 2012 Pritchard, Edwards 2015 Dehohard, Pitchard 2014	15.9	6.6 88 4.1 16 3.1 13	18.4 16.2 17	6 143 2.78 3.5 74 2.38 4.5 20 1.98	-2.50 [-4.19, -0.81] -2.20 [-4.36, -0.04] -2.20 [-4.79, 0.39]	-
U 2019 Kalteriece 2018	13.6 21.84	4.15 79 7.23 69	15.48 23.16	3.66 49 3.0N 7.27 47 1.8N	-1.88 -3.25, -0.51] -1.32 [-4.01, 1.37]	-
Subtotal (ISSN CI) Heterogeneby: Tau ² = 1.68; Ch ² = 49	20, df = 20 (7 = 0.000)	886 R; F = 588		1230 44.5%	-3.74 [-4.49, -2.99]	•]
Test for overall effect: Z = 5.77 (P < 0 1.1.2 ACCMetriks	00001)					
Chen 2018 Chen 2015 Turmensonali 2020	E.7 E.8	47 19 47 17 24 19	13.3 13.4	3.7 63 2.5N 3.3 46 2.0N 2.55 27 3.0N	-4.60 -6.54, -2.66 -4.60 -7.03, -2.17 -3.68 -5.25 -2.51	-
Petroposita 2014 Tummarapall, Wilkox 2019	13.7	5.2 100 2.73 28	17.1 14.55	4.5 84 3.0% 2.44 35 3.1%	-3.40 [-4.79, -2.01] -3.10 [-4.39, -1.81]	Ξ
Tummanapalli, Issar 2020 Brines 2018 Convesti 2015	9.86	2.47 35 4.66 60 2.19 13	14.94 12.8 12.2	2.02 35 3.48 3.4 21 2.58 3.19 13 2.38	-2.96 (-4.02, -1.90) -2.94 (-4.81, -1.07) -2.62 (-4.72, -0.52)	-
Delighard, Pitchard 2014 U 2019	14.8	3.8 13 3.51 79	16.3 13.37	3.8 20 1.9N 3.65 49 3.1N	-1.50 [-4.15, 1.15] -1.45 [-2.73, -0.17]	1
Subtotal (95% CI) Heterogenety: Tax ² = 0.97; Ch ² = 25	.43, df = 10 (7 = 0.005)	424	12.39	\$12 29.8N	-2.80 [-3.57, -2.04]	•
Test for overall effect: Z = 7.16 (P < 0 1.1.3 image)	.00001)					
bhbashi 2013 Xiorg 2018	13.46	1.23 18 0.62 79	11 15.48	2.27 57 3.4N 3.64 49 3.4N	-2.10 -2.92, -1.28 -2.02 -3.05, -0.99	-
shbash 2015 shbash 2016	8.53	4.5 153 4.2 115	9.6	2.06 47 3.5N 2.12 47 3.4N	-1.07 (-1.59, -0.15) -1.00 (-1.58, -0.02)	
Subtetal (ISSN CI) Interogenety: Tau ² = 0.03; Ch ² = 4.3 Total for exercise difference of 32.42 of 4	7, df = 4 (7 = 0.31); f -	420 - 16N		223 16.9%	-1.57 [-2.06, -1.09]	
1.1.4 Morphametric software						
Tavakoli 2013 Quatrini 2010	5.9 3.8 3.73	1.3 14 3.3 67 3.56 44	10.8 8.05 4.59	4.14 34 2.8N 2.92 10 2.3N	-4.30 (-6.01, -3.75) -4.25 (-5.85, -2.65) -0.86 (-2.96, 1.24)	-1
Subtotal (ISSN CI) Heterogenety: Tau ² = 2.90; Ch ² = 11 Taut for council affect: 7 = 3 19 /8 = 0	19, df = 2 (P = 0.004);	125		41 8.48	-3.49 [-5.63, -1.35]	•
Tetal (55% CD		1855		2013 100.0%	-3.08 [-3.58, -2.58]	
neterogenety: Tay ² = 1.64; Chi ² = 13 Test for overall effect: Z = 12.09 (F <) Test for suborous differences: Ch ² = 3	8.85, df = 39 (F < 0.600 0.60001) 15.42, df = 3 (F < 0.400	1012; F = 715 11. F = 88.25			-	-20 -10 0 10 20 More DPN Less DPN
(b)		-				
Study or Subgroup	DPN+ News [mm/mm2] 5D fo	en/mm2] Total Mean	Control (mm/mm21 SD law	n/mm2] Total Weight No	Mean Difference Random, 95% Cl Imm/mm21	Mean Difference W, Random, 95% Cl (mm/mm2)
1.2.1 CCMetrics Petroposits 2013	14.02	7.26 61	27.3	5.4 47 2.48	-13.28 (-15.47, -10.85)	
Chen 2018 Alam 2017	14.6	6.3 17 8.2 29 7 31	25.6	5.7 26 1.58 5.3 84 2.08 3.8 27 2.18	-11.00[-14.19, -7.81] -10.00[-13.05, -7.95]	=
Petroposka 2015 Azmi 2019	17	2.5 25	27.5	4.4 15 1.7N 3.5 32 2.8N	-10.50[-14.25, -4.75] -10.00[-11.58, -8.42]	E
Prochard 2014 Ahmed 2012	14.7 14 11.1	7.6 100 6.4 48 3.6 33	23.2 18.4	5.0 55 2.5N 6.3 60 2.4N 4.4 64 2.7N	-9.20[-11.80, -7.60] -9.20[-11.61, -6.79] -7.30[-8.93, -5.67]	=
Shaskanda ajah 2013 Kaltentece 2020	11.6 21.19	4 33 0.82 140	18.8	4.5 64 2.7% 0.93 30 3.2%	-7.20 (-8.95, -5.45) -5.44 (-5.82, -5.06)	7.
Kaberlece 2018 Tavakoil 2011	17.8 21.84 4.41	6.5 116 7.23 49 4.5 56	22.8 26.57 8.76	4.9 46 2.03 6.8 22 1.95 2.9 26 2.85	-5.00 (-6.85, -3.15) -4.73 (-8.04, -1.42) -4.35 (-5.78, -2.92)	-
Li 2019 Itdwards 2012	13.6	4.15 79 6.6 88	17.81 20.1	3.19 24 2.8N 6.2 61 2.5N	-4.21 (-5.78, -2.64) -4.20 (-6.28, -2.12)	-
Renz 2011 Dehohard, Fishard, Edwards 2014	12.45	4.23 13 3.3 14 4.3 39	16.2 16.15 18.1	3.56 20 2.18 4.13 20 2.38 3.7 60 2.78	-4.20 [-7.05, -1.32] -3.67 [-6.17, -1.17] -3.10 [-4.74, -1.46]	
Dehghard, Pfichard 2014 Suiteetal (95% CI)	14.8	3.1 13 1073	17	4.5 17 2.2% 800 43.0%	-2.20 [-4.92, 0.52] -6.91 [-8.06, -5.76]	•
Heterogenety: Tay = 5.45; Chr = 16 Test for overall effect: Z = 11.78 (F < 1	3.14, df = 19 (P < 0.000 0.00001)	101); I" = 88N				
1.2.2 ACCMetrics Azmi 2019 Chen 2018	7.5	2.9 29	17.8	3.5 32 2.8N	-10.30 [-11.91, -8.69]	
Petropoko 2014 Brines 2018	13.7	5.2 100 4.66 60	21.2	3.3 55 2.8N 3.2 48 2.8N	-7.50 [-8.88, -6.12] -7.24 [-8.73, -5.75]	2
Tummarapali 2020 Tummarapali, Wilcox 2019	11.25	2.4 23 2.73 28	17.43	1.83 29 2.9% 2.06 34 2.9%	-6.18 -7.37, -4.99 -5.30 -6.53, -4.87	-
Dehghard, Pitchard 2014 Ostrovski 2015	14.5	3.8 13 2.19 13	18.1 12.53	2.7 17 2.38	-3.30 -5.73, -0.87] -2.35 -4.68, -1.22]	-
LI 2019 Andersen 2018 Suburgal (SSIS CI)	11.92 12.13	3.51 79 3.63 27 418	14.66 13.9	2.31 24 2.9N 2.55 25 2.7N 194 29.5%	-2.74 [-3.95, -1.53] -1.77 [-3.47, -0.87] -5.49 [-7.03, -3.95]	
Heterogenety: Tau ² = 6.04; Ch ² = 10 Test for overall effect Z = 6.59 (P < 0	7.71, df = 10 (7 < 0.000 (00001)	(01); I ² = 91,4				•
1.2.3 imagej bhbashi 2014	8.6	4.75 55	14.2	4.97 28 2.58	-5.60 -7.83, -3.371	_
ahipashi 2013 Xiong 2018	8.9 13.46	1.23 18 0.62 79	13.3 17.81	5.3 42 2.78 3.1 24 2.98	-4.40 -6.10, -2.70 -4.35 -5.60, -3.10	
shibashi 2016 shibashi 2015 Subtotal (SSN CI)	8.53	4.2 115 4.5 153 420	15.5	3.4 43 2.50 1.9 40 3.0N 1.79 14.0N	-4.20 (-5.50, -2.90) -3.67 (-4.59, -2.25) -4.14 (-4.72, -3.56)	7
Heterogenety: Tau ² = 0.00; Ch ² = 2.1 Test for overall effect: 2 = 13.94 (F $<$	15, df = 4 (* = 0.58); i ² - 0.00001)	- (6)				
1.2.4 Morphometric software Malk 2003	5.9	1.3 14	13.5	0.3 18 3.18	-7.60 (-8.29, -6.91)	
Tavakoit 2010 Quetrini 2007 Subtotal (SSS CI)	3.8	3.3 67 3.58 44	51.21 6.14	3.63 17 2.6N 4.73 15 2.2N 50 8.0%	-7.41 [-9.31, -5.51] -2.41 [-5.03, 0.21] -6.07 [-8.64, -3.50]	
Heterogeneity: Tax ² = 4.23; Ch ² = 14 Test for overall effect: 2 = 4.63 ϕ < 0	.14, df = 2 (? = 0.0005) .00001)	i ² - 56X				•
Total (95% CI) Heterogenety: Tay ² = 4.20; Ch ² = 34	0.52, cf = 38 (P < 0.000	2036 1011; F = 89N		1423 100.0%	-6.05 [-6.77, -5.34]	+
Test for overall effect: Z = 16.50 (P <) Test for substrats differences: Off = 1	0.60001) 9.59. df = 3 (7 = 0.600	2). f = 84.7%				-we -10 0 10 20 More DPN Less DPN
(c)						
Study or Subgroup	DPN- Hean (mm/mm2) 5D (n	im/mm2) Total Mean	Control (mm/mm2) SD (mm	n/mm2) Total Weight N.:	Mean Difference Random, 95% Ci (mm/mm2)	Mean Difference W. Random, 95% Cl (mm/mm2)
Petroposius 2013 Chen 2015	20.05	6.7 50 5.1 46	27.3	5.4 47 2.13 3.7 26 2.45	-7.25 (-9.66, -4.84) -6.30 (-8.55, -4.64)	-
Petroposita 2014 Chen 2018	20.3	6.7 86 5.3 63	26.4	5.4 55 2.4N 5.3 84 2.8N	-6.10 [-8.15, -4.05] -5.50 [-7.23, -3.77]	
Alam 2017 Petroposius 2015	21.5 23.4	4.8 30 7 28	26.6	3.8 27 2.28 4.6 15 1.38	-5.10 -7.34, -2.86 -4.10 -7.58, -0.62	-
Kalteniese 2018 Delighani, Pitchard 2014	23.16	7.27 47 4.5 20	26.57	6.8 22 1.3N 3.4 17 2.0N	-3.41 [-6.93, 0.11] -2.90 [-5.45, -0.31]	-
Pritchard, Delighani 2015 U 2019	16.7 15.48	3.5 82 3.66 49	19.3 17.81	3 80 3.8N 3.19 24 2.9N	-2.60 -3.60, -1.60 -2.33 -3.97, -0.69	-
Dengkaril, Pitchard, Edwards 2014 Edwards 2012 Ahmed 2012	16	3.8 108 6 143 4.3 44	18.1 20.1 18.4	3.7 60 3.5N 6.2 61 2.7N 4.4 64 3.0N	-2.10 [-3.28, -0.92] -1.70 [-3.54, 0.14] -0.70 [-3.24, -0.74]	-
Seaskandarajah 2013 Tavakuli 2011	17.1	4.2 63 3.8 42	18.8	4.5 64 3.1N 2.9 26 3.0N	-1.70 [-3.21, -0.19] -1.16 [-2.76, 0.44]	~
Ostronski 2015 Hertz 2011 Subreau (2015 Cl)	16.9	3.79 13 3.89 12 1038	16.2 16.15	3.96 20 1.8% 4.13 20 1.7% 772 44.7%	0.70 [-1.99, 3.39] 0.97 [-1.88, 3.82] -3.12 [-4.05, -2.19]	.ŧ
Here rogeneity: Tau ² = 2.95; Ch ² = 75 Test for overall effect: Z = 6.56 (P < 0	.45, df = 17 (? < 0.0000 .00001)	ID; f = 775				
1.3.2 ACCNetrics		14 11	17.1	12 47 144		_
Chen 2015 Petroposks 2014	13.4	3.3 46 4.5 86	17.7	2.8 24 3.28 3.5 55 3.38	-4.30 -5.74, -2.86 -4.10 -5.43, -2.77	± 1
Chen 2018 Tummanapalii 2020	13.3 15.13	3.7 63 2.55 27	16.9 17.43	3.4 84 3.5N 1.83 29 3.5N	-3.60 [-4.37, -2.43] -2.30 [-3.47, -1.13]	1
r yermanapasi, Wilkox 2019 Delighari, Pitchard 2014 Andersen 2018	14.55 16.3 12.39	2.44 35 3.8 20 2.99 137	18.75 18.1 13.9	2.09 34 3.7N 2.7 17 2.4N 2.55 25 3.6N	-2.40 (-3.26, -1.14) -1.80 (-3.50, 0.30) -1.51 (-2.65, -0.37)	-
U 2018 Ostrovski 2015	13.37	3.45 49 3.19 13	14.66	2.31 24 3.3N 2.87 20 2.3N	-1.29 [-2.67, 0.09] -0.33 [-2.47, 1.81]	.1
sustatal (SSN CI) Heterogenety: Tau ² = 1.10; Ch ² = 25 Text for overall effort 7 = 5.45 for a 2	52, df = \$ (7 = 0.0005) 00001)	477 : 1 - 70%		362 31.7N	-2.63 [-3.43, -1.83]	•
133 imagej						
ishigashi 2014 Ishigashi 2016 Ishigashi 2015	10.2	1.44 23 2.12 47 2.66 47	14.2	4.97 28 2.6N 3.4 45 3.5N	-4.00 (-5.53, -2.07) -3.20 (-4.41, -1.99) -3.60 (-3.43 - 1.99)	-
Xlorg 2018 hhbashi 2013	15.45	3.44 49 2.27 57	17.81 13.3	3.1 24 3.0N 5.3 42 2.8N	-2.33 (-3.94, -6.72) -2.30 (-4.01, -0.59)	-
Subtotal (SSN CI) Heterogenety: Tax ² = 0.00; Ch ² = 2.1	18, df = 4 (7 = 0.60); f -	223	-	179 15.8%	-2.78 [-3.35, -2.22]	'
1.3.4 Norphonetric software	amo(01.)					
Tavakeil 2010 Malik 2003	8.05	4.14 34 0.9 4	11.21	3.63 17 2.3N 6.3 18 3.8N	-3.16 -5.38, -0.94 -2.70 -3.59, -1.81	-
Subtotal (SSN CI) Netwogenety: Tay ² = 0.00; Ch ² = 0.1	4.53 3. df = 2 (7 = 0.70): f •	48 - 05	6.14	50 7.8N	-2.58 [-3.48, -1.88]	•
Test for overall effect Z = 6.58 (P < 0 Testal (MSK CP	08001)	1394		THE TOTAL	281.894	
Herevogenety: Tau ² = 1.28; Ch ² = 10 Test for overall effect: Z = 11.92 (P <	9.28, 47 = 35 (7 < 0.000 0.00001)	101); P - GEN		Los I Index	-wei (*549, *640)	-20 -10 0 10 20 More DPN Less DPN
rest for subgroup differences: Chr = 0	1.72, 47 = 3 (7 = 0.87), 1	- 65				

Figure 3 | (a) Forest plots of corneal nerve fiber length (CNFL) in patients with diabetic peripheral neuropathy (DPN⁺) and without diabetic peripheral neuropathy (DNP⁻). (b) Forest plots of corneal nerve fiber length (CNFL) in patients with diabetic peripheral neuropathy (DPN⁺) and healthy control. (c) Forest plots of corneal nerve fiber length (CNFL) in patients without diabetic peripheral neuropathy (DNP⁻) and healthy control.

Definitions Working Group⁶¹; it is non-invasive, easily measured, and produces rapid results with high sensitivity^{5–7}. It allows the detection of subclinical DPN, and there is minimal overlap in corneal nerve parameters between patients with and without DPN and healthy people. In addition, CCM identifies those at risk of developing DPN^{10,11,53}.

The outcomes of the current review extend considerably the findings of a previous systematic review and meta-analysis showing a reduction in CNFD, CNBD, and CNFL in patients with and without DPN compared with controls from 13 studies with 1,680 participants⁶² and a more recent trial sequential meta-analysis which showed a reduction in CNFD, CNBD, and CNFL in patients with and without DPN compared with controls in 13 studies with 1,830 participants¹⁴.

In the present review we have included IWL which has the potential to detect earlier nerve damage^{36,63,64}, especially in patients with painful diabetic neuropathy^{28,41}.

The reliability of establishing a single estimate for the effect size of corneal nerve outcome measures from all the published studies may be affected by the inclusion of the same subjects from several studies, type of CCM used to acquire the images, the mode of image acquisition, and the image analysis tool used to quantify corneal nerve parameters. Our analysis showed that the type of software used for image analysis had no significant influence on the heterogeneity of corneal nerve outcomes. Whilst the corneal nerve measure was lower when using automated (ACCMetrics) compared with manual (CCMetrics, ImageJ) software, the magnitude of difference in corneal nerve parameters between groups was comparable^{38,65}.

Our sensitivity analysis shows no evidence of significant bias or heterogeneity (Doc S1). This was expected, given that there may be differences in corneal nerve parameters between patients with type 1 and type 2 diabetes^{5,7,13} and in relation to $HbA1c^{66}$ and glycemic variability⁶⁷, presence of metabolic syndrome⁶⁸ and hypertension or hyperlipidemia^{7,69}.

CONCLUSIONS

Corneal confocal microscopy is a rapid, non-invasive and reproducible imaging technique to quantify small nerve fiber damage. Our systematic review and meta-analysis provides robust evidence that corneal confocal

(a)

	DPN	,	D	PN-			Mean Difference	Mean Difference
Study or Subgroup	Mean [mm/mm2] SD	[mm/mm2] Tota	Mean (mm/mm2)	SD [mm/mm2]	Total	Weight	IV, Random, 95% CI [mm/mm2]	IV, Random, 95% CI [mm/mm2]
Petropoulos 2015	18.2	8 25	25		28	4.35	-6.60 I-11.38, -2.221	
Pritchard, Dehohani 2015	15.4	4.44 25	18.2	3.9	82	17.1	-2.80 [-4.73, -0.87]	
Kalteniece 2018	21.56	10 65	24.08	9.6	47	6.55	-2.52 [-6.14, 1.10]	
Subtotal (95% CI)		115			157	27.9%	-3.42 [-5.47, -1.36]	◆
Heterogeneity: Tau ^z = 0.93; 0 Test for overall effect: Z = 3.2	(h ^e = 2.67, df = 2 (P =) (6 (P = 0.001)	0.26); F = 25%						
5.1.2 ACCMetrics								
Tummanapalit, Wilcox 2019	9.6	3.04 26	14.9	2.9	35	23.4%	-5.30 [-6.78, -3.82]	•
Tummanapalii 2020	9.69	2.99 23	14.33	2.56	27	22.25	-4.64 [-6.20, -3.06]	•
Tummanapalit, Issar 2020 Sebtoral (95% CI)	11.16	2.91 35	14.58	2.67	35	26.58	-3.42 [-4.73, -2.11]	*
Heterogeneity: Tau ² = 0.45; (Test for overall effect: Z = 7.4	(h ⁴ = 3.66, df = 2 (P =) (5 (P < 0.00001)	0.16); # = 45%			21	78.8.9	-440 [-333] -370]	•
Total (95% CI)		205			754	100.05	-411/-510 -212	•
Heterogeneity: Tau ⁴ = 0.52: 0	b ² = 7.83. df = 5 (P =)	0.17): P = 36%			2.24	104.0.4	-411 [3114] - 3114]	
Test for overall effect: Z = 8.1 Test for subgroup differences	3 (P < 0.00001) Chi ² = 0.68, df = 1 (P -	- 0.41), i ⁴ - 0%						-20 -10 0 10 20 More DPN Less DPN
(b)								
	DPN		6	entrol			Mean Difference	Mean Difference
Study or Subgroup	Mean [mm/mm2] SD	[mm/mm2] Total	Mean (mm/mm2)	5D [mm/mm2]	Total	Weight	IV, Random, 95% CI [mm/mm2]	IV, Random, 95% CI [mm/mm2]
5.2.1 CCMetrics								
Kalteniece 2018	21.56	10 69	35.18	9.5	22	13.28	-13.62 [-18.24, -9.00]	
Katenlece 2020	21.6	1.7 140	35.19	2.29	30	19.2%	-13.59 [-14.46, -12.72]	
Petropoulos 2015 Petropoulos 2015	15.4	4 44 25	31.3	1.9	80	12.5%	-13.30 [-16.30, -6.30]	
Subtotal (95% CI)	13.4	259		5.5	147	62.9%	-11.62 [-15.97, -7.28]	•
Heterogeneity: Tau ² = 16.74; Test for overall effect: Z = 5.2	Chf = 40.78, df = 3.(P 4 (P < 0.00001)	< 0.00001); P = 53	×					
5.2.2 ACCMetrics								
Tummanapalii 2020	9.69	2.99 23	18.04	2.61	29	18.5X	-8.35 [-9.90, -6.80]	•
Tummanapalit, Wilcox 2019	9.6	3.04 28	17.9	2.98	34	18.6%	-8.30 [-9.81, -6.79]	÷
Subtotal (95% CI)		51			63	37.1%	-8.32 [-9.40, -7.24]	•
Test for overall effect: Z = 15	11 (P < 0.00001)	0.96K P = 0%						
Total (95% CI)		310			210	100.0%	-10.36 [-13.30, -7.42]	◆
Heterogeneity: Tau ⁴ = 11.50;	Chf = 78.78, df = 5 (P	< 0.00001); if = \$4	N					
Test for overall effect: Z = 6.5	1 (P < 0.00001)							More DPN Less DPN
Test for subgroup differences	Ch# = 2.08, df = 1 (P -	- 0.15), P = 52.0%						
(c)								
	DPN		Ce	ntrol			Mean Difference	Mean Difference
Study or Subgroup	Hean [mm/mm2] SD	[mm/mm2] Total	Mean [mm/mm2]	SD [mm/mm2]	Total	Weight	IV, Random, 95% CI [mm/mm2]	IV, Random, 95% CI [mm/mm2]
5.3.1 CCMetrics	24.08		95.40			7.65	-11 10 - 15 01 - 0 20	
Petropoulos 2015	24.08	9 24	35.18	7.2	15	6.95	-6.50 [-13.95, -6.27]	
Pritchard, Dehghard 2015	18.2	3.9 82	22.1	3.9	80	29.95	-3.90 [-5.10, -2.70]	•
Subcotal (95% CI)	CH4 - 9 71 df - 2 /2 -	157 0.015 H = 775			117	44.4%	-6.73 [-11.19, -2.26]	-
Test for overall effect: Z = 2.5	(5 (P = 0.003)							
5.3.2 ACCMetrics								
Tummanapalii 2020	14.33	2.56 27	18.04	2.61	29	28.4%	-3.71 [-5.06, -2.36]	•
Tummanapalii, Wilcox 2019 Subtotal (95% CI)	14.3	3.26 35	17.2	2.97	34 63	27.2X 55.6%	-2.90 [-4.37, -1.43] -3.34 [-4.33, -2.34]	•
Heterogeneity: $Tau^2 = 0.00$; C Test for overall effect: $Z = 6.5$	$h^{\mu} = 0.63, df = 1 (P = 0.00001)$ (7 (P < 0.00001)	0.433; P = 0%						
Total (95% CD		219			180	100.0%	-4.30 [-5.79, -2.81]	•
Heterogeneity: Taut = 1.56: 0	34 ⁴ = 11.34, df = 4 (P =	0.021: 1 = 65%			-00	2000018		
Test for overall effect: Z = 5.6 Test for subgroup differences	5 (P < 0.00001) Chr ² = 2.11, df = 1 (P -	0.15), P = 52.6%						-20 -10 0 10 20 More DPN Less DPN

Figure 4 | (a) Forest plots of inferior whorl length (IWL) in patients with diabetic peripheral neuropathy (DPN⁺) and without diabetic peripheral neuropathy (DNP⁻). (b) Forest plots of inferior whorl length (IWL) in patients with diabetic peripheral neuropathy (DPN⁺) and healthy control. (c) Forest plots of inferior whorl length (IWL) in patients without diabetic peripheral neuropathy (DNP⁻) and healthy control.

microscopy can be used to diagnose sub-clinical and established DPN.

DISCLOSURE

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Flowchart of the included studies.

Table S1 | Search details

- Table S2 | Risk of bias assessment for non-randomized studies
- Doc S1 | Methods. Risk of bias and sensitivity analysis.