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Loss of Temporal Peripapillary Retinal Nerve Fibers in Prediabetes or Type 2 Diabetes Without Diabetic Retinopathy: The Maastricht Study

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PURPOSE. The purpose of this study was to assess thinning of the peripapillary retinal nerve fiber layer (RNFL) in prediabetes or type 2 diabetes without diabetic retinopathy (DM2 w/o DRP) compared with that in individuals with normal glucose metabolism (NGM).

METHODS. We measured sectoral and mean RNFL thickness in a 3.45-mm-diameter circular scan centered on the optic nerve head, using spectral domain optical coherence tomography in 1172 participants from The Maastricht Study (a population-based cohort of individuals 59 ± 8 years of age, 47% men, 699 NGM, 186 with prediabetes, and 287 with DM2 w/o DRP). Multivariate linear regression was used to assess the association between RNFL thickness and glucose metabolism status, adjusted for age and sex.

RESULTS. In individuals with prediabetes, the temporal RNFL was thinner than that in individuals with NGM after adjustment ($\beta = -2.28 \mu\text{m}$ [95% confidence interval [CI], -4.44 to -0.13], $P = 0.04$), whereas in individuals with DM2 w/o DRP, the temporal inferior ($\beta = -3.66 \mu\text{m}$ [95% CI, -6.46 to -0.85], $P = 0.01$), the temporal superior ($\beta = -2.99 \mu\text{m}$ [95% CI, -5.95 to -0.02], $P = 0.05$), the temporal ($\beta = -2.73 \mu\text{m}$ [95% CI, -4.62 to -0.84], $P < 0.01$), and the mean RNFL ($\beta = -1.88 \mu\text{m}$ [95% CI, -3.51 to -0.26], $P = 0.02$) were thinner than those in individuals with NGM.

CONCLUSIONS. Temporal RNFL thinning is already present in individuals with prediabetes. More widespread RNFL thinning occurs in individuals with DM2 w/o DRP, that is before vascular changes are detected. This suggests preferential retinal nerve fiber layer loss in areas related to the papillomacular bundle.

Keywords: diabetic retinopathy, optic nerve head, optical coherence tomography

The prevalence of prediabetes and type 2 diabetes mellitus (DM2) continues to increase to an epidemic level.^{1,2} Diabetic retinopathy (DRP) is a severe complication of diabetes that commonly leads to vision loss and is a main cause of vision impairment and blindness among working-age adults.³ It can start insidiously, with few or no symptoms. The current gold standard for detecting diabetic retinopathy includes direct and indirect ophthalmoscopy, stereoscopic photography, and digital retinal photography. All these measurements assess signs of microvascular damage due to diabetes, such as microaneurysms, retinal ischemia, and vascular leakage. However, neurodegenerative changes around the optic nerve head, notably axonal loss leading to thinning of the peripapillary retinal nerve fiber layer (RNFL),⁴ also occur in the absence of retinal microvascular lesions.^{5–8} Retinal neurodegeneration in diabetes could therefore be considered part of diabetic retinopathy, which nevertheless is still considered a microvascular complication of diabetes.

Loss of retinal nerves in diabetes is associated with functional changes assessed by visual acuity testing,⁹ electrophysiology,^{10–13} (micro-)perimetry,^{14–16} dark adaptation test,¹⁷

contrast sensitivity test,¹⁸ and color vision test.¹⁹ Changes in RNFL thickness may explain vision-related functional changes in individuals with diabetes.

Early diabetic retinopathy includes a neurodegenerative component that can be assessed as RNFL thinning, which could start even before diabetes is clinically diagnosed. However, evidence for RNFL thinning in individuals with prediabetes is lacking. Moreover, the extent of RNFL thinning in individuals with DM2 without (w/o) DRP has still not been established in a large population. Spectral domain optical coherence tomography (SD-OCT) enables accurate measurement of mean RNFL thickness and RNFL thickness in six sectors. Establishing RNFL thinning in prediabetes by using SD-OCT could provide insights into the early pathogenic components of diabetic retinopathy.

The aim of the present study was to investigate the extent to which peripapillary RNFL thinning occurs early in diabetes by studying RNFL thinning in individuals with DM2 w/o DRP or prediabetes compared with that in individuals with normal glucose metabolism (NGM) in a large population-based cohort study.



MATERIALS AND METHODS

Study Population

In this study, we used data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology were described previously.²⁰ Briefly, the study focuses on the cause, pathophysiology, complications, and comorbidities of DM2 and is characterized by an extensive phenotyping approach. Eligible participants consisted of those between 40 and 75 years of age, who lived in the southern part of The Netherlands. Participants were recruited through mass media campaigns, from municipal registries, and from a regional Diabetes Patient Registry through mailings. Recruitment was stratified according to known DM2 status for reasons of efficiency. The present report included cross-sectional data from the first 3451 participants who completed the baseline survey between November 2010 and September 2013. The examination of each participant was performed within a time window of 3 months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of The Netherlands, on the basis of the Health Council's opinion (permit 131088-105234-PG), and is compliant with the tenets of the Declaration of Helsinki. All participants gave written informed consent.

Glucose Metabolism Status

To determine glucose metabolism, all participants, except for those who used insulin, underwent a standardized 2-hour 75-g oral glucose tolerance test after an overnight fast. For safety reasons, participants with a fasting glucose level above 11.0 mmol/L, as determined by a finger prick blood sample, did not undergo the oral glucose tolerance test. For those individuals ($n = 13$), fasting glucose level and information about diabetes medication were used to determine glucose metabolism status. Glucose metabolism was defined according to World Health Organization 2006 criteria in NGM, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), prediabetes (i.e., IFG and/or IGT), and DM2.²¹ Individuals without type 1 diabetes mellitus (DM1) taking diabetes medication were classified as having DM2.²⁰ For this study, individuals with DM1, individuals with latent autoimmune diabetes of adults, steroid-induced diabetes, and individuals who underwent a pancreas transplantation were excluded.

Ophthalmologic Measurements

Automated refraction and noncontact tonometry tests were performed in both eyes by using an automated refractor (Tonoref II; Nidek, Gamagori, Japan). Once the pupils were dilated with tropicamide, 0.5%, and phenylephrine, 2.5%, fundus photography was performed in both eyes. All fundus photographs were made with an automatic fundus camera (model AFC-230; Nidek) at 45° of at least 3 fields: 1 field centered on the optic disc, 1 field centered on the macula, and 1 temporal field positioned one disc diameter from the center of the macula. These fundus photographs were evaluated by a trained and experienced grader (EDC) in a masked fashion, and in case of any doubt or an abnormal finding, the fundus photograph was discussed with a medical retina specialist (JS). Based on these fundus photographs, the presence of diabetic retinopathy was graded according to the Diabetic Retinopathy Disease Severity Scale and the International Diabetic Retinopathy Disease Severity Scale, where no DRP = no abnormalities; mild nonproliferative DRP = microaneurysms only; moderate nonproliferative DRP = more than just

microaneurysms but less than severe nonproliferative DRP; severe nonproliferative DRP = severe intraretinal hemorrhages and microaneurysms in each of 4 quadrants and/or definite venous beading in 2 or more quadrants and/or moderate intraretinal microvascular abnormalities in 1 or more quadrants and no signs of proliferative retinopathy; and proliferative DRP = neovascularization and/or vitreous/preretinal hemorrhage.²² Next, all participants were examined using OCT (Spectralis unit and Eye Explorer version 5.7.5.0 software; Heidelberg Engineering, Heidelberg, Germany). RNFL thickness of the right eye was measured within a 3.45-mm-diameter circular scan (12°, 100 automatic real-time tracking [ART]) centered on the optic nerve head by trained research assistants according to a standard operating procedure. RNFL thickness measurements were obtained along a 360° path divided into the following sectors: temporal superior (45°), nasal superior (45°), nasal (90°), nasal inferior (45°), temporal inferior (45°), temporal (90°), and mean RNFL thickness (360°) (Figs. 1–3). The RNFL thickness was automatically calculated using OCT software (Spectralis), and the unaltered scan data were automatically exported. The peripapillary OCT scans were reviewed and scored for the presence of measurement errors (poor centering of the circular scan on the optic nerve head or incomplete OCT scan) by an experienced grader in a masked fashion, based on a predefined protocol that included examples of OCT scans. Previous diagnosis of glaucoma was assessed by questionnaire.²⁰ Participants were requested to bring all medications they used or a list from their pharmacists to the research center. Use of glaucoma medication was assessed during a medication interview where generic name, dose, and frequency were registered by trained staff.²⁰ Individuals with measurement errors, poor quality imaging (signal-to-noise ratio <20 dB), unsatisfactory ART (i.e., <90), retinal disorders (epiretinal membrane, diabetic retinopathy, or previous laser treatment), self-reported glaucoma, and glaucoma medication were excluded.

Statistical Analysis

Statistical analysis was performed using commercial software (SPSS Statistics 23, IBM, Armonk, NY, USA for Windows [Microsoft, Redmond, WA, USA]). Differences between group characteristics were tested using one-way analysis of variance (ANOVA) for continuous variables and χ^2 tests for categorical variables. Multivariate linear regression was used to analyze the association between glucose metabolism status (prediabetes and DM2 w/o DRP, the determinant) and RNFL thickness of the right eye (the outcome). We combined the categories IFG and IGT into prediabetes because analyses did not show differences between these groups (data not shown). First, a crude analysis was performed. Next, associations were adjusted for age and sex. Finally, we repeated multivariate linear regression after additional exclusion of individuals with an intraocular pressure above 21 mm Hg. Interactions between age, sex, and glucose metabolism status were tested. Results were expressed as regression coefficients (β) representing mean differences in RNFL thickness compared with individuals with NGM, with their 95% confidence intervals (CI). A P value less than 0.05 was considered statistically significant.

RESULTS

General Characteristics

Figure 4 shows the flow diagram of the study. From December 2011 to September 2013, 2164 participants from The Maastricht Study underwent RNFL measurement. Forty-one

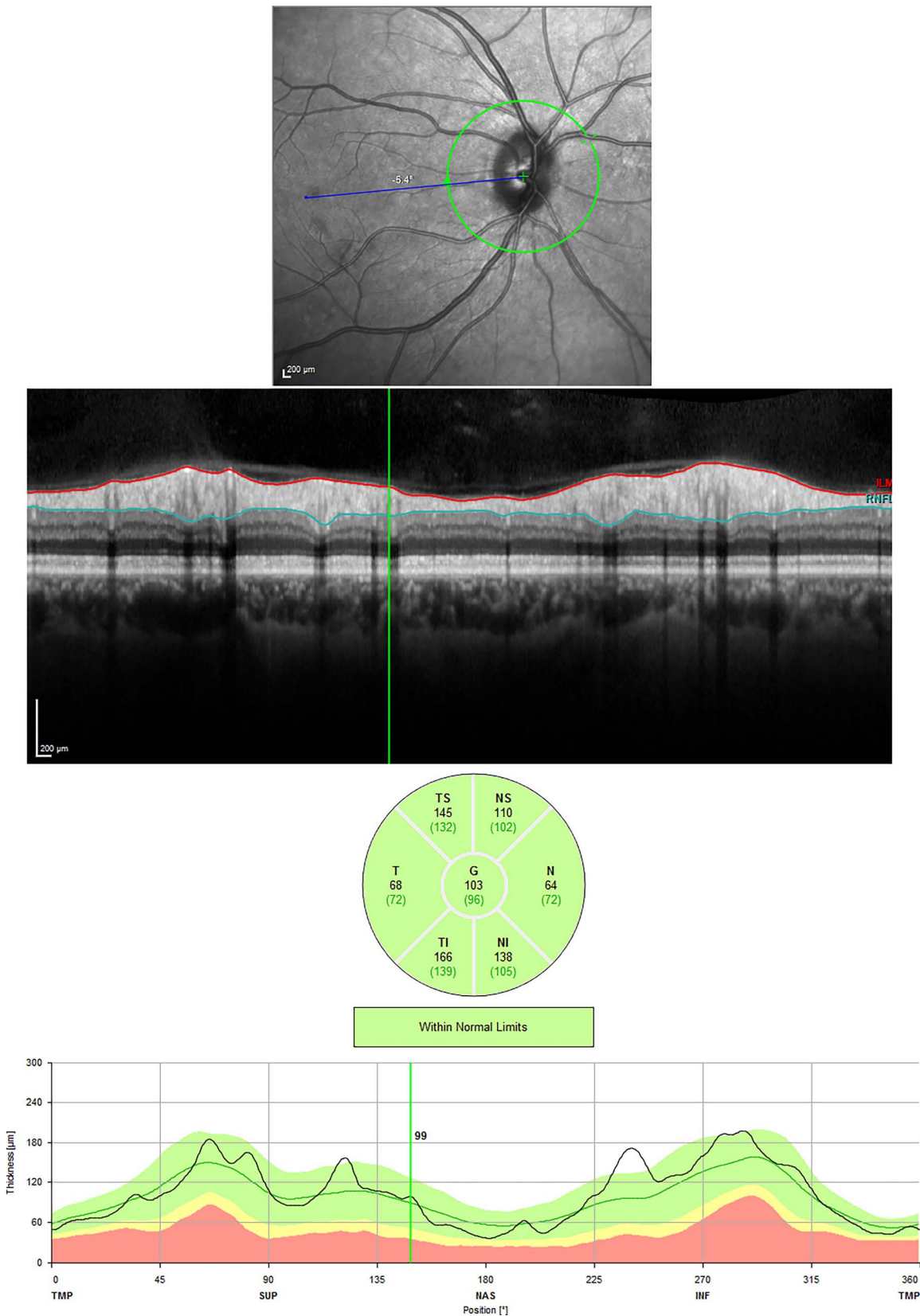


FIGURE 1. Optical coherence tomography around the optic nerve head in an individual with normal glucose metabolism, showing the distinct retinal nerve fiber layer sectors. TS, temporal superior; NS, nasal superior; N, nasal; NI, nasal inferior; TI, temporal inferior; T, temporal; G, mean RNFL. Peripapillary retinal nerve fiber layer thickness classification as defined by the Spectralis normative database of Heidelberg Engineering: *Green*, RNFL thickness within the 95th percentile of the normative database; *yellow*, RNFL thickness below the 5 percentile of the normative database; *red*, RNFL thickness within the lowest 1st percentile of the normative database.

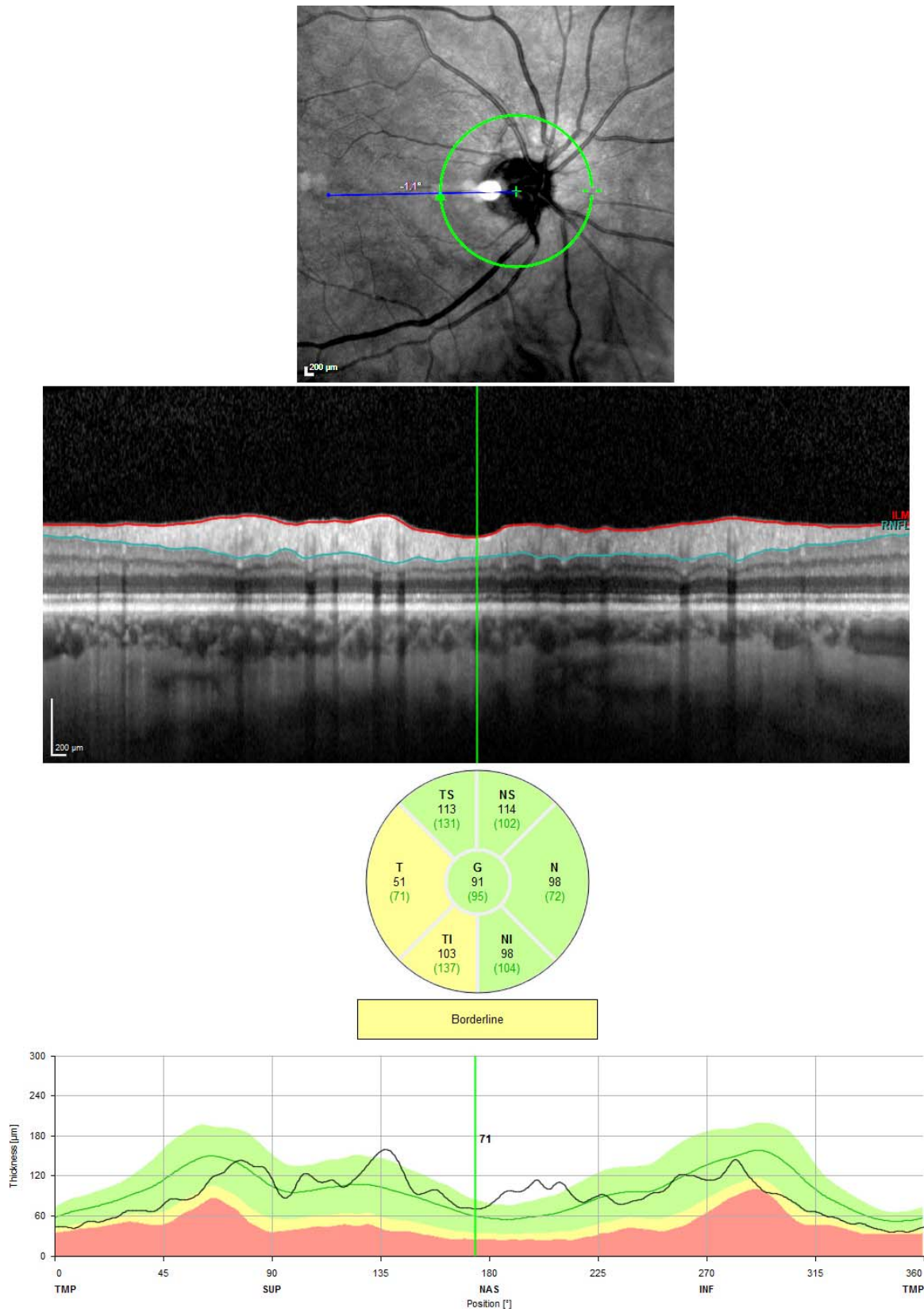


FIGURE 2. Optical coherence tomography in an individual with prediabetes, showing the thinning of the retinal nerve fiber layer in 2 sectors. Peripapillary retinal nerve fiber layer thickness classification as defined by the Spectralis normative database of Heidelberg Engineering: *Green*, RNFL thickness within the 95th percentile of the normative database; *yellow*, RNFL thickness below the 5th percentile of the normative database; *red*, RNFL thickness within the lowest 1st percentile of the normative database.

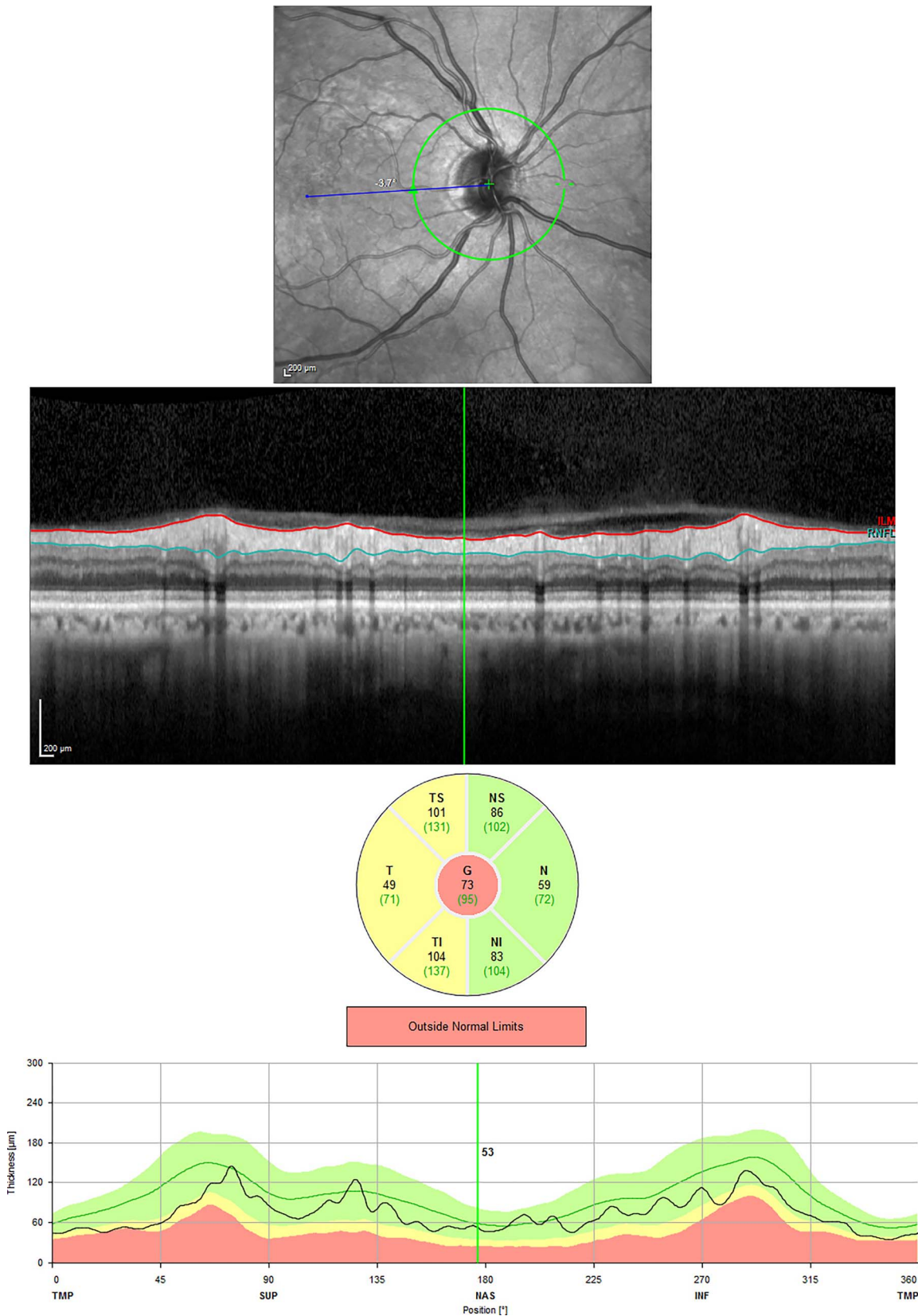


FIGURE 3. Optical coherence tomography in an individual with type 2 diabetes without diabetic retinopathy, showing the thinning of the retinal nerve fiber layer in 4 sectors. Peripapillary retinal nerve fiber layer thickness classification as defined by the Spectralis normative database of Heidelberg Engineering: *Green*, RNFL thickness within the 95th percentile of the normative database; *yellow*, RNFL thickness below the 5th percentile of the normative database; *red*, RNFL thickness within the lowest 1st percentile of the normative database.

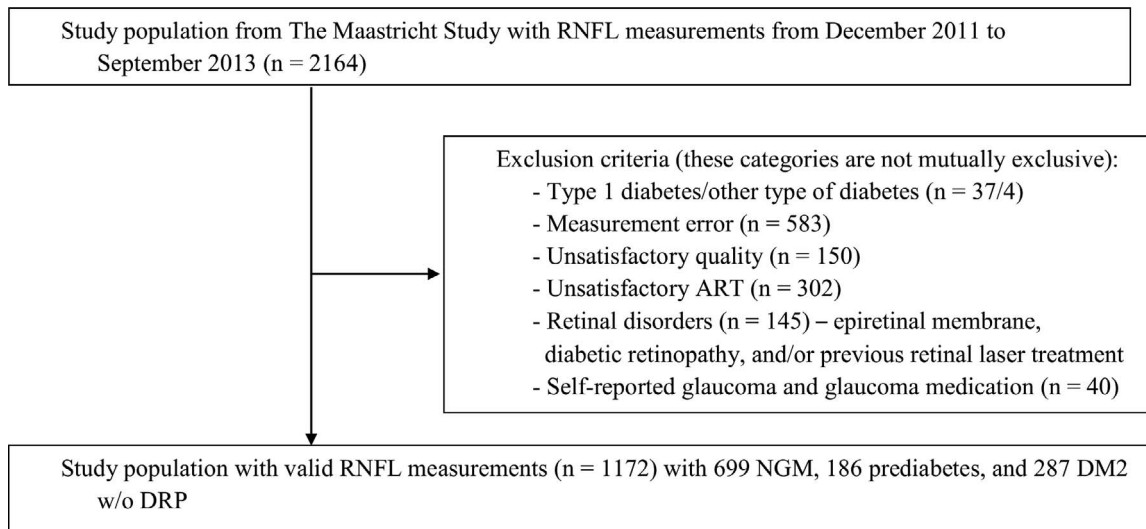


FIGURE 4. Flow diagram of the study. NGM, normal glucose metabolism; DM2 w/o DRP, type 2 diabetes without diabetic retinopathy; ART, automatic real-time tracking.

participants with DM1 or other types of diabetes were excluded from the present analysis. Participants with a measurement error ($n = 583$), an unsatisfactory quality of measurements ($n = 150$), and/or unsatisfactory ART ($n = 302$) were also excluded. In addition, individuals with retinal disorders ($n = 145$) were excluded, including those with epiretinal membrane ($n = 101$), DRP ($n = 40$), and/or previous retinal laser treatment ($n = 18$). Individuals with self-reported glaucoma and those taking glaucoma medication ($n = 40$) were also excluded. These categories are not mutually exclusive and resulted in a total exclusion of 992 participants. Thus, 1172 participants were available for crude analyses, and associations were adjusted for age and sex. Participants who were excluded due to missing values were more likely to be older (60.4 ± 8.3 vs. 58.6 ± 8.1 years of age, respectively, $P < 0.001$) and to be male (53.5% vs. 47.4% men, respectively, $P = 0.001$).

Of the 1172 participants, 699 participants had NGM (59.6%), 186 participants (15.9%) had prediabetes, and 287 participants (24.5%) had DM2 w/o DRP. There were statistically significant differences in age (60.2 ± 7.2 vs. 56.9 ± 8.0 years of age, respectively, $P < 0.001$) and sex (55.4 vs. 38.6% men, respectively, $P < 0.001$) between individuals with prediabetes and individuals with NGM. Age (61.9 ± 7.8 vs. 56.9 ± 8.0 years of age, respectively, $P < 0.001$) and sex (63.8% vs. 38.6% men, respectively, $P < 0.001$) were also significantly different

between individuals with DM2 w/o DRP and individuals with NGM.

Retinal Nerve Fiber Layer Thickness

Table 1 shows mean peripapillary RNFL thickness for each of the sectors, stratified by glucose metabolism status. In individuals with NGM, the mean peripapillary RNFL thickness was $95.8 \mu\text{m}$ (95% CI, 94.9–96.6); temporal superior thickness was $132.4 \mu\text{m}$ (95% CI, 130.8–133.9); temporal thickness was $74.3 \mu\text{m}$ (95% CI, 73.3–75.3); temporal inferior thickness was $143.3 \mu\text{m}$ (95% CI, 141.9–144.7); nasal superior thickness was $95.5 \mu\text{m}$ (95% CI, 93.7–97.4); nasal thickness was $72.0 \mu\text{m}$ (95% CI, 70.8–73.3); and nasal inferior thickness was $102.4 \mu\text{m}$ (95% CI, 100.7–104.1). In individuals with prediabetes, the temporal RNFL thickness value was significantly lower than that in individuals with NGM. In individuals with DM2 w/o DRP, the temporal superior, the temporal, the temporal inferior, and the mean RNFL thickness values were significantly lower than those in individuals with NGM.

Figure 5 shows the crude mean RNFL thicknesses and RNFL thicknesses in the temporal area for the different groups of glucose metabolism. Approximately half of the thinning observed in DM2 w/o DRP was already found in prediabetes. Thinning of RNFL thickness was more pronounced with worsening of glucose metabolism status, with a significant P value for linear trend for the mean, temporal

TABLE 1. Crude RNFL Thickness Stratified by Glucose Metabolism Status

RNFL Thickness Per Sector	Mean (95% CI), NGM ($N = 699$)	Mean (95% CI), Prediabetes ($N = 186$)	Mean (95% CI), DM2 w/o DRP ($N = 287$)	P for Trend
Mean RNFL thickness, μm	95.8 (94.9–96.6)	94.5 (93.0–96.1)	93.4 (92.1–94.6)*	<0.01
Temporal superior RNFL thickness, μm	132.4 (130.8–133.9)	130.8 (128.0–133.5)	128.7 (126.4–131.1)*	0.01
Temporal RNFL thickness, μm	74.3 (73.3–75.3)	71.4 (69.6–73.1)*	70.6 (69.0–72.2)*	<0.001
Temporal inferior RNFL thickness, μm	143.3 (141.9–144.7)	140.9 (138.0–143.7)	137.7 (135.3–140.1)*	<0.001
Nasal superior RNFL thickness, μm	95.5 (93.7–97.4)	96.5 (93.3–99.6)	94.0 (91.7–96.2)	0.41
Nasal RNFL thickness, μm	72.0 (70.8–73.3)	71.5 (69.2–73.7)	70.8 (69.2–72.4)	0.27
Nasal inferior RNFL thickness, μm	102.4 (100.7–104.1)	102.4 (99.1–105.7)	103.7 (101.0–106.4)	0.46

CI, confidence interval; DM2 w/o DRP, type 2 diabetes without diabetic retinopathy; NGM, normal glucose metabolism; RNFL, retinal nerve fiber layer.

* Boldface indicates $P < 0.05$ compared with NGM.

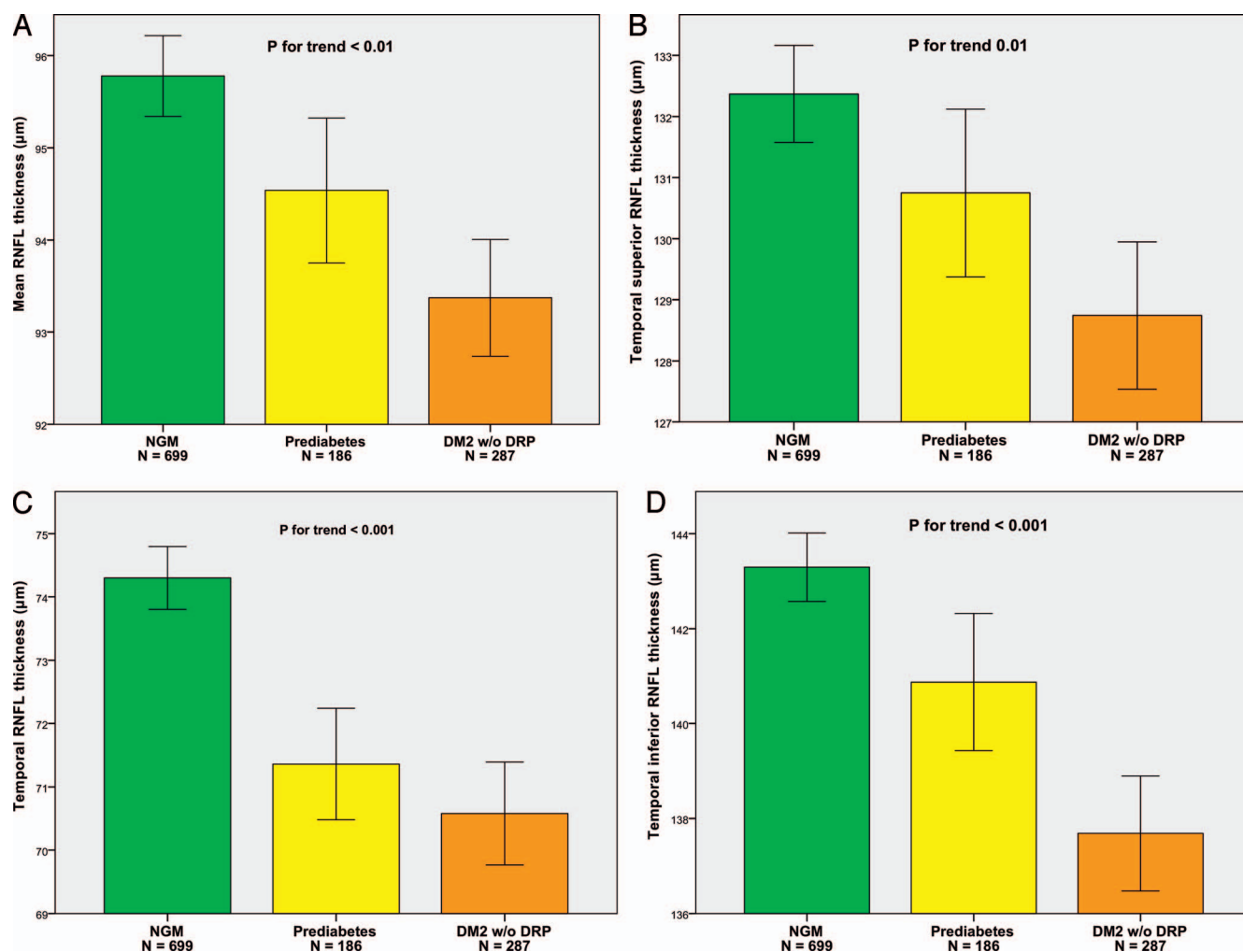


FIGURE 5. Crude mean RNFL thickness (A), temporal superior RNFL thickness (B), temporal RNFL thickness (C), and temporal inferior RNFL thickness (D) for individuals with NGM, prediabetes, and DM2 w/o DRP (mean \pm SEM). DM2 w/o DRP, type 2 diabetes without diabetic retinopathy; NGM, normal glucose metabolism; RNFL, retinal nerve fiber layer.

superior, temporal, and temporal inferior RNFL thicknesses ($P \leq 0.01$).

Table 2 shows crude analyses and associations between glucose metabolism status and the mean, temporal superior, temporal, temporal inferior, nasal superior, nasal, and nasal inferior RNFL thicknesses adjusted for age and sex. After adjustment for age and sex, the temporal RNFL ($\beta = -2.28 \mu\text{m}$ [95% CI, -4.44 to -0.13], $P = 0.04$) was significantly lower in individuals with prediabetes than in individuals with NGM. In individuals with DM2 w/o DRP, the temporal inferior ($\beta = -3.66 \mu\text{m}$ [95% CI, -6.46 to -0.85], $P = 0.01$), the temporal superior ($\beta = -2.99 \mu\text{m}$ [95% CI, -5.95 to -0.02], $P = 0.05$), the temporal ($\beta = -2.73 \mu\text{m}$ [95% CI, -4.62 to -0.84], $P < 0.01$), and the mean RNFL ($\beta = -1.88 \mu\text{m}$ [95% CI, -3.51 to -0.26], $P = 0.02$) were significantly thinner than those in individuals with NGM. Thinning of RNFL thickness was more pronounced with worsening of glucose metabolism status, with a significant P value for linear trend for the mean, temporal superior, temporal, and temporal inferior RNFL thicknesses ($P < 0.05$). After we also excluded individuals with an intraocular pressure above 21 mm Hg, the temporal RNFL was still significantly decreased in individuals with prediabetes and individuals with DM2 compared with individuals with NGM, after adjustment for age and sex (Table 3).

There were no interactions among age, sex, and glucose metabolism status ($P > 0.05$). These interaction terms were therefore left out of the model.

DISCUSSION

In this large cross-sectional population-based cohort study, we showed for the first time that peripapillary RNFL thinning occurs not only in individuals with DM2 w/o diabetic retinopathy but also in individuals with prediabetes, especially involving the temporal sector. A major part of the loss in RNFL thickness in DM2 w/o DRP was already found in prediabetes. These findings suggest that neurodegenerative changes of the retinal nerve fibers already occur at an early stage, even before DM2 is diagnosed.

This is the first study to perform an oral glucose tolerance test to assess RNFL thickness in prediabetes. To our knowledge, this is the largest study assessing neurodegenerative changes around the optic nerve head and the first study to analyze RNFL sectors instead of RNFL quadrants to more precisely define location-dependent changes. In contrast to previous studies, we also adjusted our results for age and sex, as potential confounders. Previous studies have shown that aging is correlated with neuroretinal loss.^{23–28} In our study,

TABLE 2. Comparison of RNFL Thickness in Prediabetes and DM2 Without Diabetic Retinopathy Versus Normal Glucose Metabolism*

Sector	Crude Analysis						Adjustment for Age and Sex					
	Prediabetes			DM2 w/o DRP			Trend			Prediabetes		
	β (95% CI)	P		β (95% CI)	P		P			β (95% CI)	P	
G	-1.24 (-3.06 to 0.58)	0.18		-2.41† (-3.95 to -0.86)†	<0.01		<0.01			-0.89 (-2.74 to 0.95)	0.34	
TS	-1.62 (-4.94 to 1.70)	0.34		-3.63 (-6.45 to -0.81)†	0.01		0.01			-1.20 (-4.57 to 2.18)	0.49	
T	-2.94 (-5.07 to -0.81)†	<0.01		-3.72 (-5.53 to -1.91)†	<0.001		<0.001			-2.28 (-4.44 to -0.13)†	0.04	
TI	-2.42 (-5.58 to 0.74)	0.13		-5.61 (-8.29 to -2.92)†	<0.001		<0.001			-1.13 (-4.32 to 2.06)	0.49	
NS	0.93 (-2.80 to 4.65)	0.63		-1.59 (-4.75 to 1.58)	0.33		0.41			0.48 (-3.31 to 4.27)	0.80	
N	-0.59 (-3.11 to 1.94)	0.65		-1.20 (-3.35 to 0.95)	0.27		0.27			-0.64 (-3.21 to 1.93)	0.62	
NI	0.00 (-3.70 to 3.69)	0.99		1.25 (-1.89 to 4.39)	0.43		0.46			0.30 (-3.44 to 4.04)	0.88	

β , regression coefficient; CI, confidence interval; DM2 w/o DRP, type 2 diabetes without diabetic retinopathy; G, mean RNFL thickness (μm); N, nasal RNFL thickness (μm); NI, nasal inferior RNFL thickness (μm); NS, nasal superior RNFL thickness (μm); RNFL, retinal nerve fiber layer; T, temporal RNFL thickness (μm); TI, temporal inferior RNFL thickness (μm); TS, temporal superior RNFL thickness (μm).

* Data show mean differences between RNFL thickness in prediabetes and in DM2 without diabetic retinopathy compared with normal glucose metabolism, adjusted for age and sex.

† Boldface values indicate $P < 0.05$.

TABLE 3. Comparison of RNFL Thickness in Prediabetes and DM2 Without Diabetic Retinopathy Versus Normal Glucose Metabolism, Excluding Individuals With Intraocular Pressure >21 mm Hg

Sector	Crude Analysis						Adjustment for Age and Sex					
	Prediabetes			DM2 w/o DRP			Trend			Prediabetes		
	β (95% CI)	P		β (95% CI)	P		P			β (95% CI)	P	
G	-0.97 (-2.90 to 0.96)	0.32		-2.00 (-3.67 to -0.33)†	0.02		0.02			-0.77 (-2.73 to 1.19)	0.44	
TS	-1.08 (-4.62 to 2.46)	0.55		-3.11 (-6.18 to -0.04)†	0.05		0.05			-0.84 (-4.43 to 2.76)	0.65	
T	-3.24* (-5.50 to -1.00)	<0.01		-3.61 (-5.56 to -1.65)†	<0.001		<0.001			-2.69 (-4.98 to -0.41)†	0.02	
TI	-2.12 (-5.49 to 1.25)	0.22		-5.09 (-8.01 to -2.17)†	0.001		0.001			-0.99 (-4.39 to 2.41)	0.57	
NS	1.90 (-2.04 to 5.84)	0.35		-1.23 (-4.65 to 2.18)	0.48		0.64			1.24 (-2.76 to 5.23)	0.54	
N	-0.41 (-3.09 to 2.28)	0.77		-0.54 (-2.87 to 1.79)	0.65		0.63			-0.57 (-3.30 to 2.16)	0.68	
NI	0.71 (-3.17 to 4.59)	0.72		1.56 (-1.81 to 4.92)	0.36		0.36			0.84 (-3.09 to 4.76)	0.68	

β , regression coefficient; CI, confidence interval; DM2 w/o DRP, type 2 diabetes without diabetic retinopathy; G, mean RNFL thickness (μm); N, nasal RNFL thickness (μm); NI, nasal inferior RNFL thickness (μm); NS, nasal superior RNFL thickness (μm); RNFL, retinal nerve fiber layer; T, temporal RNFL thickness (μm); TI, temporal inferior RNFL thickness (μm); TS, temporal superior RNFL thickness (μm).

* Data show mean differences between RNFL thickness in prediabetes and in DM2 without diabetic retinopathy compared with normal glucose metabolism, after exclusion of individuals with an intraocular pressure >21 mm Hg. Data were adjusted for age and sex ($N = 1042$ with 634 NGM, 166 prediabetes, and 242 DM2 w/o DRP).

† Boldface values indicate $P < 0.05$.

individuals with prediabetes were more likely to be older than individuals with NGM and therefore more prone to neuroretinal thinning, which stresses the importance of adjustment for age in the analysis. However, after adjustment for age, individuals with prediabetes still showed significant neuroretinal thinning.

We found that, in individuals with prediabetes, RNFL thinning occurred mainly in the temporal sector. RNFL thinning of the temporal sector indicates predominant damage of the papillomacular bundle. Injury to this bundle may cause central visual field defects.²⁹ The papillomacular bundle consists of parvocellular axons emanating from the ganglion cells of the macula conveying the visual information from the macula predominantly to the temporal side of the optic nerve head and through the lateral geniculate nucleus on to the visual cortex.³⁰ These axons have a fast-firing response in contrast with magnocellular axons, which have a lower firing rate and are equally distributed throughout the retina.³¹ In several neurological diseases it was observed that the thinner axons are more susceptible to injury.^{31,32} Recent studies in individuals with DM1 and DM2 have also shown significant thinning of the RNFL and the ganglion cell-inner plexiform layer of the macula, connected with the optic nerve head through the papillomacular bundle.^{33,34} Significant thinning of the macula with worsening of glucose metabolism status was also found in the same study population of The Maastricht Study (De Clerck EE, et al. *IOVS* 2016;ARVO E-Abstract 1601). Predominant damage of the papillomacular bundle also typically occurs in optic neuritis, other optic neuropathies, multiple sclerosis and other neurologic disorders.^{35–38} This could also be the case in (pre)diabetes, where the temporal sector could be more sensitive to early metabolic changes and related neurodegeneration. This vulnerability of the RNFL could also explain the increased risk of nonarteritic anterior ischemic optic neuropathy^{39–43} and glaucoma^{44–46} in individuals with diabetes. Individuals with glaucoma were excluded. Vascular occlusion and active uveitis were not present in the included participants. Individuals with Alzheimer's disease were not admitted to The Maastricht Study, as they were not able to sign the informed consent.

In individuals with DM2 w/o DRP, we demonstrated that RNFL thinning occurs not only at the temporal sector but further extends to the temporal superior and temporal inferior sectors. RNFL thinning of these sectors is consistent with previous studies using OCT in individuals with DM2 w/o DRP.^{5–7} Interestingly, a major part of the thinning visible in DM2 w/o DRP was already present in individuals with prediabetes. In addition, our results are in line with the RNFL thinning reported in a recent meta-analysis performed by our group in individuals with DM2 w/o DRP, in which there was a decrease of $-0.50\ \mu\text{m}$ (95% CI, -8.00 to 7.00) in the temporal quadrant, $-4.99\ \mu\text{m}$ (95% CI, -11.16 to 1.18) in the superior quadrant, and $-3.99\ \mu\text{m}$ (95% CI, -11.59 to 3.61) in the inferior quadrant.⁸

Molecular changes in diabetes affect neuronal cells, glial cells, and vascular cells, leading to a disruption of the neurovascular unit.⁴⁷ Because RNFL thinning is already present in individuals with DM2 w/o DRP and, particularly in individuals with prediabetes, our findings support the hypothesis that retinal neurodegenerative changes occur before signs of microvascular changes assessed with conventional techniques.⁴⁸ A recent longitudinal study demonstrating significant, progressive inner retinal thinning confirmed this hypothesis in individuals with type 1 diabetes with no or minimal DRP.⁴⁹ Also, in the sub-basal nerve plexus of the cornea, neurodegenerative changes were present in DM2 w/o DRP.^{50–53} Peripapillary RNFL thinning could be explained by the loss of axons of retinal ganglion cells. Several causes of diabetes-associated axonal loss around the optic nerve head have been suggested: oxidative stress, chronic hyperglycemia, inflammation, and

accumulation of advanced glycation end products.^{54–56} Thereafter, neuronal apoptosis and glial dysfunction could lead to a breakdown of the blood-retina barrier, vasoregression, and an impaired hemodynamic response resulting in early microvascular changes.⁵⁷ Nevertheless, the causal relationship between neuronal apoptosis and microvascular changes has not been confirmed yet.⁴⁹

SD-OCT gives two-dimensional cross-sectional scans with a resolution of 3 to 5 μm , enabling accurate measurement of the RNFL thickness in an objective, noninvasive, and repeatable way. Although RNFL thinning in individuals with prediabetes and DM2 w/o DRP was significant, this thinning, respectively, represents 4.0% and 5.0% decreases in temporal RNFL thickness, which is much smaller than changes in RNFL thinning observed in glaucoma patients.⁵⁸ This mild RNFL thinning has a substantial standard error and only 9.1% of the individuals with prediabetes and 10.8% of the individuals with DM2 w/o DRP had a temporal RNFL thickness outside the two standard deviations range of the normal mean. Therefore, the diagnostic value of peripapillary SD-OCT to identify neurodegenerative changes on an individual level seems to be limited. However, peripapillary SD-OCT can still be used to study differences between groups and to study the pathogenesis of diabetic retinopathy at an early stage. Long-term follow-up is needed to assess whether these changes are clinically relevant and whether they are a good outcome measurement for trials of neuroprotective interventions to prevent or arrest retinal neurodegeneration, as well as development and progression of the early stages of diabetic retinopathy.⁵⁹ Moreover, the presence of neurodegenerative changes around the optic nerve head in prediabetes could open new perspectives for future research in the pathogenesis of diabetic retinopathy, including neuronal and microvascular changes.

Both dysfunction of the neuronal microvasculature and intraneuronal cellular changes play a role in the development of diabetic neuropathy. Retinal neurodegeneration in diabetes could therefore, on one hand, be considered part of diabetic retinopathy but, on the other hand, as an *in vivo* biomarker for diabetic neuropathy.⁶⁰ Diagnosis of diabetes has been defined based on the fasting glucose level above which microaneurysms appear (7.0 mmol/L). Signs of neuronal dysfunction are already found in individuals with prediabetes.^{50,61–63} Once diabetes is diagnosed, this process is probably accelerated or intensified by hyperglycemia. Based on our findings, the nervous system already seems to be damaged at glucose levels that are lower than the cutoff value above which microaneurysms are visible, with a linear trend of RNFL thinning with worsening of glucose metabolism status.

Due to the cross-sectional design and because longitudinal data are not yet available, we were not able to address causal relationships. However, by comparing individuals with NGM, prediabetes, and DM2 w/o DRP, we mimicked the pathological pathway of glucose metabolism deterioration. Peripapillary OCT scans were performed by trained research assistants who went through a learning curve. Only high-quality images of the right eye were included based on a predefined protocol. In addition, after exclusion of missing values, it is possible that our sample was not truly population-based. The missing participants, however, seemed to have a higher risk of cardiovascular disease, suggesting that, if anything, RNFL thinning in the total study population of the Maastricht Study is expected to be greater than that reported here.

Future longitudinal studies should focus on possible causes and risk factors for the occurrence of peripapillary RNFL thinning in prediabetes and DM2 w/o DRP and its predictive value. Moreover, future studies should also quantify the thickness of the other peripapillary layers, because these layers can be affected differently by diabetes.⁶⁴ Because

examination of the RNFL is considered a noninvasive approach to the diagnosis of neuronal disorders,⁶⁵ future studies also need to address the relationship between RNFL thinning and cognitive decline, white matter loss, diabetic polyneuropathy,⁶⁰ and diabetic autonomic neuropathy.

In conclusion, temporal RNFL thinning is already present in individuals with prediabetes. More widespread RNFL thinning occurs in individuals with DM2 w/o DRP, that is, before vascular changes are detected. This suggests preferential retinal nerve fiber layer loss in areas related to the papillo-macular bundle.

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