(Pre)Diabetes, Greater Glycemia, and Greater Daily Glucose Variability are Associated with Lower Retinal Nerve Fiber Layer Thickness, an Index of Neurodegeneration and Precursor of Diabetic Retinopathy– The Maastricht Study

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Abstract

Objective

Diabetic retinopathy in type 2 diabetes is preceded by retinal nerve fiber layer (RNFL) thinning, an index of neurodegeneration. We investigated whether glucose metabolism status (GMS), measures of glycemia, and daily glucose variability (GV) are associated with RNFL thickness over the entire range of glucose tolerance.

Research design and methods

We used cross-sectional data from The Maastricht Study (up to 5,455 participants, 48.9% men, mean age 59.5 years and 22.7% with type 2 diabetes) to investigate the associations of GMS, measures of glycemia (fasting plasma glucose [FPG], 2-hour post-load glucose [2-h PG], HbA1c, advanced glycation endproducts [AGEs] assessed as skin autofluorescence [SAF]) and indices of daily GV (incremental glucose peak [IGP] and continuous glucose monitoring [CGM]-assessed standard deviation [SD]) with mean RNFL thickness. We used linear regression analyses and, for GMS, P for trend analyses. We adjusted associations for demographic, cardiovascular risk and lifestyle factors, and, only for measures of GV, for indices of mean glycemia.

Results

After full adjustment, type 2 diabetes and prediabetes (versus normal glucose metabolism) were associated with lower RNFL thickness (standardized beta [95%CI], respectively -0.16[-0.25;-0.08]; -0.05[-0.13;0.03]; P_{trend}=0.001). Greater FPG, 2-h PG, HbA1c, SAF, IGP and CGM-assessed SD were also associated with lower RNFL thickness (per SD, respectively -0.05 [-0.08; -0.01]; -0.06 [-0.09; -0.02]; -0.05 [-0.08; -0.02]; -0.04 [-0.07; -0.01]; -0.06 [-0.12; -0.01]; and -0.07 [-0.21; 0.07]).

Conclusion

In this population-based study, a more adverse GMS and, over the entire range of glucose tolerance, greater glycemia and daily GV were associated with lower RNFL thickness. Hence, early identification of individuals with hyperglycemia, early glucose-lowering treatment, and early monitoring of daily GV may contribute to the prevention of RNFL thinning, an index of neurodegeneration and precursor of diabetic retinopathy.

Introduction

Diabetic retinopathy, a hallmark microvascular complications of type 2 diabetes, is preceded by subtle neurodegenerative changes. Such changes include retinal nerve fiber layer (RNFL) thinning, which can be non-invasively assessed.[1-3] RNFL thinning reflects a gradual loss of retinal ganglion cell axons, which transmit visual information from the retina to the brain.[1, 2] Mechanistically, elevated glucose concentrations are thought to be toxic for retinal ganglion cells as well as for retinal endothelial and glia

cells, which contribute to local metabolic regulation.[4, 5] Thus, hyperglycemia can lead to retinal ganglion cell apoptosis and loss of retinal ganglion cell axons both directly and through impairment of endothelial and glia cell function.[6]

As postulated in the “ticking clock hypothesis”, hyperglycemia-mediated damage is thought to be a continuous (i.e., linear) process that already starts before the onset of type 2 diabetes.[7, 8] Indeed, our group observed linear associations of more adverse glucose metabolism status (GMS) and higher glycemia (estimated by various measures) with lower heart rate variability,[9] more structural brain abnormalities,[10, 11] and worse peripheral nerve function,[12] all of which are measures of neurodegeneration.

The current literature on the associations of hyperglycemia with RNFL thickness has some important limitations. First, in previous population-based studies, several important confounders were not included, such as age,[13-15] sex,[14, 15] socioeconomic status,[13-23] cardiovascular risk factors,[13-15, 19, 21-24] and lifestyle factors (e.g., alcohol use,[13, 16-19, 21] diet,[13-24] and physical activity).[13-19, 21-24] Second, no population-based studies have yet investigated whether advanced glycation endproducts (AGEs) or daily glucose variability are associated with RNFL thickness. It is important to establish to what extent hyperglycemia (including AGEs) and daily glucose variability contribute to RNFL thinning over the entire range of glucose tolerance because early diagnosis and treatment of hyperglycemia as well as novel strategies to monitor glycemic exposure may contribute to the early prevention of hyperglycemia-mediated RNFL thinning.[5, 25-27]

In view of above, we investigated, using a large, well-characterized population-based cohort study, whether more adverse GMS, greater glycemia, and greater daily glucose variability are associated with lower RNFL thickness.

Methods

Study population and design

We used data from The Maastricht Study, a prospectively designed, population-based observational cohort study. The rationale and methodology have been described previously.[28] In brief, the study focuses on the etiology, pathophysiology, complications and comorbidities of type 2 diabetes mellitus and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency.[28] The present report includes cross-sectional data of 8,005 participants who completed the baseline survey between November 2010 and September 2018. The examinations of each participant were performed within a time window of

three months. From 19 September 2016 until 13 September 2018, participants were invited to also undergo CGM.[26] During this period, a selected group of recently included participants was invited to return for CGM ('catch-up visit'). For these participants only there was a median time interval ('visit interval') of 2.1 years between CGM and all other measurements (more details are provided in the Supplemental Material). 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 (Permit 131088-105234-PG). All participants gave written informed consent.[28]

Glucose metabolism status

After an overnight fast, participants underwent a standardized seven-point oral glucose tolerance test (OGTT) as part of which venous samples were collected at 15, 30, 45, 60, 90, and 120 minutes post ingestion of a 75g glucose drink. All participants underwent an OGTT except for the participants who used insulin or had a fasting plasma glucose (FPG) concentration above 11.0 mmol/L. Based on FPG and 2-hour post-load glucose, GMS was determined as normal glucose metabolism (NGM), prediabetes (impaired fasting glucose, impaired glucose tolerance, or both), type 2 diabetes, or other types of diabetes (including type 1 diabetes) in accordance with the World Health Organization 2006 criteria.[29]

Measures of glycemia

FPG (mmol/L) and hemoglobin A1c (HbA1c; mmol/mol; %) were determined in venous plasma samples collected after an overnight fast. Two-hour post-load glucose (mmol/L) was determined in venous plasma collected at 120 minutes post glucose drink ingestion. AGEs were assessed with the AGE Reader (DiagnOptics Technologies BV, Groningen, the Netherlands). In brief, the AGE Reader is a desktop device that uses the characteristic fluorescent properties of certain AGEs to quantify their accumulation in the skin as skin autofluorescence (SAF; arbitrary units [AU]).[30] The AGE Reader illuminates a skin surface of 4 cm^2, shielded from other light, and uses the ratio of the reflection of fluorescent light (wavelength 420 to 600 nm) to non-fluorescent light (300–420 nm) to calculate SAF (more details are provided in the Supplemental Materials).

Indices of daily glucose variability

The incremental glucose peak (IGP; mmol/L), a recently validated OGTT-based index of daily glucose variability, was calculated by subtracting FPG from the maximum glucose peak value measured during a complete seven-point OGTT.[31] At the time of analysis, data on IGP were available in a subset of participants (n=2,407). We used CGM (iPro2 and Enlite Glucose Sensor; Medtronic, Tolochenaz, Switzerland) to assess daily glucose variability during a 1-week period, which was expressed as standard deviation (mmol/L) of mean sensor glucose (mmol/L).[26] CGM-assessed data were available in a subset of participants (n=622). More details on the assessment of glycemic indices with OGTT and CGM are provided in the Supplemental Methods.

RNFL thickness

We assessed peripapillary RNFL thickness (μm) in both eyes using optical coherence tomography (OCT) (Spectralis unit and Eye Explorer version 5.7.5.0 software; Heidelberg Engineering, Heidelberg, Germany; 3.45-mm-diameter circle scan, manually centered on optic nerve head, 12°, 768 voxels, 100 automatic real-time tracking). Intra- and interindividual reliability, expressed as intraclass correlation coefficients, are 0.97 and 0.96 respectively.[32] At least 15 minutes before the examination, pupils were dilated with topical 0.5% tropicamide and 2.5% phenylephrine. Experienced graders masked to clinical information on the participants reviewed the OCT scans and graded their quality. OCT images were excluded if one of the following criteria was present: scan error (i.e., incomplete scan, poor centering of the circular scan on the optic nerve head, RNFL layer incorrectly defined, or technical problem with the OCT device) or poor imaging quality (signal-to-noise ratio<15 dB).[23] If data from both eyes were available (n=2,796 participants) we averaged RNFL thickness in order to reduce measurement error. If data from only one eye were available (n=2,755 participants), we used the RNFL thickness of that eye in the analyses. More details, including on quality criteria, are shown in the Supplemental Methods.

Covariates

As described previously,[28] we assessed educational level (low, intermediate, high), socio-economic status (income level and occupational status),[33] smoking status (never, former, current), alcohol use (none, low, high), history of cardiovascular disease, and duration of diabetes by questionnaire; assessed dietary habits (“dietary intake”) with the Dutch Healthy Diet index sum score, a measure of adherence to the Dutch dietary guidelines 2015,[34] based on a validated food frequency questionnaire;[35] assessed lipid-modifying, antihypertensive, intraocular pressure-lowering, and glucose-lowering medication use as part of a medication interview; assessed weight, height, and waist circumference during a physical examination; calculated body mass index (BMI) based on body weight and height; measured office and 24-hour ambulatory blood pressure (BP); measured total daily physical activity (hours/day) with an accelerometer;[36] measured lipid profile and plasma biomarkers of low-grade inflammation[37] (i.e., high-sensitive C-reactive protein, serum amyloid A, interleukin-6, interleukin-8 and tumor necrosis factor alpha) in fasting venous blood samples; measured urinary albumin excretion in two 24-hour urine collections; calculated the estimated glomerular filtration rate (eGFR) based on serum creatinine only, since cystatin C was not presently available in all study participants; assessed presence of retinopathy in both eyes via fundus photography; and used an automated refractor and noncontact tonometer (Tonoref II; Nidek, Gamagordi, Japan) to assess spherical equivalent and intraocular pressure in both eyes. Glaucoma was defined as use of intraocular pressure-lowering medication, intraocular pressure higher than 21 mm Hg in any eye (91.3% of all participants had data on intraocular pressure available for at least 1 eye), or both. Spherical equivalent was defined as the mean spherical equivalent of both eyes (available for 91.1% of all participants) or as the spherical equivalent of the eye for which data were available.

Statistical Analysis

We used multivariable linear regression analyses to investigate the associations of GMS (entered as dummy variables of prediabetes, type 2 diabetes, or other types of diabetes versus NGM) and

standardized FPG, 2-hour post-load glucose, HbA1c, SAF, IGP, and CGM-assessed standard deviation (determinants) with standardized mean RNFL thickness (outcome).

We performed P for trend analyses to test for linear trend with more adverse GMS. To test for trend, we entered GMS into the model as an ordinal variable (i.e., GMS was coded as NGM=0, prediabetes=1, type 2 diabetes=2).In P for trend analyses we excluded participants with other types of diabetes because other types of diabetes (such as type 1 diabetes) do not constitute part of the spectrum of deterioration of GMS from NGM to prediabetes and type 2 diabetes. Then, we checked whether we could assume a linear trend by comparing the statistical variance explained by the statistical model in which GMS was entered as dummy variables to the statistical model in which GMS was entered as an ordinal variable. We used a likelihood ratio test to assess whether the amount of variance explained by both models differed statistically significantly. A P-value >0.05 indicates that both models are not different and, thus, that a linear trend can be assumed. For all analyses under study, the P-value for the likelihood ratio test was >0.05 (data not shown) and therefore a linear trend could be assumed.

Model 1 shows crude results. In model 2, we adjusted for age, sex, educational status (low, medium, high). We chose these variables because they are key potential confounders.[17] In model 3, we additionally adjusted for variables of which their status as confounder has been less firmly established (office systolic BP, use of antihypertensive medication [yes/no], waist circumference, total cholesterol / HDL cholesterol ratio, lipid-modifying medication [yes/no], smoking status [current, former, never], and alcohol consumption status [none, low, high]).[17] Then, and only for IGP and CGM-assessed standard deviation, we additionally adjusted for HbA1c or mean sensor glucose, respectively (model 3 + HbA1c; model 3 + mean sensor glucose), so that we could differentiate between daily glucose variability and average glycemia, both of which are strongly related.[38] Additionally, and only for CGM-assessed indices, we entered 'visit interval' in model 1. The associations were expressed as standardized regression coefficient (stβ) and corresponding 95% confidence interval (95%CI). Collinearity diagnostics (i.e., tolerance <0.10 and/or variance inflation factor >10) were used to detect excessive multicollinearity between covariates.

We tested for interaction to assess whether associations differed by GMS (i.e., between individuals with type 2 diabetes, individuals with prediabetes, and individuals with NGM) or by sex. For interaction analyses with GMS, we excluded participants with other types of diabetes from the interaction analyses because the number of these participants was small.

To assess the robustness of our findings we performed several sensitivity analyses. First, we repeated the analyses with additional adjustment for lifestyle factors (dietary intake, physical activity) or ocular variables (spherical equivalent, intraocular pressure).[39–41] Adjustment for these potential confounders was not included in the main analyses because data were missing for a relatively large number of participants (up to n=768 had missing data on one or more of these variables). Second, we additionally adjusted for kidney variables (eGFR and urinary albumin excretion), history of cardiovascular disease, plasma biomarkers of low-grade inflammation, retinopathy, and glaucoma. We adjusted for these

covariates in a separate model because they may be confounders but may also (in part) be mediators or descendants of the outcome.[42] Third, we performed analyses in which participants with retinopathy, glaucoma, or other types of diabetes were excluded. Fourth, we replaced IGP with other OGTT-based indices of daily glucose variability (i.e., maximum glucose peak and 1-hour post-load glucose). We did not include maximum glucose peak and 1-hour post-load glucose in the main analyses because they are known to correlate less strongly than IGP with CGM-assessed indices of daily glucose variability in GMS-stratified groups.[31] Fifth, we replaced CGM-assessed standard deviation with other CGM-assessed measures of daily glucose variability (more details are provided in the Supplemental Methods section). Sixth, we studied the association between mean sensor glucose and retinal nerve fiber layer thickness. Although the sample size of the CGM study population was relatively small (n=622), CGM-based glycemic mean sensor glucose may be less susceptible to measurement error than other measures of glycemia under study, as mean sensor glucose constitutes the average of a large number of glucose concentrations.[31, 43] Seventh, we replaced waist circumference with BMI; educational status with occupational status or income level; and office systolic BP with office diastolic BP, systolic or diastolic 24-hour ambulatory BP. Eighth, and only for SAF, we additionally adjusted the association between SAF and RNFL thickness for FPG, 2-hour post-load glucose, or HbA1c. We additionally adjusted for these measures to investigate whether the association between SAF and RNFL thickness was independent of short to middle long-term exposure to hyperglycemia. Ninth, and only for IGP and CGM-assessed standard deviation, we respectively replaced HbA1c with FPG or SAF, and mean sensor glucose with FPG, SAF, or HbA1c. Tenth, and only for CGM-assessed standard deviation, we repeated the analyses after exclusion of individuals with insufficient CGM recording days, with CGM recording data gaps, or with a visit interval. Eleventh, because of the strong correlation between CGM-assessed standard deviation and mean sensor glucose,[38] we repeated the main analysis using ridge regression, which is a valid method to counter potential instability caused by multicollinearity (additional information is provided in the Supplemental Methods).[44] Last, we studied the association between duration of diabetes and RNFL thickness. Data on duration of diabetes were only available for individuals with type 2 diabetes (n=982).

All analyses were performed with Statistical Package for Social Sciences version 25.0 (IBM SPSS, IBM Corp, Armonk, NY, USA). For all analyses, a P-value <0.05 was considered statistically significant.

Results

Selection and characteristics of the study population

Figure 1 gives an overview of the study population selection. Participants in whom OCT data were missing or of insufficient quality were excluded first (n=2,454). Next, individuals with missing data on confounders were excluded (n=96). The sample size of the final study populations depended on the availability of data on the main determinant (n=5,455 for GMS, n=982 to 5,454 for measures of glycemia, and n=622 to 2,407 for indices of daily glucose variability).

Table 1 and Supplemental Table 1 show general participant characteristics according to tertiles of RNFL thickness. Overall, participants with a thinner RNFL were more often men, were older, and had a more adverse cardiovascular risk profile. General characteristics of participants included in the study were comparable to those of participants with missing data (Supplemental Table S2).

Table 1
General study population characteristics according to tertiles of the retinal nerve fiber layer thickness in the study population with complete data on glucose metabolism status

	RNFL thickness			
	Total study population (n = 5,455)	Tertile 1 (high) (n =1,818)	Tertile 2 (middle) (n =1,819)	Tertile 3 (low) (n =1,818)
Characteristic				
Age (years)	59.5 ± 8.6	59.1 ± 8.7	59.3 ± 8.7	60.0 ± 8.5
Men	2,665 (48.9)	805 (48.9)	847 (46.6)	1,013 (55.7)
Educational level				
Low	1,914 (35.1)	669 (36.8)	651 (35.8)	594 (32.7)
Medium	1,519 (27.8)	525 (28.9)	505 (27.8)	489 (26.9)
High	2,022 (37.1)	624 (34.3)	663 (36.4)	735 (40.4)
Glucose metabolism status				
NGM	3,366 (61.7)	1,174 (64.6)	1,144 (62.9)	1,048 (57.6)
Prediabetes	820 (15.0)	266 (14.6)	280 (15.4)	274 (15.1)
Type 2 diabetes	1,239 (22.7)	370 (20.4)	383 (21.1)	486 (26.7)
Other type of diabetes	30 (0.5)	8 (0.4)	12 (0.7)	10 (0.6)
Measures of glycemia				
Fasting plasma glucose (mmol/L)*	5.9 ± 1.5	5.8 ± 1.4	5.8 ± 1.5	6.0 ± 1.7
2-hour post-load glucose (mmol/L)*	6.2 [4.9-8.6]	6.1 [4.9-8.2]	6.1 [4.9-8.5]	6.3 [5.1-9.2]
HbA1c (mmol/mol)*	39.2 ± 9.1	38.7 ± 8.5	39.1 ± 9.3	39.9 ± 9.5

Data are presented as mean ± standard deviation, median [interquartile range] or number (%).

* Data shown in the study population with complete data on fasting plasma glucose (n=5,454), 2-hour post-load glucose (n=5,180), HbA1c (n=5,449), skin autofluorescence (n=5,132), incremental glucose peak (n=2,407), and CGM-assessed standard deviation (n=622).

Abbreviations: CGM, continuous glucose monitoring; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; NGM, normal glucose metabolism; RNFL, retinal nerve fiber layer; AU, arbitrary units

	RNFL thickness			
HbA1c (%)*	5.7 ± 0.8	5.7 ± 0.8	5.7 ± 0.8	5.8 ± 0.9
Skin autofluorescence (AU)*	2.2 ± 0.5	2.1 ± 0.5	2.2 ± 0.5	2.2 ± 0.5
Indices of daily glucose variability				
Incremental glucose peak (mmol/L)*	4.1 [2.7-6.5]	3.9 [2.6-5.9]	4.1 [2.8-6.5]	4.4 [2.8-7.0]
CGM-assessed standard deviation (mmol/L)*	0.86 [0.68-1.21]	0.86 [0.67-1.18]	0.85 [0.67-1.17]	0.88 [0.70-1.29]
Use of glucose-modifying medication, yes vs. no	927 (17.0)	266 (14.6)	289 (15.9)	372 (20.5)
Waist circumference, men (cm)	100.4 ± 11.6	100.2 ± 11.7	99.9 ± 11.7	101.0 ± 11.5
Waist circumference, women (cm)	89.1 ± 12.5	89.2 ± 12.8	88.9 ± 12.1	89.2 ± 12.8
Total-to-HDL cholesterol ratio	3.4 [2.8-4.2]	3.3 [2.7-4.1]	3.3 [2.8-4.2]	3.4 [2.8-4.2]
Use of lipid-modifying medication, yes vs. no	1,687 (30.9)	535 (29.4)	541 (29.7)	611 (33.6)
Office systolic blood pressure (mmHg)	133.2 ± 17.7	132.0 ± 18.0	133.0 ± 17.6	134.5 ± 17.5
Office diastolic blood pressure (mmHg)	75.5 ± 9.8	75.0 ± 9.8	75.3 ± 9.7	76.4 ± 9.9
Use of antihypertensive medication, yes vs. no	1,983 (36.4)	601 (33.1)	637 (35.0)	745 (41.0)
Smoking status				
Never	2,101 (38.5)	717 (39.4)	684 (37.6)	700 (38.5)
Former	2,666 (48.9)	843 (44.4)	911 (50.1)	912 (50.2)
Current	688 (12.6)	258 (14.2)	224 (12.3)	206 (11.3)
Alcohol consumption				
None	995 (18.2)	344 (18.9)	356 (19.6)	295 (16.2)

Data are presented as mean ± standard deviation, median [interquartile range] or number (%).

* Data shown in the study population with complete data on fasting plasma glucose (n=5,454), 2-hour post-load glucose (n=5,180), HbA1c (n=5,449), skin autofluorescence (n=5,132), incremental glucose peak (n=2,407), and CGM-assessed standard deviation (n=622).

Abbreviations: CGM, continuous glucose monitoring; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; NGM, normal glucose metabolism; RNFL, retinal nerve fiber layer; AU, arbitrary units

	RNFL thickness			
Moderate	3,181 (58.3)	1,070 (58.9)	1,042 (57.3)	1,069 (58.8)
High	1,279 (23.4)	404 (22.2)	421 (23.1)	454 (25.0)
RNFL thickness (µm)	94.8 ± 10.8	106.0 ± 6.3	95.2 ± 2.5	83.2 ± 6.8
Data are presented as mean ± standard deviation, median [interquartile range] or number (%).				
* Data shown in the study population with complete data on fasting plasma glucose (n=5,454), 2-hour post-load glucose (n=5,180), HbA1c (n=5,449), skin autofluorescence (n=5,132), incremental glucose peak (n=2,407), and CGM-assessed standard deviation (n=622).				
Abbreviations: CGM, continuous glucose monitoring; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; NGM, normal glucose metabolism; RNFL, retinal nerve fiber layer; AU, arbitrary units				

Glucose metabolism status and RNFL thickness

After full adjustment (model 3), a more adverse GMS was associated with lower RNFL thickness (standardized beta [95%CI], type 2 diabetes versus NGM -0.16 [-0.25; -0.08]; prediabetes versus NGM -0.05 [-0.13; 0.03], P for trend =0.001; Table 2 and Figure 2).

Table 2
Associations of glucose metabolism status, measures of glycemia, and indices of daily glucose variability with RNFL thickness

		RNFL, per SD			
		Model 1	Model 2	Model 3	Model 3 + HbA1c/MSG
	Number of participants	stβ (95% CI)	stβ (95% CI)	stβ (95% CI)	stβ (95% CI)
Glucose metabolism status					
Prediabetes versus NGM	5,455	-0.07 (-0.15; 0.003)	-0.05 (-0.12; 0.03)	-0.05 (-0.13; 0.03)	NA
Type 2 diabetes versus NGM	5,455	**-0.19 (-0.25; -0.12)**	**-0.16 (-0.26; -0.09)**	**-0.16 (-0.25; -0.08)**	NA
Measures of glycemia					
Fasting plasma glucose, per SD	5,454	**-0.07 (-0.09; -0.04)**	**-0.05 (-0.08; -0.02)**	**-0.05 (-0.08; -0.01)**	NA
2-hour post-load glucose, per SD	5,180	**-0.07 (-0.09; -0.04)**	**-0.06 (-0.09; -0.03)**	**-0.06 (-0.09; -0.02)**	NA
HbA1c, per SD	5,449	**-0.06 (-0.09; -0.03)**	**-0.05 (-0.08; -0.02)**	**-0.05 (-0.08; -0.02)**	NA
Skin autofluorescence, per SD	5,132	**-0.06 (-0.08; -0.03)**	**-0.04 (-0.04; -0.01)**	**-0.04 (-0.07; -0.01)**	NA
Indices of daily glucose variability					
Incremental glucose peak, per SD	2,407	**-0.09 (-0.13; -0.05)**	**-0.07 (-0.11; -0.03)**	**-0.06 (-0.11; -0.01)**	**-0.06 (-0.12; -0.01)**
CGM-assessed standard deviation, per SD	622	**-0.09 (-0.18; -0.01)**	**-0.09 (-0.17; -0.001)**	-0.08 (-0.17; 0.01)	-0.07 (-0.21; 0.07)

RNFL, per SD
Standardized regression coefficient (stβ) represents the difference in RNFL thickness in SD for type 2 diabetes or prediabetes versus NGM or per SD greater measure of glycemia or index of daily glucose variability. In the GMS, fasting plasma glucose, 2-hour post-load glucose, HbA1c, and skin autofluorescence study populations, 1 SD corresponds with 10.8 µm for RNFL thickness, 1.5 mmol/L for fasting plasma glucose, 4.0 mmol/L for 2-hour post-load glucose, 0.8% or 9.1 mmol/mol for HbA1c, and 0.5 AU for skin autofluorescence. For incremental glucose peak, 1 SD corresponds with 11.1 µm for RNFL thickness and 2.9 mmol/L for incremental glucose peak. For the CGM-assessed standard deviation, 1 SD corresponds with 10.8 µm for RNFL thickness and 0.58 mmol/L for CGM-assessed standard deviation.
Bold denotes P<0.05.
Variables entered in the models in addition to glucose metabolism status, measures of glycemia, or indices of daily glucose variability: model 1: none (crude results); model 2: age, sex, and educational status (low, medium, high); model 3: model 2 + office systolic blood pressure, total cholesterol to HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication (yes/no), waist circumference, smoking status (current, ever, never), and alcohol consumption status (none, low, high). In addition, only for incremental glucose peak, model 3 was additionally adjusted for HbA1c (model 3 + HbA1c), and only for CGM-assessed standard deviation, model 3 was additionally adjusted for MSG (model 3 + MSG). Additionally, and only for CGM-assessed SD, we entered ‘visit interval’ in model 1.
Abbreviations: AU, arbitrary unit; CI, confidence interval; CGM, continuous glucose monitoring; GMS, glucose metabolism status; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; NA, not applicable; NGM, normal glucose metabolism; SD, standard deviation; RNFL, retinal nerve fiber layer; MSG, mean sensor glucose.

Measures of glycemia and RNFL thickness

After full adjustment (model 3), greater FPG, 2-hour post-load glucose, HbA1c, and SAF were statistically significantly associated with lower RNFL thickness (per SD, respectively -0.05 [-0.08; -0.01]; -0.06 [-0.09; -0.02]; -0.05 [-0.08; -0.02]; and -0.04 [-0.07; -0.01]; Table 2 and Figure 2).

Indices of daily glucose variability and RNFL thickness

After full adjustment (model 3), greater IGP was statistically significantly associated with lower RNFL thickness (per SD, -0.06 [-0.11; -0.01]; Table 2 and Figure 2). The association remained statistically significant after additional adjustment for HbA1c (per SD, -0.06 [-0.12; -0.01]). After full adjustment (model 3), CGM-assessed standard deviation was also negatively associated with RNFL thickness, but not statistically significantly (per SD, -0.08 [-0.17; 0.01]). The association was similar after further adjustment for mean sensor glucose.

Interaction analyses

GMS and sex did not modify any of the associations under study. All P-values for interaction are shown in Supplemental Table S4.

Additional analyses

Quantitatively similar results were observed in a range of sensitivity analyses and are presented in the Supplemental Results section.

Discussion

The present population-based study had two main findings. First, a more adverse GMS, greater glycemia (estimated from FPG, 2-hour post-load glucose, HbA1c, and SAF) and greater daily glucose variability (estimated from IGP and CGM-assessed standard deviation) were all linearly and –except for CGM-assessed standard deviation– statistically significantly associated with lower RNFL thickness. Second, the associations between indices of daily glucose variability and RNFL thickness did not materially change after additional adjustment for measures of glycemia.

Our findings are in line with and extend observations from most previous studies.[13, 19–24] The present study is the first large population-based study to comprehensively report associations of GMS, measures of glycemia, and indices of daily glucose variability with RNFL thickness, and also adjust for an extensive set of potential confounders. Additionally, the present study is the first to present associations of SAF, duration of diabetes, and indices of daily glucose variability with RNFL thickness.

Mechanistically, the linearity of the associations of GMS, measures of glycemia, and indices of daily glucose variability with RNFL thickness most likely reflects the increasing loss of retinal ganglion cells due to both hyperglycemia-induced neurotoxicity and impairment of functioning of retinal cells that contribute to metabolic regulation.[4, 5] Such impairment of metabolic regulation can predispose retinal ganglion cells to ischemia.[5, 45] Importantly, retinal ganglion cells are thought to be highly susceptible to ischemia, since they are highly active and have an energy demand that exceeds that of brain cells.[45]

These findings extend our previous work on the “ticking clock hypothesis”,[5, 9–12] which postulates that hyperglycemia-induced microvascular and neuronal deterioration is a continuous, gradual process that starts in prediabetes, progresses with the onset of type 2 diabetes, and continues during type 2 diabetes.[8] Indeed, we observed that the regression estimate for prediabetes was in between the estimate for type 2 diabetes and the reference category (i.e., NGM), and was directionally and numerically comparable to our previous findings.[9–12] However, the association between prediabetes and RNFL thickness was not statistically significant, which is most likely due to insufficient statistical power. We, therefore, additionally tested for a linear trend with GMS deterioration by using the statistically more powerful P for trend analysis, [46] which was consistent with a linear decrease in RNFL thickness with more adverse GMS. In support, all measures of glycemia, regardless of whether they reflect shorter (i.e., FPG, 2-h PG, and HbA1c) or longer (i.e., SAF and duration of diabetes) exposure, were consistently linearly associated with RNFL thickness.

Similarly, a likely explanation why the association between CGM-assessed standard deviation and RNFL thickness was not statistically significant is that the statistical power to detect any such association was too low.[47] Indeed, we observed that the association between IGP and RNFL thickness, which included almost fourfold the number of participants (n=2,407 versus n=622), was statistically significant. Moreover, the strength of the associations of IGP and CGM-assessed standard deviation with RNFL thickness were numerically analogous.

A probable explanation why the association between daily glucose variability and RNFL thickness was not materially altered after additional adjustment for measures of average glycemia is that daily glucose variability, measures of glycemia, and GMS represent different underlying constructs.[26] While daily glucose variability reflects oscillating glucose levels, other measures under study reflect exposure to average chronic levels of glycemia. Mechanistically, substantial glucose fluctuations entail hyperglycemic peaks, hypoglycemic nadirs (in individuals with type 2 diabetes treated with agents that can induce hypoglycemia), or both, which are thought to be potent inducers of retinal ganglion cell apoptosis.[26, 45] Whereas hyperglycemic peaks may be highly neurotoxic, hypoglycemic nadirs likely hamper retinal ganglion cell metabolism as their key nutrient is glucose.[45]

Our findings can have several implications for clinical practice. First, the strength of the association between type 2 diabetes and RNFL thickness corresponds with 15 years of aging and, thus, indicates that with respect to neurodegeneration substantial “additional aging” occurs in individuals with type 2 diabetes (Supplemental Table S14 shows how this comparison was calculated). Second, RNFL thickness may be a biomarker for the identification of individuals at risk of retinopathy. Use of RNFL thickness measurement is feasible because RNFL thickness assessment is non-invasive,[2] relatively inexpensive[2] and easier to perform than other tests of early neuronal dysfunction such as 24-hour electrocardiogram,[9] magnetic resonance imaging,[10, 11] or electromyography.[12] Indeed, RNFL thickness has been found to be a promising early biomarker for other neurodegenerative diseases which are in part of a vascular origin (e.g., Alzheimer’s disease).[48] Third, early glycemic control, possibly already in prediabetes, is likely crucial in the early prevention of microvascular complications.[5] Last, our findings add to growing evidence that control of daily glucose variability besides mean glucose concentrations may be important to prevent microvascular complications.[49, 50]

Strengths of this study are 1) the large size of this population-based cohort with oversampling of individuals with type 2 diabetes, which enabled accurate comparison of individuals with and without diabetes; 2) the extensive number of potential confounders that were considered; 3) the use of state-of-the-art and novel methods (e.g., CGM)[26] to assess all variables included in this study; and 4) the considerable number of additional analyses, which generally yielded consistent findings.

The study has certain limitations. First, due to the cross-sectional nature of the study, causal inferences should be made with caution.[51] Mechanistically, hyperglycemia may not only lead to neurodegeneration but the reverse may also be true, thus causing a vicious cycle. Intact neurovascular interaction is required for normal microvascular function and impaired microvascular function may aggravate hyperglycemia.[5, 52] Second, we may have underestimated the strength of the associations of GMS, measures of glycemia, and daily glucose variability with RNFL thickness if such an association was similar or stronger in participants that were excluded from the study population (who generally tend to be less healthy).[53] The 2-hour post-load glucose and IGP results are most susceptible to this form of selection bias, as no data was available in individuals with the most therapy-intensive diabetes because they were excluded from undergoing an OGTT. Such range restriction may lead to underestimated associations.[53] Third, a single

OGTT may misclassify GMS, especially in individuals with prediabetes. Because individuals classified with prediabetes based on their first OGTT are relatively more prone to receive a NGM classification based on their second OGTT,[54] this would likely lead to an underestimation of the association with RNFL thickness in the prediabetes group. Fourth, although we took an extensive set of confounders into account, we cannot fully exclude bias due to unmeasured confounding (e.g., environmental factors such as air pollution).[55] Fifth, due to the relatively low numbers of participants with data on CGM-based glycemic indices (n=622)), and –to a lesser extent– IGP (n=2,407), statistical power of analyses with these determinants was reduced compared to statistical power of analyses with GMS and measures of glycemia (n=5,132 to n=5,455).[47] Last, we studied Caucasian individuals aged 40-75 years with access to high-quality diabetes care. Therefore, the generalizability of our results to other populations requires further study.

Conclusions

In summary, the present population-based study demonstrated that adverse GMS, greater glycemia, and greater daily glucose variability are associated with lower RNFL thickness, i.e., neurodegeneration, independent of demographics, cardiovascular risk factors, and lifestyle risk factors. Hence, these results suggest that RNFL thickness may be an early biomarker for the identification of individuals at risk of diabetic retinopathy. Additionally, the combination of early glycemic monitoring and early glucose-lowering treatment, possibly already in prediabetes, may contribute to the prevention of RNFL thinning and, ultimately, diabetic retinopathy.

Declarations

Ethical approval and consent to participate

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 (Permit 131088-105234-PG).

Consent for publication

Not applicable

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Author Contributions

F.C.T.vd.H. and Y.D.F. contributed to conception and design, participated in acquisition of data, analyzed and interpreted data, drafted the manuscript (with C.D.A.S., N.C.S., M.C.G.J.B., R.M.A.H., and J.S.A.G.S.), revised the manuscript critically for important intellectual content, and provided final approval of the version to be published. F.C.T. vd.H and Y.D.F. also are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. I.W.M.F., A.A.K., P.C.D., S.J.P.M.E., T.J.M.B., C.A.B.W., M.T.S., C.J.H.vd.K., M.M.J.v.G., A.W., and C.G.S. contributed to conception and design, revised the manuscript critically for important intellectual content, and provided final approval of the version to be published.

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Figures

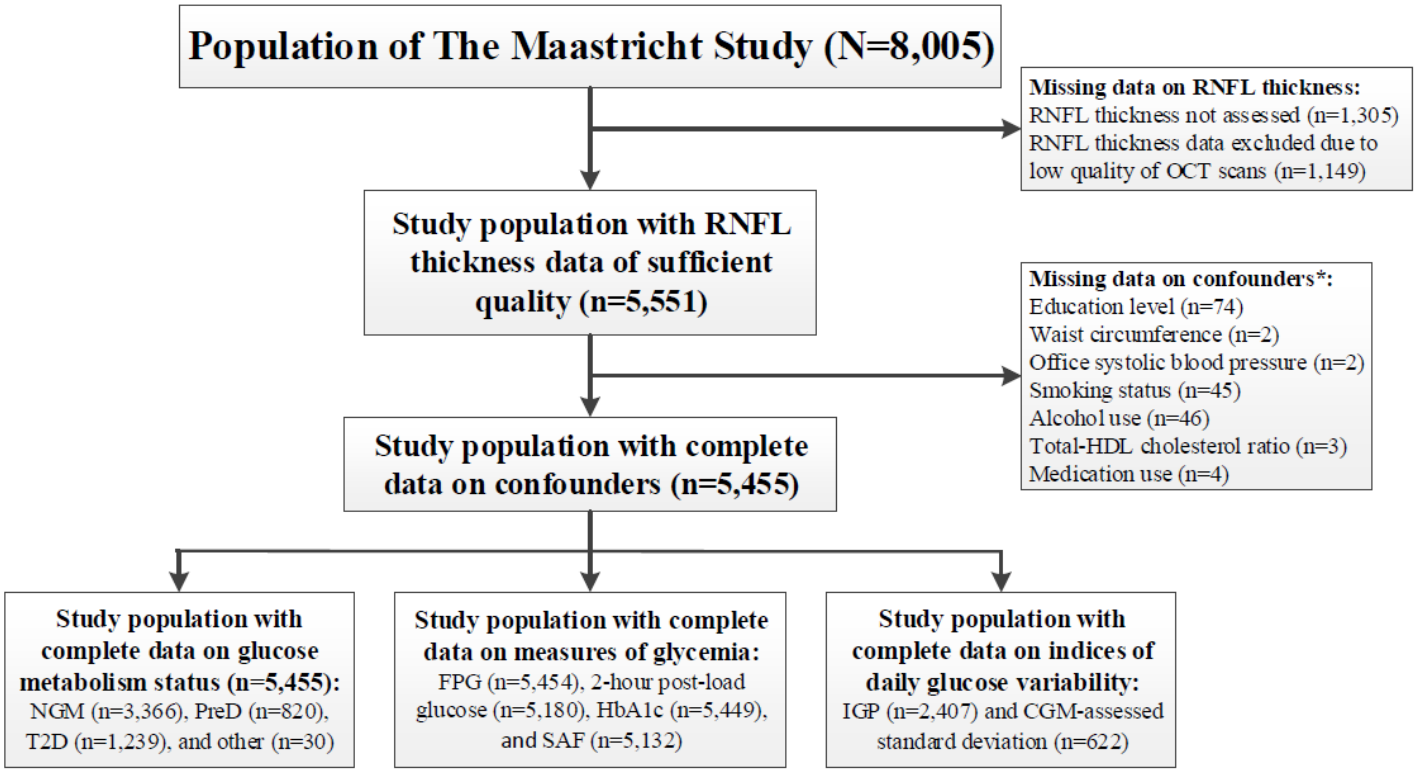

Figure 1

RNFL study population selection * Not mutually exclusive Abbreviations: OCT, optical coherence tomography; RNFL, retinal nerve fiber layer; HDL, high density lipoprotein; NGM, normal glucose metabolism; PreD, prediabetes; T2D, type 2 diabetes; FPG, fasting plasma glucose; HbA1c, hemoglobin A1C; SAF, skin autofluorescence; IGP, incremental glucose peak; CGM, continuous glucose monitoring.

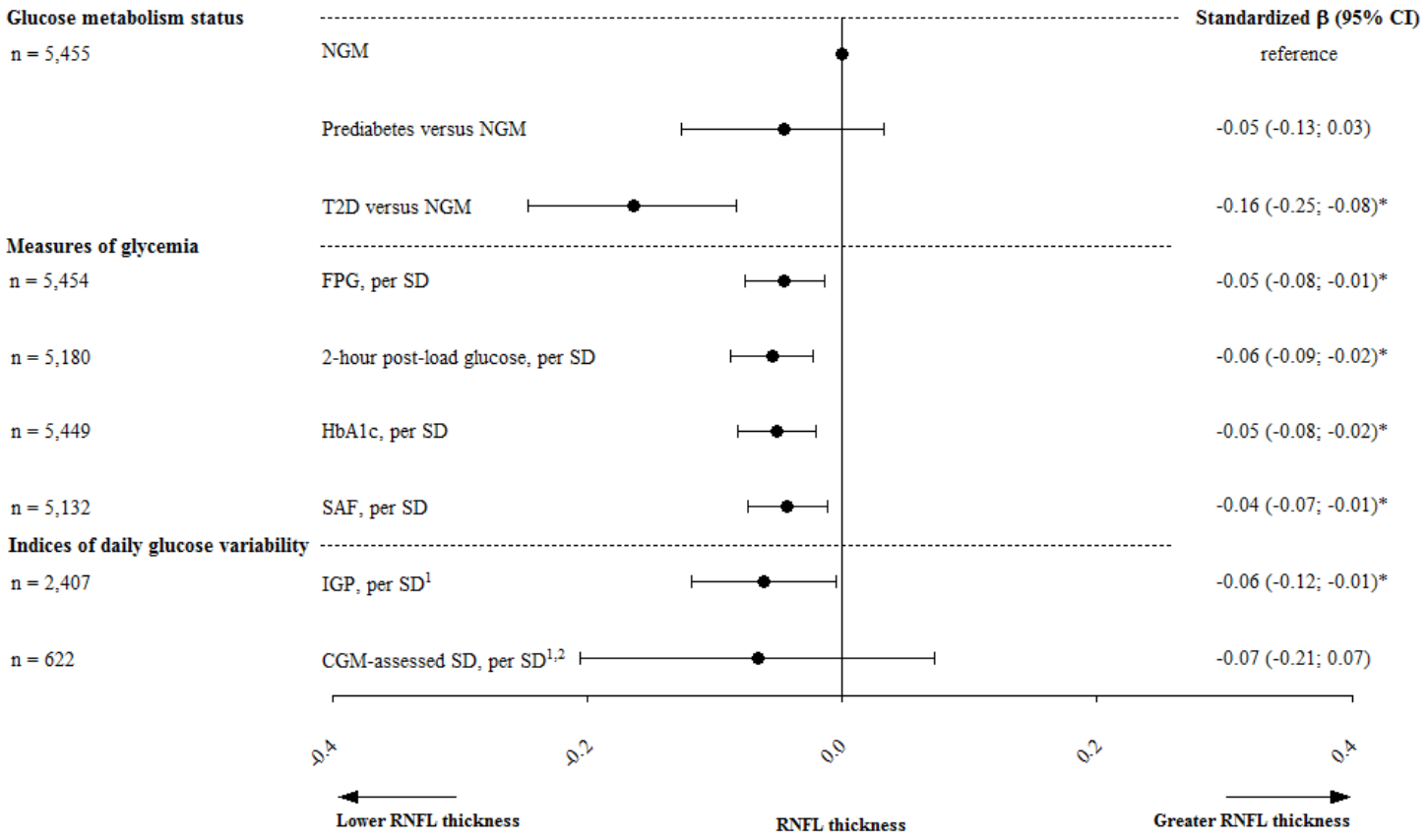

Figure 2

Associations between glucose metabolism status, measures of glycemia and indices of daily glucose variability with RNFL thickness (per SD) Standardized regression coefficient (stβ) represents the difference in RNFL thickness in SD for type 2 diabetes or prediabetes status versus NGM status, or per SD greater measure of glycemia or index of daily glucose variability. In the GMS, fasting plasma glucose, 2-hour post-load glucose, HbA1c, and SAF study populations, 1 SD corresponds with 10.8 µm for RNFL thickness, 1.5 mmol/L for fasting plasma glucose, 4.0 mmol/L for 2-hour post-load glucose, 0.8% or 9.1 mmol/mol for HbA1c, and 0.5 AU for SAF. For incremental glucose peak, 1 SD corresponds with 11.1 µm for RNFL thickness and 2.9 mmol/L for incremental glucose peak. For CGM-assessed standard deviation, 1 SD corresponds with 10.8 µm for RNFL thickness and 0.58 mmol/L for CGM-assessed standard deviation. Variables entered in the models in addition to glucose metabolism status, measures of glycemia, or indices of daily glucose variability: age, sex, and educational status (low, medium, high), office systolic blood pressure, total cholesterol to HDL cholesterol ratio, use of antihypertensive or lipid-modifying medication (yes/no), waist circumference, smoking status (current, ever, never), and alcohol consumption status (none, low, high). * Indicates statistically significant (P<0.05). 1 The associations of indices of daily glucose variability with RNFL thickness were additionally adjusted for HbA1c (IGP) or mean sensor glucose (CGM-assessed SD). 2 Additionally, and only for CGM-assessed SD, we entered ‘visit interval’ in model 1. Abbreviations: SD, standard deviation; CI, confidence interval; NGM, normal glucose metabolism; T2D, type 2 diabetes; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; SAF, skin autofluorescence; IGP, incremental glucose peak; CGM, continuous glucose monitoring.

Supplementary Files

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