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Correlation of retinal neurodegeneration with measures of peripheral autonomic neuropathy in type 1 diabetes

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ABSTRACT.

Purpose: **To evaluate the relationship of neuroretinal layer thickness with sensitive measures of cardiovascular autonomic neuropathy in diabetic patients with non-proliferative diabetic retinopathy (NPDR).**

Methods: **Twenty-seven eyes of 27 patients with type 1 diabetes presenting with mild-to-moderate NPDR were compared to 27 healthy control (HC) eyes matched for age and gender. The total macular volume (TMV) and the volumes of individual neurosensory layers in the macula were analysed from spectral domain optical coherence tomography using automated layer segmentation. Cardiovascular autonomic regulation was assessed by short-term power spectrum analysis of heart rate variability (HRV) before, during and after an orthostatic challenge.**

Results: **The patients had an age of 46 ± 12 years and diabetes since 28 ± 9 years. Diastolic and mean arterial pressure was lower in the patients compared to HCs. TMV ($r = 0.58$, $p = 0.002$), inner plexiform layer volume (IPLV; $r = 0.39$, $p = 0.047$) and inner nuclear layer volume (INLV; $r = 0.60$, $p = 0.001$) were associated with reduced recovery of low-frequency (LF) spectral power of HRV after orthostatic load in diabetic patients but not in HCs. The response of LF spectral power during the orthostatic manoeuvre was blunted in patients compared to HCs ($p = 0.02$). Diabetes duration was negatively associated with TMV and INLV, whereas IPLV was significantly reduced in eyes with moderate NPDR compared to HCs.**

Conclusion: **The results indicate a correlation between inner retinal tissue loss and diminished autonomic regulation in type 1 diabetic patients with mild-to-moderate NPDR. The observed changes can be interpreted as congruent early signs of retinal and systemic neuropathy in diabetes.**

Key words: cardiovascular autonomic neuropathy – diabetes mellitus – diabetic retinopathy – heart rate variability – optical coherence tomography

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Introduction

Diabetic retinopathy (DR) is often noticed as the earliest complication in diabetes, and its clinical evaluation is usually based on pathological changes of retinal vessels detectable in biomicroscopy, fundus photography and fluorescein angiography. Accordingly, it is known from experimental findings that microvascular changes on a histological level and blood flow alterations in the various levels of the ocular angioarchitecture commence before the emergence of clinically established DR (Pemp & Schmetterer 2008). Early alterations in the diabetic retina also include a reduced vascular reactivity to retinal stimulation (Garhöfer et al. 2004). However, comparisons with extra-ocular vessel reactivity and with systemic stimuli indicate that this impairment not only is due to vascular dysfunction but also may be influenced by a regulatory impairment of the retinal neurovascular complex (Pemp et al. 2009a,b). In addition, numerous studies have demonstrated that subclinical signs of functional impairment as seen in electrophysiological tests (Caputo et al. 1990; Juen & Kieselbach 1990; Palmowski et al. 1997; Lopes de Faria et al. 2001) and with different psychophysical testing methods (Realini et al. 2004; Stavrou & Wood 2005; Gualtieri et al. 2011; Jackson et al. 2012) are detectable in diabetic patients

with early clinical signs and to a lesser extent also in patients without DR. These findings suggested primary changes in neural function of the retina in diabetes, which cannot be explained only by an altered retinal circulation. In recent years, there is accumulating evidence that retinal neurodegeneration is present early in diabetes mellitus and occurs even before the development of clinically detectable microvascular DR (Simó & Hernández 2012; De Clerck et al. 2015; Sohn et al. 2016). Using optical coherence tomography (OCT), subtle atrophic changes in retinal thickness could be detected non-invasively in diabetic patients with early retinopathy and to a lesser extent also in patients without retinopathy (Biallosterski et al. 2007; Oshitari et al. 2009). More specifically, the neuroretinal tissue loss in the diabetic macula seems to involve mainly the perifoveolar inner retinal layers, where these layers are originally thickest (van Dijk et al. 2009, 2012; Cabrera DeBuc & Somfai 2010; Chhablani et al. 2015; Sohn et al. 2016). These findings corroborate experimental data which suggest that neuronal apoptosis in the retina is an early and persistent event in diabetes (Abu-El-Asrar et al. 2004; Barber et al. 2011).

It is well known that diabetes can cause peripheral nerve fibre loss which results in different forms of peripheral diabetic neuropathy including dysfunction of motor, sensory and autonomic fibres (American Diabetes Association and American Academy of Neurology 1988). Conflicting opinions exist on whether large myelinated, small myelinated and unmyelinated autonomic nerve fibres are damaged in parallel by diabetes (Levy et al. 1987; Ziegler et al. 1987, 1988; Spitzer et al. 1997; Orlov et al. 2012). Currently, there is evidence that small myelinated and unmyelinated fibres may be affected earlier than large fibres (Guy et al. 1985; Hendriksen et al. 1993; Sumner et al. 2003). Among autonomic dysfunctions in diabetes, cardiovascular autonomic neuropathy (CAN) is the most intensively studied and clinically important form. The prevalence of confirmed CAN is around 20% in diabetic patients and increases up to 65% with age and diabetes duration (Spallone et al. 2011). Reduced heart rate variation is the earliest indicator of CAN and therefore one of the earliest signs of peripheral diabetic neuropathy (Ziegler 1994; Tesfaye et al. 2010).

The purpose of this study was to provide insight into common pathomechanisms of retinal and systemic diabetic neuropathy. Therefore, we aimed at studying diabetic patients who were prone to have subclinical but detectable neuropathy in both systems and evaluated possible associations of neuroretinal layer measurements with sensitive measures of cardiovascular autonomic regulation in patients with early DR and long-standing type 1 diabetes.

Materials and Methods

The study was conducted at the Department of Ophthalmology and at the Department of Clinical Pharmacology of the Medical University of Vienna. Institutional Review Board (IRB)/Ethics Committee approval was obtained before the study started. It was carried out in adherence to the tenets of the Declaration of Helsinki and Good Clinical Practice guidelines. The study was registered in the Clinical Trial Database of the National Institute of Health (NCT00880139).

Patient inclusion and evaluation

Twenty-seven adult patients with type 1 diabetes, 17 males and 10 females, diagnosed since more than 1 year were included in this study. A total of 45 patients were screened. Eighteen patients had no DR or featured other ocular pathologies than non-proliferative DR including macular oedema and diabetic proliferations. Hence, they did not meet the inclusion criteria and were not included in the study. The eyes of diabetic patients were compared to the eyes of a group of 27 healthy subjects matched for age and gender. After written informed consent was obtained, all participants underwent a detailed screening for medical history, height and weight measurement, measurement of resting arterial blood pressure, assessment of best-corrected visual acuity (BCVA) using standardized illuminated logarithmic visual acuity charts ('ETDRS' Charts, Precision Vision, La Salle, IL, USA) and a complete ophthalmologic examination according to the protocol. Diabetic retinopathy (DR) was classified as mild or moderate according to the criteria established in the Early Treatment Diabetic Retinopathy Study (ETDRS; Early Treatment Diabetic Retinopathy Study Research Group 1991) based on seven standard field colour fundus photographs taken by a digital camera connected to a fundus camera (FF 450; Carl Zeiss Meditec AG, Jena, Germany) after pupil dilation using tropicamide eye drops (Mydriaticum 'Agepha', Agepha, Vienna, Austria). Patients with microaneurysms and at least one definite additional haemorrhage, hard exudate or cotton wool spot were classified as having mild DR, whereas eyes with moderate microaneurysms or haemorrhages in four standard fields or severe microaneurysms or haemorrhages in one field or eyes with intraretinal microvascular abnormalities in up to three standard fields were classified as moderate DR eyes. Patients without or with more severe stages of DR, patients with active macular oedema or a previous treatment with intravitreal injections or laser and patients or subjects with other eye diseases or a refractive error of more than 6 diopters were not included in the study. In the healthy controls, left and right eyes were evenly chosen for further measurements, whereas in the diabetic patients, the eye with more signs of DR based on the clinical findings was measured. Blood samples were taken from diabetic patients to measure their glucose and haemoglobin A1c (HbA1c) levels. Sensomotoric neuropathy was excluded by patellar tendon reflex test and by measuring the intensity of residual vibration perception using a Rydell–Seiffert tuning fork.

Optical coherence tomography of neurosensory layers

Spectral domain OCT (Spectralis OCT; Heidelberg Engineering, Heidelberg, Germany) was carried out in the central 20 degree macular area of one eye of each patient. This fourth-generation OCT system has an optical depth resolution of 7 μm and lateral resolution of 14 μm in tissue and enables non-invasive cross-sectional imaging of ocular structures with high detail (Castro Lima et al. 2011). Proprietary noise reduction of the device reduces the axial A-scan resolution to a digital resolution of 3.5 μm/pixel. The transverse digital resolution depends on the

amount of registered A-scans and can be adjusted. The device produces up to 40 000 A-scans per second and simultaneously images the fundus with a 30 degree infrared confocal scanning laser ophthalmoscope (SLO). The built-in real-time eye movement tracking system recognizes features in the SLO image including blood vessels and the optic disc. This enables stabilization of the scan coordinates in relation to the retina coordinates and minimizes motion artefacts during image acquisition. Activated eye-tracking was used to average multiple repeated OCT scans at each line of the macular cube scan protocol, whereupon single B-scan frames were only added to the averaged OCT image, if the scanning position matched the original position in the SLO image. The eye-tracking feature allows for a significant reduction of background noise and visualization of retinal microstructures with enhanced contrast and reduced variance (Pemp et al. 2013). A built-in viewing software (HRA Viewing Module version 6.7.17; Heidelberg Engineering) was used to automatically segment the retinal nerve fibre layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL) and photoreceptor layer (PRL) of all obtained B-scans. Thus, the three-dimensional mapping of individual retinal layers was achieved. For quantitative analysis, the central macular area was overlaid with a circular ETDRS grid consisting of three concentric circles with diameters of 1, 3 and 6 mm. By this means, the total macular volume and volumes of individual neurosensory retinal layers were obtained in the central area of 6 mm using the built-in automated layer segmentation.

Heart rate variability

Cardiovascular autonomic regulation was assessed by standardized short-term power spectrum analysis of heart rate variability (HRV) using a commercially available system (VariaCardio TF5; Advanced Medical Diagnostics Group, Leeds, UK). High-resolution single-channel electrocardiography was recorded by a precordial electrode. The signal was transferred to a receiver connected to a personal computer and displayed online. R-R intervals were identified with a sampling rate of 1000 Hz. Artefacts were identified and labelled automatically by the recording software, and a specific algorithm inserted beat-to-beat intervals throughout an artefact period to preserve the timing relationships of the adjacent, uncorrupted heart rate data. Thus, three measurements collecting 256 seconds of artefact-free measurements each were taken before, during and after an orthostatic load (positions: supine1–standing–supine2). Spectral analysis of HRV based on fast Fourier transformation was displayed online as three-dimensional running spectra and stored for further analysis. The system automatically analyses frequency-domain HRV parameters as total spectral power, spectral power of the low-frequency band (LF; 0.05–0.15 Hz) and spectral power of the high-frequency band (HF; 0.15–0.40 Hz) in each position. To evaluate HRV changes in the LF band during the orthostatic manoeuvre, LF spectral power was expressed as proportion of individual total power in normalized units, in accordance with current guidelines and recommendations in the literature (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996, Perini & Veicsteinas 2003). Reduced LF and HF spectral power at rest, a diminished increase of normalized LF spectral power during orthostasis and a reduced normalization after an orthostatic challenge are sensitive signs of blunted autonomic cardiovascular control (Bellavere et al. 1992; Howorka et al. 2010). Participants avoided caffeinated beverages, smoking, alcohol and large meals for at least 2 hr before the assessment. Heart rate variability (HRV) was measured after a resting period of 10 min. Capillary blood glucose was measured in all patients before the test to exclude hypoglycaemia and marked hyperglycaemia.

Statistical analysis

Numerical data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare baseline data between patients and healthy subjects. To control for possible confounding effects of detected blood pressure differences on HRV, ANCOVA including diastolic and mean arterial blood pressure as covariates was used for comparison of baseline spectral power between patients and controls. The same covariates were also included in a general linear model for repeated measurements, which was applied to compare group data from repeated measurements of HRV. Pearson's product–moment correlation coefficients were calculated to assess correlations between the measured parameters of OCT and HRV in both groups. Subgroup analysis was conducted with an unpaired *t*-test. A p-value of 0.05 was considered the level of significance for all calculations. The statistical analysis was conducted using the software STATISTICA® (Release 6.1; StatSoft Inc., Tulsa, OK, USA).

Results

Baseline characteristics of the investigated patients and healthy controls are presented in Table 1. Age, visual acuity, body mass index, pulse rate and systolic arterial pressure were similar in both groups. All patients and controls had normal blood pressure and intraocular pressure, but diastolic pressure and mean arterial pressure were significantly lower and intraocular pressure was slightly higher in the patient group. All patients had type 1 diabetes with a mean disease duration of 28 ± 9 years, presented either mild ($n = 15$) or moderate ($n = 12$) non-proliferative DR and showed a normal sensomotoric function in the screening tests conducted.

The OCT measurements detected no significant difference between the eyes of diabetic patients and healthy controls. The spectral power of HRV at baseline was significantly reduced in diabetic patients in both the LF and HF bands. During the orthostatic manoeuvre, normalized LF spectral power showed a significantly blunted response in the diabetic patients compared to healthy controls ($p = 0.02$; Fig. 1A). The reaction of HF spectral power was not reduced in the diabetic patients ($p = 0.5$; Fig. 1B).

The correlation coefficients between OCT and HRV regulation in the LF band after orthostasis are presented in Table 2.

The OCT measurements of the total macular volume, macular IPL volume and INL volume were significantly correlated with normalized LF spectral power difference in position supine1

Table 1. Characteristics of patient eyes and healthy control eyes.

	Diabetes patients (n = 27)	Healthy controls (n = 27)	p-Values
Age (years)	46 ± 12	41 ± 20	0.3
Diabetes duration (years)	28 ± 9	–	–
HbA1c (%)	7.6 ± 0.9	–	–
Body mass index (kg/m²)	24.8 ± 3.2	24.1 ± 3.9	0.6
Systolic blood pressure (mmHg)	121 ± 16	126 ± 13	0.3
Diastolic blood pressure (mmHg)	66 ± 13	78 ± 11	**0.004**
Mean arterial blood pressure (mmHg)	85 ± 14	99 ± 9	**0.002**
Pulse rate (min^{-1})	75 ± 12	73 ± 7	0.7
Visual acuity (logMAR)	−0.04 ± 0.06	−0.06 ± 0.11	0.4
Visual acuity (Snellen equivalent)	20/18	20/18	–
Intraocular pressure (mmHg)	14.2 ± 2.1	12.9 ± 1.9	**0.02**
Macular volume (mm³)	8.66 ± 0.45	8.76 ± 0.30	0.4
Retinal nerve fibre layer volume (mm³)	0.95 ± 0.11	0.92 ± 0.09	0.3
Ganglion cell layer volume (mm³)	1.07 ± 0.12	1.09 ± 0.10	0.7
Inner plexiform layer volume (mm³)	0.87 ± 0.09	0.90 ± 0.07	0.2
Inner nuclear layer volume (mm³)	0.93 ± 0.09	0.94 ± 0.05	0.4
Outer plexiform layer volume (mm³)	0.81 ± 0.06	0.82 ± 0.05	0.3
Outer nuclear layer volume (mm³)	1.73 ± 0.19	1.80 ± 0.11	0.1
Photoreceptor layer volume (mm³)	2.30 ± 0.08	2.27 ± 0.07	0.2
Baseline LF spectral power ($mseconds^2$)	474 ± 495	1199 ± 1144	–
Baseline LF spectral power (ln [$mseconds^2$])	5.60 ± 1.26	6.54 ± 1.14	**0.007**
Baseline HF spectral power ($mseconds^2$)	344 ± 363	2160 ± 2538	–
Baseline HF spectral power (ln [$mseconds^2$])	5.17 ± 1.35	6.89 ± 1.47	**0.0002**

HF = high-frequency band [0.15–0.40 Hz]; LF = low-frequency band [0.05–0.15 Hz].
Results are presented as mean ± SD.
Bold values indicate significant p-values ($p < 0.05$, ANCOVA in spectral power comparisons including diastolic and mean arterial blood pressure as covariates and ANOVA in all other comparisons; absolute spectral power was not compared due to skewness of data).

minus supine2 (Fig. 2), indicating a reduced recovery of HRV LF spectral power after orthostatic load in patients with reduced inner neuroretinal layers. In contrast to these findings, the healthy subjects showed no correlation between inner retinal layers and LF spectral power regulation after orthostasis. Macular volumes of the outer neurosensory layers OPL, ONL and PRL were also not associated with orthostatic HRV regulation in both groups.

In the diabetic patients, disease duration was negatively associated with the total macular volume ($r = -0.47$, $p = 0.01$) and INL volume ($r = -0.38$, $p = 0.050$), but not with baseline LF spectral power ($r = -0.19$, $p = 0.3$) or LF spectral power difference after orthostasis ($r = -0.15$, $p = 0.5$). HbA1c was also not correlated with these HRV parameters ($r = -0.16$, $p = 0.4$ and $r = -0.07$, $p = 0.7$, respectively) nor with macular volume ($r = 0.00$, $p = 1.0$). A subgroup analysis carried out *post hoc* showed no significant difference in HRV or OCT measurements between patients with mild DR and patients with moderate DR but revealed a significant reduction of IPL volume in eyes of patients with moderate non-proliferative DR compared to healthy control eyes (0.84 ± 0.11 versus 0.90 ± 0.07 mm³, $p = 0.047$, unpaired t-test).

Discussion

Our results show significant correlations of macular OCT measurements of the inner retinal layers with HRV parameters sensitive to CAN in diabetic patients with early DR but not in healthy controls. Although statistical comparison with healthy controls did not prove a significant reduction in macular OCT over the whole patient group, total macular volume and inner retinal layer volume decreased significantly with diabetes duration and eyes with moderate DR had a reduced inner retinal layer volume compared to healthy controls. Both findings indicate a progressive loss of neuroretinal tissue during the course of the disease. In addition, most patients had a reduced HRV at rest in both the LF and the HF bands and a blunted reaction of HRV during the orthostatic challenge in the LF band indicating simultaneous presentation of the earliest signs of CAN including an affected reactivity in the sympathetic system. Taken together, these findings show a parallel degeneration of ocular and peripheral neural tissue in diabetes on a subclinical level. To our knowledge, this is the first description of an association of macular neurodegeneration with systemic autonomic neuropathy in diabetes. Earlier studies using OCT have found associations between moderate and severe sensomotoric diabetic polyneuropathy with reduced peripapillary RNFL in the inferior quadrant (Shahidi et al. 2012) and with decreased average RNFL thickness in the macula, while GCL + IPL thickness was not reduced (Salvi et al. 2015; Srinivasan et al. 2016).

Our results are in good accordance with other reports that found mainly the inner retinal layers affected by the subtle atrophic changes in diabetes (Lopes de Faria et al. 2002; Biallosterski et al. 2007; van Dijk et al. 2009, 2012; Oshitari et al. 2009; Cabrera DeBuc & Somfai 2010; Chhablani et al. 2015; Carpineto et al. 2016; Ng et al. 2016). An increased apoptosis of neuronal and glial cells in the retina has been demonstrated earlier in post-mortem eyes of diabetic patients (Barber et al. 1998; Sohn et al. 2016), and apoptosis may be one of the mechanisms contributing to neuroretinal tissue loss in diabetes. Thereby, triggered retinal neurodegeneration may also participate in the development of early microvascular changes occurring in DR such as the breakdown of the blood–retina barrier, vasoregression and neurovascular coupling impairment (Simó & Hernández 2012). However, the causal relationship between diabetic retinal neurodegeneration and microangiopathy is not yet fully understood. Subclinical retinal microcirculatory alterations and dysregulation could as well induce or enhance neurodegenerative mechanisms. Whether the observed correlations of neurodegenerative signs in the retina and in the autonomic nervous system in diabetic patients are results of the same pathomechanisms or only concurrent signs of chronic disease is also not entirely clear from our results. Different parts of the nervous system could be affected differently by neurodegenerative, microvascular and/or subclinical inflammatory processes during the course of the disease, and all these

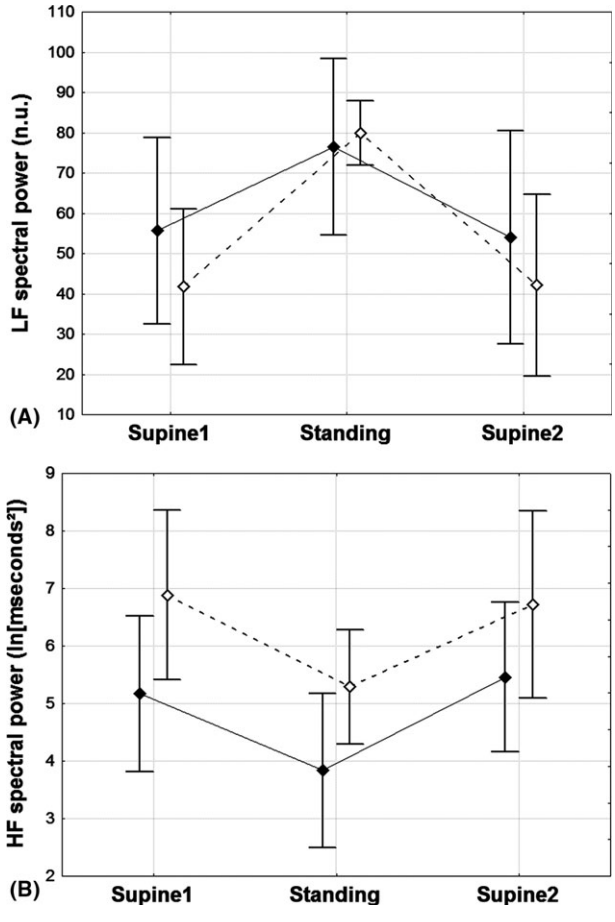

Fig. 1. Normalized low-frequency spectral power of heart rate variability (A) and high-frequency spectral power (B) in diabetic patients (black diamonds) and in healthy controls (white diamonds) before, during and after the orthostatic challenge; p-values (repeated-measures general linear model including the covariates diastolic and mean arterial pressure): (A) p = 0.02, (B) p = 0.5; results are presented as mean ± SD; HF = high frequency; LF = low frequency; n.u. = normalized units.

Table 2. Correlation analysis between macular OCT and HRV regulation after orthostasis.

	ΔLF spectral power (supine1−supine2) (n.u.)			
	Diabetes patients		Healthy controls	
OCT volume (mm³)	*r*	p-Value	*r*	p-Value
Total macula	**0.58**	**0.002**	−0.05	0.8
Retinal nerve fibre layer	0.19	0.3	0.22	0.3
Ganglion cell layer	0.34	0.08	−0.12	0.5
Inner plexiform layer	**0.39**	**0.047**	−0.18	0.4
Inner nuclear layer	**0.60**	**0.001**	−0.25	0.2
Outer plexiform layer	0.05	0.8	−0.17	0.4
Outer nuclear layer	0.17	0.4	−0.01	0.9
Photoreceptor layer	0.27	0.2	0.15	0.5

HRV = heart rate variability; LF = low-frequency band [0.05–0.15 Hz]; n.u. = normalized units; OCT = optical coherence tomography; *r* = Pearson's correlation coefficient,
Bold values indicate significant correlations ($p < 0.05$, unpaired *t*-tests).

mechanisms may have an influence on each other.

Peripheral polyneuropathy is a frequent complication in diabetes and is usually diagnosed by sensory tests or electrophysiology. Whereas the underlying damage to large fibres is mostly detected in later phases of the disease, the earliest neural damage in diabetes may take place in small myelinated and unmyelinated nerve fibres including the autonomic (sympathetic and parasympathetic) nerves (Guy et al. 1985; Hendriksen et al. 1993; Sumner et al. 2003). Assessment of HRV is a standardized, non-invasive method for quantification of autonomic function and thus recommended for the evaluation of subclinical and manifest CAN in diabetes (Pumprla et al. 2002; Perini & Veicsteinas 2003; Spallone et al. 2011). The measurement is based on the analysis of the sinus rhythm modulations by peripheral autonomic reflex mechanisms. These include the arterial baroreceptor reflex, which influences sympathetic activity contributing to the LF spectral power of HRV, and the respiratory sinus arrhythmia due to vagus nerve suppression during inhalation, which is represented by oscillations in the HF band. Thus, the modulating effect of both autonomic components can be measured separately from the HRV power spectrum to detect CAN signs in short-term recordings at rest and during a short-term orthostatic load (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996; Howorka et al. 2010) as illustrated by our results in patients with diabetes. Generally, spectral analysis of HRV appears to be the method of choice to assess early autonomic dysfunction in diabetes as it shows higher sensitivity than traditional methods (Bellavere et al. 1992; Lagi et al. 1994).

Most of the earlier reports of retinal neurodegeneration in diabetes could only analyse the total macular thickness (Biallosterski et al. 2007; Oshitari et al. 2009; De Clerck et al. 2015) or a combined measurement of GCL and IPL (van Dijk et al. 2009; Cabrera DeBuc & Somfai 2010; Chhablani et al. 2015; Carpineto et al. 2016; Ng et al. 2016; Sohn et al. 2016; Srinivasan et al. 2016). Technical improvements in OCT and image analysis now enable detailed automated analysis of intraretinal layers separately with high accuracy, at least if tissue borders are not substantially disturbed. Retinal segmentation produces more detailed information about patterns of atrophic changes in different ocular diseases and also in systemic neurodegenerative disorders using retinal layer measurements with OCT as a non-invasive biomarker. It seems reasonable to

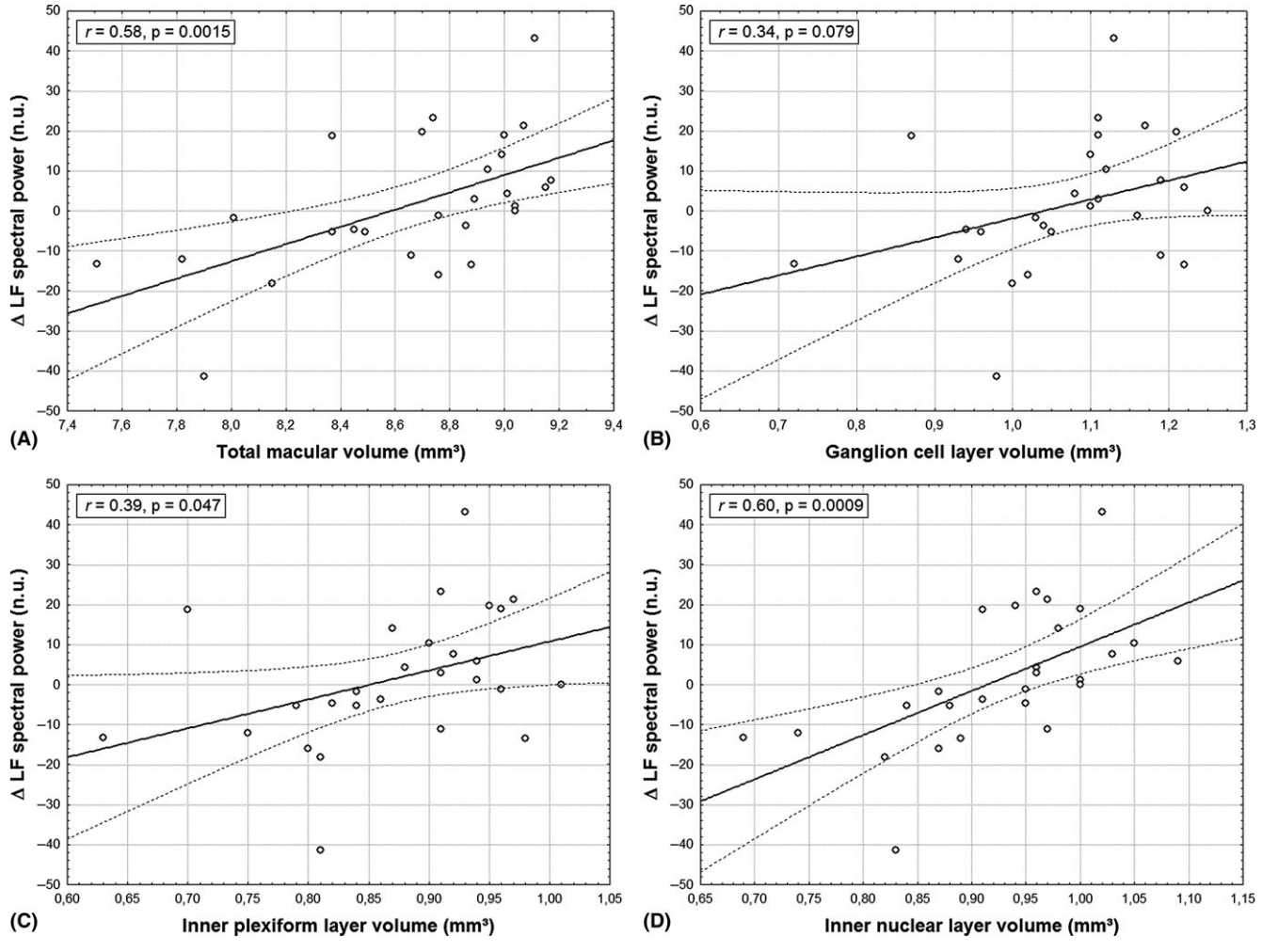

Fig. 2. Correlation analysis of OCT total macular volume (A) and inner retinal layer volumes (B–D) with normalized low-frequency spectral power difference (ΔLF) in HRV of position supine1 minus supine2; HRV = heart rate variability; n.u. = normalized units; OCT = optical coherence tomography.

monitor the neurodegenerative component of DR with high-resolution OCT. More long-term investigations will, however, be necessary to ascertain the level of atrophic changes over time in individual patients with diabetes and may also help to identify protective systemic factors, visual prognosis and efficacy of potentially neuroprotective treatments.

Some limitations of this study need to be considered: first, this is a study in a relatively small group of patients. A larger number of participants possibly would have detected a more apparent difference in neuroretinal layers between groups. Also, the included patients had heterogeneous levels of DR and the subgroups were different in size. This was partly due to our approach to select the eye with more signs of DR for measurement. In addition, patients without clinical DR were not included in this study. The detected associations between the analysed parameters in our patient group might therefore not apply to diabetic patients without DR. Finally, neuroretinal function was not measured specifically in this study. Hence, we cannot state any correlation between retinal and systemic neuronal function.

In conclusion, the present study indicates that patients with long-standing type 1 diabetes and non-proliferative DR show associations of inner retinal tissue loss with diminished peripheral autonomic nerve function. The observed changes can be interpreted as congruent early signs of retinal and systemic neuropathy in diabetes.

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