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Type 2 diabetes and cardiac autonomic neuropathy screening using dynamic pupillometry

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Abstract

Aim—To determine if changes in pupillary response are useful as a screening tool for diabetes and to assess whether pupillometry is associated with cardiac autonomic neuropathy.

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Competing interests

None declared.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Comparison of pupillometry values according to sociodemographic variables.

AUTHOR: Please supply the supporting information file for Table S1.

Methods—We conducted a cross-sectional study with participants drawn from two settings: a hospital and a community site. At the community site, individuals with newly diagnosed diabetes as well as a random sample of control individuals without diabetes, confirmed by oral glucose tolerance test, were selected. Participants underwent an LED light stimulus test and eight pupillometry variables were measured. Outcomes were diabetes, defined by oral glucose tolerance test, and cardiac autonomic dysfunction, determined by a positive readout on two of four diagnostic tests: heart rate response to the Valsalva manoeuvre; orthostatic hypotension; 30:15 ratio; and expiration-to-inspiration ratio. The area under the curve, best threshold, sensitivity and specificity of each pupillometry variable was calculated.

Results—Data from 384 people, 213 with diabetes, were analysed. The mean ($\pm$ SD) age of the people with diabetes was 58.6 ($\pm$ 8.2) years and in the control subjects it was 56.1 ($\pm$ 8.6) years. When comparing individuals with and without diabetes, the amplitude of the pupil reaction had the highest area under the curve [0.69 (sensitivity: 78%; specificity: 55%)]. Cardiac autonomic neuropathy was present in 51 of the 138 people evaluated (37.0%; 95% CI 28.8–45.1). To diagnose cardiac autonomic neuropathy, two pupillometry variables had the highest area under the curve: baseline pupil radius [area under the curve: 0.71 (sensitivity: 51%; specificity: 84%)], and amplitude of the pupil reaction [area under the curve: 0.70 (sensitivity: 82%; specificity: 55%)].

Conclusions—Pupillometry is an inexpensive technique to screen for diabetes and cardiac autonomic neuropathy, but it does not have sufficient accuracy for clinical use as a screening tool.

Introduction

There were an estimated 366 million people worldwide living with Type 2 diabetes mellitus in 2011, and ~80% of these people were in low- and middle-income countries [1]. Approximately 50% of people with diabetes remain undiagnosed [2,3], especially in developing countries, until further complications become clinically evident.

An early consequence of diabetes is autonomic dysfunction [4], which is often subclinical. Autonomic neuropathy is one of the least recognized complications of diabetes, but is of great clinical significance because of the potential cardiovascular, gastrointestinal, sudomotor and ocular autonomic neuropathy complications [5,6]. There are several tests available to assess autonomic dysfunction, but these tests require well-trained personnel and equipment, are time-consuming, and require active patient participation. Pupillometry, however, is a non-invasive and rapid screening method for autonomic dysfunction that does not require technical expertise and has the potential to improve screening for diabetes and its complications without the need to obtain a blood test or multiple screening tests for cardiac autonomic dysfunction [7].

As the pupillary response is controlled by both the parasympathetic and the sympathetic divisions of the autonomic nervous system, changes in pupillary responses to external light can provide an indirect means to assess the integrity of neuronal pathways controlling pupil size [8]. Previous studies have shown that people with diabetes have a smaller resting pupil size and smaller reflex amplitude than those without this condition, even before the disease is clinically apparent [8–10].

The aim of the present study was firstly to determine whether a change in pupillary responses was useful as a screening tool for Type 2 diabetes and could distinguish individuals with and without diabetes, including the estimation of sensitivity and specificity of different measures of pupillary function. Secondly, we aimed to assess whether pupillometry variables were associated with manifestations of cardiac autonomic neuropathy.

Methods

Ethics

The independent institutional review boards of three centres, the University Peruana Cayetano Heredia, the Hospital Nacional Cayetano Heredia and A.B. PRISMA, all located in Lima, Peru, approved the study protocol. Informed consent was obtained from participants before starting fieldwork activities.

Study design and setting—We conducted a cross-sectional study among people with and without diabetes drawn from two settings in Lima, Peru: (1) the Endocrinology Division of the Hospital Nacional Cayetano Heredia, a tertiary specialized hospital in the north of Lima; and (2) Pampas de San Juan de Miraflores, a peri-urban community located 25 km south of Lima's city centre. In the latter setting, a sub-sample of participants from the population-based CRONICAS cohort study, described elsewhere [11], was invited to participate.

The decision to establish two enrolling sites was so that (1) at the hospital facility, we could ensure the participation of individuals with documented shorter and longer duration of diabetes and (2) from the community site, we were able to select participants with diabetes in the general population, as well as participants without diabetes, who served as a comparison group. Participants with diabetes from the community site were expected to have a shorter time of disease awareness (<2 years), whereas participants without diabetes were randomly selected from all the individuals previously evaluated from the cohort study. The fieldwork phase of the study was conducted between March 2012 and July 2012.

Population study

People aged 40–75 years who provided consent were eligible for inclusion in the study. Potential participants were excluded from the study if they had neurological conditions, such as Grave's disease, Parkinson's disease, Alzheimer's disease or multiple sclerosis; or ocular complications, such as corneal lesions, glaucoma or severe cataracts, as these conditions might interfere with the proper interpretation of pupillometry results. Patients from the hospital facility were consecutively enrolled to obtain similar numbers of individuals with diabetes diagnosed for < 10 years and > 10 years.

In the community, all participants identified from the CRONICAS cohort study with diabetes were invited to participate. A subset of participants from the CRONICAS cohort study without diabetes was randomly selected, and were confirmed to be in the group without diabetes by performing an oral glucose tolerance test.

Procedures

All patients completed a brief questionnaire to provide general information (sex, age, duration of diabetes and current medications). Height and weight were measured to calculate BMI using standard procedures. We also measured systolic and diastolic blood pressure in triplicate using an automatic monitor Omron HEM-780 (Omron, Kyoto, Japan), previously validated for adult populations [12,13]. Patients also provided urine samples for semi-quantitative measurements of albumin levels using ChemStrip® Micral Test strips (Roche Diagnostics, Indianapolis, IN, USA).

Blood sampling

Blood sampling was planned and conducted according to study site and diabetes status. At both the community site and the hospital facility, all participants had a venous blood sample drawn from a trained nurse to measure HbA_{1c} levels.

For the participants without diabetes in the community setting, in addition to the HbA_{1c} measurements, a 75-g oral glucose tolerance test using standard techniques [14] was performed to confirm the absence of diabetes. From the oral glucose tolerance test results, we determined those without diabetes (comparison group), those with impaired glucose tolerance and those with newly diagnosed diabetes, and participants were assigned to a diabetes and non-diabetes group accordingly; those with impaired glucose tolerance were excluded from further analyses.

Plasma glucose levels were measured using an enzymatic colorimetric method (PAP; Modular P-E/Roche-Cobas, Grenzach-Whylen, Germany). HbA_{1c} was measured using high-performance liquid chromatography (D10; BioRad, Munich, Germany).

Clinical evaluations

All patients with diabetes from both study sites were invited to return for a second visit to receive an assessment of the fundus of the eye by a certified ophthalmologist after pupil dilation with tropicamide.

Cardiac autonomic neuropathy tests were performed in participants with diabetes from the hospital facility only. All the evaluations were conducted by an internal medicine physician using standardized techniques as recommended by the American Diabetes Association [15]. Tests for autonomic dysfunction such as heart rate response to the Valsalva manoeuvre, systolic blood pressure response to standing (orthostatic hypotension), heart rate response to standing (30:15 ratio), and expiration-to-inspiration ratio were conducted.

Pupillometry testing

Pupillometry was performed on all participants by one assessor. Testing consisted of application of obscured goggles with LED light stimulus for repeated pupillary light reflex stimulation. Patients were subjected to a light intensity no greater than a typical fluorescent light bulb for 20 cycles. Testing took ~5 min. A member of the study team remained with the patient as he/she underwent the pupillometric examination. All analysis was performed

using LABVIEW-based software (LABVIEW 9.0, program View Pupil Data T2; Steven Moore, Mt Sinai School of Medicine, New York City, NY, USA).

Various pupillometry values were captured, as previously described in the literature [10]: baseline pupil radius (R1), measured in mm after 2 min of adaptation to darkness; latency for onset of constriction, in seconds; maximum constriction ratio (R2), measured in mm; amplitude of the pupil reaction, defined as the difference of baseline pupil radius and maximum constriction ratio (R1–R2), measured in mm; latency to maximum constriction, in seconds; re-dilation latency, in seconds; time to 75% re-dilation, in seconds; and R2/R1 ratio.

Outcome variables and other variables

Diabetes and cardiac autonomic neuropathy were the outcomes of interest. As per oral glucose tolerance test guidelines, participants were considered as having diabetes if blood glucose levels were ≥ 200 mg/dl after glucose challenge, and as having impaired glucose tolerance if blood glucose levels were ≥ 140 and <200 mg/dl [16,17]. An oral glucose tolerance test was not conducted in those who already had a clinical diagnosis of diabetes, as described above (see blood sampling procedures). HbA_{1c} not meeting target levels ($< 7\%$) was indicative of patients at a higher risk of microvascular diabetes complications according to criteria set by the American Diabetes Association [18].

Cardiac autonomic neuropathy was defined as positive results for two or more of the proposed tests, as described in detail elsewhere [15]. Heart rate response to the Valsalva manoeuvre was obtained by the participant exhaling into a mouthpiece of a manometer to 40 mmHg for 15 s during ECG monitoring. In this test, healthy subjects were expected to develop tachycardia and peripheral vasoconstriction during strain and an overshoot bradycardia and rise in blood pressure with release. The normal ratio of longest RR to shortest RR should be >1.2 . For the systolic blood pressure response to standing, systolic blood pressure is measured in the supine participant, and then again after 2 min of standing. A normal response is a fall of <10 mmHg, a borderline response is a fall of 10–29 mmHg, and an abnormal response is a fall of >30 mmHg with symptoms. For the heart rate response to standing tests the 30:15 ratio was made during continuous ECG monitoring; the RR interval was measured at beats 15 and 30 after standing. Normally, tachycardia is followed by reflex bradycardia. The normal 30:15 ratio is >1.03 . Finally, the expiration-to-inspiration ratio was performed with the participant at rest and supine and taking into account the participant's age; heart rate was monitored by ECG while the participant breathed in and out at 6 breaths per min: a difference in heart rate of < 10 beats per min is abnormal.

Other variables included in the analyses were: sex, age (40–49, 50–59, 60–69 or ≥ 70 years), number of years with a diagnosis of diabetes (< 10 and ≥ 10 years), BMI (<25 kg/m², 25 and <30 kg/m² and ≥ 30 kg/m²), hypertension (systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg and/or current anti-hypertensive treatment [19]), microalbuminuria, and retinopathy. Patients were defined as having microalbuminuria if microalbumin levels were >20 mg/l. Retinopathy was determined by an ophthalmologist through examination of the fundus of both eyes to examine the blood vessel architecture after dilation with tropicamide 1%.

Sample size and power calculation

Assuming a 5% significance level, with > 150 participants in each group, the study had >80% power to detect a sensitivity of 75% (or specificity of 75%) with a precision in the estimates of 7.5%. In addition, with this sample size, the study had > 80% power to detect an area under the curve (AUC) of up to 90%.

Statistical analysis

Comparisons between studied groups were performed using Student's *t*-test or a chi-squared test, as appropriate. Pupillometry variables, as numerical values, were compared as a screening tool for diabetes and cardiac autonomic neuropathy, and the AUC was calculated. According to the Youden index [20,21], the best threshold for each pupillometry variable was selected, reporting sensitivity and specificity, as well as likelihood ratios, positive and negative. In addition, the 'rocreg' command in STATA was used to verify the effect of some variables (e.g. age), in the receiver-operator curve calculations. In addition, some extra analyses, according to site and participants characteristics were also performed. STATA 11 for Windows (STATA Corp, College Station, TX, USA) was used for analysis.

Results

Study population

A total of 424 participants were enrolled from both the hospital and community study sites. Of these, 40 were excluded from the analysis: one who was aged < 40 years, and one aged > 75 years, and 38 because of errors in pupillometry readings yielding unavailable results; therefore, data from 384 participants [mean ($\pm$ SD) age 57.6 ($\pm$ 8.3) years, 196 (51%) women] were analysed.

A total of 165 participants with diabetes were recruited at the Hospital Nacional Cayetano Heredia, of whom 46% had been diagnosed for 10 years. At the community study site, 40 participants with a previous diagnosis of diabetes were identified from the CRONICAS cohort study and were included without an oral glucose tolerance test. A random sample of 179 subjects without a previous diagnosis of diabetes underwent an oral glucose tolerance test and 8/179 (4.5%, 95% CI 1.9–8.6) were further classified as having diabetes, 20/179 (11.2%, 95% CI 7.0–16.7) as having impaired glucose tolerance and 151/179 as without diabetes. Detailed characteristics of the population according to diagnosis and study site are shown in Table 1.

Pupillometry variables as a screening tool for diabetes

Data from 213 participants with diabetes were compared with those from 151 participants without diabetes. There was strong evidence of differences in several pupillometry variables between diabetes groups (Table 2), yet the AUC of these values was not > 0.70 (Table 3). Amplitude of the pupil reaction had an AUC of 0.69, and using this variable's information the best threshold (5.97) had a sensitivity of 77.5% and a specificity of 55.4%. When diagnostic ability of pupillometry was compared according to study setting, results did not differ from the results presented (data not shown).

Pupillometry variables as a screening tool for cardiac autonomic neuropathy

Clinical cardiac autonomic neuropathy evaluations, conducted among hospital participants only, were completed in 138/165 participants with diabetes and 68 (49.6%) were positive according to the 30:15 ratio only, 67 (48.6%) were positive according to the Valsalva manoeuvre test, 29 (21.0%) were positive according to the expiration-to-inspiration ratio, and only four (2.9%) were positive according to the systolic blood pressure response to standing. Overall, cardiac autonomic neuropathy was positive in 51/138 participants (37.0%, 95% CI 28.8–45.1), and only duration of disease ≥ 10 years was associated with cardiac autonomic neuropathy ($P < 0.001$).

As shown in Table 2, there was strong evidence of differences in baseline pupil radius and amplitude of the pupil reaction between cardiac autonomic neuropathy groups, and they appeared to be potential markers for cardiac autonomic neuropathy screening with AUCs of 0.71 and 0.70, respectively (Table 4). In the case of baseline pupil radius, the best threshold was 21.79, with a sensitivity of 50.6% and a specificity of 84.3%; whereas amplitude of the pupil reaction had a threshold of 4.88, a sensitivity of 81.6% and specificity of 54.9%.

Other relevant findings among individuals with diabetes

There were differences between some pupillometry variables and age, time of disease among participants with diabetes, and BMI categories (Table S1). In addition, about half of the participants with Type 2 diabetes were obese (54.2% in the community setting and 42.4% in the hospital). Approximately 70% of participants with diabetes did not meet the HbA_{1c} target levels, and these findings were similar when comparing individuals from both study sites ($P = 0.27$). Hypertension was present in 44/213 participants with diabetes (20.7%), with no differences between study sites ($P = 0.21$). Rates of microalbuminuria were higher among the participants with diabetes from the community at 83.3%, compared with 47.3% of hospital participants ($P < 0.001$). In addition, 41/198 (20.7%) of all participants with diabetes presented some degree of retinopathy, with no apparent differences according to study site ($P = 0.30$).

Discussion

The results of the present study showed that there were important differences in several pupillometry variables after a well-established diagnosis of diabetes and cardiac autonomic neuropathy; however, most of the pupillometry variables had poor accuracy as a screening tool, with low sensitivity and specificity. Notably, amplitude of pupil reaction was the single best variable with reasonable accuracy, enabling it to be considered as a screening tool for diabetes and cardiac autonomic neuropathy. The likelihood ratios for all the pupillometry variables were very close to values compatible with minimal change in the likelihood of disease. According to the literature, likelihood ratios of > 10 and < 0.1 provide strong evidence to rule in and rule out diagnoses, respectively, in most circumstances [22].

The rationale for the present study was to identify a simple-to-use, low-cost, laboratory-free, bloodless technique that could allow an estimation of both diabetes and cardiac autonomic neuropathy in a non-specialized clinical facility, in particular, in resource-poor settings. Yet,

pupillometry remains a niche technique that enables a crude assessment of the integrity of the autonomic nervous system, and changes in pupillometry variables within a patient over time may indicate the development of cardiac autonomic neuropathy that can be validated by a medical examination. Moreover, the American Diabetes Association recommends an initial assessment of cardiac autonomic neuropathy at the time of diabetes diagnosis, and then annual follow-up examinations; however, the time used for this evaluation can be demanding [18]. Thus, future studies that track pupillometry responses over time in patients with diabetes will provide us with data on the feasibility of using pupillometry for this purpose.

The present study also found a high rate of individuals with diabetes not meeting HbA_{1c} control targets, as previously reported [3,23]. This was particularly prevalent among hospital-based participants, where almost half of participants had > 10 years of disease. Consistent with previous studies [24,25], we found high rates of microalbuminuria in the present study. We found higher rates of microalbuminuria in the population cohort than in the hospital. This may be attributable to the previous lack of awareness of their albumin status before this study, and therefore a lower likelihood of treatment. This observation is important because, for limited resource settings, it puts into context and identifies treatment gaps in relation to the evidence generated from different trials, which have shown that significant reduction in albumin levels in urine follow after achieving an HbA_{1c} target of <6.5% [26–28], but such change has not been observed for retinopathy or neuropathy [29]. Intensive glycaemic control is therefore needed to avoid the microvascular complications of diabetes, and this goal must be reached as soon as the diagnosis is made. Whether pupillometry may have a role in the monitoring of patients with diabetes for the development of complications remains an area for further exploration.

The strengths of the present study include the use of a well-established clinical diagnosis of diabetes as well as the use of oral glucose tolerance test for classifying those without diabetes as our control subjects [16]. This strength, together with leveraging from the population-based sample of the CRONICAS cohort study alongside recruitment in a clinical facility, enhanced the selection of well-defined comparison groups, and enabled us to select participants with diabetes of short and long duration. The study also has some limitations. For example, its results are not transferable because participants, especially those with diabetes in the hospital, were not randomly selected; nevertheless estimations of diabetes prevalence are within range of those reported in previous studies in the same area [30]. In addition, the proportion of overweight and obese people in the group of healthy participants was higher than in the general Peruvian population (e.g. 39.1 vs. 16.0% obese) [31]. Insulin resistance is associated with obesity [32], and hence potentially with autonomic dysfunction; therefore, this avenue of research, possibly using population-based and longitudinal study designs, could provide further insights into the interplay between insulin resistance and obesity with autonomic dysfunction, either as an independent or diabetes-mediated entity. We also excluded from pupillometry analysis participants from the community who were found to have impaired glucose tolerance, as there were not enough participants with impaired glucose tolerance to give the study high enough power for statistical analysis. Future studies that include more participants would be able to make comparisons among groups with impaired glucose tolerance, with diabetes and without diabetes; however, for

the purposes of the present study, we wanted to investigate whether there were statistically significant differences between people with and without diabetes to encourage the use of pupillometry as a screening tool for diabetes or cardiac autonomic neuropathy.

Although pupillometry can be a simple and inexpensive technique, useful for diabetic autonomic neuropathy, and some of its variables might distinguish between individuals with diabetes from those without diabetes as well as identifying those with and without cardiac autonomic neuropathy, its accuracy for discrimination as a screening tool is insufficient. Cardiac autonomic neuropathy presents a significant cause of morbidity and mortality in patients with diabetes [33], and is associated with a high risk of cardiac arrhythmias and sudden death [34,35]; however, once cardiac autonomic neuropathy has developed, implementation of tight glycaemic control will not improve patient outcomes. A simple screening tool to predict which patients with diabetes will go on to develop cardiac autonomic neuropathy could be useful, therefore, for targeting intervention efforts and ultimately to reduce progression to cardiac autonomic neuropathy and achieve lower mortality. In the present study we selected people with diabetes that had both long and short duration of disease, as previous studies have shown that the risk of cardiac autonomic neuropathy increases with the duration of diabetes, and we wanted to capture a comprehensive group to study differences in pupillometry and cardiac autonomic neuropathy. In addition, previous studies have shown pupillometry variables such as pupil size and dilation velocity were negatively correlated with age, and our data corroborated these findings [36]. Future studies that explore whether the differences in pupillary light reflexes are able to identify patients with diabetes that will progress to cardiac autonomic neuropathy are needed to determine whether pupillometry is a valid tool for this purpose. As pupillary autonomic dysfunction may occur early in diabetes [10], its assessment may well have a role as part of screening, evaluation and monitoring of adherence to diabetes treatment, and in the assessment of specific diabetes-related complications, taking advantage, in particular, of future technological developments. Alterations to the autonomic nerve system can be non-homogenous [8], and thus a better exploration of the pupil and its innervation is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What's new?

- Rapid, easy-to-implement, point-of-care, bloodless and laboratory-free screening tools would advance the field of diabetes. Pupillometry is such a tool and was performed following an automated technique and using a portable computer to minimize measurement error, including operator-dependent bias.
- The usefulness of pupillometry as a screening tool for Type 2 diabetes in clinical and community settings has not been previously explored. The results from this study showed that pupillometry was not good enough to recommend its usage as a screening tool.
- Our results do not exclude the usefulness of pupillometry for neuropathy diagnosis or for monitoring and patient follow-up at clinical encounters.

Figure 1. Pupillometry variables and cardiac autonomic neuropathy: receiver–operator characteristic (ROC) curves using (a) baseline pupil radius, and (b) amplitude of the pupil reaction.

Table 1

Characteristics of the population according to diagnosis and study site

	Community setting*		Hospital facility	<i>P</i>
	Group without diabetes (<i>n</i> = 151)	Group with diabetes (<i>n</i> = 48)	With diabetes (<i>n</i> = 165)	
Sex: male, <i>n</i> (%)	83 (55.0)	24 (50.0)	76 (46.1)	0.29
Mean (SD) age	56.1 (8.6)	57.7 (7.9)	58.9 (8.3)	0.01
Age, <i>n</i> (%)				0.009
40–49 years	40 (26.5)	8 (16.7)	23 (13.9)	
50–59 years	66 (43.7)	20 (41.7)	63 (38.2)	
60–69 years	31 (20.5)	18 (37.5)	60 (36.4)	
70 years	14 (9.3)	2 (4.1)	19 (11.5)	
Mean (SD) years of diabetes	-	3.8 (5.0)	8.6 (6.3)	
Years of diabetes, <i>n</i> (%)[†]				< 0.001
10 years		2 (5.0)	76 (46.1)	< 0.001
Mean (SD) BMI	29.2 (5.8)	30.6 (4.3)	29.3 (5.2)	0.27
BMI, <i>n</i> (%)				
< 25 kg/m ²	31 (20.5)	5 (10.4)	26 (15.8)	
25 and < 30 kg/m ²	61 (40.4)	17 (35.4)	69 (41.8)	0.31
30 kg/m ²	59 (39.1)	26 (54.2)	70 (42.4)	
Hypertension, <i>n</i> (%)	16 (10.6)	13 (27.8)	69 (41.8)	< 0.001
Mean (SD) HbA_{1c}	5.8 (0.3)	8.4 (2.3)	8.7 (2.2)	< 0.001
HbA_{1c} 7.0%, <i>n</i> (%)[†]		31 (64.6)	120 (72.7)	0.28
Microalbuminuria, <i>n</i> (%)	18 (12.1)	21 (43.8)	33 (20.0)	< 0.001
Retinopathy, <i>n</i> (%)[†]	-	5/35 (14.3)	36/163 (22.1)	0.30
Cardiac autonomic dysfunction, <i>n</i> (%)	-	-	51/138 (37.0%)	-

Results may not add up due to missing values.

* Classification of diabetes status was based on oral glucose tolerance test results.

[†] Comparisons were performed considering only information from individuals with diabetes according to setting.

Table 2
Comparison of pupillometry variables by diabetes and cardiac autonomic neuropathy status

Pupillometry variable	Non-diabetes (<i>n</i> = 151)	Diabetes (<i>n</i> = 213)	<i>P</i>	Without cardiac autonomic neuropathy (<i>n</i> = 87)	With cardiac autonomic neuropathy (<i>n</i> = 51)	<i>P</i>
Baseline pupil radius, mm	21.96 (3.31)	20.22 (3.87)	<0.0001	21.11 (3.69)	18.30 (3.90)	0.0001
Amplitude of the pupil reaction, mm	6.72 (1.23)	5.74 (1.79)	<0.0001	6.01 (1.49)	4.77 (1.93)	0.0002
Ratio R2/R1	0.69 (0.05)	0.72 (0.06)	<0.0001	0.71 (0.06)	0.75 (0.08)	0.01
Constriction ratio	0.52 (0.07)	0.48 (0.09)	<0.0001	0.49 (0.08)	0.44 (0.12)	0.01
Latency to maximum constriction, s	0.80 (0.06)	0.79 (0.07)	<0.0001	0.79 (0.06)	0.77 (0.06)	0.12
Latency for onset of constriction, s	0.23 (0.04)	0.24 (0.10)	0.10	0.23 (0.06)	0.26 (0.15)	0.21
Time to 75% re-dilation, s	0.99 (0.19)	0.91 (0.21)	0.0001	0.92 (0.21)	0.83 (0.19)	0.02
Re-dilation latency, s	0.70 (0.06)	0.70 (0.06)	0.82	0.70 (0.06)	0.69 (0.06)	0.20

Data are mean (SD) values.

* Comparisons were performed using a *t*-test for independent samples.

Table 3
Pupillometry variables by diabetes status: receiver–operator curve analysis and area under the curve results

Pupillometry variable	AUC	Best threshold	Sensitivity, %	Specificity, %	LR+	LR–
Baseline pupil radius	0.63	20.06	70.9	48.4	1.37	0.60
Amplitude of the pupil reaction	0.69	5.97	77.5	55.4	1.74	0.41
Ratio R2/R1	0.63	0.74	35.7	88.1	2.99	0.73
Constriction ratio	0.63	0.45	88.1	35.7	1.37	0.33
Latency to maximum constriction (s)	0.51	0.72	92.7	13.6	1.07	0.54
Latency for onset of constriction (s)	0.57	0.27	22.5	92.7	3.09	0.84
Time to 75% re-dilation (s)	0.62	0.79	87.4	35.2	1.35	0.36
Re-dilation latency	0.51	0.64	89.4	16.0	1.06	0.66

AUC, area under the curve.
Sensitivity, specificity, LR+ and LR– were calculated for the best thresholds for the pupillometry variables.

Pupillometry variables by cardiac autonomic neuropathy among patients with diabetes: receiver–operator curve analysis and area under the curve results

Table 4

Pupillometry variable	AUC	Best threshold	Sensitivity, %	Specificity, %	LR+	LR–
Baseline pupil radius	0.71	21.79	50.6	84.3	3.22	0.59
Amplitude of the pupil reaction	0.70	4.88	81.6	54.9	1.81	0.34
Ratio R2/R1	0.62	0.73	60.8	58.6	1.47	0.67
Constriction ratio	0.62	0.40	92.0	29.4	1.30	0.27
Latency to maximum constriction (s)	0.58	0.79	57.5	58.8	1.40	0.72
Latency for onset of constriction (s)	0.58	0.26	45.1	77.0	1.96	0.71
Time to 75% re-dilation (s)	0.62	0.80	66.7	52.9	1.42	0.63
Re-dilation latency	0.57	0.70	57.5%	58.8%	1.40	0.72

AUC, area under the curve.

Sensitivity, specificity, LR+ and LR– were calculated for the best thresholds for the pupillometry variables.