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Association Between Glycemic Variability and Pupillary Autonomic Function in Type 2 Diabetes: A Cross-Sectional Study

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Abstract

Background: Although glycated hemoglobin (HbA1c) reflects average glycemia, it does not capture glucose fluctuations. Glycemic variability has been linked to cardiovascular autonomic neuropathy, but its association with pupillary autonomic function in type 2 diabetes remains unclear. We examined this association using continuous glucose monitoring (CGM) and dynamic pupillometry.

Methods: We conducted a single-center, cross-sectional analysis of 53 patients with type 2 diabetes from a prospective cohort. All had a phakic study eye, at least 10 days of valid CGM data, and no use of medications known to affect the pupillary light reflex. Glycemic variability was quantified as the standard deviation (CGM SD) and coefficient of variation (%CV) of sensor glucose. Eye-tracking pupillometry assessed five indices, including the sympathetically influenced re-dilation ratio (D1%) and the predominantly parasympathetically mediated relative constriction amplitude (AMP%). We used Pearson correlations and further examined the D1%–glycemic variability associations using multivariable linear regression adjusted for age, sex, diabetes duration, and HbA1c.

Results: The mean age was 64.5 ± 8.6 years, and mean HbA1c was 7.1 ± 0.6%. D1% correlated positively with CGM SD ($r = 0.316$, $P = 0.021$) and %CV ($r = 0.302$, $P = 0.028$), but not with mean glucose or time in range. Both associations remained after adjustment (CGM SD: $\beta = 0.229$, $P = 0.005$; %CV: $\beta = 0.303$, $P = 0.024$) and across post hoc adjustment sets accounting for constriction amplitude, cardiac autonomic function, peripheral neuropathy, and retinopathy stage. In unadjusted analyses, AMP% was not significantly correlated with any CGM metric, and HbA1c was not significantly correlated with any pupillary index. The unadjusted D1% associations did not survive correction for multiple comparisons.

Conclusions: Greater CGM-derived glycemic variability was associated with a higher pupillary re-dilation ratio after adjustment for HbA1c and other covariates, whereas no corresponding association was observed for constriction amplitude. These exploratory findings require confirmation in larger, longitudinal studies before their physiological and clinical significance can be established.

Keywords: Glycemic variability; Continuous glucose monitoring; Pupillometry; Pupillary light reflex; Autonomic nervous system; Type 2 diabetes

Background

Diabetic autonomic neuropathy is a common but frequently underdiagnosed complication of diabetes mellitus, associated with substantial morbidity and increased mortality [1,2]. Pupillary dynamics are governed by the opposing actions of the parasympathetic and sympathetic nervous systems, and dynamic pupillometry provides a noninvasive means of probing autonomic function [3]. In type 2 diabetes, pupillary light reflex (PLR) abnormalities—reduced baseline pupil size, decreased constriction amplitude, and prolonged latency—can appear before clinically evident retinopathy [4–7]. Parasympathetic impairment was traditionally thought to precede sympathetic dysfunction [8], although detailed PLR studies indicate that the sympathetic pathway is also affected [9].

Chronic hyperglycemia, conventionally assessed by glycated hemoglobin (HbA1c), is an established risk factor for the development of diabetic complications [10]. Nevertheless, HbA1c provides only an average measure of glucose control over several months and fails to capture day-to-day or intraday glucose fluctuations. Advances in continuous glucose monitoring (CGM) have highlighted glycemic variability—often quantified by the standard deviation (SD) or coefficient of variation (%CV) of sensor glucose—as a risk factor for microvascular and macrovascular complications [11], as well as cardiovascular autonomic neuropathy in both type 1 and type 2 diabetes, beyond the effects of sustained hyperglycemia alone [12–14]. Because the pupillary light reflex is under autonomic control, glycemic variability might therefore affect pupillary function.

The primary aim of this cross-sectional study was to determine whether CGM-derived glycemic variability is associated with dynamic pupillary light reflex function in patients with type 2 diabetes. In addition, we secondarily explored the associations of the individual pupillary

indices with other diabetes-related characteristics, including disease duration, diabetic nephropathy, cardiac autonomic function, and peripheral nerve conduction.

Methods

Study Design and Participants

This cross-sectional analysis was conducted within the Hyogo Diabetes Hypoglycemia Cognition Complications (HDHCC) study, a prospective cohort investigating associations between CGM-derived metrics and chronic complications in patients with diabetes [15]. The study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Hyogo Medical University Hospital (Approval No. 4250), and was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000032143). All methods were performed in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all participants.

This cross-sectional secondary analysis included HDHCC participants who were referred to the Department of Ophthalmology between January 2021 and March 2026. Because pupillometry had been performed as part of the cohort's ophthalmic assessment, eligibility for the present analysis was determined retrospectively by review of the ophthalmology and internal-medicine records of the pupillometry visit. Inclusion criteria were a confirmed diagnosis of type 2 diabetes and at least 10 days of valid CGM data. Exclusion criteria, assessed in the right eye, were (1) prior intraocular surgery (i.e., a pseudophakic lens) or panretinal photocoagulation; (2) other ocular disease that could affect the pupillary light reflex, such as glaucoma, optic neuritis, or severe macular disease; and (3) use of topical mydriatic or miotic agents, or systemic medications that may modify the pupillary light reflex, including agents with anticholinergic,

sympathomimetic, α- or β-adrenergic blocking, or opioid activity, consistent with prior pupillographic protocols [16,17].

Of these 223 referred participants, 131 had a good-quality right-eye recording; 66 were then excluded (no type 2 diabetes, $n = 23$; pseudophakic study eye, $n = 18$; panretinal photocoagulation, $n = 2$; <10 days of CGM, $n = 23$), leaving 65 eligible patients. Of these, 12 receiving autonomic-active medications (α-adrenergic blocking agents, $n = 10$; β-blocker, $n = 1$; tramadol, $n = 1$) were excluded, yielding 53 phakic eyes of 53 patients (Figure 1).

Continuous Glucose Monitoring

CGM was performed with FreeStyle Libre Pro (Abbott Diabetes Care, Alameda, CA, USA). Participants wore the sensor on the back of the upper arm for up to 14 days while maintaining their usual diet and activities. Only participants with at least 10 days of valid data were analyzed. Standard CGM metrics were derived according to international consensus recommendations [18]: mean glucose, time in range (TIR, 70–180 mg/dL), time above range (TAR, >180 mg/dL), time below range (TBR, <70 mg/dL), and glycemic variability, expressed as the standard deviation of sensor glucose (CGM SD) and the coefficient of variation (%CV; CGM SD / mean glucose × 100).

Dynamic Pupillometry

The dynamic pupillary light reflex was measured with a screen-based eye-tracking system (Tobii Pro Spectrum; Tobii Pro AB, Danderyd, Sweden) at a sampling frequency of 300 Hz, following a previously validated protocol [16]. Testing was performed in a dimly lit room, with the participant seated 60 cm from a 23.8-inch monitor. After 2 minutes of dark adaptation, a full-

screen white-light stimulus (13 cd/m²) was presented for 1 second while the pupillary response was recorded. Blink artifacts were removed and corrected by linear interpolation, and the signal was smoothed with a 10-point moving-average filter. Five parameters were extracted from the waveform (Figure 2): (1) the baseline pupil diameter (BPD), the mean diameter during the 1-s pre-stimulus period; (2) the constriction latency, the interval from stimulus onset to the onset of reflex constriction; (3) the relative constriction amplitude (AMP%), calculated as (BPD − minimum pupil diameter) / BPD, which is predominantly mediated by the parasympathetic nervous system; (4) the maximum constriction velocity (MCV), the peak velocity of pupillary constriction, likewise mediated predominantly by the parasympathetic nervous system; and (5) the re-dilation ratio (D1%), the pupil diameter 3.5 s after stimulus offset divided by BPD, which is sympathetically influenced, as re-dilation proceeds through both withdrawal of parasympathetic tone and sympathetic activation via the superior cervical ganglion [16]. Constriction latency was determined from the same 300-Hz waveform, to which a 7-point median filter was additionally applied before moving-average smoothing to suppress residual blink spikes. The pre-stimulus trend was modeled by linear regression of pupil diameter over the 0.5 s preceding stimulus onset, and latency was estimated by two complementary methods: in the tangent method, as the intersection of this trend with the tangent to the constriction curve at the point of maximum constriction velocity; in the threshold method, as the first point at which the diameter fell ≥0.1 mm below the extrapolated trend and reached ≥0.3 mm below it within the following 0.3 s. When both estimates lay within a physiologically plausible range (150–550 ms) and differed by ≤80 ms, their mean was used (n = 48; median absolute difference, 18 ms [interquartile range, 10–27 ms]); when only one was plausible, that estimate was used (n = 2, both threshold estimates after a distorted tangent fit). Recordings in which neither estimate was

plausible were inspected visually, and in all 3, a blink or pupillary instability at stimulus onset precluded reliable determination; these were treated as missing.

Clinical and Laboratory Measurements

Clinical characteristics (age, sex, diabetes duration, body mass index, and blood pressure) and laboratory data (HbA1c and estimated glomerular filtration rate, measured in fasting blood samples) were extracted from the Department of Diabetes, Endocrinology and Clinical Immunology outpatient records of a visit on the same day as, or within a few days of, the pupillometry examination. Microvascular complications and medication use were obtained from medical records. Diabetic retinopathy was defined as the presence of mild non-proliferative diabetic retinopathy or worse in the measured right eye, graded according to the International Clinical Diabetic Retinopathy severity scale. Diabetic neuropathy was defined as the presence of either neuropathic symptoms on clinical interview or a sural nerve conduction abnormality graded as mild or worse on a point-of-care nerve conduction device (DPN-Check; graded as normal, mild, moderate, or severe). Diabetic nephropathy was staged according to the Japanese classification of diabetic nephropathy; stage 2 or higher (microalbuminuria or worse) was regarded as diabetic nephropathy. Sural nerve conduction velocity and sensory nerve action potential amplitude were measured bilaterally with a point-of-care nerve conduction device (DPN-Check) and are reported as the mean of both legs. Cardiovascular autonomic function was assessed by the coefficient of variation of the R–R interval (CVRR) on resting electrocardiography. CVRR was analyzed in 46 patients after excluding 2 with unreliable electrocardiographic recordings.

Statistical Analysis

Continuous variables are presented as mean ± SD and categorical variables as numbers and percentages. Associations between the pupillary indices and the glycemic, clinical, autonomic, and peripheral-nerve measures were assessed with the Pearson correlation coefficient (point-biserial correlation for the binary nephropathy variable). Normality was assessed with the Shapiro–Wilk test; D1% deviated from a normal distribution ($P = 0.002$), whereas CGM SD and %CV did not ($P = 0.38$ and $P = 0.66$, respectively). To assess robustness to distributional assumptions and influential observations, correlations involving D1% were additionally examined after log-transformation, after exclusion of an influential outlier, and with the Spearman rank correlation. The association between CGM SD and constriction latency was likewise examined with the Spearman rank correlation. These correlations were exploratory and involved multiple comparisons; they were therefore interpreted as hypothesis-generating, with a Bonferroni-adjusted threshold reported. Analyses were performed in Python 3.11 (scipy 1.18, statsmodels 0.14), with a two-sided $P < 0.05$ considered statistically significant.

Results

Baseline Characteristics

The analysis comprised 53 Japanese patients with type 2 diabetes (Table 1). The mean age was 64.5 ± 8.6 years, and 34 patients (64.2%) were men. The mean diabetes duration was 13.3 ± 11.0 years, and the mean HbA1c was 7.1 ± 0.6%. Diabetic retinopathy was present in 12 patients (22.6%), diabetic neuropathy in 9 (17.0%), and diabetic nephropathy (stage ≥2) in 13 (24.5%). Insulin therapy was used by 8 patients (15.1%).

Pupillary Responses and CGM Parameters

Descriptive statistics for the CGM and pupillometry parameters are shown in Table 1.

Among the pupillary indices, D1% was positively correlated with CGM SD ($r = 0.316$, $P = 0.021$; Figure 3a), whereas it was not correlated with mean glucose or the time-in-range metrics (Table 2), indicating specificity for glycemic variability rather than overall glycemic level. D1% was similarly correlated with %CV ($r = 0.302$, $P = 0.028$), which was unrelated to mean glucose ($r = -0.033$). Greater CGM SD, but not %CV ($r = -0.218$, $P = 0.13$), was also associated with a shorter constriction latency ($r = -0.298$, $P = 0.036$), whereas baseline pupil diameter, constriction amplitude, and maximum constriction velocity were unrelated to any CGM metric. The D1%–CGM SD association remained significant after log-transformation of CGM SD ($r = 0.315$, $P = 0.022$) and after exclusion of a single influential observation ($r = 0.385$, $P = 0.005$), and was directionally consistent with the Spearman correlation ($\rho = 0.270$, $P = 0.051$). AMP% was not associated with any CGM metric (Table 2; Figure 3b), whereas D1% and AMP% were themselves inversely correlated ($r = -0.556$, $P < 0.001$), reflecting in part their shared dependence on baseline pupil diameter. Constriction latency was not correlated with the other pupillary indices (all $P > 0.40$), indicating that it captured a feature distinct from the other measures.

Pupillary Indices and Non-CGM Measures

Beyond glycemic variability, the pupillary indices showed distinct associations with clinical, autonomic, and peripheral-nerve measures (Table 3). Longer diabetes duration was associated with lower AMP% ($r = -0.453$, $P < 0.001$) and lower MCV ($r = -0.559$, $P < 0.001$), and diabetic nephropathy (stage ≥2) with a smaller baseline pupil diameter ($r = -0.433$, $P = 0.001$); neither

was associated with the other indices. HbA1c was not correlated with any pupillary index (all $P > 0.25$), even though HbA1c and CGM SD were themselves correlated ($r = 0.546$, $P < 0.001$). Neither cardiac autonomic function (CVRR) nor sural nerve conduction velocity was significantly associated with any pupillary index (all $P > 0.10$). Among these correlations, those of AMP% and MCV with diabetes duration and of BPD with nephropathy remained below the Bonferroni-adjusted threshold ($\alpha = 0.002$), whereas the others should be regarded as exploratory.

Independent Associations Between Glycemic Variability and Autonomic Function

Given the associations observed between D1% and both glycemic variability metrics, this relationship was examined further with multivariable linear regression adjusting for other characteristics (age, sex, HbA1c, and diabetes duration). The relationship was modeled bidirectionally, with D1% and CGM SD each entered as the dependent variable in turn (Model 1 and Model 2, respectively); multicollinearity was assessed with the variance inflation factor, and Model 1 was repeated with log-transformed D1%. Both models were then repeated with %CV in place of CGM SD. Multivariable linear regression results are presented in Table 4 (panel A, CGM SD; panel B, %CV). All VIF values were below 1.7 (maximum, 1.62), indicating no meaningful multicollinearity. The residuals of Model 1 deviated from normality (Shapiro–Wilk $P = 0.006$), whereas those of Model 2 did not ($P = 0.92$).

In Model 1 (dependent variable, D1%), CGM SD was significantly and positively associated with D1% ($\beta = 0.229$; 95% CI, 0.074 to 0.385; $P = 0.005$), indicating that greater glycemic variability is independently linked to enhanced pupillary re-dilation. Higher HbA1c was associated with lower D1% ($\beta = -2.630$; $P = 0.046$). The overall model was significant (adjusted $R^2 = 0.137$; $P = 0.035$).

In Model 2 (dependent variable, CGM SD), D1% was significantly and positively associated with CGM SD (β = 0.691; 95% CI, 0.224 to 1.158; $P = 0.005$). Higher HbA1c was also strongly associated with increased CGM SD (β = 9.667; $P < 0.001$). This model showed higher explanatory power (adjusted $R^2 = 0.377$; $P < 0.001$). In the robustness check using log-transformed D1% as the dependent variable in Model 1, the positive association with CGM SD remained significant (β = 0.0026; $P = 0.005$), confirming the robustness of the findings. With %CV in place of CGM SD (Table 4, panel B), the association was concordant in both directions (Model 1: β = 0.303; 95% CI, 0.041 to 0.565; $P = 0.024$; Model 2: β = 0.341; 95% CI, 0.046 to 0.636; $P = 0.024$). Unlike in the CGM SD models, HbA1c was associated with neither D1% nor %CV ($P = 0.39$ and $P = 0.15$), and the overall fit of both models was weaker (adjusted R^2 = 0.080 and 0.078; model $P = 0.11$ and 0.12).

The CGM SD–D1% association was robust across post hoc adjustments for constriction amplitude, cardiac autonomic function (CVRR), peripheral neuropathy, and retinopathy stage (Additional file 1: Table S1). AMP% was itself independently associated with D1% but not with CGM SD, and its inclusion attenuated the CGM SD coefficient by 19% without eliminating it; CVRR, peripheral neuropathy, and retinopathy stage were not independently associated with D1%. In change-in-estimate analyses, HbA1c was the only meaningful confounder—its inclusion strengthened the CGM SD coefficient, indicating negative confounding—and both backward and forward selection converged on a parsimonious model containing CGM SD and HbA1c. The %CV–D1% association was likewise robust to these adjustments (β = 0.267 to 0.353; all $P < 0.03$), and both selection procedures retained %CV alone (Additional file 1: Table S2).

Discussion

In this cross-sectional analysis of 53 patients with type 2 diabetes, greater CGM-derived glycemic variability, whether expressed as its absolute (CGM SD) or mean-normalized (%CV) magnitude, was independently associated with enhanced pupillary re-dilation (D1%), after adjustment for age, sex, diabetes duration, and HbA1c. In contrast, the parasympathetically mediated constriction amplitude (AMP%) was not associated with any CGM metric. These findings suggest that glycemic variability is related to a change in pupillary autonomic function that appears preferential for the sympathetic pathway and independent of mean glycemia as reflected by HbA1c.

The direction and physiological basis of this association merit careful interpretation. Established diabetic autonomic neuropathy is typically characterized by progressive denervation and, in the pupil, by reduced and slowed responses [1,6,7]; a purely neurodegenerative process would be expected to blunt, rather than enhance, re-dilation. Pupillary re-dilation, however, is not a pure sympathetic measure: it is driven both by withdrawal of parasympathetic tone—which dominates the early phase and classically precedes sympathetic change—and by sympathetic activation, which emerges later [16,19]. An enhanced re-dilation (higher D1%) with greater glycemic variability is therefore most consistent with a functional, potentially early stage of autonomic dysregulation in which parasympathetic suppression coincides with emerging sympathetic activation, rather than with fixed sympathetic overactivity. This hypothesis is consistent with the accompanying finding that higher CGM SD was associated with a shorter constriction latency but not with the amplitude or maximum velocity of constriction, indicating a brisker, more dynamic reflex rather than a stronger parasympathetic response. It also aligns with evidence that acute glucose fluctuations provoke oxidative stress [20] and may promote

sympathetic activation, with the recognized sympathetic overactivity of the dysmetabolic state [21], and with reports that insulin itself acutely raises sympathetic nerve activity independently of glycemia [22]. The sympathetic dysregulation may thus manifest as overactivity, reflected in increased re-dilation, rather than only as reduced dilation, and the re-dilation phase provides a specific, noninvasive readout of the sympathetic component—an interpretation supported by the elevation of the same index, and its correlation with the low-frequency heart-rate–variability band, in a non-neurodegenerative, stress-related chorioretinal condition [16]. The metabolic drivers that differentially affect the parasympathetic and sympathetic pathways remain incompletely understood.

Beyond this primary association, the other pupillary indices related to markers of cumulative disease rather than to glycemic variability, with different components of the response mapping onto different facets of the disease (Table 3). Lower constriction amplitude (AMP%) and lower maximum constriction velocity were associated with longer diabetes duration, and a smaller baseline pupil diameter with diabetic nephropathy—a smaller resting pupil being a recognized sign of sympathetic denervation in more advanced disease [6,9]. In cross-section, this pattern parallels the recognized progression from early, functional autonomic dysregulation toward the parasympathetic decline and structural sympathetic denervation of cumulative disease and microvascular complications; an analogous sequence—early parasympathetic impairment and a relative sympathetic predominance preceding sympathetic denervation—is well described for cardiovascular autonomic neuropathy [1,2]. These associations were exploratory, and only those of AMP% and maximum constriction velocity with duration and of baseline pupil diameter with nephropathy remained below the Bonferroni-adjusted threshold.

Our findings connect two largely separate lines of research. Glycemic variability has been linked to diabetic autonomic neuropathy, but that evidence has come almost entirely from cardiovascular reflex and heart-rate–variability testing—in which greater variability has been associated with reduced heart-rate variability, R–R interval variation, and baroreflex sensitivity, in part independently of HbA1c [12–14,23,24]—whereas dynamic pupillometry has typically been applied to more established disease and has emphasized reduced, slowed responses [4–9]. To our knowledge, no previous study has directly linked CGM-derived glycemic variability to dynamic pupillary autonomic function in type 2 diabetes. Moreover, whereas a previous pupillometric study found parasympathetic constriction measures to be more prominently affected than re-dilation in relation to diabetic retinopathy severity [3]—a marker of more advanced structural disease—we observed the opposite pattern, an enhancement of the sympathetic re-dilation component associated with dynamic glucose fluctuations, potentially reflecting a distinct or earlier, functional process.

The adjustment variables were selected because each is plausibly related to pupillary function or glycemic variability: pupillary re-dilation declines with age [25]; sex influences the light reflex [26], diabetes duration reflects cumulative glycemic exposure [1], and HbA1c indexes mean glycemia. Despite these potential pathways, the positive association between glycemic variability and D1% persisted after adjustment for all four, and was already evident before adjustment ($r = 0.316$ for CGM SD and 0.302 for %CV). The association was consistent across bidirectional models and robust to potential artifacts and confounders: it was attenuated but remained significant after adjustment for constriction amplitude (AMP%; $P = 0.005$)—so it is not merely an artifact of parasympathetic constriction depth—and was independent of a cardiovascular autonomic index (CVRR), peripheral neuropathy, and retinopathy stage, none of

which was itself associated with D1%. This suggests that D1% conveys sympathetic information not reflected in conventional cardiac autonomic testing or in structural diabetic complications. The two variability measures also clarify the role of mean glycemia. Because CGM SD scales with mean glucose, adjustment for HbA1c strengthened its association with D1%, consistent with negative confounding; %CV—the variability measure favored by international consensus [18], which normalizes SD to the mean and was unrelated to mean glucose and HbA1c—showed a concordant association that did not depend on this adjustment. D1% therefore appears to track the magnitude of glucose fluctuations per se rather than the overall glycemic level. Because HbA1c did not track this variability-associated sympathetic change, HbA1c alone may underestimate the autonomic burden of glucose fluctuations, and a simple, noninvasive pupillary index such as D1% could serve as a candidate marker [27]—though any screening application requires prospective, outcome-based confirmation.

This study has important limitations. Its cross-sectional design precludes causal or temporal inference, and reverse or shared-cause explanations cannot be excluded. It was a retrospective, exploratory, single-center analysis of a modest sample ($n = 53$) of Japanese patients. The main univariate association was modest ($r = 0.316$; $P = 0.021$); although it was robust to log-transformation and influential-observation exclusion, it did not reach the Bonferroni-adjusted threshold for the multiple correlations examined, and a chance finding cannot be excluded. The models using %CV had weaker overall fit than those using CGM SD, and the association with constriction latency was observed only for CGM SD and was not confirmed by the Spearman rank correlation ($\rho = -0.247$, $P = 0.083$). Pupillometry relied on a single right-eye measurement, mechanistic pathways were not assessed, and the absence of an association with AMP% should not be taken as evidence of preserved parasympathetic function. Moreover, pupillometry cannot

separate increased neural activity from altered receptor sensitivity, nor partition the parasympathetic-withdrawal and sympathetic contributions to re-dilation; the pupillary changes are thus functional signatures rather than direct measures of sympathetic nerve activity. Finally, this analysis characterized the association between a pupillary functional index and glycemic variability rather than diagnosing or grading autonomic neuropathy, so the pupillary measures should be interpreted as functional correlates, not as evidence of established autonomic damage. Accordingly, these findings are hypothesis-generating and require confirmation in larger, prospective, and ideally multicenter studies.

Conclusions

In this cross-sectional study of patients with type 2 diabetes, greater CGM-derived glycemic variability was independently associated with enhanced pupillary re-dilation, whereas the parasympathetically mediated constriction amplitude was not. Because this pupillary signal was independent of HbA1c, assessment based on HbA1c alone may overlook the autonomic impact of glucose fluctuations. Dynamic pupillometry may thus offer a noninvasive means of capturing this information, and prospective, longitudinal studies are warranted to establish its clinical value.

List of abbreviations

AMP%: Relative constriction amplitude

BPD: Baseline pupil diameter

CGM: Continuous glucose monitoring

CI: Confidence interval

%CV: Coefficient of variation of sensor glucose

CVRR: Coefficient of variation of R–R intervals

D1%: Re-dilation ratio

HbA1c: Glycated hemoglobin

HDHCC: Hyogo Diabetes Hypoglycemia Cognition Complications

IOL: Intraocular lens

MCV: Maximum constriction velocity

PLR: Pupillary light reflex

SD: Standard deviation

SNCV: Sural nerve conduction velocity

TAR: Time above range

TBR: Time below range

TIR: Time in range

UMIN: University Hospital Medical Information Network

VIF: Variance inflation factor

Declarations

Ethics approval and consent to participate

This study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Hyogo Medical University Hospital (Approval No. 4250). Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available because they contain personal clinical information, but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

M. Ito conceived and designed the study, acquired, analyzed, and interpreted the data, and wrote the manuscript. F.G. contributed to the study design and critically revised the manuscript. Y.Y., H.F., and H.K. contributed to the study design. M.O., C.I., K.O., M. Inoue, K.T., and Y.K. contributed to the study design and data acquisition. All authors reviewed and approved the final manuscript.

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482

Figure Legends

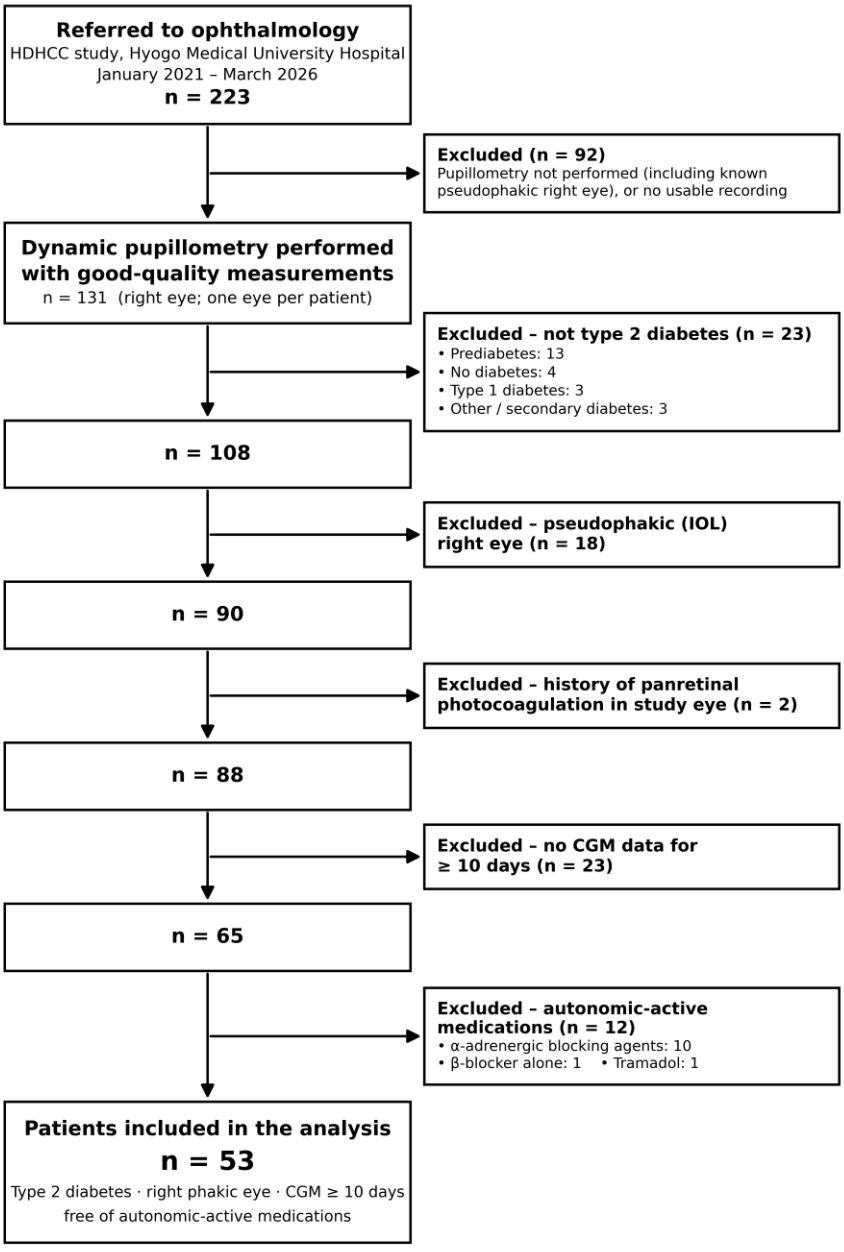
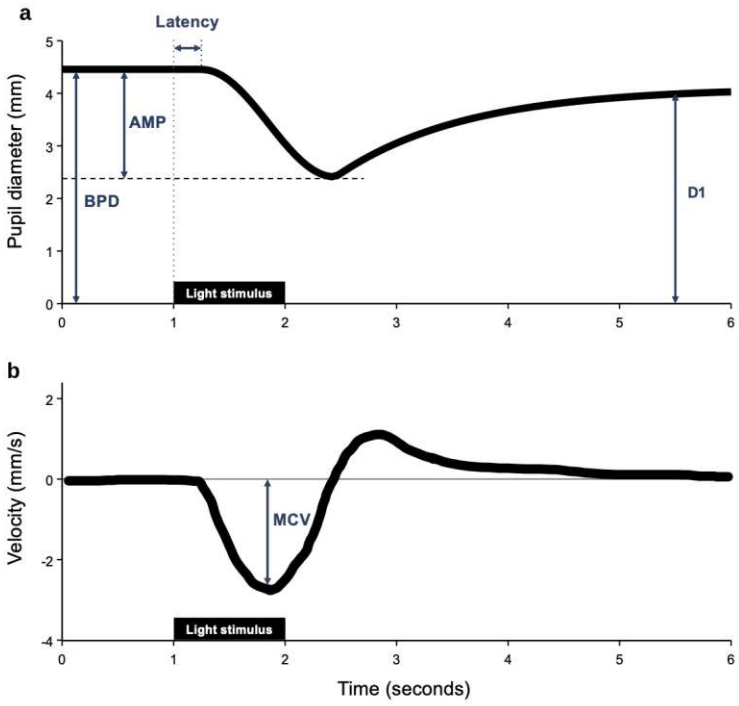


Figure 1. Enrollment flow of study participants. The analysis comprised 53 patients. CGM, continuous glucose monitoring; HDHCC, Hyogo Diabetes Hypoglycemia Cognition Complications; IOL, intraocular lens.



488

489 **Figure 2.** Schematic pupillary light reflex illustrating the measured parameters. (a) Pupil

490 diameter and (b) constriction velocity over time. BPD, baseline pupil diameter; Latency, interval

491 from light onset to the onset of constriction; AMP, constriction amplitude (BPD − minimum

492 diameter); D1, re-dilation pupil diameter at 5.5 seconds (3.5 s after stimulus offset); MCV,

493 maximum constriction velocity. AMP% and D1% denote AMP and D1 expressed as a

494 percentage of BPD.

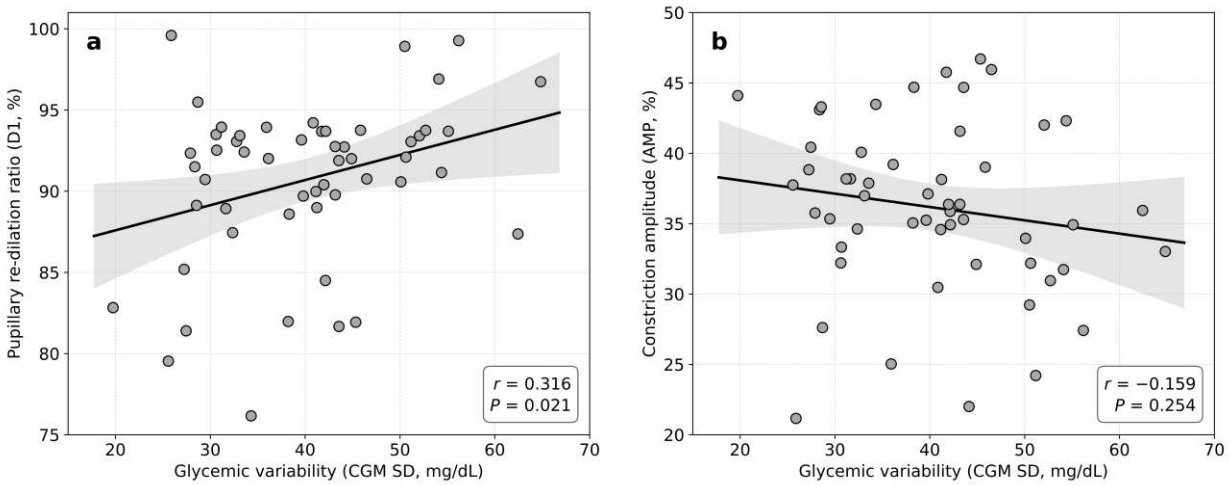


Figure 3. Association of pupillary autonomic indices with glycemic variability. Scatterplots show the sympathetically influenced re-dilation ratio (D1%; a) and the parasympathetically mediated constriction amplitude (AMP%; b) plotted against the standard deviation of continuous glucose monitoring (CGM SD). Solid lines indicate linear regression, and shaded areas indicate 95% CIs; *r* and *P* denote Pearson correlation coefficients and two-sided *P* values ($n = 53$). CGM, continuous glucose monitoring.

Table 1. Baseline characteristics and continuous glucose monitoring and dynamic pupillometry parameters (N = 53).

Characteristic	Value
Age, years	64.5 ± 8.6 (46–77)
Male sex, n (%)	34 (64.2)
Body mass index, kg/m²	25.3 ± 3.8 (19.6–41.4)
Diabetes duration, years	13.3 ± 11.0 (0–44)
HbA1c, %	7.1 ± 0.6 (5.9–8.7)
Diabetic retinopathy, n (%)	12 (22.6)
Diabetic neuropathy, n (%)	9 (17.0)
Diabetic nephropathy (stage ≥2), n (%)	13 (24.5)
Sural nerve conduction velocity, m/s	53.1 ± 6.0 (38–63)
Sural nerve action potential amplitude, μV	12.3 ± 5.7 (3.0–31.5)
Coefficient of variation of R–R intervals (CVRR), %	3.1 ± 1.6 (0.4–7.2)
Treated with insulin, n (%)	8 (15.1)
Non-insulin glucose-lowering agents, n (%)	40 (75.5)
Diet and exercise only, n (%)	5 (9.4)
Continuous glucose monitoring	
Mean glucose, mg/dL	146.8 ± 26.4 (103.5–213.8)
Standard deviation (CGM SD), mg/dL	40.2 ± 10.2 (19.7–64.8)
Coefficient of variation (%CV), %	27.4 ± 5.3 (16.7–42.8)
Time in range 70–180 mg/dL, %	76.5 ± 17.3 (25.6–99.3)
Time above range >180 mg/dL, %	22.3 ± 17.9 (0.5–74.4)
Time below range <70 mg/dL, %	1.1 ± 2.0 (0.0–9.2)
Dynamic pupillometry	
Baseline pupil diameter, mm	4.57 ± 0.81 (2.60–6.48)
Constriction latency, ms	305 ± 49 (175–526)
Constriction amplitude (AMP%), %	36.2 ± 6.0 (21.2–46.7)
Maximum constriction velocity, mm/s	3.89 ± 0.81 (2.20–6.52)
Re-dilation ratio (D1%), %	90.7 ± 5.0 (76.2–99.6)

Data are mean ± SD (range) unless otherwise indicated (n [%] for categorical variables). HbA1c, hemoglobin A1c; CGM, continuous glucose monitoring; AMP%, relative constriction amplitude; D1%, re-dilation ratio 3.5 s after light offset. Diabetic nephropathy stage ≥2 corresponds to microalbuminuria or worse (Japanese classification). Constriction latency was available in 50, CVRR in 46, and sural nerve conduction in 50 participants.

Table 2. Pearson correlations of pupillary indices with continuous glucose monitoring parameters (N = 53).

Pupillary index	Mean glucose	CGM SD	%CV	TIR 70–180	TAR >180	TBR <70
BPD	−0.035	−0.112	−0.134	0.143	−0.118	−0.181
Latency	−0.194	−0.298*	−0.218	0.145	−0.152	0.105
AMP%	−0.200	−0.159	−0.007	0.157	−0.179	0.235
MCV	−0.137	−0.090	−0.019	0.169	−0.166	0.021
D1%	0.119	0.316*	0.302*	−0.175	0.156	0.119

*Values are Pearson correlation coefficients (r); *P < 0.05, **P < 0.01, ***P < 0.001. Analyses included 53 patients, except constriction latency (n = 50). Of the 30 correlations, none survived Bonferroni correction (α = 0.0017); the associations of CGM SD and %CV with D1% (P = 0.021 and P = 0.028, respectively) and of CGM SD with latency (P = 0.036) are considered exploratory. BPD, baseline pupil diameter; AMP%, relative constriction amplitude; MCV, maximum constriction velocity; D1%, re-dilation ratio; TIR, time in range; TAR, time above range; TBR, time below range.*

Table 3. Pearson correlations of pupillary indices with non-CGM clinical, autonomic, and peripheral-nerve measures (N = 53).

Pupillary index	HbA1c	Duration	Nephropathy	CVRR	SNCV
BPD	−0.080	−0.096	−0.433**	0.095	−0.082
Latency	−0.161	−0.085	0.111	0.044	−0.041
AMP%	−0.158	−0.453***	0.002	0.242	0.096
MCV	−0.145	−0.559***	−0.198	0.221	0.095
D1%	−0.062	0.042	0.071	−0.139	0.056

*Values are Pearson correlation coefficients (r); *P < 0.05, **P < 0.01, ***P < 0.001. Analyses included 53 patients, except constriction latency (n = 50), CVRR (n = 43–46), and sural nerve conduction velocity (SNCV; n = 48–50). Of the 25 correlations, those of AMP% and MCV with diabetes duration and of BPD with nephropathy remained below the Bonferroni-adjusted threshold (α = 0.002). Nephropathy denotes stage ≥2 (point-biserial correlation). BPD, baseline pupil diameter; AMP%, relative constriction amplitude; MCV, maximum constriction velocity; D1%, re-dilation ratio; Duration, diabetes duration; CVRR, coefficient of variation of R–R intervals; SNCV, sural nerve conduction velocity.*

Table 4. Bidirectional multivariable regression between D1% and CGM SD (A) or %CV (B) (N = 53).

Variable	β	95% CI	P value
A. Glycemic variability expressed as CGM SD			
Model 1 — dependent variable: D1%			
CGM SD	0.229	0.074 to 0.385	0.005
Age	0.091	−0.073 to 0.255	0.271
Sex	−1.231	−4.005 to 1.543	0.376
HbA1c	−2.630	−5.214 to −0.046	0.046
Diabetes duration	0.033	−0.102 to 0.168	0.627
Adjusted R^2 = 0.137; overall model P = 0.035			
Model 2 — dependent variable: CGM SD			
D1%	0.691	0.224 to 1.158	0.005
Age	0.098	−0.189 to 0.386	0.494
Sex	−0.561	−5.411 to 4.290	0.817
HbA1c	9.667	5.947 to 13.388	<0.001
Diabetes duration	−0.118	−0.351 to 0.115	0.312
Adjusted R^2 = 0.377; overall model P < 0.001			
B. Glycemic variability expressed as %CV			
Model 1 — dependent variable: D1%			
%CV	0.303	0.041 to 0.565	0.024
Age	0.105	−0.064 to 0.274	0.217
Sex	−1.845	−4.702 to 1.013	0.200
HbA1c	−0.964	−3.211 to 1.283	0.393
Diabetes duration	0.042	−0.100 to 0.184	0.557
Adjusted R^2 = 0.080; overall model P = 0.111			
Model 2 — dependent variable: %CV			
D1%	0.341	0.046 to 0.636	0.024
Age	0.053	−0.128 to 0.234	0.558
Sex	1.306	−1.757 to 4.369	0.395
HbA1c	1.732	−0.617 to 4.082	0.145
Diabetes duration	−0.117	−0.264 to 0.030	0.115
Adjusted R^2 = 0.078; overall model P = 0.116			

All models were adjusted for age, sex, HbA1c, and diabetes duration. Sex was coded as male = 1, female = 0. All variance inflation factors were below 1.7 (maximum, 1.62 in panel A and 1.37 in panel B). β, unstandardized regression coefficient; CI, confidence interval; %CV, coefficient of variation of sensor glucose; CGM SD, standard deviation of sensor glucose; D1%, re-dilation ratio.

Additional files

Additional file 1 (PDF). Table S1. Independent association between D1% and CGM SD across adjustment sets. Table S2. Independent association between D1% and %CV across adjustment sets.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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