# Autonomic Nerve Testing Predicts the Development of Complications

## A 12-year follow-up study

ANN M. MAGUIRE, MB, BOA, BCH[1,2]
MARIA E. CRAIG, PHD[1,2,3]
ANNE CRAIGHEAD[1]
ALBERT K.F. CHAN, M APP STAT[1]
JANINE M. CUSUMANO[1]
STEPHEN J. HING, MBBS[1]
MARTIN SILINK, MD[1,2]
NEVILLE J. HOWARD, MBBS[1]
KIM C. DONAGHUE, PHD[1,2]

**OBJECTIVE** — Cardiac autonomic nerve tests have predicted increased mortality in adults with diabetes, predominantly due to nephropathy, cardiac disease, and hypoglycemia. The significance of subclinical autonomic nerve test abnormalities has not been systematically studied in adolescents. We aimed to reassess an adolescent cohort, whose autonomic nervous system had been tested 12 years earlier by both pupillometry and cardiovascular tests.

**RESEARCH DESIGN AND METHODS** — From 1990 to 1993, adolescents with type 1 diabetes ($n = 335$) were assessed for autonomic neuropathy (median age 14.7 years [interquartile range 13.0–16.8], duration of diabetes 6.3 years [4.0–9.6], and A1C 8.3% [7.5–9.4]). Between 2003 and 2005, contact was made with 59% of the original group. Individual assessment 12 years later included completion of a validated hypoglycemia unawareness questionnaire ($n = 123$) and urinary albumin-to-creatinine ratio ($n = 99$) and retinal ($n = 102$) screening, as well as analysis of reports from external doctors ($n = 35$).

**RESULTS** — At baseline, there was no difference in age, duration of diabetes, or complications between those who participated in the follow-up phase ($n = 137$) and those who did not participate ($n = 196$). However, baseline A1C was lower in the follow-up participants (8.2 vs. 8.5% for participants vs. nonparticipants, respectively, $P = 0.031$). At 12 years of follow-up, 93% were aware and 7% were unaware that they had hypoglycemia; 32 (31%) had no retinopathy, but 10% required laser therapy, and 80 (81%) had no microalbuminuria. Small pupil size at baseline was independently associated with the development of microalbuminuria (odds ratio 4.36 [95% CI 1.32–14.42], $P = 0.016$) and retinopathy (4.83 [1.3–17.98], $P = 0.019$) but not with the development of hypoglycemia unawareness. There was no association with baseline cardiovascular tests and the development of complications 12 years later.

**CONCLUSIONS** — In this study, we found an association between baseline pupillometry tests and the presence of microalbuminuria and retinopathy at 12 years of follow-up. This suggests that pupillometry abnormalities may be early indicators of patients who are at high risk of future microvascular disease.

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During the 1970s, Ewing et al. (1) presented a landmark study that reported a predictive relationship between symptomatic autonomic neuropathy and mortality in adults with type 1 diabetes. Deaths 5 years later were predominantly due to associated vascular disease (1), which other authors (2) have considered to be an explanation for the increased mortality related to abnormal cardiac autonomic tests. Deaths were also due to hypoglycemia and "sudden death" (1). Owing to the era of the Ewing et al. study, A1C was not measured; therefore, it was not controlled for, and questions remained unanswered. These questions include whether increased mortality was directly related to glycemic control (i.e., a shared mechanism of damage causing both neuropathy and cardiovascular disease by accumulation of advanced glycation end products) or whether there may be an additional mechanism of damage (e.g., autonomic neuropathy compounding the effect of poor glycemic control on mortality).

Subsequent studies have attempted to clarify the importance of asymptomatic cardiac autonomic nerve (CAN) function abnormalities, defined either by conventional cutoffs or as the lower quartile of the study population (3–5). Patients with normal electrocardiograms and with no underlying renal disease were reported to have increased mortality when CAN test abnormalities were present (23 vs. 3% in those with normal tests) (5). A meta-analysis of 15 studies examining the relationship between CAN dysfunction and the risk of mortality reported that the relative risk of death was 3.45 when two CAN tests were abnormal compared with 1.2 when only one CAN test was abnormal. However, only some of the studies included in this meta-analysis attempted to control for other known risk factors (4). More recently, in the Hoorn Study (3), increased mortality was reported in adult diabetic patients whose autonomic function tests were in the lower quartile of the distribution, even after adjusting for potential confounders, which is a phenomenon not seen in the nondiabetic control subjects.

Although pupillary function is more sensitive and reproducible than CAN testing in young individuals (6,7), the predictive ability of pupillometry testing has not been longitudinally studied. In a cross-sectional analysis, abnormal pupillary function appeared to precede early renal

From the [1]Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, Sydney, New South Wales, Australia; the [2]Discipline of Paediatrics and Child Health, University of Sydney, Sydney, New South Wales, Australia; and the [3]School of Women's and Children's Health, University of New South Wales, Sydney, New South Wales, Australia.

Address correspondence and reprint requests to Dr. Ann Maguire, Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, Locked Bag 4001, Sydney, NSW 2145, Australia. E-mail: annm4@chw.edu.au.

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**Abbreviations:** CAN, cardiac autonomic nerve.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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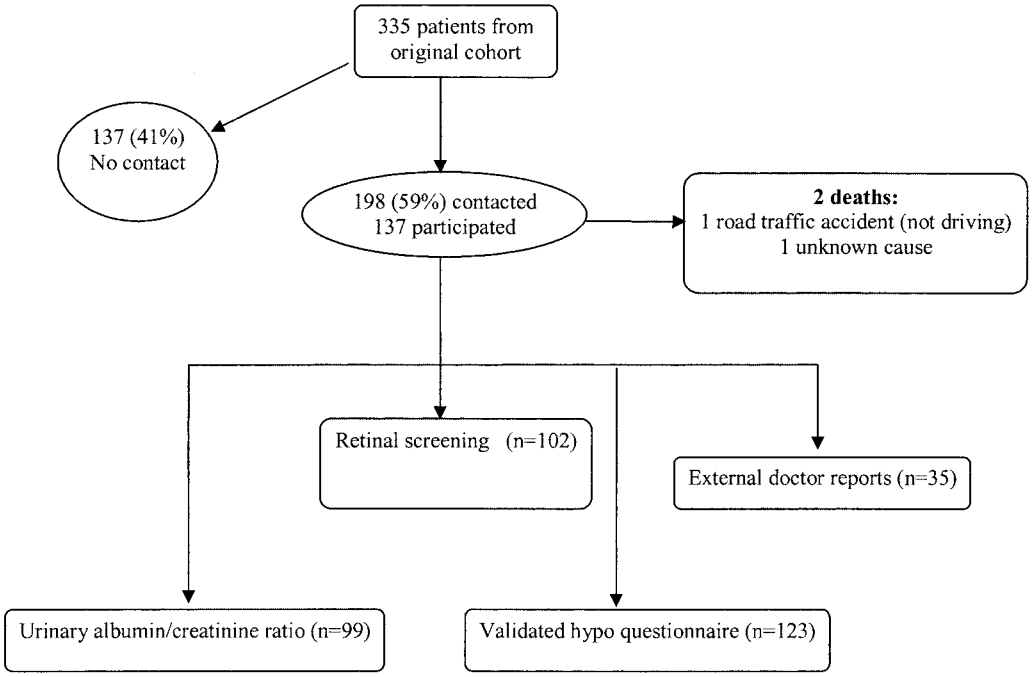


**Figure 1—***Study recruitment and follow-up during 2003–2005 in young adults initially seen in 1990–1993.*

disease in adolescents, suggesting that autonomic dysfunction may contribute to vascular disease (8).

In this longitudinal study, we aimed to examine vascular and nonvascular outcomes in a young-adult cohort 12 years after initial autonomic testing and to evaluate the predictive value of autonomic nerve testing for future development of complications. The cohort had undergone pupillometry and cardiovascular testing and retinal and microalbuminuria screening during adolescence. We hypothesized that subclinical autonomic nerve abnormalities could predict other long-term microvascular complications independent of glycemic control.

## RESEARCH DESIGN AND METHODS

— From 1990 to 1993, autonomic nerve testing was performed in 335 adolescents with type 1 diabetes. Initial assessment and follow-up of this cohort was approved by the ethics committee at The Children's Hospital at Westmead. Outcomes examined at follow-up were renal disease, retinal disease, and hypoglycemic awareness. Patients were offered diabetes complications assessment at The Children's Hospital at Westmead between 2003 and 2005. The first contact was by postal mail, and initial nonresponders were then contacted by phone. If patients declined to participate, or were unable to attend, permission was requested to obtain microvascular complication screening results from other treating doctors. Contact was made with 198 (59%) of the original group; of these, 137 consented to participate in the study. Recruitment and follow-up numbers are shown in Fig. 1.

At the beginning of the study, cardiovascular autonomic nerve function was assessed by three tests of heart rate variation (maximum-minimum heart rate during deep breathing, Valsalva, and lying-standing change) and one of blood pressure (change from lying to standing) (adapted from Ewing et al.) (1). Reference ranges for cardiovascular tests were obtained from 122 nondiabetic control subjects (9). Pupillary autonomic function at baseline and reference ranges were assessed using an infrared computerized pupillometer, as previously described (7,9). The fifth percentile reference range limits used to define abnormal cardiovascular and pupillary tests are described in Table 1 (9).

At follow-up, hypoglycemic awareness was assessed by a validated hypoglycemia unawareness questionnaire ($n$ =

**Table 1—***Comparison of baseline characteristics of participants and nonparticipants*

| | Participants | Nonparticipants | *P* value |
|---|---|---|---|
| *n* | 137 | 196 | |
| Age (years) | 14.5 | 14.9 | 0.22 |
| Diabetes duration (years) | 7.1 | 6.2 | 0.31 |
| Complications (%) | | | |
| Retinopathy | 47 | 40 | 0.47 |
| Any pupillary variable <5th percentile* | 20 | 25 | 0.26 |
| Any cardiac variable <5th percentile† | 23 | 27 | 0.42 |
| Microalbuminuria (AER) | 6 | 7 | 0.66 |
| A1C (%) | 8.2 | 8.5 | 0.031 |

*Abnormal resting pupil diameter (<5.3 mm), reflex amplitude (<1.7 mm), and maximum constriction velocity (<5.0 mm/s). †Abnormal deep breathing heart rate variation [<34.791 − (0.9708 × age)], Valsalva ratio (<1.48), 30/15 ratio (<1.09), and systolic blood pressure drop (>14 mmHg). AER, albumin excretion rate.

123) (10). Hypoglycemia awareness was scored from 0 to 10 (≤2, aware; 3–6, partially aware; and ≥7, unaware).

Retinopathy was assessed using stereoscopic fundal photography of seven fields in 102 patients. The stereophotographs were taken with a Topcon Fundus Camera (TRC 50-VT; Tokyo Optical, Tokyo, Japan) after dilatation of the pupils with 1% cyclopentolate and 2.5% phenylephrine. Nonsimultaneous photographic pairs were taken of seven standardized fields in each eye and then viewed with a Donaldson Stereoviewer, providing a three-dimensional representation of the fundus, enabling microaneurysms to be more easily distinguished from hemorrhages and artifacts. The Early Treatment Diabetic Retinopathy Study adaptation of the modified Airlie House classification of diabetic retinopathy was used. Mild retinopathy was defined as ≤31, moderate retinopathy was 41–51, and severe retinopathy was ≥61 or any patients who had required laser therapy. The presence or absence of retinopathy as an outcome variable was assessed in the regression analysis. For a further 35 patients, the presence of retinopathy was determined based on external doctors' reports.

Urinary albumin-to-creatinine ratio was measured in 99 patients. Albumin was measured by competitive chemiluminescence immunoassay by the IMMULITE analyzer (Diagnostic Products, Los Angeles, CA). Creatinine was measured by Jaffe reaction, Dimension ARX (Dade Behring, Newark, DE). Albumin-to-creatinine ratio (in milligrams per millimole) was calculated by the formula [albumin (mg/l)/creatinine (μmol/l)] × 1,000. Microalbuminuria was defined as an albumin-to-creatinine ratio >2.5 mg/mmol.

### Statistical analysis

Results are reported as median (interquartile range). Statistical analysis was performed using SAS (version 8; SAS Institute, Cary, NC). Wilcoxon's rank-sum tests were used to compare continuous variables between two groups.

Logistic regression was used to assess the association between the outcome (complications at follow-up) and potential explanatory variables. The predictors considered were cardiovascular and pupillometry tests (both as categorical [quartiles] and continuous variables), age, diabetes duration, A1C, blood pressure percentile, and BMI SD scores as continuous variables. Significant variables are reported as odds ratios (ORs) and 95% CIs.

**RESULTS** — Patient recruitment is shown in Fig. 1. Of those families who were contacted, there were two deaths (one road traffic accident [patient was not driving] and one unknown cause). Seventy-three patients (37%) reported active follow-up with an adult endocrinologist.

At baseline assessment, the median age was 14.7 years (95% CI 13.0–16.8), duration of diabetes 6.3 years (4.0–9.6), and A1C 8.3% (7.5–9.3); at follow-up, the respective values were 26 years (24.7–28.7), 18.9 years (16.1–21.7), and 7.9% (7.3–8.9). Baseline age, duration of diabetes, and complications were similar between the 137 participants (those who participated in the follow-up phase) and 196 nonparticipants (see Table 1). However, baseline A1C was lower (8.2 vs. 8.5%, respectively, $P = 0.031$).

The median follow-up time was 12.5 years (95% CI 11.8–13.0). No patient had overt nephropathy, was on renal dialysis, or had received a renal transplant. Nineteen patients (19%) had elevated albumin-to-creatinine ratios, which ranged from 2.58 to 23.75 mg/mmol.

Of the 102 patients who had retinal screening results at follow-up, 10 (10%) had severe retinopathy requiring laser therapy, 45 (44%) had mild retinopathy, 15 (15%) had moderate retinopathy, and 32 (31%) had no retinopathy. Of the 70 patients with retinopathy, 19 had retinopathy detected on external doctors' reports. Although the prevalence of retinopathy was higher in those screened at our institution (73%), versus the external doctors' reports (59%), the proportions did not differ significantly ($P = 0.17$).

Hypoglycemia unawareness was present in 7% of patients, while 38% were partially aware and 55% completely aware of all hypoglycemic episodes. In univariate analysis, patients with resting pupil diameter in the lower quartile at baseline were more likely to develop microalbuminuria (OR 2.89 [95% CI 1.02–8.15], $P = 0.045$) and retinopathy (4.43 [1.22–16.11], $P = 0.024$) at follow-up (Table 2).

In a multiple logistic regression model after adjusting for baseline A1C and duration of diabetes, resting pupil diameter in the lower quartile (<4.7 mm) predicted the development of both microalbuminuria (OR 4.36 [95% CI 1.32–14.42], $P = 0.016$) and retinopathy (4.83 [1.3–17.98], $P = 0.019$) (Fig. 2). A pupillometry test in the lower quartile at baseline was not associated with baseline retinopathy or microalbuminuria.

Baseline cardiovascular tests in the lower quartile were not associated with retinopathy or microalbuminuria 12 years later (17 vs. 21% for baseline vs. follow-up, respectively, $P = 0.63$). Neither cardiovascular nor pupillometry tests (abnormal or lower quartile) were associated with the development of hypoglycemia unawareness at follow-up.

**CONCLUSIONS** — This longitudinal study identifies a predictive relationship between small pupil size, a marker of early autonomic neuropathy, and the presence of microalbuminuria and retinopathy 12 years later. This relationship persisted after adjusting for glycemic control. Such an association was not identified for conventional Ewing's test of cardiovascular autonomic function.

We previously reported (6,7) that pupillometry was more sensitive in detecting subclinical autonomic neuropathy than cardiovascular tests in adolescents with type 1 diabetes. More recently, these pupillary tests have been compared in a similar group of young adults (median age 22.8 years and duration of diabetes 16 years) (13). While all pupillary variables differed, none of the conventional Ewing's battery of cardiovascular tests differed from age-matched control subjects, which further justifies the choice of autonomic nerve tests in our current study. Some of the mathematically derived linear time domain parameters and nonlinear parameters of heart rate variability did differ between patients and control subjects (13).

Another Australian study (8) of adolescents also found an association between pupillometry abnormalities (resting pupil diameter) and microalbuminuria but not cardiovascular tests. The lack of an association between subclinical cardiovascular test dysfunction (in contrast to pupillary dysfunction) and the future development of microvascular complications could be explained by the differential sensitivities of pupillometry and cardiovascular tests in the assessment of autonomic function. The lack of an association between baseline pupillometry and baseline retinopathy or microalbuminuria suggests that pupillometry abnormalities predate detectable retinopathy and microalbuminuria.

**Table 2—*Risk factors for the outcomes of retinopathy and microalbuminuria using univariate analysis***

| Predictor | Outcome at follow-up | |
|---|---|---|
| | Retinopathy | Microalbuminuria |
| Autonomic test variables at baseline | | |
| Deep breathing HR variation | 0.98 (0.94–1.03) | 0.98 (0.92–1.04) |
| Deep breathing HR variation in LQ | 1.20 (0.46–3.11) | 1.64 (0.57–4.72) |
| Valsalva ratio | 0.48 (0.20–1.17) | 0.72 (0.23–2.28) |
| Valsalva ratio in LQ | 1.73 (0.62–4.84) | 1.59 (0.53–4.78) |
| 30/15 ratio | 1.55 (0.28–8.58) | 2.29 (0.31–17.19) |
| 30/15 ratio in LQ | 1.24 (0.46–3.34) | 1.48 (0.50–4.43) |
| Systolic BP change | 1.03 (0.97–1.08) | 0.97 (0.91–1.04) |
| Systolic BP change in LQ | 0.57 (0.23–1.42) | 0.53 (0.14–1.99) |
| Any cardiovascular variable in LQ | 1.31 (0.55–3.14) | 0.83 (0.29–2.34) |
| Resting pupil diameter* | 0.43 (0.21–0.87), $P = 0.018$ | 0.42 (0.21–0.87), $P = 0.019$ |
| Resting pupil diameter in LQ* | 4.43 (1.22–16.11), $P = 0.024$ | 2.89 (1.02–8.15), $P = 0.045$ |
| Reflex amplitude | 0.48 (0.15–1.47) | 0.45 (0.12–1.65) |
| Reflex amplitude in LQ | 0.89 (0.35–2.26) | 1.64 (0.57–4.72) |
| Maximum constriction velocity | 1.01 (0.72–1.41) | 0.82 (0.56–1.22) |
| Maximum constriction velocity in LQ | 1.12 (0.43–2.91) | 1.30 (0.44–3.85) |
| Any pupillary variable in LQ* | 1.95 (0.83–4.56) | 3.94 (1.20–12.92), $P = 0.024$ |
| Physiological characteristics at baseline | | |
| Age | 0.92 (0.77–1.10) | 0.92 (0.74–1.14) |
| Duration of diabetes | 0.998 (0.89–1.13) | 0.995 (0.86–1.15) |
| A1C* | 1.64 (1.06–2.52), $P = 0.026$ | 2.08 (1.29–3.35), $P = 0.0026$ |
| Systolic BP percentile | 0.996 (0.98–1.01) | 1.01 (0.99–1.03) |
| Diastolic BP percentile | 1.02 (0.998–1.03) | 1.01 (0.99–1.03) |
| BMI SD score | 1.45 (0.81–2.59) | 1.01 (0.50–2.04) |
| Physiological characteristics at follow-up | | |
| Age | 0.99 (0.80–1.24) | 0.87 (0.67–1.14) |
| Duration of diabetes | 1.03 (0.90–1.19) | 0.95 (0.81–1.12) |
| A1C* | 1.75 (1.19–2.56), $P = 0.0043$ | 1.72 (1.12–2.65), $P = 0.014$ |
| Systolic BP percentile* | 0.996 (0.98–1.01) | 1.03 (1.004–1.05), $P = 0.023$ |
| Diastolic BP percentile | 1.000 (0.98–1.02) | 1.01 (0.98–1.03) |
| BMI SD score | 0.70 (0.32–1.54) | 1.16 (0.51–2.67) |

Data are ORs (95% CIs). *P* values are included when significant; *$P < 0.05$. BP, blood pressure; HR, heart rate; LQ, lower quartile.

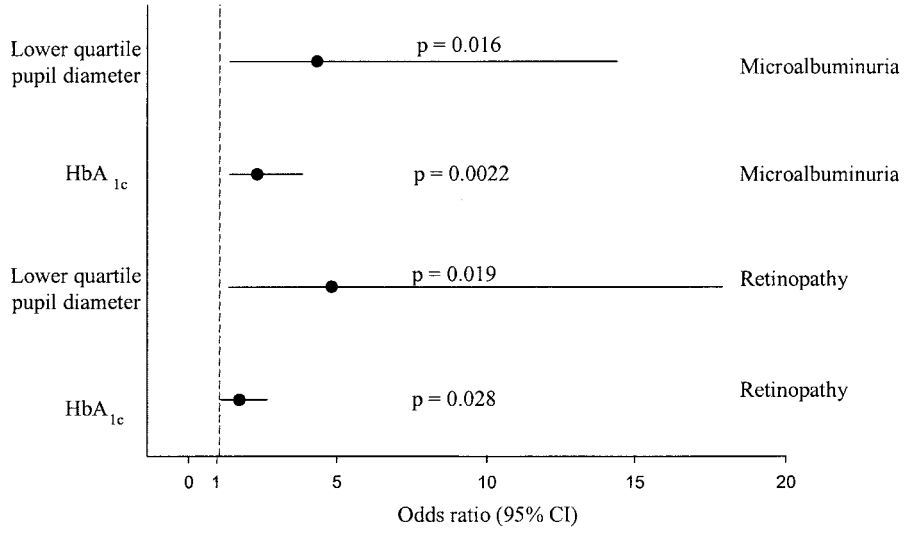


**Figure 2—*Results of multiple logistic regression for predictors of microalbuminuria and retinopathy at follow-up in young adults. Resting pupil diameter in the lower quartile (<4.7 mm) and A1C at baseline were significant predictors.***

It was surprising to find no association between subclinical autonomic neuropathy at baseline and future self-reported hypoglycemia unawareness. The questionnaire that we used had been validated and was found to be a reliable estimation of hypoglycemia unawareness in adults (mean age 38 years) with type 1 diabetes (10). Those with reduced awareness on the retrospective self-reported questionnaire had significantly fewer symptoms and were less likely to detect blood glucose levels <3.9 mmol/l when prospectively recording blood glucose symptoms/estimations on personal handheld computers (10). It is possible that the younger age of our study group (26 years), in combination with social/cultural differences, may have prevented the accurate reporting of hypoglycemia in our group. In addition, as only seven patients reported hypoglycemia unaware-

ness, our sample size may have been too small to detect an association.

The pathological mechanism linking subclinical neuropathy and future microvascular complications has not been fully clarified. Early glycemic damage to the autonomic nerves may, in parallel, directly damage the retinal and glomerular blood vessels. However, as pupillary dysfunction predicted microvascular complications even after adjusting for glycemic control, we postulate that early autonomic nerve dysfunction affects vascular tone and results in changes to both glomerular and retinal blood flow, thus predisposing to microalbuminuria and retinopathy. Other researchers have hypothesized that damage to the autonomic nerves supplying the renal vasculature may lead to increased renal blood flow, glomerular hyperfiltration, and, thus, microalbuminuria (14). Similarly, increased retinal blood flow during acute hyperglycemia and an overall decreased retinal blood flow in type 1 diabetes may contribute to the development of retinopathy (15). Neurally mediated changes in retinal blood flow may further contribute to the development of retinopathy.

The exact neurological mechanism of damage to the pupillary light reflex (mediated by both sympathetic and parasympathetic nerve fibers) in patients with type 1 diabetes is unclear. The balance between parasympathetic and sympathetic nerve function determines pupil size. Small pupillary diameter may be due to decreased sympathetic or increased parasympathetic activity. Pharmacological testing using cocaine drops have demonstrated sympathetic pathway damage. However, another study (16) reported no difference in the response to cocaine between diabetic and nondiabetic control subjects, whereas pilocarpine caused greater constriction in diabetic patients, suggesting parasympathetic denervation hypersensitivity. In support of initial damage being sympathetic, Hoeldtke et al. (17) have found that sudomotor responses due to acetylcholine are redistributed, i.e., increased in the forearm and decreased in the foot, in diabetic subjects with poor glycemic control.

As baseline A1C was lower in participants than nonparticipants (8.2 vs. 8.5%), we acknowledge that there may be a bias in the follow-up group. However, our clinical experience suggested that the follow-up group attended for a variety of reasons (to obtain a second opinion about existing complications or for an opportunity for reengagement with health services) and were not exclusively the "best" patients. A study of the transition process in the U.K. showed that clinic attendance for young adults with type 1 diabetes fell from 98% pre-transfer to 61% after 2 years (18). Therefore, although it is concerning, it is not surprising that only 37% of our contacted cohort (~8 years after transition) had active follow-up with an endocrinologist. It is also possible that some of the patients without retinopathy (on external doctors' reports) did have retinopathy, as we cannot be sure of the quality assurance and reproducibility of retinal photography from external sources. External retinopathy screening methods may not be as sensitive as our own retinal screening, given that 73% had retinopathy when examined by us compared with 59% in the externally examined group. However, the difference in proportions was not statistically significant.

While the relationship between cardiac autonomic neuropathy and microalbuminuria has been described in cross-sectional studies of children with type 1 diabetes (19) and adults with type 2 diabetes (14), our longitudinal study using pupillometry also extends these findings to retinopathy. Our results support the idea that the changes in vascular tone cause damage, in addition to coexisting glycemic damage. With the benefit of an adolescent cohort, we were able to study autonomic neuropathy at an earlier stage of the disease process. Although pupillometry assessment was not performed in the DCCT (Diabetes Control and Complications Trial), it is clear from the DCCT that neuropathy progression can be prevented by improving glycemic control. Therefore, the effect of improved glycemic control on early pupillometry abnormalities needs to be ascertained. In the meantime, it would be prudent to consider these patients at higher risk of developing microalbuminuria and retinopathy.

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