Am. J. Trop. Med. Hyg., 94(6), 2016, pp. 1290–1298
doi:10.4269/ajtmh.14-0775

Pupillary Light Reflexes are Associated with Autonomic Dysfunction in Bolivian Diabetics but not Chagas Disease Patients

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Abstract. Autonomic dysfunction is common in Chagas disease and diabetes. Patients with either condition complicated by cardiac autonomic dysfunction face increased mortality, but no clinical predictors of autonomic dysfunction exist. Pupillary light reflexes (PLRs) may identify such patients early, allowing for intensified treatment. To evaluate the significance of PLRs, adults were recruited from the outpatient endocrine, cardiology, and surgical clinics at a Bolivian teaching hospital. After testing for Chagas disease and diabetes, participants completed conventional autonomic testing (CAT) evaluating their cardiovascular responses to Valsalva, deep breathing, and orthostatic changes. PLRs were measured using specially designed goggles, then CAT and PLRs were compared as measures of autonomic dysfunction. This study analyzed 163 adults, including 96 with Chagas disease, 35 patients with diabetes, and 32 controls. PLRs were not significantly different between Chagas disease patients and controls. Patients with diabetes had longer latency to onset of pupil constriction, slower maximum constriction velocities, and smaller orthostatic ratios than nonpatients with diabetes. PLRs correlated poorly with CAT results. A PLR-based clinical risk score demonstrated a 2.27-fold increased likelihood of diabetes complicated by autonomic dysfunction compared with the combination of blood tests, CAT, and PLRs (sensitivity 87.9%, specificity 61.3%). PLRs represent a promising tool for evaluating subclinical neuropathy in patients with diabetes without symptomatic autonomic dysfunction. Pupillometry does not have a role in the evaluation of Chagas disease patients.

INTRODUCTION

Cardiac autonomic dysfunction is a progressive disorder observed in several chronic diseases including Chagas disease and diabetes. In both Chagas disease and diabetes, patients with autonomic dysfunction face higher mortality, either from arrhythmias in Chagas disease or myocardial infarctions in diabetes.[1,2] Several conventional autonomic testing (CAT) protocols exist to evaluate cardiovascular responses to provocative tests like the Valsalva maneuver, orthostatic change, and deep breathing.[1,3–6] Though autonomic dysfunction is not the direct cause of death, CAT can identify Chagas disease patients with abnormal cardiac conduction and can identify subclinical autonomic dysfunction before symptom onset.[7,8] Diabetic patients also develop subclinical autonomic dysfunction affecting multiple organ systems, which can precede overt peripheral neuropathy, cardiac arrhythmias, and myocardial infarctions.[9] CAT is unfortunately limited by the fact that many patients cannot participate in these tests, either due to compromised extremities or functional status from diabetes or due to heart failure in Chagas disease. Furthermore, diabetic autonomic dysfunction identified by CAT is frequently too advanced for changes in medical management to impact associated mortality rates.[10]

Evaluation of pupillary light reflexes (PLRs) may allow earlier evaluation of autonomic function in these patients by observing changes in different nervous pathways. Sympathetic innervation relaxes the ciliary body of the iris causing pupillary dilation, which affects baseline pupil radius and velocity of redilation after light exposure. Similarly, parasympathetic innervation causes contraction of the circular muscle of the iris, which affects amplitude of pupillary reaction and maximum constriction velocity after light exposure (Figure 1).[11–13] PLR abnormalities precede CAT abnormalities and symptomatic neuropathy in diabetes but have not been evaluated in Chagas disease.[11,14,15] If PLRs could differentiate people with these diseases, they would be useful screening tools to identify patients who would benefit from intensified treatment. To evaluate PLR variation between healthy controls, Chagas disease patients, and patients with diabetes, we conducted a cross-sectional study of autonomic function by PLR and CAT among urban outpatients at a hospital with high rates of both diseases.

MATERIALS AND METHODS

Ethical approval. This study was approved by the institutional review boards of Johns Hopkins University, Asociación Benéfica PRISMA, and the Hospital Universitario Japonés. Informed consent was obtained from all participants prior to participation. After providing informed written consent, patients were included based upon known disease status.

Site and participants. This study was conducted at Hospital Universitario Japonés, a public hospital in Santa Cruz, Bolivia, serving ~60% of the city's 1.1 million people. Vector-borne transmission of the parasite responsible for Chagas disease

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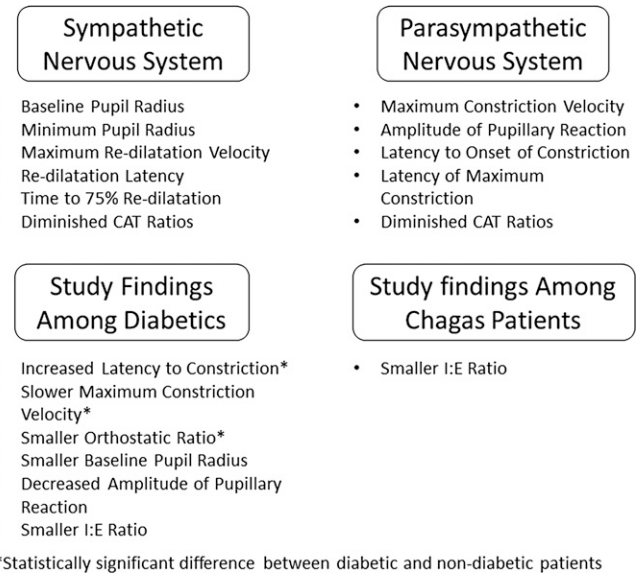

FIGURE 1. Pupillary light reflex parameters, associated autonomic control, and study findings.

does not occur within urban Santa Cruz, though migration from endemic areas is common.

Participants with Chagas disease and diabetes were recruited from the combined waiting room of the hospital's adult outpatient specialty clinics, where cardiology, endocrinology, and surgery patients register. Control patients without diabetes or Chagas disease were recruited while awaiting general medicine and surgical clinics. All patients were screened for voluntary participation and were not matched by age or sex. Patients were excluded if they were younger than 18, older than 70, or had systemic or ophthalmologic disease known to interfere with PLRs (including myasthenia gravis, multiple sclerosis, amyloidosis, Parkinson's disease, Alzheimer's disease, pseudotumor cerebri, retinopathy, optic neuritis, binocular blindness, glaucoma, recent eye trauma, cataracts, prior ocular surgery, binocular corneal scarring, bilateral pterygium, or anisocoria). Patients using ophthalmologic beta-blockers were excluded from analysis, but Chagas patients and patients with diabetes taking oral beta-blockers were included ($N = 3$). Healthy controls taking any oral medications were excluded. No Chagas patients recruited had concurrent heart failure. Patients with both Chagas disease and diabetes were excluded from primary analysis and later evaluated in secondary analysis comparing dually diagnosed patients to those with either Chagas disease or diabetes alone. Tobacco and coca leaf are commonly used stimulants in Bolivia. Patients were not excluded for historical use of either, but refrained from use on the day of autonomic testing.

Clinical evaluation. Medical history and physical examinations were obtained along with electrocardiogram (ECG) and chest X-ray for all patients. This allowed for Kuschnir class determination, which measures heart failure status in Chagas disease. All included patients were either Kuschnir class 0 (no symptoms, normal ECG, and normal chest X-ray) or Kuschnir class 1 (no symptoms, normal chest X-ray, but abnormal ECG). Laboratory testing included 8-hour fasting serum glucose, hemoglobin A1c, and serologic testing for *Trypanosoma cruzi*. As recruitment occurred in 2008, diabetes was defined as fasting serum glucose ≥ 126 mg/dL on two separate occasions, random serum glucose ≥ 200 mg/dL with symptoms, or hemoglobin A1c ≥ 7% (53 mmol/mol), rather than the more recent definition of ≥ 6.5% (48 mmol/mol).[16] Diabetic patients were predominantly type 2 and insulin use was rare ($N = 2$). Chagas disease was confirmed by positive results on both *T. cruzi* lysate enzyme-linked immunosorbent assay (ELISA) (Weiner Laboratory, Rosario, Argentina) and indirect hemagglutination assay (Lemos Laboratory, Buenos Aires, Argentina). When tests were discordant, a recombinant *T. cruzi* ELISA (Weiner Laboratory) served as tiebreaker. Control patients were those who tested negative for both Chagas disease and diabetes.

Conventional autonomic testing. Participants were scheduled for autonomic testing from 8 AM to 12 PM after an 8-hour fast, 8-hour sleep, and abstention from caffeine, coca leaf, and tobacco. Patients confirmed these criteria before testing. Respiratory variation of heart rate was performed during six deep breaths lasting 10 seconds each while lying supine. Continuous ECG rhythm strips (lead II) were analyzed for the longest and shortest RR intervals during each breath. Mean inspiratory and expiratory heart rates (longest and shortest intervals, respectively) were calculated, with each patient's ratio defined as the inspiratory-to-expiratory ratio (I : E ratio). After resting for 5 minutes, participants performed a 15-second sustained Valsalva maneuver. The ratio of the longest RR interval immediately following Valsalva and the shortest RR interval during the subsequent minute was defined as the Valsalva ratio, which was abnormal if ≤ 1.20, reflecting blunted variation.[17,18] After resting 5 minutes supine, patients rose to standing as fast as possible (orthostatic testing). The ratio between the 30th RR interval and the 15th RR interval immediately after standing was defined as the orthostatic ratio (elsewhere called the 30:15 ratio), which is abnormal if < 1.0.[19] Because these ratios measure the relationship between sympathetic and parasympathetic tone, abnormalities in either system can blunt CAT measurements.

Pupillary light reflexes. PLRs were evaluated with custom-built pupillometers based on laptop-based video eye movement systems.[20,21] Participants wore form-fitting motorcycle goggles, which occlude the left eye and house a digital video camera that images the right eye at 200 frames/second (Firefly MV, Point Grey Research, Inc., Richmond, BC, Canada). The right eye was illuminated by two infra-red light-emitting diodes (LEDs) (HSDL 4200, Agilent Technologies, Inc., Santa Clara, CA) at a wavelength of 875 nm, which is invisible to the subject. An infrared pass filter (FIL-IR730/10, Allthings.com.au) at the back of the camera lens prevents imaging of visible wavelengths from the LEDs. A wideband "hot" mirror reflects the image onto the camera lateral to the subject's field of view. A 40 × 40 mm cell phone backlight display (LCD-08842, SparkFun Electronics) is supported 30 mm from the eye and contains six white LEDs with programmable flash duration and brightness and a diffuser to provide even illumination. The goggles occlude all ambient light and weigh 60 g. The goggle-mounted camera was connected via firewire to a laptop running Windows XP (Microsoft Corp., Seattle, WA) and LabVIEW (National Instruments, Austin, TX).

After CAT, patients rested for 5 minutes before putting on PLR goggles, which were calibrated using a monochrome image to clearly delineate the pupil from the sclera. Patients then experienced 120 seconds in total darkness to establish

baseline pupil size. Next, they experienced 20 light pulses at an intensity of 30 cd/m^2, lasting 100 ms each and separated by random intervals of 4.5–5.5 seconds of darkness. Pupil size was determined by both center-of-mass and ellipse-fit methods at a rate of 200 Hz.[20–22] Both measures yield identical results, so center-of-mass calculations were used when pupil images were not obstructed by eyelids or lashes, and ellipse-fit calculations were used for obstructed images.

PLR parameters calculated included: 1) baseline pupil radius—initial pupil radius before the light stimuli; 2) minimum pupil radius—minimum pupil radius after the light stimulus before redilation; 3) latency to onset of constriction—time between onset of light stimulus and initiation of pupillary constriction; 4) maximum constriction velocity—maximum rate of change in pupil size during constriction following light stimulus; 5) amplitude of pupillary reaction—difference between initial pupil diameter and minimum pupil diameter after light stimulus; 6) latency of maximum constriction—time from onset of light stimulus to minimum pupil diameter, 7) redilation latency—time between cessation of light stimulus and initiation of pupil redilation, which can be negative if the pupil dilates before cessation of light stimulus; 8) maximum redilation velocity—maximum rate of change in pupil size during redilation; and 9) time to 75% redilation—time required after cessation of light stimulus for the pupil to redilate to 75% of the initial pupil diameter. Mean values of each parameter were calculated for each participant. Constriction ratio was calculated as minimum pupil radius divided by baseline pupil radius.[23]

Statistical analysis. Data were entered using Microsoft Access 2007 (Microsoft Corp.), then cleaned and analyzed using STATA Version 11.0 (StataCorp LP, College Station, TX). Demographic and PLR differences were compared using two-tailed *t* tests or analysis of variance, as appropriate. Adjusted mean PLR parameters were calculated from multivariate linear regressions including sex, age, and use of any of the following medications (defined as a single variable): statins, spironolactone, diuretics, amiodarone, warfarin, calcium channel blockers, aspirin, angiotensin-converting enzyme inhibitors, beta-blockers, or proton-pump inhibitors. Multivariate linear regressions were constructed comparing mean PLR values between diabetic patients, Chagas disease patients, and controls according to CAT results. Chagas disease patients and controls were pooled as "nondiabetes" to differentiate PLR variables between patients with and without diabetes. The heteroskedasticity-consistent estimator of standard errors was used to reduce standard deviation variability from relatively small sample size.[24] Patients with both diabetes and Chagas disease were not included in primary analysis, but compared with diabetic patients without Chagas disease in secondary analysis.

Multivariate logistic regression models were constructed to correlate adjusted PLR parameters with abnormal CAT results. To identify parameters that best correlated with laboratory-confirmed diabetes, thereby indicating "diabetes complicated by autonomic dysfunction," PLR variables were dichotomized using cut points maximizing area under the receiver operating characteristic curve (AUC). Multivariate logistic regressions were constructed with demographic, clinical, and dichotomized PLR variables against diabetes status. PLR parameters with $P < 0.30$ in bivariate analysis were included in multivariate logistic regressions models with backward stepwise construction using $P < 0.25$ as a cutoff. Clinical variables affecting autonomic test parameters such as age and sex were also included. A clinical prediction score—the average pupillary pulsed light evaluation (APPLE) response score—was constructed to identify patients with "diabetes complicated by autonomic dysfunction," defined as "abnormal PLRs in a diabetic patient." The score assigned points equal to the lowest common denominator of logistic regression coefficients, rounded to increments of the nearest 0.5.[25] Discrimination ability and calibration were evaluated by AUC and the Hosmer–Lemeshow statistic, respectively. The APPLE response score was internally validated using bootstrap aggregation of AUC across 200 replications, and an optimism-corrected AUC was calculated to assess for overfitting.[26]

RESULTS

Patient characteristics. Initial recruitment included 192 patients, 29 of whom were excluded due to ophthalmologic problems, leaving 163 patients for analysis: 96 patients with Chagas disease, 35 with diabetes, and 32 controls (Table 1). Overweight and obesity were common among the study population (32.7% and 38.7%, respectively), though body mass index was not significantly different between controls, patients with diabetes, and Chagas disease patients. Smoking was uncommon (9.2% of patients) and did not differ significantly between groups. Patients with diabetes were more likely to be older, hypertensive, take medications, and have a higher waist-to-hip ratio than those with Chagas disease or controls. They were also more likely to be male, obese, and smokers, though these results were not statistically significant. Total pack-years smoked was not different between groups. Though both patients with diabetes and Chagas disease patients were more likely to take medications than controls, only statin use was statistically significantly different between patients with diabetes and nonpatients with diabetes. Only three of 163 patients used beta-blockers, which was not different between groups. Among patients with diabetes, two (5%) took only insulin, 27 (64%) took only oral hypoglycemic medicines, and six (14%) used both.

Autonomic testing results. Results of both PLRs and CAT parameters are presented in Table 2 (unadjusted results) and Table 3 (adjusted results). Compared with both patients with Chagas disease and healthy controls, diabetic patients had smaller baseline pupil radius, increased latency to constriction, slower maximum constriction velocity, and decreased amplitude of pupillary reaction, which together demonstrate blunting of both sympathetic and parasympathetic pathways (Table 2). Though not statistically significant, patients with Chagas disease also tended to have smaller amplitude of pupillary reaction and maximum redilatation velocity compared with controls. The orthostatic ratio and the I : E ratio were both statistically significantly different between patients with diabetes and controls. Both ratios were largest in controls, smallest in patients with diabetes, and intermediate in Chagas disease, suggesting more autonomic dysfunction among patients with diabetes than Chagas disease patients by both tests (Table 2). The rate of abnormal orthostatic or Valsalva results was not statistically significant between groups for either ratio. After adjusting for age, sex, and medications taken, patients with diabetes had longer mean latency to onset of constriction and slower maximum constriction

TABLE 1
Patient demographics and clinical characteristics

Variable	Controls (N = 32)		Diabetic patients (N = 35)		Chagas patients (N = 96)		P value*
	n	%	n	%	n	%	
Female sex	19	59.4	23	34.3	69	71.9	0.40
Age (years)†	31	39.8 (12.7)	35	50.7 (10.0)	96	46.7 (10.5)	< 0.01
Body mass index (kg/m^2)‡	31	27.5 (7.3)	34	29.7 (6.5)	91	27.3 (6.7)	0.41
Normal weight (18.5–24.9 kg/m^2)	8	25.8	6	17.7	26	28.6	0.66
Overweight (25.0–29.9 kg/m^2)	10	32.3	11	32.3	32	35.2	
Obese (> 30.0 kg/m^2)	13	41.9	17	50.0	33	36.3	
Waist: hip ratio‡	31	0.89 (0.09)	34	0.91 (0.06)	90	0.90 (0.09)	0.14
High waist: hip ratio§	20	64.5	31	91.2	58	64.4	0.01
Current smoking	3	9.4	6	17.1	6	6.3	0.16
Smoking history (pack-years)‡	32	0 (0.3)	34	0 (2.5)	91	0 (0)	0.25
Hypertension	1	4.0	11	34.4	21	23.9	0.02
Coronary heart disease	1	3.6	3	8.6	4	4.4	0.60
Kuschnir class 1		NA		NA	59	61.5	NA
Took any regular medications¶	0	0.0	29	82.9	32	33.3	< 0.01
Calcium channel blockers	0	0.0	0	0.0	3	3.1	0.57
Beta-blockers	0	0.0	1	2.86	2	2.08	1.00
ACE inhibitors	0	0.0	6	17.1	16	16.7	0.02
Spironolactone	0	0.0	2	5.7	3	3.1	0.48
Diuretics	0	0.0	2	5.7	5	5.2	0.57
Aspirin	0	0.0	3	8.6	7	7.3	0.29
Anticoagulants	0	0.0	0	0.0	2	2.1	1.00
Statins	0	0.0	5	14.3	1	1.0	< 0.01
Thyroid supplements	0	0.0	1	2.9	1	1.0	0.66
Digoxin	0	0.0	0	0.0	2	2.1	1.00
Amiodarone	0	0.0	0	0.0	5	5.2	0.22
Contraceptives	0	0.0	1	2.9	4	4.2	0.82

ACE = angiotensin-converting enzyme; ANOVA = analysis of variance; NA = not applicable.
* P value represents ANOVA between all three groups (those with diabetes, those with Chagas disease, and those with neither).
† % Column represents mean (standard deviation).
‡ % Column represents median (interquartile range).
§ High waist-to-hip ratio was defined as ≥ 0.90 for males and ≥ 0.85 for females.
¶ Included any of the following medications: calcium channel blockers, beta-blockers, ACE inhibitors, spironolactone, diuretics, aspirin, anticoagulants, statins, thyroid supplements, digoxin, amiodarone, or contraceptives.

TABLE 2
Mean pupillary light reflex (PLR) and conventional autonomic test (CAT) parameters, by Chagas disease and diabetes status

	(A) Control patients			(B) Chagas disease patients			(C) Diabetic patients			P value*		
	N	Mean	95% CI	N	Mean	95% CI	N	Mean	95% CI	A vs. B	A vs. C	B vs. C
PLR parameters†												
Baseline pupil radius	30	23.79	22.72, 24.87	94	23.00	22.22, 23.78	34	21.10	19.78, 22.42	0.30	< 0.01	0.01
Latency to onset of constriction	30	0.21	0.19, 0.22	94	0.22	0.21, 0.22	34	0.26	0.23, 0.29	0.21	< 0.01	< 0.01
Maximum constriction velocity	29	−3645.1	−4086.2, −3204.0	91	−3318.9	−3490.2, −3147.5	33	−2771.1	−3034.2, −2508.0	0.10	< 0.01	< 0.01
Amplitude of pupillary reaction	30	7.61	6.98, 8.25	94	6.93	6.57, 7.29	34	6.57	5.97, 7.17	0.09	< 0.01	0.33
Latency of maximum constriction	30	0.77	0.76, 0.79	94	0.78	0.76, 0.80	34	0.77	0.74, 0.80	0.70	0.93	0.76
Redilation latency	30	0.68	0.66, 0.69	94	0.69	0.67, 0.71	34	0.68	0.65, 0.71	0.63	0.69	0.92
Maximum redilation velocity	30	1394.9	724.5, 2065.3	94	1244.0	865.3, 1622.7	34	1065.40	435.7, 1695.2	0.02	0.21	0.74
Time to 75% redilation	30	1.00	0.93, 1.08	94	0.98	0.94, 1.02	34	0.96	0.89, 1.03	0.59	0.22	0.60
Constriction ratio	30	0.54	0.50, 0.57	94	0.52	0.50, 0.54	34	0.51	0.48, 0.54	0.50	0.14	0.59
Reaction ratio	30	0.68	0.67, 0.69	94	0.69	0.67, 0.71	34	0.68	0.65, 0.71	0.60	0.95	0.64
CAT parameters												
Orthostatic ratio	31	1.26	1.21, 1.31	87	1.21	1.18, 1.24	34	1.14	1.09, 1.19	0.11	< 0.01	0.02
≤ 1.0	0	0.0‡	–	2	2.3‡	0.3, 8.1	3	8.8‡	1.9, 23.7	1.00§	0.24§	0.13§
> 1.0	31	100.0‡	–	85	97.7‡	91.9, 99.7	31	91.2‡	76.3, 98.1			
I : E ratio	31	1.22	1.18, 1.27	91	1.18	1.15, 1.20	34	1.16	1.12, 1.20	0.07	0.05	0.44
Valsalva ratio	30	1.41	1.32, 1.49	87	1.33	1.28, 1.39	34	1.31	1.23, 1.39	0.15	0.10	0.66
≤ 1.2	8	26.7‡	12.3, 45.9	24	27.6‡	18.5, 38.2	13	38.2‡	22.2, 56.4	1.00§	0.43§	0.28§
< 1.2	22	73.3‡	54.1, 87.7	63	72.4‡	61.8, 81.5	21	61.8‡	43.6, 77.8			

95% CI = 95% confidence interval.
* P value calculated by T-tests between each pair of disease profile groups (A, B, C), except as specified by the symbol "§" indicating use of a Fischer's exact test.
† Units for pupillometric variables were pixels for static distances, seconds for time, and pixels per second for velocity metrics.
‡ Instead of a mean value, this number represents the percentage of patients with disease profile with CAT value as described.

TABLE 3
Adjusted mean pupillary light reflex (PLR) and conventional autonomic test (CAT) parameters,* by Chagas disease and diabetes status

	(A) Control patients			(B) Chagas disease patients			(C) Diabetic patients			*P* value†		
	N	Mean	95% CI	*N*	Mean	95% CI	*N*	Mean	95% CI	A vs. B	A vs. C	B vs. C
PLR parameters‡												
Baseline pupil radius	30	29.16	26.65, 31.68	94	29.40	26.88, 31.92	34	28.04	25.14, 30.94	0.75	0.22	0.06
Latency to onset of constriction	30	0.16	0.13, 0.2	94	0.17	0.14, 0.21	34	0.21	0.17, 0.25	0.50	0.02	0.01
Maximum constriction velocity	29	−5253.8	−6150.8, −4356.7	91	−5134.0	−5837.2, −4430.9	33	−4741.90	−5520.4, −3963.3	0.51	0.02	0.03
Amplitude of pupillary reaction	30	8.66	7.32, 10	94	8.12	6.78, 9.47	34	7.95	6.4, 9.49	0.18	0.14	0.65
Latency of maximum constriction	30	0.74	0.69, 0.78	94	0.75	0.69, 0.81	34	0.74	0.67, 0.8	0.63	0.97	0.66
Redilation latency	30	0.64	0.59, 0.69	94	0.65	0.59, 0.7	34	0.64	0.58, 0.71	0.61	0.83	0.79
Maximum redilation velocity	30	1131.6	−35.6, 2298.8	94	1021.6	245.0, 1798.1	34	814.96	−443.2, 2073.2	0.09	0.24	0.77
Time to 75% redilation	30	0.87	0.72, 1.02	94	0.83	0.68, 0.98	34	0.80	0.63, 0.98	0.41	0.24	0.52
Constriction ratio	30	0.50	0.43, 0.57	94	0.48	0.41, 0.55	34	0.47	0.39, 0.55	0.39	0.26	0.60
Reaction ratio	30	0.70	0.64, 0.76	94	0.72	0.66, 0.78	34	0.71	0.64, 0.78	0.31	0.71	0.56
CAT parameters												
Orthostatic ratio	31	1.39	1.28, 1.51	87	1.37	1.26, 1.49	34	1.32	1.19, 1.45	0.48	0.05	0.07
≤ 1.0	0	0.0§	–	2	1.5§	0, 65.6	3	4.8§	0, 89.7	–	–	0.27
> 1.0	31	100.0§	–	85	98.5§	34.4, 99.9	31	95.2§	10.3, 99.9			
I : E ratio	31	1.39	1.28, 1.51	91	1.37	1.26, 1.48	34	1.37	1.25, 1.49	0.37	0.43	0.92
Valsalva ratio	30	1.57	1.39, 1.74	87	1.54	1.34, 1.73	34	1.52	1.32, 1.72	0.58	0.48	0.76
≤ 1.2	8	27.6§	6.5, 67.8	24	21.0§	4.4, 60.3	13	31.4§	6, 76.6	0.48	0.76	0.25
< 1.2	22	72.4§	32.2, 93.5	63	79.0§	39.7, 95.6	21	68.6§	23.4, 94			

95% CI = 95% confidence interval.
*Adjusted for age, sex, and use of any of the following medications: calcium channel blockers, beta-blockers, angiotensin-converting enzyme inhibitors, spironolactone, diuretics, aspirin, anticoagulants, statins, thyroid supplements, digoxin, amiodarone, or contraceptives.
†*P* values calculated by T-tests between each pair of disease profile groups (A, B, C).
‡Units for pupillometric variables were pixels for static distances, seconds for time, and pixels per second for velocity metrics.
§Instead of a mean value, this number represents the percentage of patients with disease profile with CAT value as described.

velocities than Chagas and control patients with a trend toward smaller baseline pupil radius and smaller orthostatic ratios (Table 3). No significant difference was found between PLR values among patients with Chagas disease and controls.

After adjustment for age, sex, and medication use, diabetic patients had longer latency to onset of pupillary constriction, slower maximum constriction velocity, smaller orthostatic ratio, and nonsignificant trends toward smaller baseline pupil radius

TABLE 4
Adjusted means of pupillary light reflex and conventional autonomic testing parameters, by diabetes status*

	Patients without diabetes			Patients with diabetes			
	N	Mean	95% CI	*N*	Mean	95% CI	*P* value
Pupillometric parameters†							
Baseline pupil radius	124	29.28	26.88, 31.68	34	27.97	25.11, 30.83	0.06
Latency to onset of constriction	124	0.17	0.13, 0.21	34	0.21	0.16, 0.26	< 0.01
Maximum constriction velocity	120	−5191.28	−5788.25, −4594.32	33	−4772.83	−5478.33, −4067.33	0.02
Amplitude of pupillary reaction	124	8.55	7.60, 9.50	34	8.04	6.91, 9.18	0.07
Latency of maximum constriction	124	0.74	0.67, 0.81	34	0.73	0.65, 0.82	0.72
Redilation latency	124	0.64	0.58, 0.71	34	0.64	0.57, 0.72	0.87
Maximum redilation velocity	124	1393.94	809.79, 2455.67	34	1377.69	681.77, 2073.62	0.92
Time to 75% redilation	124	0.85	0.71, 0.99	34	0.82	0.65, 0.99	0.39
Constriction ratio	124	0.49	0.42, 0.55	34	0.48	0.39, 0.55	0.46
Reaction ratios	124	0.71	0.65, 0.77	34	0.71	0.64, 0.78	0.72
Autonomic parameters							
Orthostatic ratio	118	1.39	1.29, 1.48	34	1.33	1.21, 1.44	0.05
≤ 1.0‡	2	0.8	0.0, 45.6	3	3.5	0.0, 85.2	0.16
> 1.0‡	116	99.2	54.4, 100.0	31	96.5	24.8, 100.0	
Valsalva ratio	117	1.55	1.38, 1.73	34	1.53	1.32, 1.74	0.65
≤ 1.2‡	32	24.4	5.8, 62.8	13	33.8	6.8, 78.1	0.32
> 1.2‡	85	75.6	37.2, 94.2	21	66.2	21.9, 93.2	
I : E ratio	122	1.38	1.30, 1.47	34	1.38	1.28, 1.48	0.76

95% CI = 95% confidence interval.
*Adjusted for sex, age, and use of the following medications: statins, spironolactone, diuretics, amiodarone, warfarin, calcium channel blockers, aspirin, angiotensin-converting enzyme inhibitors, beta-blockers, and proton-pump inhibitors.
†Units for pupillometric variables were pixels for static distances, seconds for time, and pixels per second for velocity metrics.
‡Percentage (95% confidence interval), otherwise mean (95% CI).

TABLE 5
Adjusted means of pupillary light reflex parameters* by conventional autonomic test results in diabetic patients†

Variable	Normal I : E/orthostatic/Valsalva ratio			Abnormal I : E/orthostatic/Valsalva ratio			*P* value
	N	Mean	95% CI	*N*	Mean	95% CI	
Baseline pupil radius	20	28.44	21.88, 35.01	13	26.00	19.24, 32.77	0.06
Latency to onset of constriction	20	0.22	0.05, 0.39	13	0.28	0.11, 0.46	0.04
Maximum constriction velocity	20	−3850.6	−5371.6, −2329.5	12	−3183.2	−4769.1, −1606.24	0.04
Amplitude of pupillary reaction	20	6.26	3.95, 8.56	13	5.58	3.21, 7.95	0.14
Latency of maximum constriction	20	0.67	0.56, 0.78	13	0.66	0.55, 0.78	0.78
Redilation latency	20	0.58	0.46, 0.69	13	0.60	0.46, 0.68	0.75
Maximum redilation velocity	20	1446.9	−725.3, 3619.1	13	2002.9	−232.9, 4238.8	0.19
Time to 75% redilation	20	0.43	0.21, 0.65	13	0.41	0.18, 0.63	0.66
Constriction ratio	20	0.37	0.26, 0.47	13	0.37	0.26, 0.47	0.91
Reaction ratio	20	0.81	0.73, 0.88	13	0.80	0.72, 0.88	0.95

95% CI = 95% confidence interval.
*Units for pupillometric variables were pixels for static distances, seconds for time, and pixels per second for velocity metrics.
†Adjusted for age, sex, and use of selected medications (statins, spironolactone, diuretics, angiotensin-converting enzyme inhibitors, beta-blockers, and proton-pump inhibitors).

and smaller amplitude of pupillary reaction than nonpatients with diabetes, indicating both sympathetic and parasympathetic dysfunction among patients with diabetes (Table 4). Evaluation of patients diagnosed with both Chagas disease and diabetes ($N = 43$) revealed a slightly shorter latency to onset of constriction after adjustment for age, sex, and medication use than among diabetic patients without Chagas disease (0.18 versus 0.21 seconds, $P = 0.046$) and a trend toward smaller Valsalva ratios among those with both diagnoses. Among diabetic patients, Chagas disease status did not significantly affect I : E ratio ($P = 0.448$), Valsalva ratio ($P = 0.216$), or orthostatic ratio ($P = 0.94$). No other statistically significant difference or trend was found that differentiated patients with diabetes with and without Chagas disease or that differentiated Chagas disease patients with or without diabetes (data not shown).

Correlation between PLRs and conventional autonomic tests. Multivariate logistic regressions compared each PLR parameter and the presence of diabetes or Chagas disease to abnormal CAT results. No significant differences were found for any PLR parameter between patients with diabetes with normal CAT results and patients with diabetes with abnormal CAT results. Patients with diabetes with abnormal Valsalva ratios trended toward decreased baseline pupil radius (25.95 versus 28.73 pixels, $P = 0.06$) and slower maximum constriction velocity (−4261.1 versus −4957.8 pixels/second, $P = 0.09$) compared with patients with diabetes with normal Valsalva ratios, suggesting both sympathetic and parasympathetic dysfunction. Similarly, no significant differences were found for any PLR parameter between nondiabetic patients with abnormal CAT results and nondiabetic patients with normal CAT results.

After adjustment for age, sex, and use of medications, PLR parameters were compared with each individual CAT variable as well as with a composite variable of "any abnormal CAT result." Among patients with diabetes, those with "any abnormal CAT results" were noted to have longer latency to onset of constriction, slower maximum constriction velocity, as well as nonsignificant trends toward smaller baseline pupil radius, smaller amplitude of pupillary reaction, and faster maximum redilatation velocity, suggesting mixed sympathetic and parasympathetic dysfunction in these patients (Table 5). No PLR parameters were even modestly correlated with abnormal CAT among nonpatients with diabetes (data not shown).

TABLE 6
Average pupillary pulsed light evaluation response score using pupillary light reflexes to identify diabetes complicated by pupillary autonomic dysfunction*

Variable	β-coefficient (95% CI)	Adjusted† OR (95% CI)	Points
Baseline pupillary radius			
≤ 22.80	0.00	1.00	0
> 22.80	0.98 (−0.13, 2.09)	4.24 (1.67, 10.75)	2
Latency to onset of constriction			
≤ 0.22	0.00	1.00	0
> 0.22	1.44 (0.51, 2.38)	3.79 (1.55, 9.29)	3
Maximum constriction velocity			
≤ −3260.39	0.00	1.00	0
> −3260.39	0.69 (−0.45, 1.83)	1.99 (0.64, 6.25)	1.5
Sex			
Male	0.00	1.00	0
Female	0.17 (−0.76, 1.09)	1.18 (0.47, 2.98)	0.5
Age			
≤ 47 years	0.00	1.00	0
> 47 years	0.52 (−0.38, 1.42)	1.69 (0.69, 4.14)	1
AUC			
Uncorrected AUC‡	0.77 (0.70, 0.85)		
Optimism-corrected AUC‡	0.71 (0.62, 0.79)		

AUC = area under the ROC curve; 95% CI = 95% confidence interval; OR = odds ratio; ROC = receiver operating curve.
*Units for pupillometric variables were pixels for static distances, seconds for time, and pixels per second for velocity metrics.
†Adjusted for the other parameters included in the logistic regression model (parameters shown).
‡Area under the ROC curve (95% CI).

TABLE 7
APPLE response score as a clinical predictor of diabetes, sensitivity, specificity, and likelihood ratios (LRs)

Cutoff points	Sensitivity (%)	Specificity (%)	Correctly classified (%)	LR+	LR−
≥ 5.0	93.9	54.6	63.2	2.07	0.11
≥ 5.5	87.9	61.3	67.1	2.27	0.20
≥ 6.0	81.8	63.9	67.8	2.26	0.28
≥ 6.5	78.8	66.4	69.1	2.34	0.32
≥ 7.0	72.7	68.1	69.1	2.28	0.40
≥ 7.5	51.5	79.0	73.0	2.45	0.61
≥ 8.0	45.5	81.5	73.7	2.46	0.67

APPLE = average pupillary pulsed light evaluation; LR+ = positive likelihood ratio, LR− = negative likelihood ratio.

Development of APPLE response clinical score to differentiate patients with and without diabetes. Table 6 presents the PLR parameters that found to be significantly associated with diabetes as the APPLE response score. This score was defined as the finding of abnormal PLR in diabetic patient, indicating "diabetes complicated by autonomic dysfunction" that was not necessarily observed on CAT. AUC for this model was 0.77 (95% confidence interval [CI] 0.70, 0.85), which fell to 0.71 (95% CI 0.62, 0.79) after optimism correction. The final model was not improved by including CAT variables. Selected cutoff values are presented in Table 7 with their respective sensitivity, specificity, and positive and negative likelihood ratios for the identification of patients with "diabetes complicated by pupillary autonomic dysfunction." Those patients with APPLE response scores ≥ 5.5 are 2.27 times as likely as those with lower scores to have "diabetes complicated by pupillary autonomic dysfunction" (sensitivity: 87.9%, specificity: 61.3%). Increased likelihood of "diabetes complicated by pupillary autonomic dysfunction" was found with larger APPLE response scores at the cost of sensitivity and negative predictive value.

DISCUSSION

This study represents the first comparison of PLRs and CAT in patients with Chagas disease and the first attempt to define PLR cut points to identify diabetic autonomic dysfunction. Though patients with Chagas disease can develop autonomic dysfunction, PLRs could not differentiate patients with Chagas disease from controls, nor could they differentiate Chagas disease patients with normal CAT results from those with abnormal CAT results in this study.[8]

Trypanosoma cruzi localizes to the heart and intestines and contributes to parasympathetic dysfunction at those sites over time.[27,28] Over the last few decades, several mechanisms have been proposed through which Chagasic cardiac and gastrointestinal dysfunction may occur. These include direct damage to the tissue by live parasites, so-called "bystander" damage caused to the tissue through host defenses targeting the parasite, and autoimmune destruction of these tissues by a combination of cellular and humoral immune responses to cardiac proteins released when the parasite damages cardiac myocytes. The relative significance of these mechanisms has been debated for years. At a minimum, there is a poor correlation between the degree of cardiac dysfunction and parasite burden found on histopathologic evaluation that suggests more to the story than simple parasite persistence.[29,30] On the other hand, antibodies have been identified in Chagas disease that target beta-adrenergic receptors, but despite a biologically plausible association with autonomic dysfunction, these have not been shown to be associated with either the extent of cardiac disease nor unique to Chagasic cardiomyopathy.[30,31] One major aspect of this debate is its implication for treatment decisions, since an autoimmune pathophysiology would minimize the role for specific antiparasitic therapy. This discussion has recently grown more significance in light of the BENEFIT trial published last year, which confirmed that benznidazole treatment reduced parasite detection by polymerase chain reaction, but found no difference in cardiac events after treatment of cardiac Chagas disease patients with mild heart disease.[32] As the topic continues to be debated, it is reasonable to consider that some combination of the multiple proposed mechanisms is at play, but for the time being, the data presented here suggest that the autonomic pathways controlling PLRs are not affected in Chagas disease, and PLRs are not a useful tool for the evaluation of Chagas disease patients.

In contrast, diabetic patients were found to have multiple differences in their PLRs compared with both Chagas disease patients and controls. Unlike Chagas disease, diabetes causes global autonomic impairment.[1] Patients with diabetes in this study were found to have autonomic dysfunction measured by both CAT and PLRs (Table 4). The decreased baseline pupil radius among diabetic patients reflects altered sympathetic tone, which was not found among those with Chagas disease, consistent with the pathophysiology of each condition. On the other hand, latency to onset, maximum constriction velocity, and amplitude of pupillary reaction reflect more parasympathetic tone, which would be expected in Chagas disease if it affected the pupils, though no significant association was found.[11–13] Diabetic patients with CAT abnormalities demonstrated PLR abnormalities that were more advanced than diabetic patients without CAT abnormalities, but when diabetic patients develop CAT abnormalities, it is too late to reverse autonomic dysfunction even with tight glycemic control.[10]

No single test of cardiac autonomic function has been shown to have sufficient sensitivity and specificity to be used alone to evaluate dysfunction among diabetic patients.[6,9] Although CAT has demonstrated differences between patients with and without Chagas disease, PLRs did not reliably differentiate patients with and without autonomic dysfunction by CAT.[8,12] This was true for both Chagas disease and for diabetes and likely reflects the larger extent of autonomic dysfunction required to develop truly abnormal CAT values. Patients with Chagas disease, for example, are known to have lower Valsalva ratios than healthy controls, but a recent meta-analysis found the average Valsalva ratio among Chagas patients to be 1.74, which is well within the limits of normal.[3,33] Likewise, previous studies have demonstrated differences between PLR parameters of healthy and diabetic subjects, and PLR abnormalities can be found among patients with diabetes with and without abnormalities on CAT.[14,15,34–36] Abnormal PLRs have been associated with future microvascular disease and may be associated with future CAT abnormalities.[15,37] By the time CAT abnormalities develop in diabetes, even tight glycemic control will not reduce mortality,[10] suggesting that abnormal CAT findings may be a threshold effect rather than a point along the reversible spectrum of morbidity. If dysfunction is tolerated in multiple organs up to the

point that irreversible damage occurs and the risk of adverse events increases, surrogate measures like abnormal PLRs might identify patients in need of intense glycemic control before irreversible damage sets in. In the interest of finding an endpoint upstream from irreversible cardiac autonomic dysfunction, this study attempted to develop a diagnostic model for asymptomatic autonomic dysfunction in patients with diabetes.

The model presented in this article (the APPLE response score) uses a combination of three pupillometric parameters: baseline pupil radius, latency to onset of constriction, and maximum constriction velocity, along with age and sex for evaluating patients with diabetes. Although the score was derived from evaluation of patients with laboratory-confirmed diabetes, the construction of this model means that the risk score measures not likelihood of "having diabetes" but of "having diabetes complicated by autonomic neuropathy." This score will need to be validated in prospective cohorts to determine its utility in predicting cardiac autonomic dysfunction and macrovascular complications. Once validated, PLRs and the APPLE response score may be preferred over CAT for the evaluation of autonomic dysfunction due to shorter test duration (< 2 minutes versus ~20 minutes in this protocol), minimal training required of clinic staff, and its association with microvascular complications such as retinopathy and nephropathy that can be prevented by tight glycemic control.

This cross-sectional study had several important limitations. Patients with diabetes tend to be diagnosed after several years without symptoms, so many patients with currently well-controlled disease have unknown amounts of previous damage. This cross-sectional study could not, therefore, determine how long patients had been affected by either Chagas disease or diabetes, and it could not evaluate adequacy of prior diabetes control. Since autonomic function measurements by both CAT and PLRs are indicators of cumulative damage, this study cannot speculate on the extent to which time with disease affects PLR or CAT variables.[38] Likewise, the exclusion of patients with significant eye disease likely excluded advanced diabetic patients with poor autonomic function. The exclusion of people using medications such as eye drops that directly affect PLRs may also limit generalizability of these data to patients taking those medications. Many patients with either diabetes or heart failure take medications such as beta-blockers that directly affect autonomic activity. No patients in this study had heart failure and only three took beta-blockers. Medication effects were not found to significantly alter PLRs in this study, but practitioners should be cautious in generalizing these results until they are confirmed in a population with higher rates of medication use, such as in the United States. Though different PLR results were apparent between patients with and without diabetes, the fact that no statistically significant differences were found on CAT between Chagas disease patients and controls may reflect a small control group, which would mask differences between patients with and without Chagas disease. Additionally, the pupillometer in this study was developed by the authors and is not available outside of clinical protocols.[21] Multiple devices are available commercially at costs from $3,000 to $30,000. As more data are generated about PLRs, standardized devices may become more available, thereby increasing their clinical application. Finally, this cross-sectional study could not provide any test of the prognostic role of PLRs or the APPLE response score for future cardiac complications, both of which remain areas for future research.

Despite these limitations, evaluation of PLRs using the risk score presented here represents a promising screening tool for the evaluation of subclinical diabetic autonomic dysfunction that appears to be more sensitive than CAT. This testing can be easily performed in the clinic or at public health fairs with little time, a computer, and minimally trained staff. Though diabetes itself is easily diagnosed using hemoglobin A1c and fasting glycemia, the risk score developed in this study may help identify diabetic patients with subclinical autonomic dysfunction who need more aggressive therapy earlier than evaluation with CAT. In particular, this approach could have implications for developing world and rural health care settings such as Bolivia with high rates of diabetes (6.29%) and limited resources to perform widespread laboratory-based screening programs.[39] As a chronic condition affecting so many people in lower and middle income countries, autonomic dysfunction associated with diabetes and Chagas disease merits further attention so that testing and potential treatment options can become more widely available. Pupillometry does not appear to have a role in the evaluation of autonomic dysfunction in patients with Chagas disease.

Received December 2, 2014. Accepted for publication January 16, 2016.

Published online April 4, 2016.

Acknowledgments: We thank the study participants, the staff of Hospital Universitario Japonés and the Laboratory of Infectious Diseases Research at Universidad Peruana Cayetano Heredia, including the Chagas Working Group in Bolivia and Peru, without whom this study could never have been conducted. Members of the Chagas Disease Working Group in Peru and Bolivia include Corina Espada, Emilene Flores, Jorge Flores, Carlos La Fuente, Estela Lopez, Margot Ramirez, Nur Selum, and Walter Selum.

Financial support: This work was supported by the National Institutes of Health Fogarty International Clinical Scholars Program. Additional funding for pupillometer development and salary support for Drs. Moore and Morris was provided by National Aeronautics and Space Administration grant NNX12AM25G.

Disclaimer: The funders had no role in study design, data collection and analysis, manuscript preparation, or decision to publish the results.

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