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Assessment of the relationship between dynamic pupillometry and exercise heart rate recovery among healthy subjects

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Abstract. – OBJECTIVE: Dynamic pupillometry (DP) is a simple, non-invasive computerized technique for assessment of pupillary light response which provides data concerning the balance of both branches of the autonomous nervous system (ANS). Heart rate (HR) recovery (HRR) after graded exercise reflects cardiac autonomic activity and predicts cardiovascular events. In this study, we aimed to evaluate the utility of DP as a predictor of cardiac autonomic activity assessed by HRR.

PATIENTS AND METHODS: A total of 62 consecutive healthy subjects (mean age = 33.7 ± 8.6 years, 39 males and 23 females) were enrolled. Pupil diameters (R0, R1, R2 and R%): latency (Lc), amplitude (Ac), velocity (Vc) and duration of pupil contraction (Tc): latency (Ld), velocity (Vd) and duration of pupil dilatation (Td) were measured in DP. HRR indices were calculated by subtracting 1st (HRR1), 2nd (HRR2) and 3rd (HRR3) minute HR from the maximal HR during treadmill exercise stress test.

RESULTS: HRR1 was 32.9 ± 8.0 bpm, HRR2 was 55.1 ± 11.6 bpm and HRR3 was 58.3 ± 12.7 bpm, respectively. Correlation analysis revealed significant positive correlations of HRR1 with Vc (r = 0.660, p = 0.001), Ac (r = 0.559, p = 0.001) and Vd (r = 0.412, p = 0.001). HRR had significant negative correlations with Lc (r = -0.442, p = 0.001), R% (r = -0.384, p = 0.002) and Ld (r = -0.286, p = 0.025). Vc [β = 3.995 (1.040 to 6.951, 95% CI, p = 0.009)] and Lc [β = -0.032 (-0.056 to -0.008, 95% CI, p = 0.01)] were found to be significant independent predictors of HRR1.

CONCLUSIONS: Pupillary autonomic functions assessed by DP correlates with cardiac autonomic functions evaluated by HRR. Among the DP parameters analyzed, Vc and Lc were independent predictors of cardiac autonomic functions.

Key Words:
Autonomous nervous system, Dynamic pupillometry, Heart rate recovery.

Introduction

In recent years, a marked interest has emerged about the physiological or pathophysiologic features and clinical evaluation of the autonomic nervous system (ANS) regulation of the heart rate (HR)[1,2]. Cardiac autonomic dysfunction (CAUD) has been associated with increased all-cause mortality, death from cardiovascular disease and sudden cardiac death[3,4]. One of the most frequently used diagnostic and prognostic tool for evaluating CAUD is heart rate recovery (HRR) after graded exercise[1,5]. Exercise is associated with increased sympathetic and decreased parasympathetic activity and the period of recovery after maximum exercise is characterized combination of sympathetic withdrawal and parasympathetic reactivation. Therefore, HRR reflects autonomic activity and predicts cardiovascular events and mortality not only in cardiovascular system disorders but also in various systemic disorders[6-8].

Dynamic pupillometry (DP) is a novel, standardized, fully automated system for evaluating pupillary autonomic nerve activity[9,10]. The pupil is a convenient and accessible terminal station for studying autonomic function because the parasympathetic and sympathetic nervous systems innervate constrictor and dilator muscles of the iris[9-11]. Therefore, the infrared DP which uses

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pupillary light reflex allows independent evaluation of both branches of pupillary autonomic activity[11-13]. DP has been evaluated in patients with hypertension[10], heart failure[11], obstructive sleep apnea[12], diabetes mellitus[14] and neurological diseases[15,16]. However, correlation and association of DP indices with cardiac autonomic activity has not been assessed before. In this study, we aimed to investigate the clinical utility of DP indices as a predictor of cardiac autonomic functions via HRR.

Patients and Methods

Study Population and Data Collection

A total of 62 (mean age = 33.7 ± 8.6 years, 39 males and 23 females) consecutive healthy subjects (volunteers such as healthy participants from our outpatient clinics, physicians and nurses) were enrolled in our study. During participation and data collection 4 (6.1%) cases were excluded. Exclusion criteria were arterial hypertension in three cases and diabetes mellitus in one case. All subjects had no systemic conditions with known ocular involvement, use of medication and topical eye treatment. All participants had a normal visual acuity (20/20) and had no history of ocular abnormalities.

Data collection was carried out by face-to-face interviews of subjects by two trained researchers. Demographic features, smoking habits, and use of medications were interviewed using a questionnaire. All subjects underwent a complete physical examination, and their height and weight were recorded. BMI was calculated as weight divided by square of height (kg/m^2).

After initial data collection, all participants underwent EST and DP. These two studies were performed by blinded researchers to results of one another. The current study complies with the Declaration of Helsinki. Informed consent was obtained from all participants and the study was approved by the Research Ethics Committee.

Treadmill Exercise Testing

A treadmill EST was conducted in all patients using modified Bruce protocol. All subjects achieved an exercise time of more than 6 minutes, and a final HR of at least 85% of age-predicted maximal HR response. After achieving peak workload, all patients spent 3-minutes recovery without a cool-down period. HRR indices were calculated by subtracting 1^{st}, 2^{nd}, 3^{rd} minute HR from the maximal HR obtained during EST and designated as HRR1, HRR2, and HRR3. Exercise capacity was measured in metabolic equivalent levels (METs) at peak exercise. Duration of the EST, maximal HR, systolic blood pressure at rest (SBP) and maximum exercise (SBP_{ME}); diastolic blood pressure at rest (DBP) and maximum exercise (DBP_{ME}) were recorded.

Dynamic Pupillometry

Monocular DP analysis was performed for each eye (darkness adaptation 300 seconds, duration of 90 seconds, sampling frequency = 30 Hz) with Metrovision MonPack one. Software which is provided in the DP device automatically outlines pupillary contour on the images, ensuring the accuracy of the measurements (accuracy of measurements of pupil diameter = 0.1 mm) under controlled illumination conditions. The DP device stimulator had near-infrared illumination (950 nm) and a high-resolution near-infrared image sensor which allows measurement of pupil diameter even in absolute darkness. From the DP analysis of response to visual stimulus: pupil diameters (initial, R0; maximum, R1; minimum, R2; R2/R0 expressed as R%): latency (Lc), amplitude (Ac), velocity (Vc) and duration of pupil contraction (Tc): latency (Ld), velocity (Vd) and duration of pupil dilatation (Td) were measured. DP analysis and HRR measurement are represented in Figure 1.

Statistical Analysis

Distribution of data was assessed by using a one-sample Kolmogorov-Smirnov test. Data are demonstrated as mean ± SD for normally distributed continuous variables, median (minimum-maximum) for skew-distributed continuous variables, and frequencies for categorical variables. Univariate linear regression analysis was performed to evaluate the effects of DP variables on HRR indices. Predictors obtained from univariate analysis were further checked with multivariate linear regression analysis. Pearson's correlation analysis was used in order to assess the relationship between the DP variables and HRR indices. Spearman's correlation analysis was used for skew-distributed continuous DP variables in order to evaluate the relationship with HRR indices. Statistical analysis of the data was conducted using SPSS 15 (SPSS Inc., Chicago, IL, USA) and two-tailed $p < 0.05$ was considered statistically significant.

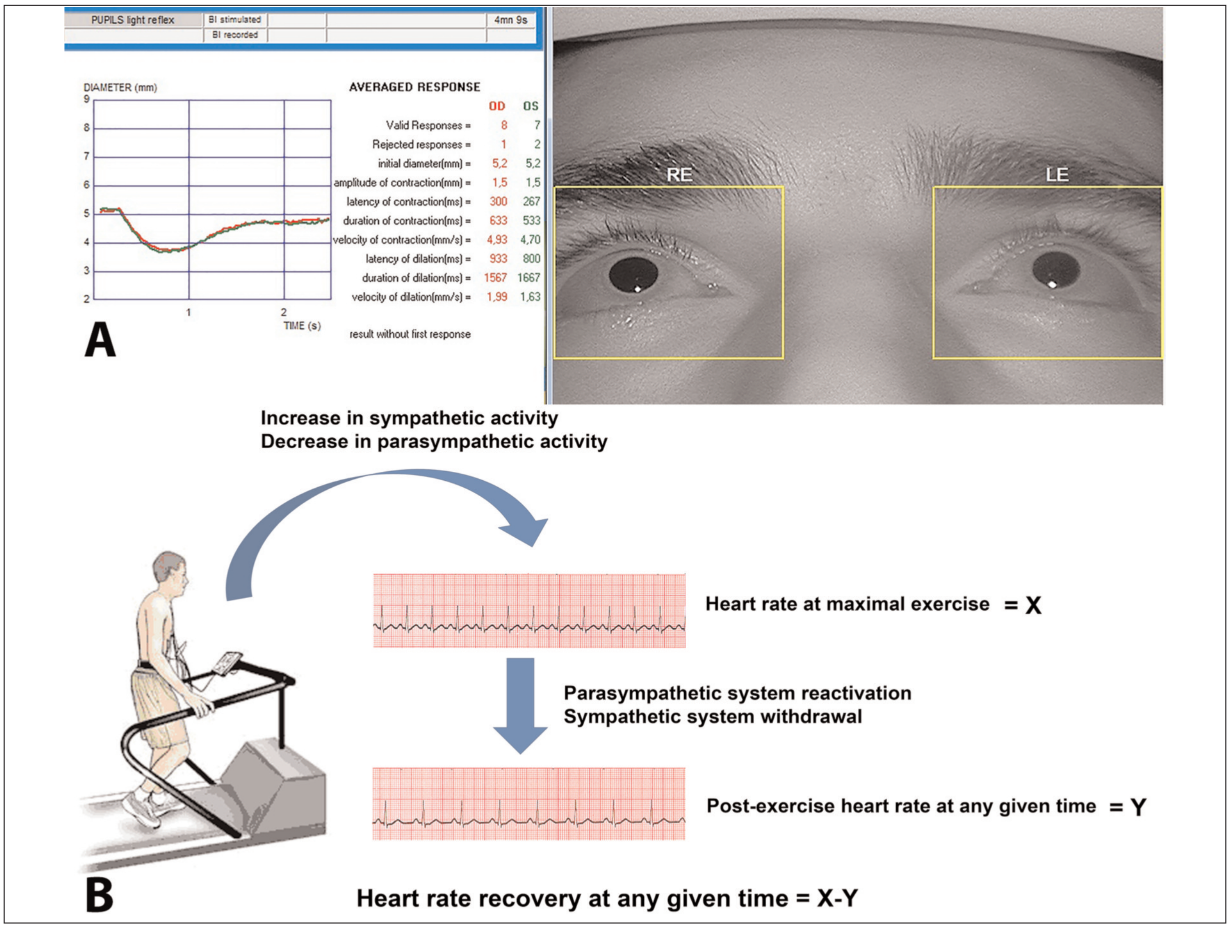

Figure 1. Dynamic pupillometry analysis *(A)* and *(B)* HRR measurement are shown.

Results

Of the 62 participants (mean age = 33.7 ± 8.6 years, 39 males and 23 females), 14 (22.6%) were currently smokers. The mean BMI of the study group was 23.0 ± 1.9 kg/m^2, SBP was 111.3 ± 12.8 mmHg and DBP was 68.2 ± 8.9 mmHg. Mean HRR1 was 34.5 ± 7.7 bpm, HRR2 was 45.8 ± 6.6 bpm and HRR3 was 61.2 ± 9.6 bpm, respectively. Treadmill exercise test parameters of participants are represented in Table I.

Pupil sizes were determined 4.40 ± 0.93 mm for R0, 4.88 ± 0.84 mm for R1, 3.28 ± 0.76 mm for R2, respectively. Detailed DP indices and correlation with HRR are shown in Table II. Correlation analysis revealed significant positive correlations of HRR1 with Vc (r = 0.660, p = 0.001), Ac (r = 0.559, p = 0.001) and Vd (r = 0.412, p = 0.001). There were significant negative correlations of HRR1 with Lc (r = -0.442, p = 0.001), R% (r = -0.384, p = 0.002) and Ld (r = -0.286, p = 0.025) (Figure 2).

Table I. Treadmill exercise test parameters of participants.

Variable	Participants (n = 62)
Duration of exercise, min	9.8 ± 1.9
Peak exercise capacity (METs)	12.0 ± 2.0
Maximal heart rate, bpm	177.0 ± 8.5
SBP_{ME}, mmHg	148.8 ± 10.8
DBP_{ME}, mmHg	75.5 ± 13.3
HRR1, bpm	34.5 ± 7.7
HRR2, bpm	45.8 ± 6.6
HRR3, bpm	61.2 ± 9 .6

Numeric variables with a normal distribution were presented as the mean ± standard deviation. Abbreviations: bpm, beats per minute; HRR1-3, heart rate recovery indices (see text for full description); DBP_{ME}, diastolic blood pressure at maximum exercise; METs, metabolic equivalent levels; SBP_{ME}, systolic blood pressure at maximum exercise.

Table II. Dynamic pupillometry measurements of participants and correlations with HRR indices.

Variable	Participants (n=62)	Correlation coefficient (r)			Linear regression analysis (β)	
		HRR1	HRR2	HRR3	Univariate	Multivariate
R0, mm	4.40 ± 0.93				1.710	
R1, mm	4.88 ± 0.84				2.122	
R2, mm	3.28 ± 0.76				-0.103	
R%	0.74 (0.60-0.92)	**-0.384**			**-6.355	-1.853
Lc, ms	267 (133-333)	**-0.442**	**-0.316**		****-0.057**	*-0.032
Ld, ms	800 (633-1500)	**-0.286**			***0.019**	-0.014
Dc, ms	533 (333-1167)				***0.016**	0.019
Dd, ms	1616 (733-1867)				-0.007	
Ac, mm	1.60 (1.20-2.30)	**0.559**			****15.729**	-3.350
Vc, mm/s	4.96 ± 1.15	**0.660**			****4.338**	***3.995**
Vd, mm/s	1.75 (1.26-5.22)	**0.412**			****5.859**	1.114

Numeric variables with a normal distribution were presented as the mean ± standart deviation. *Abbreviations*: Ac, amplitude of contraction; Lc, latency of contraction; Ld, latency of dilatation; Dc, duration of contraction; Dd, Duration of dilatation; Vc, velocity of contraction; Vd, velocity of dilatation. **$p < 0.01$: *$p < 0.05$.

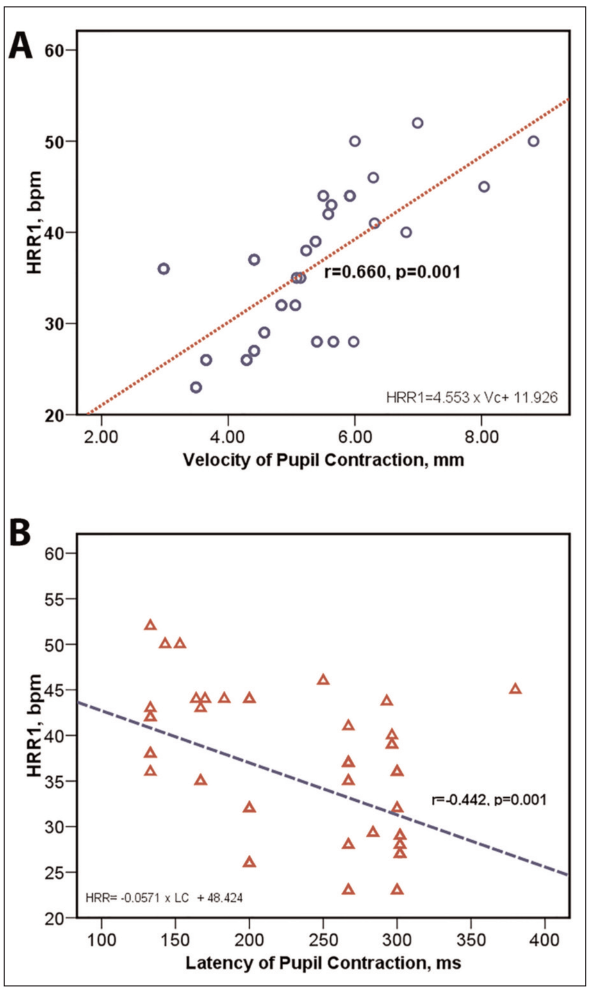

Figure 2. Correlation analysis of HRR with *(A)* velocity of pupil contraction, *(B)* latency of pupil contraction. R = correlation coefficient.

Effects of age, gender, smoking status, BMI and DP variables on HRR indices were examined in a univariate linear regression analysis separately. R% (β = -6.355, p = 0.001), Ac (β = 15.729, p = 0.001), Vc (β = 4.338, p = 0.001), Lc (β = -0.057, p = 0.001), Dc (β = 0.016, p = 0.001), Ld (β = 0.019, p = 0.001), and Vd (β = 5.859, p = 0.001) were found to be significant predictors of HRR1. These significant variables were further analyzed in multivariate linear regression model. Vc [β = 3.995 (1.040 to 6.951, 95% CI, p = 0.009)] and Lc [β = -0.032 (-0.056 to -0.008, 95% CI, p = 0.01)] were found to be significant independent predictors of HRR1.

Discussion

The main findings of this study are as follows: (1) pupillary autonomic functions assessed by DP correlates with cardiac autonomic functions evaluated by HRR, (2) among the measured DP indices, Vc and Lc were found to be significant independent predictors of cardiac autonomic functions. To the best of our knowledge, it is the first study that evaluated the utility of DP as a predictor of cardiac autonomic activity assessed by HRR. Importantly, DP indices were found to be correlated and associated with HRR1.

Sympathetic and parasympathetic limbs play an important role in modulating both intrinsic and actual HR[2,17]. Parasympathetic activity reduces the HR via vagal nerve. In contrast, sympathetic activity accelerates the HR via circulating catecholamines and neural release of norepinephrine[18,19]. Likewise, ANS have modulatory role on dynamic change in pupil size. Both sphincter and dilator muscles of the iris receive dual innervations from the two branches of the ANS[11,12]. Parasympathetic fibers supply the iris sphincter and cause reduction of pupil size by contraction of the muscle. Meanwhile, the sphincter had sympathetic innervations which are capable of inhibiting pupil contraction by relaxation of the sphincter muscle. This inhibition is primarily via beta-adrenergic receptors. Pupil dilation via sympathetic inhibition of the sphincter is about one-third of the maximum physiological dilation. On the other side, sympathetic innervation causes contraction of iris dilator muscle via alpha-adrenergic receptors which ends up with the increase in pupil diameter[12,13]. DP is a non-invasive, standardized and fully automated system for assessing ANS activity of pupils. Characteristic V-shaped light response recorded during DP is divided into 3 parts: first part reflects parasympathetic activation; second part consists both sympathetic and the parasympathetic activity; and third one signifies sympathetic activity alone[11-13] (Figure 3). From the first part, Ac and Vc are indicators of parasympathetic activity. In accordance with this data, we found that Ac and Vc have significant positive correlations with HRR1. Because of sympathetic withdrawal and parasympathetic reactivation in the process of HRR, indicators of indicators of parasympathetic activity were found to be positively correlated. Importantly, Vc was found to independently predict cardiac autonomic activity assessed by HRR1. Therefore, Vc can be used as an indicator of parasympathetic modulation on the heart.

On the other hand, R0, R% and Lc are mainly under sympathetic control and can be used as an indicator of sympathetic activity. Complying with this, we found that R% and Lc have negative correlations with cardiac autonomic activity assessed by HRR1. Furthermore, Lc was found to be an independent predictor of HRR1. Consequently, Lc can be used as an indicator of sympathetic modulation on the heart. Remaining parameters, Ld and Vd carries the effect of both sympathetic and parasympathetic activity. There was also a significant positive correlation between Vd and HRR1 in our analysis.

Conclusions

As a conclusion, pupillary autonomic functions assessed by DP correlates with cardiac autonomic functions evaluated by HRR. Among the DP parameters analyzed, Vc and Lc were

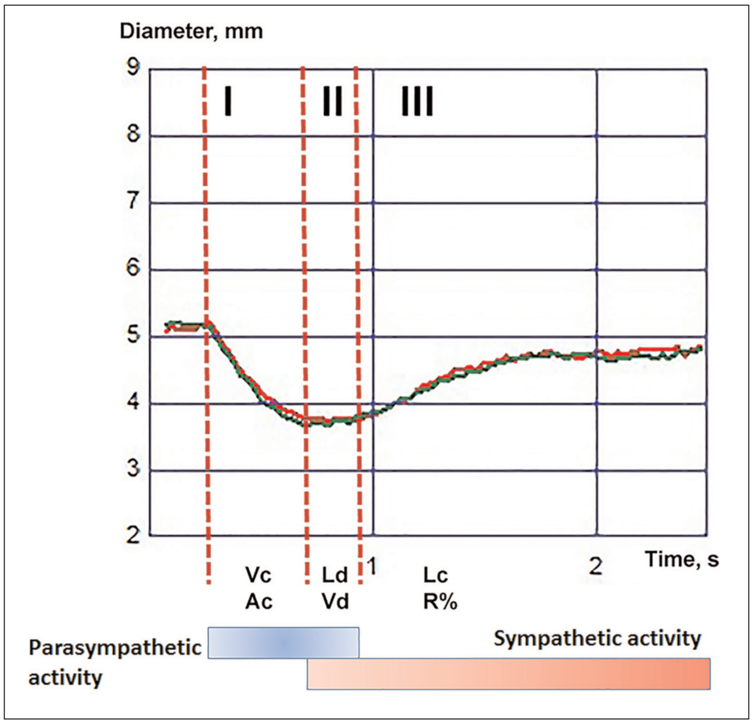

Figure 3. ***A,*** Characteristic V-shaped response of dynamic pupillometry. Ac, amplitude of contraction; Lc, latency of contraction; Ld, latency of dilatation; Vc, velocity of contraction; Vd, velocity of dilatation.

found to be significant independent predictors of cardiac autonomic functions. Considering the physiological mechanisms and results, Vc can be used as an index of parasympathetic activity and Lc can be used as an index of sympathetic modulation on the heart. Further studies should be performed for a better understanding of these two autonomic systems interrelations and application of DP indices in CAUD and different clinical settings in cardiology.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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