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Diabetic retinopathy is associated with early autonomic dysfunction assessed by exercise-related heart rate changes

C.K. Kramer[1], C.B. Leitão[1], M.J. Azevedo[1], F.B. Valiatti[2], T.C. Rodrigues[1], L.H. Canani[1] and J.L. Gross[1]

[1]Serviço de Endocrinologia, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil
[2]Divisão de Retina e Vítreo, Hospital Banco de Olhos de Porto Alegre, Porto Alegre, RS, Brasil

Correspondence to: C.K. Kramer, Serviço de Endocrinologia, Hospital de Clínicas de Porto Alegre, UFRGS, Rua Ramiro Barcelos, 2350, Ed. 12, 4º andar, 90035-003 Porto Alegre, RS, Brasil
Fax: +55-51-2101-8777. E-mail: ckkramer@terra.com.br

Diabetic retinopathy has been associated with cardiac autonomic dysfunction in both type 1 and type 2 diabetes mellitus (DM) patients. Heart rate (HR) changes during exercise testing indicate early alterations in autonomous tonus. The aim of the present study was to investigate the association of diabetic retinopathy with exercise-related HR changes. A cross-sectional study was performed on 72 type 2 and 40 type 1 DM patients. Autonomic dysfunction was assessed by exercise-related HR changes (Bruce protocol). The maximum HR increase, defined as the difference between the peak exercise rate and the resting rate at baseline, and HR recovery, defined as the reduction in HR from the peak exercise to the HR at 1, 2, and 4 min after the cessation of the exercise, were determined. In type 2 DM patients, lower maximum HR increase (OR = 1.62, 95%CI = 1.03-2.54; P = 0.036), lower HR recovery at 2 (OR = 2.04, 95%CI = 1.16-3.57; P = 0.012) and 4 min (OR = 2.67, 95%CI = 1.37-5.20; P = 0.004) were associated with diabetic retinopathy, adjusted for confounding factors. In type 1 DM, the absence of an increase in HR at intervals of 10 bpm each during exercise added 100% to the odds for diabetic retinopathy (OR = 2.01, 95%CI = 1.1-3.69; P = 0.02) when adjusted for DM duration, A1c test and diastolic blood pressure. In conclusion, early autonomic dysfunction was associated with diabetic retinopathy. The recognition of HR changes during exercise can be used to identify a high-risk group for diabetic retinopathy.

Key words: Type 2 diabetes mellitus; Type 1 diabetes mellitus; Heart rate changes; Diabetic retinopathy

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Introduction

Hypertension is a well-known risk factor for diabetic retinopathy (1). Even minor blood pressure (BP) increases during ambulatory BP monitoring might influence the development of diabetic retinopathy (2), probably due to decreased vasomotor control of the circulation of the retinal vessels allowing even small alterations in BP to impact the retina.

Diabetic retinopathy has been associated with cardiac autonomic dysfunction in both type 1 (3) and type 2 diabetes mellitus (DM) patients (4). Heart rate (HR) changes related to exercise testing (5,6) may detect early alterations in autonomous tonus and are a major predictor of sudden death and cardiac mortality in healthy and post-myocardial infarct patients (7-9).

Therefore, the aim of the present study was to investigate the association of diabetic retinopathy with a specific HR profile related to exercise testing in DM patients in the absence of both ischemic heart disease and clinical autonomic neuropathy.

Patients and Methods

Patients

A cross-sectional study was performed on type 1 and type 2 DM patients diagnosed according to American Diabetes Association criteria (10) and regularly attending the DM outpatient clinic at Hospital de Clínicas de Porto Alegre, Brazil, since 1994. Patients were selected from a cohort of 270 type 2 DM (11) and 349 type 1 DM patients based on the presence of funduscopic evaluation by an ophthalmologist and exercise electrocardiograph testing without evidence of myocardial ischemia. The exclusion criteria were age less than 18 years (type 1 DM), creatinine >1.5 mg/dL, other renal diseases, presence of persistent cardiac arrhythmia, abnormal sensitive peripheral tests (Achilles tendon reflexes, vibration or sensory perception tests), autonomic symptoms and orthostatic hypotension. The autonomic symptoms evaluated were chronic diarrhea, syncope or vasomotor symptoms and orthostatic hypotension was defined as systolic BP drop of ≥20 mmHg or diastolic BP ≥10 mmHg at 1 min in the standing position after decubitus.

The study protocol was approved by the Ethics Committee of the hospital, and written informed consent was obtained from all patients.

Clinical evaluation

Patients underwent an interview and clinical examination to record demographic and anthropometrical data, as previously described (11,12). BP was measured twice on the right arm to the nearest 2 mmHg after a 10-min rest using a standard mercury sphygmomanometer (phases I and V of Korotkoff sounds) on decubitus. BP was also recorded 1 min after standing.

Fundus eye examination was performed by an experienced ophthalmologist after mydriasis and diabetic retinopathy was classified using the scale developed by the Global Diabetic Retinopathy Group (13,14). The diabetic retinopathy level was based on the most severe degree of retinopathy in the worst eye affected. For the purpose of this study, patients were grouped according to the presence or absence of any degree of diabetic retinopathy.

Cardiovascular autonomic evaluation

Exercise electrocardiography was conducted according to the standard Bruce protocol utilizing a computerized database (15). Cardioactive medications were stopped one week before the exam.

Data on symptoms, HR and rhythm, BP and estimated workload in metabolic equivalents (METs) were collected midway through each stage of the exercise protocol, at peak exercise and at 1, 2, and 4 min after cessation of exercise. METs were estimated according to standard tables and are equal to 3.5 mL of oxygen uptake per kilogram of body weight per minute.

HR increase was defined as the difference between the peak exercise rate and resting rate at baseline. HR recovery was defined as the reduction in the rate from the peak exercise level to the rate at 1, 2, and 4 min after cessation of exercise. The BP increase was defined as the difference between the peak exercise BP and resting BP; recovery BP was defined as the reduction in mmHg from the peak of exercise level to the BP at 1, 2, and 4 min after the cessation of the exercise.

A subset of type 1 DM patients (N = 22) performed cardiac autonomic system evaluation by conventional non-invasive tests: the Valsalva maneuver, beat-to-beat HR variation, the HR response to standing, postural fall in blood pressure, and the sustained handgrip test as previous described (16,17).

Laboratory methods

Urinary albumin excretion rate (UAER) was measured in 24-h sterile urine samples by immunoturbidimetry in at least two samples collected over the preceding 6 months (MicroAlb Sera-Pak® immuno microalbuminuria; Roche®, USA). The results are reported in µg/min and the mean intra-assay and interassay variation coefficients are 4.5 and 7.6%, respectively. Glycated hemoglobin (A1c test) was measured by high-performance liquid chromatography (reference range 4.7-6.0%; Merck-Hitachi 9100, Germany). Fasting plasma glucose was measured by the glucose-peroxidase colorimetric enzymatic method (Biodiagnostica, Brazil). Creatinine was measured by the Jaffé method and serum total cholesterol and triglycerides were measured by enzymatic-colorimetric methods (Merck Diagnostica; Boeringher Mannheim, Germany), HDL cholesterol by homogeneous direct method (autoanalyzer, ADVIA 1650; Siemens Healthcare Diagnostics, Germany). LDL cholesterol was calculated using the Friedewald formula (18).

Statistical analysis

The Student *t*-test or the chi-square test were used to compare clinical and laboratory data. Quantitative variables not having a normal distribution were log transformed. Correlations were performed using the Pearson's chi-square or Spearman's rank correlation depending on the distribution of variables. Multiple logistic regression models were constructed with diabetic retinopathy as the dependent variable, adjusted for A1c test, DM duration, systolic BP, age, UAER, and METs. In each model, one

variable of HR profile was included: HR recovery at 2 min, HR recovery at 4 min or maximum increase in HR. The HR variables in the multiple analyses were categorized at intervals of 10 bpm each. Data are reported as mean ± SD, except for triglycerides and UAER, which are reported as median (range). $P < 0.05$ (two-tailed) were considered to be significant.

Results

Seventy-two type 2 DM patients and 40 type 1 were included. Clinical and laboratory characteristics of the patients are presented in Table 1. Among type 2 DM patients, 22 (30.5%) had some degree of diabetic retinopathy. Patients with diabetic retinopathy had higher A1c values and office BP levels than those without diabetic retinopathy. DM duration, lipid profile, UAER, body mass index, waist circumference, and smoking habit were not different between groups. Among type 1 DM patients, 21 (52.3%) had some degree of diabetic retinopathy. Patients with diabetic retinopathy were older than those without. BP levels, glycemic control, DM duration, lipid profile, UAER, body mass index, waist circumference, and smoking habit did not differ between groups.

In type 2 DM patients, resting HR was higher in the patients with diabetic retinopathy, but the HR increase during exercise was lower than in those without diabetic retinopathy (Table 2). The HR recovery at 1 and 2 min after exercise was similar between groups, while at 4 min after exercise the diabetic retinopathy group had lower HR recovery than the group without diabetic retinopathy. The peak systolic and diastolic BP levels during exercise did not differ between groups nor did the maximum BP increment. The systolic BP recovery 2 min after exercise was higher in patients with diabetic retinopathy but diastolic BP recovery was not different between patients with and without diabetic retinopathy.

There was no difference in MET levels achieved in each group (8.0 ± 2.1 *vs* 8.2 ± 2; $P = 0.61$) and all patients reached at least 85% of the maximal estimated HR frequency.

Based on multivariate logistic regression analysis, increased resting HR, lower HR increase and slower HR recovery at 2 and 4 min were associated with diabetic retinopathy (Table 3) before and after adjustment for A1c test, DM duration, systolic BP, age, albuminuria, and METs.

The same pattern of altered HR variability during exercise observed in type 2 DM patients with diabetic retinopathy was observed in type 1 DM: the HR increase during exercise was greater in the group without diabetic retinopathy (100 ± 22 *vs* 79 ± 16 bpm; $P = 0.001$; Table 2). At 2 min after exercise, HR recovery was lower in patients with diabetic retinopathy (47 ± 26 *vs* 62 ± 15 bpm; $P = 0.03$) than in those without diabetic retinopathy. The same pat-

Table 1. Clinical and laboratory characteristics as a function of the presence of diabetic retinopathy in type 2 and type 1 diabetic patients.

	Type 2 diabetes retinopathy		Type 1 diabetes retinopathy	
	Absent (N = 50)	Present (N = 22)	Absent (N = 19)	Present (N = 21)
Male subjects (N)	27 (54%)	13 (59%)	10 (53%)	14 (67%)
Age (years)	55.8 ± 9	53.7 ± 9	39.5 ± 10	50.4 ± 8.5*
Diabetes duration (years)	8.7 ± 6.8	10.7 ± 6.0	21.5 ± 11.7	27 ± 8.9
Body mass index (kg/m^2)	28.6 ± 4.1	29.6 ± 4.4	24.3 ± 4.0	23.8 ± 3.9
Waist circumference (m)	0.97 ± 0.1	0.98 ± 0.1	86.5 ± 9.7	83.6 ± 11.6
Smoking (N)	25 (50%)	8 (36%)	2 (11%)	3 (14%)
Systolic blood pressure (mmHg)	130 ± 15	138 ± 11*	115 ± 19	123 ± 11
Diastolic blood pressure (mmHg)	78 ± 9	83 ± 7*	75 ± 12	78 ± 7
Glycated hemoglobin (%)	6.7 ± 0.9	8.2 ± 1.5*	7.5 ± 1	8.2 ± 1.4
Fasting plasma glucose (mg/dL)	133 ± 43	165 ± 50*	154 ± 68	185 ± 105
Total cholesterol (mg/dL)	195 ± 31	186 ± 44	179 ± 23	171 ± 32
High density cholesterol (mg/dL)	51 ± 16	47 ± 11	52 ± 12	52 ± 13
Low density cholesterol (mg/dL)	115 ± 29	106 ± 35	104 ± 20	100 ± 20
Triglycerides (mg/dL)	129 (39-974)	152 (50-555)	83 (42-348)	83 (32-534)
Urinary albumin excretion rate (µg/min)	8 (0.1-1004)	14 (0.11-810)	6 (0.3-45)	9 (0.1-501)
Creatinine (mg/dL)	0.8 ± 0.2	0.7 ± 0.2	1.0 ± 0.2	1.2 ± 0.7

Data are reported as means ± SD or number (%) except for triglycerides and urinary albumin excretion rate, which are reported as median (range). *$P < 0.05$ compared to absence of retinopathy (Student *t*-test for continuous variable; chi-square test for categorical variables).

tern was observed at 4 min after exercise (60 ± 20 *vs* 77 ± 14 bpm; P = 0.005). The BP parameters analyzed during exercise were similar between groups. Also, lower HR increase during exercise and slower HR recovery at 2 min were associated with diabetic retinopathy adjusted for DM duration, A1c test and diastolic BP (Table 3).

The parameters of HR changes during exercise correlated with conventional autonomic cardiovascular tests in type 1 DM patients. The mean HR variability during Valsalva maneuver was 2.4 ± 2.8 bpm and during standing was 1.34 ± 0.77 bpm. The HR recovery at 2 and 4 min of exercise correlated with HR variability during Valsalva maneuver (2 min: r = 0.679; P = 0.005; 4 min: r = 0.637; P = 0.01). The HR recovery at 4 min and HR maximum increase during exercise correlated with HR variability in standing tests (HR at 4 min: r = 0.653; P = 0.01; HR increment: r = 0.561; P = 0.03).

Discussion

This study detected an independent association between diabetic retinopathy and high resting HR, low HR increase and recovery related to exercise. An alteration of the autonomic system in patients with diabetic retinopathy

Table 2. Exercise testing variables as a function of the presence of diabetic retinopathy in type 2 and type 1 diabetic patients.

	Type 2 diabetes retinopathy		Type 1 diabetes retinopathy	
	Absent (N = 50)	Present (N = 22)	Absent (N = 19)	Present (N = 21)
Resting heart rate (bpm)	82 ± 12	89 ± 10*	79 ± 17	83 ± 14
Peak exercise heart rate (bpm)	159 ± 15	152 ± 17	179 ± 14	162 ± 15*
Heart rate increase (bpm)	77 ± 17	64 ± 18*	100 ± 22	79 ± 16*
Heart rate recovery at 1 min (bpm)	13 ± 11	11 ± 10	11 ± 16	14 ± 17
Heart rate recovery at 2 min (bpm)	49 ± 11	42 ± 15	62 ± 15	47 ± 26*
Heart rate recovery at 4 min (bpm)	60 ± 12	53 ± 14*	77 ± 14	60 ± 20*
Resting systolic blood pressure (mmHg)	130 ± 15	138 ± 11*	115 ± 19	123 ± 11
Resting diastolic blood pressure (mmHg)	78 ± 9	83 ± 7*	75 ± 12	78 ± 7
Peak systolic blood pressure (mmHg)	177 ± 21	182 ± 25	170 ± 31	171 ± 24
Peak diastolic blood pressure (mmHg)	79 ± 8	83 ± 13	76 ± 16	73 ± 9
Systolic blood pressure increase (mmHg)	47 ± 18	55 ± 20	54 ± 26	48 ± 22
Diastolic blood pressure increase (mmHg)	0.4 ± 7	0.5 ± 11	1.2 ± 10	-4 ± 9
Systolic blood pressure recovery at 2 min	14 ± 23	24 ± 16*	9 ± 15	7 ± 17
Diastolic blood pressure recovery at 2 min	0.6 ± 8	0.7 ± 7	-2 ± 9	-0.4 ± 6
Total metabolic equivalent	8.2 ± 2	8 ± 2.1	11.2 ± 3	10 ± 2.3

Data are reported as means ± SD. Heart rate recovery = maximum heart rate minus heart rate at 1, 2, and 4 min after exercise cessation. Blood pressure recovery = maximum blood pressure level minus blood pressure at 2nd min after exercise cessation. *P ≤ 0.05 compared to absence of retinopathy (Student *t*-test).

Table 3. Multivariate analysis of heart rate at stress test and the presence of diabetic retinopathy in type 2 and type 1 diabetes.

	Type 2 diabetes retinopathy[a]			Type 1 diabetes retinopathy[b]		
	OR	95%CI	P	OR	95%CI	P
Resting heart rate (bpm)	1.1	1-1.1	0.005	1.0	0.96-1.05	0.69
Peak exercise heart rate (bpm)	0.95	0.9-1	0.07	0.9	0.85-0.98	0.01
Heart rate increase (bpm)	0.90	0.85-0.96	0.002	0.9	0.9-0.99	0.02
Heart rate recovery at 1 min (bpm)	0.98	0.93-1.0	0.62	1.02	0.98-1.07	0.26
Heart rate recovery at 2 min (bpm)	0.93	0.88-0.99	0.02	0.96	0.92-1.00	0.07
Heart rate recovery at 4 min (bpm)	0.91	0.85-0.97	0.006	0.93	0.88-0.99	0.02

[a]Adjusted for glycated hemoglobin A1c test, diabetes mellitus duration, systolic blood pressure, age, albuminuria, and metabolic equivalents (METs). [b]Adjusted for glycated hemoglobin A1c test, diabetes mellitus duration and diastolic blood pressure. Heart rate recovery = maximum heart rate minus heart rate at 1, 2, and 4 min after exercise cessation.

may explain these data as suggested by correlation of these HR changes with cardiovascular autonomic test variables.

The association between diabetic retinopathy and autonomic dysfunction has been suggested by previous studies that evaluated the autonomic system by the cardiovascular tests described by Ewing et al. (16). Krolewski et al. (3), in a cohort of type 1 DM patients followed for 15 to 21 years, showed a relationship between abnormal cardiac autonomic tests and proliferative diabetic retinopathy. Moreover, abnormalities in autonomic ocular function were associated with the development of diabetic retinopathy in a prospective study of 335 type 1 DM patients (19). In type 2 DM, autonomic cardiac neuropathy as assessed by R-R interval variability and resting HR was also associated with the severity of diabetic retinopathy (4,20). Different from the present study, these studies included patients with the clinical manifestations of autonomic dysfunction. Moreover, patients also had ischemic heart disease that could lead to alterations in autonomic function (21). In the present study, the patients included did not have ischemic heart disease or clinical manifestation of autonomic neuropathy, suggesting that alterations in HR profile associated with exercise occur earlier than clinically evident autonomic dysfunction.

The evaluation of HR changes related to exercise is a feasible and simple test to perform and has a rational basis to evaluate the autonomic system. During exercise, a combination of increased sympathetic activity and vagal withdrawal increases HR and the arterial baroreflex is reset in relation to workload (6,22). These coordinated changes in the autonomic system are responsible for the changes in BP and HR during and after exercise. HR recovery after exercise testing is correlated with HR variability in healthy elderly men (23) and acetylcholinesterase inhibition improves HR recovery after exercise in heart failure patients (24), suggesting that the HR profile during exercise could be an early marker of decreased vagal cardiac tonus. This premise is reinforced by data from the present study that showed a strongly significant ($r = 0.679$, $P = 0.005$) correlation of HR recovery after exercise with HR variability on cardiovascular autonomic tests in patients with type 1 DM.

One possible explanation is that autonomic dysfunction might be related to diabetic retinopathy pathogenesis through alterations in the BP pattern. We have reported that alterations in BP pattern in type 1 DM patients were associated with diabetic retinopathy (2), and a blunt fall in nocturnal diastolic BP was related to higher sympathetic activity (17,25). These data suggest that alterations in BP pattern are associated with autonomic dysfunction. Moreover, autonomic dysfunction may affect the autoregulation of retina vessels, allowing an abnormal BP pattern or small elevations to have a deleterious impact on the retina.

A possible limitation of this study could be attributed to its cross-sectional design that precludes a causal relationship between the HR exercise profile and diabetic retinopathy development.

Early autonomic dysfunction, assessed by HR changes related to exercise, was associated with diabetic retinopathy. The recognition of HR changes during exercise, a feasible and simple index, might be a practical tool to identify a high-risk group for the presence of diabetic retinopathy.

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