

Myocardial Dysfunction and Adrenergic Cardiac Innervation in Patients With Insulin-Dependent Diabetes Mellitus

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Background. Insulin-dependent diabetes mellitus (IDDM) is associated with an increased incidence of heart failure due to several factors, and in some cases a specific cardiomyopathy has been suggested.

Objectives. This study sought to assess the mechanisms of exercise-induced left ventricular (LV) dysfunction in asymptomatic patients with IDDM in the absence of hypertensive or coronary artery disease.

Methods. Fourteen consecutive patients with IDDM were enrolled (10 men, 4 women; mean [\pm SD] age 28.5 ± 6 years); 10 healthy subjects matched for gender (7 men, 3 women) and age (28.5 ± 3 years) constituted the control group. LV volume, LV ejection fraction (LVEF) and end-systolic wall stress were calculated by two-dimensional echocardiography at rest and during isometric exercise. LV contractile reserve was assessed by post-extrasystolic potentiation (PESP) obtained by transesophageal cardiac electrical stimulation and dobutamine infusion. Myocardial iodine-123 metaiodobenzylguanidine (MIBG) scintigraphy was performed to assess adrenergic cardiac innervation.

Results. Diabetic patients were classified into group A ($n = 7$), with an abnormal LVEF response to handgrip ($42 \pm 7\%$), and group B ($n = 7$), with a normal response ($72 \pm 8\%$). Baseline LVEF

was normal in both group A and B patients ($60 \pm 6\%$ vs. $61 \pm 7\%$, $p = \text{NS}$). In group A patients, the LV circumferential wall stress-LVEF relation showed an impairment in LVEF disproportionate to the level of LV afterload. No significant changes in LVEF occurred during dobutamine ($60 \pm 6\%$ vs. $64 \pm 10\%$, $p = \text{NS}$), whereas PESP significantly increased LVEF ($60 \pm 6\%$ vs. $74 \pm 6\%$, $p < 0.001$); PESP at peak handgrip normalized the abnormal LVEF ($42 \pm 7\%$ vs. $72 \pm 5\%$, $p < 0.001$); and MIBG uptake normalized for body weight or for LV mass was lower than that in normal subjects (1.69 ± 0.30 vs. 2.98 ± 0.82 cpm/MBq per g, $p = 0.01$) and group B diabetic patients (vs. 2.79 ± 0.94 cpm/MBq per g, $p = 0.01$). Finally, a strong linear correlation between LVEF at peak handgrip and myocardial MIBG uptake normalized for LV mass was demonstrated in the study patients.

Conclusions. Despite normal contractile reserve, a defective blunted recruitment of myocardial contractility plays an important role in determining exercise LV dysfunction in the early phase of diabetic cardiomyopathy. This abnormal response to exercise is strongly related to an impairment of cardiac sympathetic innervation.

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Patients with insulin-dependent diabetes mellitus (IDDM) appear to have an increased likelihood of developing congestive heart failure (1,2). Possible mechanisms for myocardial dysfunction include multiple factors, such as a combination of altered substrate metabolism (3), coronary macrovascular and microvascular angiopathy and hypertensive disease (4). However, cardiac problems in diabetic patients cannot always be attributed to these causes, and a specific cardiomyopathy has been suggested (5) as a causal factor producing the increase in cardiac mortality and morbidity. The concept of cardiomyop-

athy implies a time-dependent development of cardiac muscle disease, which includes a period when symptoms and abnormal signs are not present. Subclinical left ventricular (LV) systolic (6) or diastolic (7) dysfunction, or both, may be observed at rest and more often during exercise. The relation between LV dysfunction induced by exercise and contractile function represents a very intriguing problem. In fact, it has been demonstrated (8) that an abnormal LV ejection fraction (LVEF) response to exercise cannot be equated with the presence of abnormal myocardial contractile reserve; and more recently, a defective inotropic recruitment has been shown (9) to play an important role in determining an abnormal left ventricular response to exercise despite a normal inotropic reserve. In light of these observations, the aim of the present study was to investigate the mechanisms of exercise-induced LV dysfunction in asymptomatic patients with IDDM. In particular, we related LV contractile function, assessed by post-extrasystolic

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Abbreviations and Acronyms

ECG	= electrocardiographic
IDDM	= insulin-dependent diabetes mellitus
I-123 MIBG	= iodine-123 metaiodobenzylguanidine
LV	= left ventricular
LVEF	= left ventricular ejection fraction
PESP	= post-extrasystolic potentiation
ROI	= region of interest

potentiation (PESP) and dobutamine infusion, to cardiac adrenergic function measured by assessing adrenergic myocardial innervation using scintigraphic imaging with iodine-123 metaiodobenzylguanidine (MIBG).

Methods

Study patients. We studied 14 consecutive asymptomatic patients with IDDM (10 men, 4 women; mean [\pm SD] age 28.5 ± 6 years) who fulfilled the following inclusion criteria: 1) no overt long-term diabetic complications; 2) no overt heart disease; 3) blood pressure <150 mm Hg systolic and <90 mm Hg diastolic; 4) no major additional risk factors for coronary artery disease (i.e., blood cholesterol concentration ≤ 5.2 mmol/liter, no smoking); 5) absence of electrocardiographic (ECG) signs and clinical history of myocardial infarction; 6) no angina pectoris; 7) absence of ischemic ST segment changes induced by isotonic and isometric exercise; 8) no LV wall motion abnormalities induced by isometric exercise; 9) no evidence of significant obstruction in coronary vessels by coronary angiography; 10) serum creatinine <1.2 mg/dl; and 10) no assumption of any drugs known to affect cardiac function or to interfere with myocardial MIBG uptake.

In addition, 10 healthy control subjects matched for gender (7 men, 3 women), body mass index (23.6 ± 1.4 vs. 23.1 ± 1.3 g/m², $p = \text{NS}$) and age (28.5 ± 3 years) were recruited to perform PESP isometric exercise and myocardial MIBG scintigraphy.

Study protocol. The study protocol was approved by the local ethics committee, and informed consent was obtained from all participants. Assessment of the study cohort included 1) assessment of retinal and renal long-term diabetic complications; 2) determination of the degree of metabolic control; 3) coronary angiography; 4) LV function study at rest and during isometric exercise by quantitative two-dimensional echocardiography; 5) assessment of LV contractile function at rest by dobutamine infusion and PESP; 6) evaluation of LV contractile function during isometric exercise by PESP; 7) evaluation of autonomic cardiac innervation by MIBG scintigraphy.

Assessment of degree of metabolic control. All participants were asked to fast for at least 12 h before the blood sampling. Blood samples were taken without cuffing to determine plasma glucose, hemoglobin A1c, electrolytes and creatinine levels and lipid profile. Patients with IDDM were all able to self-monitor

their plasma glucose levels at least three times a day, both in the fasting condition and 2 h after principal meals. A complete lifestyle questionnaire to obtain medical histories, parental history of cardiovascular disease, information on smoking habits and physical activity was completed by each patient, and answers were discussed with one of the physicians (A.A.). The metabolic variables are reported in Table 1.

Assessment of retinal and renal long-term diabetic complications. The fundus oculi was examined by a trained ophthalmologist, and each patient was classified according to the presence or absence of diabetic retinopathy; 24-h microalbuminuria was measured for 3 days.

MIBG scintigraphy. Myocardial MIBG scintigraphy was performed in diabetic patients and control subjects. Thyroid blockade was achieved with potassium iodide (Lugol's solution) for several days before the study. Participants were instructed to avoid tea, coffee and alcoholic beverages on the day of the study. Otherwise, diabetic patients consumed their regular diets, and insulin was administered as usual. After a 30-min rest period, a dose of 100 to 185 MBq of I-123 MIBG was slowly infused intravenously. A dynamic acquisition was performed 1 h after injection in the left anterior oblique position (40°) for a better visualization of the left ventricle by means of a large field of view gamma camera (Siemens Medical System) equipped with a high resolution low energy collimator. The set of scintigraphic images consisted of 10 consecutive frames, each of 6 min, for a duration of 1 h, recorded and processed on a dedicated computer (Max Delta, Microvax Digital). Equivalent regions of interest (ROIs) of the left ventricle and lung were established to calculate both the heart/lung ratio and myocardial I-123 MIBG washout and to assess adrenergic cardiac innervation. The following variables were measured: 1) I-123 MIBG myocardial half-life (washout), expressed in minutes and determined from heart and lung curves; 2) heart/lung ratio, measured from equivalent ROIs referred to LV and lung tissue uptake; 3) sympathetic myocardial innervation, evaluated through the calculation of cpm/administered dose, normalized for body weight (expressed as cpm/MBq per kg) or for LV myocardial mass (expressed as cpm/MBq per g) calculated by two-dimensional echocardiography.

Echocardiographic analysis. Echocardiographic examinations were performed with a Hewlett-Packard 77030A phase array ultrasonoscope and a 2.5- or 3.5-MHz transducer. Echocardiographic studies were coded and read by two independent observers (R.S., G.F.) in blinded manner, with no knowledge of to the patient's identity and experimental condition. Echocardiographic analysis was performed using the digitized cine loop method (Prevue III System, Nova Microsonics, Inc.). Wall motion and myocardial thickening were detected by examining echocardiographic images of the left ventricle obtained in the apical four- and two-chamber views and in the parasternal long- and short-axis views. LV wall motion was analyzed by repeated viewing. Agreement of interobserver analysis for segmental asynergy was seen in 98% of the segments visualized; discrepancies were resolved by consensus. LV volumes

Table 1. Clinical Characteristics of Study Patients and Control Group*

Pt No./Gender	Age (yr)	Duration of IDDM (yr)	BMI (kg/m ²)	HbA _{1c} (%)	FPG (mg/dl)	AER (mg/day)	Diabet Retinop	Na (mEq/liter)	K (mEq/liter)	IR (U/day)
Group A (n = 7)										
1/F	46	12	20.9	8.1	12.3	5	A	140	4.3	42
2/F	26	11	21.3	8.7	16.4	10	P	132	5.1	24
3/M	31	6	22.0	9	5.8	6	A	141	4.6	34
4/M	27	11	23.9	8.3	8.4	9	A	141	4.6	49
5/M	23	2	24.2	7.5	4.2	28	A	145	4	22
6/M	31	7	21.0	7.4	2.1	5	A	137	4.4	74
7/M	24	10	23.4	8.6	16	2	P	138	4.6	36
Mean	29.7	8.4	22.3	8.2†	9.3	9.2		139.1	4.5	40.1
SD	7.8	3.5	1.4	0.6	5.7	8.6		4.0	0.3	17.6
Group B (n = 7)										
8/M	26	14	22.2	7.9	11	15	A	142	4.2	39
9/M	27	7	25.3	8.5	6	8	A	143	4.1	18
10/M	29	7	25.4	8.9	10	8	A	141	4.2	56
11/M	28	10	23.4	7.6	4	10	A	142	4.3	35
12/M	26	6	24.2	9	6.4	12	P	142	5.7	56
13/F	20	2	24.2	8	12	6	A	142	4	28
14/F	36	12	22.8	8.4	8.2	5	A	139	3.9	60
Mean	27.4	8.2	23.9	8.3†	8.2	9.1		143	4.3	41.7
SD	4.7	4.0	1.2	0.5	2.9	3.4		2.7	0.6	16
Normal subjects (n = 10)										
Mean	28.5		23.6	5.2	7.7	8		141	4.3	
SD	3		1.4	0.5	1.2	2		2	0.3	

*p = NS for intergroup patient analysis. †p < 0.01 versus normal subjects. A = absent; AER = albumin excretion rate; BMI = body mass index; Diabet Retinop = diabetic retinopathy by fundus oculi examination; FPG = fasting plasma glucose; Group A = Diabetic patients with abnormal left ventricular response to exercise; Group B = Diabetic patients with normal left ventricular response to exercise; Hb = hemoglobin; IDDM = insulin-dependent diabetes mellitus; IR = insulin requirement; P = present; Pt = patient.

were calculated by an ellipsoid biplane area-length method (10). LVEF was derived as end-diastolic volume minus end-systolic volume divided by end-diastolic volume. LV endocardial echocardiograms in the apical four- and two-chamber views, for a minimum of two to four cardiac cycles, were digitized at end-diastole (R wave peak) and end-systole (time of smallest cavity area) by two independent observers. A discrepancy >10 ml for LV volume required the analysis of the echocardiographic tracing by a third observer; agreement was achieved by consensus. However, interobserver and intraobserver variability for LV area ($r = 0.94$ and $r = 0.98$, respectively) and for LV length ($r = 0.95$ and $r = 0.96$, respectively) was acceptable. LV mass was calculated by multiplying LV myocardial volume by the specific weight of the myocardium (1.05) (11). Calibration of the carotid pulse tracings was performed at rest and during isometric exercise, with assignment of systolic blood pressure to the peak and diastolic blood pressure to the nadir of the tracing (12). Linear interpolation to the level of the incisura was then performed to estimate end-systolic pressure. LV end-systolic circumferential wall stress (kdynes/cm²) was calculated by $S = (1.332PD/2h)/(1 - h/D - D2/2L2)$, where P = end-systolic pressure (simultaneous with LV echocardiographic measurements); D = LV end-systolic short-axis diameter (in parasternal short-axis view); h = LV wall thickness; and 1.332 is the factor to convert

from mm Hg to kdynes/cm² (13). Wall stress was calculated at rest, during handgrip testing and during dobutamine infusion.

Isometric exercise. Handgrip testing was performed with the patient supine. Arterial pressure was measured every 30 s by an oscillometric method (Nippon Colin Co. Ltd.), and LV function was continuously monitored by two-dimensional echocardiography. Maximal voluntary contraction was determined by means of a handgrip dynamometer. Isometric exercise was performed for 3 min at 40% maximal voluntary contraction. Patients were instructed to avoid performing the Valsalva maneuver during handgrip. Left ventricular function was assessed before and every 30 s during exercise. Heart rate and electrocardiographic tracings were continuously monitored.

Post-extrasystolic potentiation. A quadripolar electrode catheter for transesophageal cardiac stimulation (interelectrode distance 2 to 3 cm) was passed through the nares into the distal esophagus. The lead was secured where the unipolar atrial electrogram, recorded from the middle electrodes, exhibited the greatest amplitude and the most rapid deflection. In this position, consistent and constant atrial capture was achieved by means of a programmable stimulator with a pulse width of 10 ms and an amplitude of 15 mA. A bipolar single atrial extrastimulus was delivered from the two middle electrodes every seventh sensed, spontaneous sinus beat. At the

baseline assessment, the coupling interval between the spontaneous sinus beat and the induced extrasystole was progressively decreased by 10-ms increments from 500 to 300 ms. The post-extrasystolic beat was then allowed to occur spontaneously according to the patient's intrinsic rhythm. During the procedure, LV volumes were monitored by echocardiography, and only beats without significant changes in LV end-diastolic volume (increase <10% from control value) were considered for analysis. PESP was repeated at peak handgrip using a coupling interval ranging from 400 to 300 ms. The procedures produced no complications or significant patient discomfort.

Dobutamine echocardiography. Dobutamine echocardiography was performed 1 h after the PESP procedure. A quad screen, continuous loop was used to simultaneously compare rest images with dobutamine and recovery images. Dobutamine was infused intravenously by an infusion pump at 10 $\mu\text{g}/\text{kg}$ for 5 min. Patients underwent continuous ECG monitoring, and blood pressure was recorded every 30 s. LV wall motion, LV volumes and LVEF were assessed before and at the fifth minute of infusion.

Coronary angiography. Coronary cineangiography was performed in all study patients using the Judkins technique; all examinations were evaluated in blinded manner.

Tests for cardiovascular autonomic nervous function. Assessment was performed at least 3 h after the last insulin injection and included standard autonomic function tests by measuring heart rate variability during three standardized Valsalva manoeuvres (blowing against 40 mm Hg for 15 s), heart rate variability on deep breathing and heart rate variability and blood pressure variability on lying to standing (14).

Laboratory analysis. Plasma glucose was assayed using a glucose analyzer (Beckman). Hemoglobin A_{1c} was determined with a high performance liquid chromatographic method (15). Microalbuminuria was determined by the method of Keen and Chlouverakis (16). Sodium and potassium were measured with an automated assay.

Statistical analysis. Results are expressed as mean value \pm SD. Multiple comparisons were performed by two-way repeated measures analysis of variance, followed by the Fisher protected least significant difference test. Correlations and regressions were determined by a linear least squares method. A p value ≤ 0.05 by the two-tailed test was considered to indicate statistical significance.

Results

Patients with IDDM were classified into two groups on the basis of their response to isometric exercise: group A included patients with an abnormal LVEF response to exercise (defined as a decline >0.05 U); group B included patients with a normal LV response (increase or no change in LVEF at peak handgrip). Women were equally distributed between groups A and B (40% vs. 42%, p = NS).

Baseline LV function. Baseline characteristics of normal subjects and study patients are shown in Table 2. Measurements of LV end-diastolic volume (64 ± 9 ml/m², range 54 to

Table 2. Baseline and Peak Handgrip Variables

	Normal Subjects (mean \pm SD)	Diabetic Patients	
		Group A (mean \pm SD)	Group B (mean \pm SD)
Baseline			
HR (beats/min)	62 \pm 14	67 \pm 9	70 \pm 14
SBP (mm Hg)	122 \pm 7	122 \pm 12	133 \pm 17
DBP (mm Hg)	76 \pm 7	74 \pm 7	75 \pm 11
LVEDVI (ml/m ²)	64 \pm 9	66 \pm 12	63 \pm 10
LVESVI (ml/m ²)	26 \pm 6	29 \pm 4	28 \pm 8
LVEF (%)	60 \pm 9	60 \pm 6	61 \pm 7
LVESS (kdynes/cm ²)	220 \pm 49	229 \pm 52	218 \pm 64
Peak handgrip			
HR (beats/min)	89 \pm 16	81 \pm 10	83 \pm 12
SBP (mm Hg)	154 \pm 30	156 \pm 15	158 \pm 18
DBP (mm Hg)	90 \pm 19	95 \pm 14	100 \pm 14
LVEDVI (ml/m ²)	80 \pm 9	76 \pm 12	82 \pm 15
LVESVI (ml/m ²)	18 \pm 6	43 \pm 7*	22 \pm 10†
LVEF (%)	78 \pm 8	42 \pm 7*	72 \pm 8†
LVESS (kdynes/cm ²)	328 \pm 54	364 \pm 48	336 \pm 60

*p < 0.001, normal subjects versus diabetic patients. †p < 0.001, Group A versus Group B. DBP = diastolic blood pressure; HR = heart rate; LVEDVI = left ventricular end-diastolic volume index; LVEF = left ventricular ejection fraction; LVESS = left ventricular end-systolic stress; LVESVI = left ventricular end-systolic volume index; SBP = systolic blood pressure.

74) and LVEF ($60 \pm 9\%$, range 57% to 70%) in the control group were used to determine the mean and the 95% confidence limits of normal values. In diabetic patients, the baseline mean values of LV end-diastolic volume and LVEF did not differ compared with those of control subjects. Three patients (two in group A, one in group B) had an enlarged left ventricle, as indicated by an LV end-diastolic volume index greater than the upper normal limit. All patients showed a normal LVEF. Heart rate and systolic and diastolic blood pressures did not differ between diabetic patients and control subjects.

LV response to handgrip (Table 2). Myocardial ischemia was not induced by isometric exercise as assessed by the appearance of wall motion abnormalities or ST segment changes. Heart rate and systolic blood pressure increased at peak exercise in both groups of diabetic patients and in normal subjects without significant differences. LVEF response to handgrip in the control group was characterized by a >0.05 -U increase in all but two subjects, who showed no change during isometric exercise (mean baseline value $60 \pm 9\%$ vs. mean peak value $78 \pm 8\%$, p < 0.005). Seven of the diabetic patients had a >0.05 -U decline in LVEF (group A); peak LVEF was significantly lower than the baseline value ($42 \pm 7\%$ vs. $60 \pm 6\%$, p < 0.001). The remaining seven diabetic patients had a normal LV response to handgrip (five had a significant increase, whereas two showed no change, in LVEF [group B]). At peak handgrip, LVEF was greater in group B than in group A patients ($72 \pm 8\%$ vs. $42 \pm 7\%$, p < 0.001), whereas LV end-diastolic volume, heart rate and systolic and diastolic blood pressures did not differ. LVEF values at rest did not differ in patients with an abnormal response to isometric

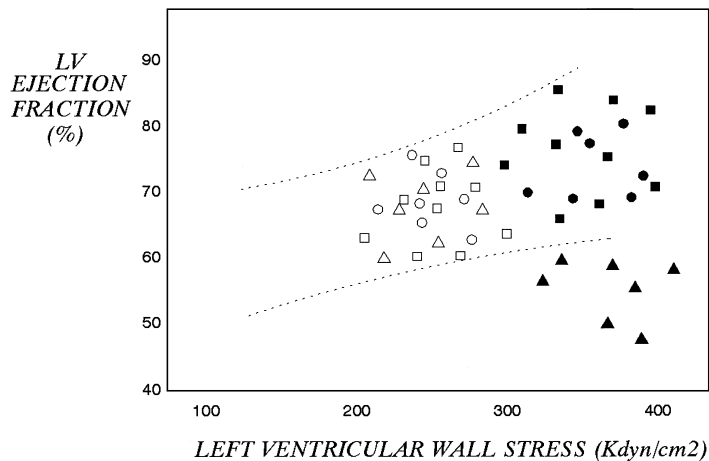


Figure 1. Relation between LVEF and LV circumferential end-systolic wall stress at rest and at peak handgrip. **Open symbols** = baseline values in normal subjects (**squares**), group A patients (**triangles**) and group B patients (**circles**); **solid symbols** = values at peak exercise in normal subjects (**squares**), group A patients (**triangles**) and group B patients (**circles**); **dashed lines** = 95% confidence limit of relation in normal subjects.

exercise, in patients with a normal response and in normal subjects ($60 \pm 6\%$, $61 \pm 7\%$ and $60 \pm 9\%$ respectively).

Circumferential wall stress-LVEF relation. When control points obtained at rest and during handgrip were considered, the relation between LV circumferential wall stress and LVEF during exercise was linear for normal subjects, with a correlation coefficient of 0.82. As shown in Figure 1, values for group A patients were downwardly displaced and fell below the 95% confidence interval, showing an impairment of ejection performance that was disproportionate to the degree of afterload during handgrip.

PESP potentiation (Table 3). PESP significantly increased LVEF both in group A ($60 \pm 6\%$ vs. $74 \pm 6\%$, $p < 0.001$) and in group B patients ($61 \pm 7\%$ vs. $72 \pm 5\%$, $p < 0.001$). To further clarify the etiology of LV dysfunction by exercise in diabetic patients, PESP was performed at peak handgrip. PESP significantly increased LVEF at peak exercise in group A ($72 \pm 5\%$ vs. $42 \pm 7\%$, $p < 0.001$), whereas no significant changes occurred in group B ($75 \pm 4\%$ vs. $72 \pm 5\%$, $p = \text{NS}$). In group A patients, potentiated peak handgrip LVEF did not differ from potentiated values at rest ($75 \pm 4\%$ vs. $72 \pm 5\%$, $p = \text{NS}$).

Dobutamine echocardiography. On dobutamine administration, mean LVEF improved significantly ($74 \pm 5\%$ vs. $61 \pm 7\%$, $p = 0.001$) in group B patients, but no significant changes

occurred in group A patients ($64 \pm 10\%$ vs. $60 \pm 6\%$, $p = \text{NS}$) (Fig. 2). LV asynergies did not appear during dobutamine infusion in either group of patients.

Cardiovascular autonomic nervous function. Traditional autonomic function tests did not reveal any differences between the two groups of diabetic patients or between patients and normal subjects.

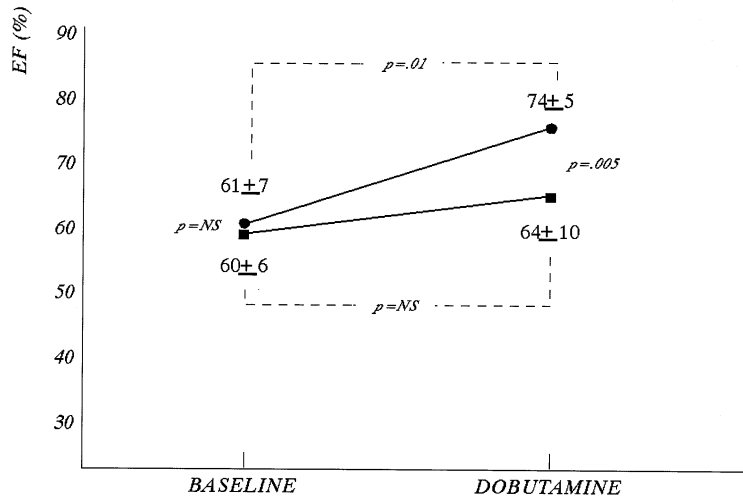
MIBG scintigraphy (Fig. 3). MIBG myocardial half-life was reduced in diabetic patients compared with that in control subjects (332 ± 68 vs. 472 ± 40 min, $p < 0.05$), with groups A and B exhibiting similar values (330 ± 44 vs. 334 ± 67 min, $p = \text{NS}$). Calculation of cpm/administered dose did not reveal differences between normal subjects and group B diabetic patients when values were normalized for body weight (4.97 ± 0.92 vs. 5.09 ± 0.85 cpm/kg, $p = \text{NS}$) or for LV myocardial mass (2.98 ± 0.82 vs. 2.79 ± 0.94 cpm/g, $p = \text{NS}$). MIBG uptake normalized for body weight or for LV myocardial mass was significantly lower in group A diabetic patients with an abnormal LV response to handgrip than in both normal subjects (3.82 ± 1.03 vs. 4.97 ± 0.92 cpm/MBq per kg, $p < 0.05$; 1.69 ± 0.30 vs. 2.98 ± 0.82 cpm/MBq per g, $p = 0.01$) and group B patients with a normal LV response to exercise (3.82 ± 1.03 vs. 5.09 ± 0.85 cpm/MBq per kg, $p < 0.05$; 1.69 ± 0.30 vs. 2.79 ± 0.94 cpm/MBq per g, $p = 0.01$). Finally, the

Table 3. Left Ventricular Function During Inotropic Stimulation by Post-Extrasystolic Potentiation or Dobutamine Infusion

	Group A (mean \pm SD)			Group B (mean \pm SD)		
	Base	PESP	Dob	Base	PESP	Dob
LVEDVI (ml/m ²)	66 \pm 12	68 \pm 9	68 \pm 9	63 \pm 10	64 \pm 8	60 \pm 10
LVESVI (ml/m ²)	29 \pm 4	18 \pm 5*†	24 \pm 5	28 \pm 8	18 \pm 4*	16 \pm 6‡
LVEF (%)	60 \pm 6	74 \pm 6*†	64 \pm 10	61 \pm 7	72 \pm 5*	74 \pm 5‡§
LVES (kdynes/cm ²)	229 \pm 52	—	236 \pm 48	218 \pm 64	—	229 \pm 58
HR (beats/min)	67 \pm 9	—	72 \pm 12	70 \pm 14	—	74 \pm 18
SBP (mm Hg)	122 \pm 12	—	128 \pm 19	133 \pm 17	—	144 \pm 25
DBP (mm Hg)	74 \pm 7	—	85 \pm 12	85 \pm 11	—	85 \pm 20

* $p < 0.001$ versus baseline (Base) values. † $p < 0.005$, post-extrasystolic potentiation (PESP) versus dobutamine (Dob). ‡ $p < 0.001$, dobutamine versus baseline values. § $p < 0.005$. Other abbreviations as in Table 2.

Figure 2. Changes in LVEF induced by dobutamine inotropic stimulation in study patients. **Squares** = Group A; **circles** = Group B.



relation between LVEF at peak handgrip and myocardial uptake normalized for LV mass exhibited a significant negative correlation ($y = 10x + 31$, $r = 0.68$, $p = 0.0063$, SD 9.86) (Fig. 4).

Coronary angiography. Significant (>50%) coronary stenosis could not be detected in any patient by selective coronary angiography.

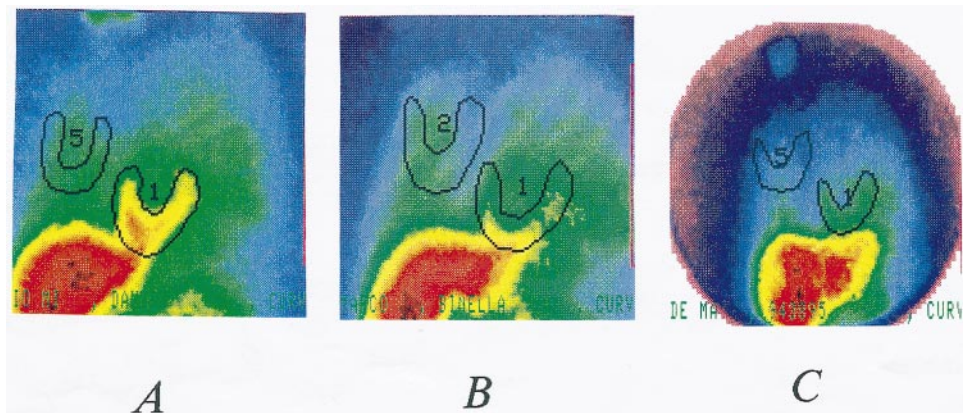
Discussion

The major finding of the present study is that the abnormal LV response to isometric exercise in patients with IDDM without apparent long-term diabetic complications is strongly related to a functional impairment of cardiac sympathetic innervation.

Myocardial contractility assessment by PESP. In the present study, cardiac inotropism was stimulated by two interventions (PESP and dobutamine) acting with different mechanisms. PESP, as part of the force-interval relation represents an inotropic stimulus clearly independent of variations in diastolic ventricular filling because it has been demonstrated in the isovolumetrically contracting heart (17). In the ejecting

ventricle, when the premature beat is followed by a compensatory pause, LV end-diastolic volume may be augmented, and this increased preload contributes to the enhanced performance. To minimize these changes in preload, only potentiated beats without significant changes in LV end-diastolic volume were evaluated in the present clinical study. Moreover, the maximal rate of ventricular pressure can be affected if afterload falls sufficiently. However, it does not appear likely that this would occur in the intact patient, given the relatively short test interval used to evaluate the force-interval relation. This assumption is supported by cardiac catheterization studies in which the plateau of restitution was sustained for >1,000 ms. During this period, aortic diastolic pressure would have fallen significantly, yet the maximal rate of rise of pressure appears to have been unaffected (18). Finally, calcium availability at the level of the myofibrils is a critical determinant of PESP, but the interaction at the beta-adrenoceptor and increases in intracellular cyclic adenosine monophosphate (cAMP) concentration play little direct role in PESP. Thus, PESP is a potent contractile stimulus dependent on the sarcoplasmic reticulum function and on the calcium sensitivity of contractile

Figure 3. Scintigraphic images obtained for three individual patients: (A) normal cardiac MIBG uptake; (B) heterogeneity in MIBG distribution; and (C) severe reduction in cardiac MIBG uptake. **Numbers** = ROIs of LV myocardial wall (area 1) and lung background (areas 2 and 5).



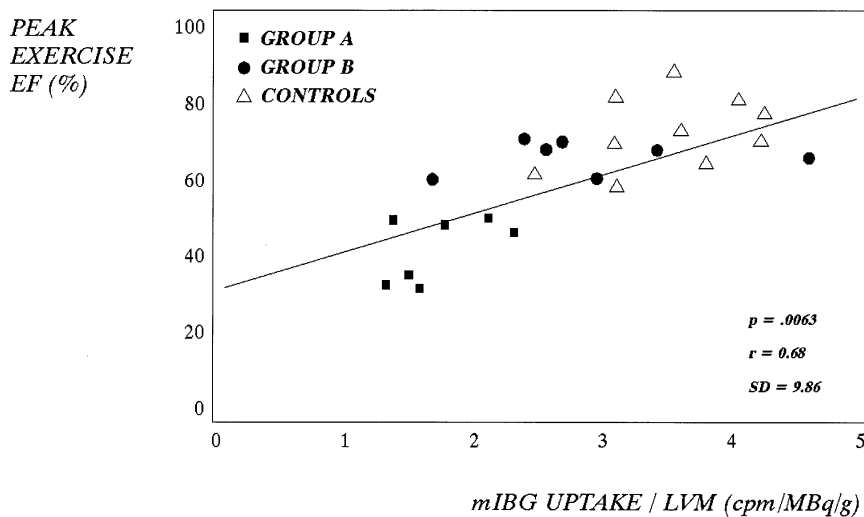


Figure 4. Linear relation between peak hand-grip LVEF (EF) and myocardial MIBG uptake normalized for left ventricular mass (LVM).

proteins (19). In contrast, the inotropic stimulus induced by dobutamine is mediated by the interaction with myocyte adrenoceptor and by the secondary increase in intracellular cAMP.

Muscle and sympathetic fiber function in diabetic heart.

Several previous studies have demonstrated an abnormal response of LVEF to exercise in a large subset of young adult patients with IDDM (20-23), although baseline myocardial contractility was normal (8,9). In our study patients, contractile reserve, defined as the capability to improve LV pump function by PESP, was normal in all study patients regardless of LVEF response to exercise. However, dobutamine infusion increased LVEF in diabetic patients with a normal response to exercise but failed to improve LVEF in diabetic patients with LV dysfunction induced by exercise. The afterload-LVEF relation has been used to examine myocardial contractility in isolated papillary muscle and in clinical investigations (24-26). The response of the left ventricle to a sustained increase in afterload during exercise in normal subjects is described by a linear correlation: The increase in LV afterload is matched by an adequate increase in myocardial contractility. Sustained static muscular contraction increases heart rate and blood pressure, imposing a pressure load on the left ventricle. Activation of fast-twitch fibres during static contraction stimulates unmyelinated free nerve endings in the contracted muscle, inducing an arterial pressure reflex, which results in an important increase in arterial blood pressure and LV afterload (27,28). Moreover, a twofold increase in plasma norepinephrine and a fivefold increase in plasma epinephrine were demonstrated during handgrip exercise. These changes in plasma catecholamine levels may represent the basis of chronotropic and inotropic response (29). LVEF values for Group A patients with IDDM were downwardly displaced and fell below the 95% confidence interval described by the control group values, indicating an inadequate increase in myocardial contractility at each comparable level of afterload (Fig. 1). Nevertheless, these diabetic patients with an abnormal LVEF

response to exercise were able to normalize their response by PESP inotropic stimulation performed at peak handgrip. Thus, a defective recruitment of contractility seems to be a major cause of LV dysfunction during exercise in these patients. Moreover, the strong relation between the value of LVEF at peak handgrip and MIBG myocardial uptake (Fig. 4) demonstrates that the inadequate contractile response during isometric exercise may be related to impaired function of cardiac sympathetic nerve fibers. The blunted response to inotropic dobutamine stimulation in these patients with IDDM offers additional evidence supporting this hypothesis. PESP can normalize LVEF at peak handgrip because this inotropic stimulus is largely independent of the integrity of adrenergic receptors (19,30). The altered adrenergic innervation is also suggested by the rapid washout of MIBG from the myocardium in patients with IDDM with an abnormal LV response to exercise compared with both to normal subjects and patients with IDDM with a normal LV response to exercise. These data in humans are in agreement with the defective inotropic response to catecholamines demonstrated in animal studies (31-33) and extend the findings of Kreiner et al. (34), who, by using MIBG scintigraphy, recently showed that sympathetic myocardial dysinnervation is much more common in patients with IDDM than previously thought and that subclinical LV dysfunction is related to disturbances in adrenergic cardiac innervation.

As shown in animal models (32,33), the sympathetic nervous system appears to be activated during the early stages of diabetes on the basis of reports of elevated plasma catecholamine levels. Prolonged exposure to catecholamines in humans induces a downward regulation of adrenergic receptors and alterations in adrenergic nervous fibers in the myocardium (35). A decrease in serum T3 levels, hyperglycemia and insulin deficiency are additional possible factors contributing to the abnormalities in cardiac innervation in diabetes (36).

As demonstrated by coronary angiography, myocardial ischemia was ruled out as a major cause of exercise LV dysfunction in our study patients. In fact, LV dysfunction induced by exercise

was always of the global, diffuse form, without regional asynergy. This finding is also strengthened by the lack of angina and ST segment changes during exercise, in accordance with a previous study (37) in which thallium imaging revealed no perfusion defects associated with LV dysfunction in IDDM. Finally, the normal increase in LV end-diastolic volume index at peak hand-grip indicates that venous return was not a limiting factor in abolishing the normal LVEF response during exercise.

Traditionally, detection of autonomic dysfunction has been based on abnormal responses in heart rate or blood pressure to various bedside maneuvers. This approach has significant limitations in that it does not directly assess sympathetic cardiac innervation, lacks the ability to examine specific anatomic regions and is nonquantitative and thereby subject to large measurement variability. Our study demonstrates that there is an important difference in sensitivity between the traditional approaches, which were unable to demonstrate cardiac autonomic dysfunction, and the findings obtained both by myocardial MIBG uptake, which indicated a significant cardiac autonomic nervous impairment in patients with IDDM with an abnormal LV response to exercise.

Conclusions. The present study showed that a subset of uncomplicated young patients with IDDM exhibit an abnormal LV response to exercise despite normal contractility and that defective inotropic recruitment plays an important role in determining exercise LV dysfunction. Results of MIBG scintigraphy and dobutamine infusion revealed functional impairment of cardiac sympathetic nerve fibers as a major cause of this blunted contractile response during exercise. Finally, these results suggest that abnormalities in the cardiac beta-adrenergic system in diabetes may contribute to LV dysfunction before the appearance of irreversible damage to contractile machinery and overt heart failure.

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