Abnormal Myocardial Perfusion and Contractile Recruitment during Exercise in Type 1 Diabetic Patients

ROLDANO SCOGNAMIGLIO, M.D., CHRISTIAN NEGUT, M.D., SAULA VIGILI DE KREUTZENBERG, M.D., MONICA PALISI, M.D., ANTONIO TIENGO, M.D., ANGELO AVOGARO, M.D.

Metabolic Cardiology Unit, Division of Metabolic Diseases, Department of Clinical and Experimental Medicine, University of Padua, Padua, Italy

Summary

Background: No data are available on the relationship between myocardial perfusion and left ventricular (LV) function in type 1 diabetes mellitus (T1DM), which may constitute a factor explaining the progressive contractile dysfunction to the overt phase of diabetic cardiomyopathy.

Hypothesis: This study was undertaken to test whether myocardial perfusion abnormalities are present at rest and during exercise and whether they are related to contractile dysfunction in T1DM.

Methods: Twenty-two patients with T1DM, aged 32 ± 8.3 years, without macro- or microvascular complications, and 10 controls, aged 31 ± 3 years, were studied. Left ventricular function and myocardial perfusion were assessed by two-dimensional and myocardial contrast echocardiography at rest and during handgrip (HG).

Results: Fourteen patients with T1DM showed a decline in LV ejection fraction (LVEF) during HG (Group 1) while 8 had a normal response (Group 2). Both basal myocardial blood volume (MBV) and velocity (β) were normal in T1DM. During exercise, MBV and β increased and were associated with

an increase in myocardial blood flow (MBF) in controls. In T1DM, β did not change and MBV increased only in Group 2, while this increase was not observed in Group 1 (controls: 14.9 ± 2.3 vs. Group 1: 7.6 ± 1.6 , $p < 0.001$; and vs. Group 2: 10.2 ± 2.8 , $p < 0.001$), β (0.86 ± 0.12 vs. 0.68 ± 0.14 , $p < 0.001$; and vs. 0.67 ± 0.15 , $p < 0.001$). A correlation between the ratio exercise MBF/resting MBF and LVEF at peak exercise in T1DM was observed ($r = 0.805$, $p < 0.001$).

Conclusions: A large proportion of patients with T1DM exhibit abnormalities in myocardial adaptable capacity to match an acute overload, which are related to a defective increase in myocardial perfusion.

Key words: myocardium, perfusion, function, type 1 diabetes, exercise

Introduction

A major complication of type 1 diabetes mellitus (T1DM) is the development of cardiomyopathy, a condition characterized by defects of contractile function and heart failure in the absence of significant coronary artery disease, systemic hypertension, or other etiologic factors.^{1,2} Several defects have been proposed to explain the pathogenesis of diabetic cardiomyopathy, including a reduced adrenoceptor myocardial density, alterations in contractile proteins, impaired calcium cellular movements, and defective energy metabolism.^{1–5} Several anatomical and functional abnormalities of the microcirculation were recognized in the human diabetic heart without obstructive coronary atherosclerosis or coexisting arterial hypertension.⁶ Moreover, it has been shown that DM is associated with both reduced coronary flow reserve^{7–9} and impaired endothelial-dependent epicardial coronary vasodilation.¹⁰ No data are available on the relationship between myocardial perfusion and left ventricular (LV) function in T1DM that may constitute a factor leading to progressive contractile dysfunction.

Address for reprints:

Roldano Scognamiglio, M.D.
Cardiologia Metabolica
Divisione di Malattie del Metabolismo
Dipartimento di Medicina Clinica e Sperimentale
Policlinico Universitario
via Giustiniani 2
35128 Padova, Italy
e-mail: r.scognamiglio@unipd.it

Received: April 9, 2004

Accepted with revision: October 25, 2004

In this study we investigated the relationship between myocardial function and perfusion in T1DM without micro- or macrovascular complications at rest and during an acute LV overload by quantitative two-dimensional (2-D) and myocardial contrast echocardiography.

Methods

Study Patients (Table I)

Twenty-two consecutive patients with T1DM (10 men and 12 women, aged 32 ± 8.3 years), who were C-peptide negative, lean (body mass index [BMI], mean $\pm$ standard deviation [SD], 23 ± 2 kg/m²) were studied. The duration of diabetes was 12 ± 3 years. Insulin requirement was 43 ± 11 units/day. All patients were nonsmokers. Exclusion criteria were proliferative retinopathy, blood pressure > 150 mmHg systolic (SBP) and > 85 mmHg diastolic (DBP), history of myocardial infarction, $> 50\%$ diameter reduction of coronary vessels by selective angiography, serum creatinine > 1.2 mg/dl, microalbuminuria > 20 mg/day, abnormal bedside autonomic nervous system function tests, and inadequate echocardiographic examinations to assess LV function and myocardial perfusion. Ten healthy subjects matched for gender (6 men, 4 women), BMI (24 ± 2 kg/m²) and age (31 ± 3 years) constituted the controls.

The study protocol was approved by the Local Ethical Committee, and informed consent was obtained from all participants. All study patients underwent the following assessments: retinal and long-term diabetic complications, degree of metabolic control and lipid profile, autonomic dysfunction by traditional clinical tests, selective coronary angiography, and left ventricular ejection fraction (LVEF) and myocardial per-

fusion at rest and during handgrip (HG). Patients were studied in fasting state at their spontaneous plasma glucose concentration before breakfast.

Echocardiographic Analysis

Studies were coded and read by two independent observers blinded to the patient's identity and experimental condition. Left ventricular volumes were calculated by an ellipsoid area-length method.¹¹ Left ventricular EF was derived as end-diastolic minus end-systolic volume divided by end-diastolic volume. Inter- and intraobserver variability for LV area ($r = 0.92$ and $r = 0.96$, respectively) and for LV length ($r = 0.94$ and $r = 0.96$, respectively) was acceptable.

Isometric Exercise

During HG, arterial pressure was measured every 30 s by an oscillometric method. Maximal voluntary contraction was determined by means of a dynamometer. Exercise was performed for 3 min at 40% maximal voluntary contraction. Heart rate and electrocardiographic tracings were monitored continuously.

Contrast Echocardiography

Myocardial contrast echocardiography was performed in apical four-chamber view using a continuous infusion¹²⁻¹⁵ with an end-systolic trigger. Signal intensity was measured by densitometry in myocardial regions of the left ventricle and in the cavity. Background-subtracted myocardial signal intensity (SI) was plotted over the increasing trigger intervals for the determination of the rate constant (β), which provides a measure of flow velocity, and the myocardial plateau intensity (MPI), which correlates to capillary cross sectional area and to my-

TABLE I Baseline characteristics of the study population

	Controls (n = 10)	T1DM Group 1 (n = 14)	T1DM Group 2 (n = 8)	p Value
Sex (men/women)	6/4	6/7	4/5	NS
Age (years)	31 ± 3	32 ± 8.1	32 ± 8.3	NS
BMI (kg/m ²)	24 ± 2	22 ± 3	23 ± 2	NS
Diabetes duration (years)	—	13 ± 2	12 ± 4	—
Insulin requirement/day (IU)	—	43 ± 11	42 ± 13	—
Glucose (mg/dl)	86 ± 4	217 ± 20	215 ± 23	< 0.001
HbA _{1c} (%)	4.8 ± 0.6	8.2 ± 0.6	8.3 ± 0.5	< 0.01
Triglyceride (mg/dl)	96 ± 13	104 ± 14	100 ± 16	NS
Total cholesterol (mg/dl)	179 ± 12	200 ± 7	196 ± 9	NS
LDL cholesterol (mg/dl)	106 ± 21	121 ± 30	117 ± 35	NS
HDL cholesterol (mg/dl)	54 ± 6	60 ± 5	61 ± 4	NS

Numbers are mean $\pm$ SD, p value for the comparison between controls and the two groups (1 and 2) of type 1 diabetic patients; no significant differences exist between Group 1 and Group 2 type 1 diabetic patients.

Abbreviations: T1DM = type 1 diabetes mellitus, BMI = body mass index, Hb hemoglobin, LDL = low-density lipoprotein, HDL = high-density lipoprotein, NS = not significant, SD = standard deviation.

ocardial blood volume. The product ($\beta \times \text{MPI}$) represents a dimensionless index of myocardial blood flow (MBF).¹⁵ An intensity versus dose curve from the LV cavity was plotted to obtain the dose at which the relation was linear; this dose was used in the contrast echocardiographic studies. Absence of any change in myocardial video intensity over five successive frames by visual assessment indicated the steady state (2–3 min after starting the infusion). Digitized studies were coded and read by two independent observers blinded to the patient's identity and the order of the study. An index of mean global myocardial perfusion was calculated by adding the values of regional MBF and dividing this value by the number of analyzed LV segments. In this study, the degree of inter- and intraobserver correlations for measurements of myocardial blood volume (MBV) ($r = 0.94$, and $r = 0.96$, respectively) and β ($r = 0.95$, and $r = 0.96$, respectively) was acceptable.

Statistical Analysis

Results are expressed as mean $\pm$ SD. Comparison between resting and peak HG values and between groups were made with the Student's *t*-test (paired or unpaired, as appropriate). Multiple comparisons were performed with repeated measure

analysis of variance (ANOVA), followed by the Fisher protected least significant difference test. Correlation was determined by a linear squares method. For all statistical analysis we used the Statistical Package for Social Sciences (version 10.1) (SPSS Inc., Chicago, Ill., USA). A *p* value ≤ 0.05 by the two-tailed test was considered to indicate statistical significance.

Results

Baseline Left Ventricular Function and Myocardial Perfusion (Table II)

All diabetic patients had a normal baseline LVEF and were classified into two groups on the basis of LV response to HG: Group 1 included patients with an abnormal response to exercise (decline > 0.05 U in LVEF); Group 2 included patients with a normal response (increase or no change in LVEF). Measurements of LV end-diastolic volume index (LVEDVI) (62 ± 8 ml/m², range 53–71) and LVEF ($60 \pm 3.6\%$, range 55–72) in the controls were used to determine mean and 95% confidence limits of normal values. In T1DM, baseline LVEDVI and LVEF did not differ from controls. Four patients (three in Group 1 and

TABLE II Baseline and peak handgrip variables in controls and type 1 diabetic patients

	Controls (n = 10)	T1DM Group 1 (n = 14)	T1DM Group 2 (n = 8)
Baseline			
HR (beats/min)	64 $\pm$ 12	68 $\pm$ 9	69 $\pm$ 10
SBP (mmHg)	125 $\pm$ 8	122 $\pm$ 10	129 $\pm$ 11
DBP (mmHg)	75 $\pm$ 7	74 $\pm$ 7	75 $\pm$ 5
LVEDVI (ml/m ²)	62 $\pm$ 8	66 $\pm$ 12	64 $\pm$ 10
LVESVI (ml/m ²)	25 $\pm$ 6	28 $\pm$ 4	26 $\pm$ 8
LVEF (%)	60 $\pm$ 3.6	58 $\pm$ 3.8	59 $\pm$ 5.6
MBV	8.5 $\pm$ 1.2	8 $\pm$ 0.9	8.8 $\pm$ 2
β	0.66 $\pm$ 0.12	0.67 $\pm$ 0.10	0.67 $\pm$ 0.12
MBF	5.27 $\pm$ 0.14	5.36 $\pm$ 0.09	5.29 $\pm$ 0.11
Peak handgrip			
HR (beats/min)	90 $\pm$ 15	88 $\pm$ 10	87 $\pm$ 12
SBP (mmHg)	156 $\pm$ 30	158 $\pm$ 16	158 $\pm$ 18
DBP (mmHg)	90 $\pm$ 19	92 $\pm$ 14	96 $\pm$ 14
LVEDVI (ml/m ²)	80 $\pm$ 9	80 $\pm$ 12	82 $\pm$ 15
LVESVI (ml/m ²)	20 $\pm$ 7	46 $\pm$ 7 ^a	30 $\pm$ 10 ^{a, b}
LVEF (%)	72 $\pm$ 6	44.9 $\pm$ 5.6 ^a	63 $\pm$ 7.9 ^{a, b}
MBV	14.9 $\pm$ 2.3	7.6 $\pm$ 1.6 ^a	10.2 $\pm$ 2.8 ^{a, b}
β	0.86 $\pm$ 0.12	0.68 $\pm$ 0.14 ^a	0.67 $\pm$ 0.15 ^a
MBF	12.8 $\pm$ 0.27	5.46 $\pm$ 0.22 ^a	6.83 $\pm$ 0.42 ^{a, b}
Exercise MBF/resting MBF	2.42 $\pm$ 0.19	1.01 $\pm$ 0.14 ^a	1.29 $\pm$ 0.15 ^{a, b}

Numbers are mean $\pm$ SD.

Group 1 or 2 patients versus controls: ^a *p* < 0.001.

Group 1 versus Group 2: ^b *p* < 0.01.

Abbreviations: HR = heart rate, SBP = systolic blood pressure, DBP = diastolic blood pressure, LVEDVI = left ventricular end-diastolic volume index, LVESVI = left ventricular end-systolic volume index, LVEF = left ventricular ejection fraction, MBV = myocardial blood volume, β = mean microbubble velocity, MBF = myocardial blood flow = MBV $\times$ β ; T1DM = type 1 diabetes mellitus.

one in Group 2) had an LVEDVI greater than the upper normal limit. All patients showed normal LVEF. Heart rate, SBP, and DBP did not differ between T1DM and controls.

Cardiac index (CI) and LV end-diastolic pressure (LVEDP), measured during cardiac catheterization, were normal in all diabetic patients without difference between Groups 1 and 2 (CI: 5.2 ± 0.9 vs. 5 ± 1 l/min/m², $p = \text{NS}$; LVEDP: 8 ± 2.4 vs. 8 ± 2 mmHg, $p = \text{NS}$). Atherosclerotic irregularities in the coronary artery walls of diabetic patients were infrequent and equally distributed between Groups 1 (2/14, 14%) and 2 (1/8, 12.5%). Stenosis $> 50\%$ in major epicardial coronary segments was an exclusion criterion. Duration of diabetes (8.4 ± 3.4 vs. 8.2 ± 4 years, $p = \text{NS}$) and HbA_{1c} (8.2 ± 0.6 vs. $8.3 \pm 0.5\%$, $p = \text{NS}$) were similar in the two groups of diabetic patients.

Myocardial contrast echocardiographic studies showed that both baseline β (0.66 ± 0.12 vs. 0.67 ± 0.14 , $p = \text{NS}$) and MBV (8.5 ± 1.2 vs. Group 1 8 ± 0.9 , and vs. Group 2 8.8 ± 2 , $p = \text{NS}$) did not differ between control subjects and T1DM. As a consequence, baseline MBF was normal in T1DM.

Changes in Left Ventricular Function and Myocardial Perfusion during Handgrip (Table II)

Heart rate and SBP increased at peak HG in both groups of diabetic patients and controls without significant differences. The LVEF response to HG in controls was characterized by a > 0.05 U increase in all but two subjects, who showed no significant changes (60 ± 3.6 vs. $72 \pm 6\%$, $p < 0.005$). Fourteen of the patients with T1DM had a > 0.05 U decline in LVEF (Group 1); peak LVEF was significantly lower than the baseline value (44.9 ± 5.6 vs. $58 \pm 3.8\%$, $p < 0.001$). The remaining eight patients with T1DM (Group 2) had a normal LV response to HG (three had a significant increase whereas five showed no change in LVEF); peak LVEF did not differ significantly from baseline (63 ± 7.9 vs. $59 \pm 5.6\%$, $p = \text{NS}$). At peak HG, LVEF was greater in Group 2 than in Group 1 patients (63 ± 7.9 vs. $44.9 \pm 5.6\%$, $p < 0.001$), whereas LVEDVI, HR, SBP, and DBP did not differ. Peak HG LVEF was lower in Group 2 T1DM compared with controls (63 ± 7.9 vs. $72 \pm 6\%$, $p < 0.01$). During isometric exercise, β values (derived from the time-intensity curve) showed significant increase from resting values in controls (0.66 ± 0.12 vs. 0.86 ± 0.12 , $p < 0.001$), while they did not change in T1DM (Group 1: 0.67 ± 0.1 vs. 0.68 ± 0.14 , $p = \text{NS}$; Group 2: 0.67 ± 0.12 vs. 0.67 ± 0.15 , $p = \text{NS}$). In controls, MBV increased during exercise (8.5 ± 1.2 vs. 14.7 ± 2.3 , $p < 0.001$). Group 1 patients had no changes in MBV from baseline to peak HG (8 ± 0.9 vs. 7.6 ± 1.6 , $p = \text{NS}$). In Group 2, MBV increased during the acute overload imposed by HG (8.8 ± 2 vs. 10.2 ± 2.8 , $p < 0.01$). Exercise MBV and MBF were significantly higher in Group 2 compared with Group 1 ($p < 0.01$). In both groups of patients, exercise MBV and MBF were lower than in controls ($p < 0.001$). Thus, the capacity to increase MBF during LV acute overload as expressed by the ratio exercise-MBF/baseline-MBF is significantly reduced in T1DM (Fig. 1). Figures 2 to 4 show some examples of representative contrast echocardiograms at baseline and

during HG in Groups 1 and 2 of diabetic patients and in controls. Finally, in T1DM, LVEF at peak exercise significantly correlates with the capacity to increase MBF during LV acute overload as expressed by the ratio exercise-MBF/baseline-MBF ($r = 0.805$, $p < 0.001$) (Fig. 5).

Discussion

This study shows that patients with T1DM without coronary artery disease and microvascular complications have normal myocardial perfusion at rest. However, the capacity for increasing myocardial perfusion during a condition of acute overload induced by isometric exercise is impaired, causing a blunted or absent improvement in LV myocardial performance. This impaired myocardial perfusion response during acute overload may be related to several anatomical^{16, 17} and functional abnormalities^{18–20} described in the coronary microcirculation of T1DM without coronary artery disease. Our findings also suggest that even early stages of diabetic heart disease may be associated with abnormalities in coronary flow regulation, particularly in response to stress. In normal subjects, the increase in afterload during exercise is matched by an adequate increase in myocardial contractility. Activation of fast-twitch fibers during static contraction stimulates unmyelinated free nerve endings in the contracted muscle, inducing an arterial pressure reflex that results in an important increase in arterial blood pressure and LV afterload.²¹ Changes in plasma catecholamine levels and increases in coronary blood flow and myocardial perfusion represent the basis of chronotropic and inotropic response. In T1DM, the capacity for increasing myocardial perfusion is impaired to a different degree, causing different responses of the left ventricle to the augmented afterload. Similar changes in plasma catecholamine levels are associated with cold. The cold pressor test, which activates the sympathetic nervous system, induces dilation of coronary arteries in normal subjects and constriction of

Fig. 1 Myocardial blood flow (MBF) reserve (exercise-MBF/resting-MBF) in controls (n = 10), Group 1 (n = 14), and Group 2 (n = 8) type 1 diabetes mellitus (T1DM). Data are expressed as mean $\pm$ standard deviation (SD). * $p < 0.001$ for the comparison between controls and both groups of T1DM, and † $p < 0.001$ between the two groups of T1DM.

FIG. 2 Actual myocardial contrast echocardiography images, at rest (A) and at peak handgrip (B), in an individual type 1 diabetic patient from Group 2 (normal response in left ventricular function during handgrip) and the corresponding acoustic video intensity versus time plots (C). The plateau of the time intensity curves is higher during handgrip evaluation (dotted line) compared with resting condition (solid line). This result indicates increase in myocardial blood volume during isometric exercise. The increase in myocardial perfusion does not reach normal values, as is the case in the control patient (see Fig. 4). Pulsing interval (PI) versus background-subtracted video-intensity (VI) plots were generated, and they were fitted to the following exponential function: $y = A(1 - e^{-\beta t})$, where y is the VI at a PI of t , A is the plateau where VI represents myocardial blood volume, and β represents the mean microbubble velocity.¹³

FIG. 3 Actual myocardial contrast echocardiography images, at rest (A) and at peak handgrip (B), in an individual type 1 diabetic patient from Group 1 (decrease in left ventricular function during handgrip) and the corresponding acoustic video intensity versus time plots (C). The plateau of the time intensity curves is lower during handgrip evaluation (dotted line) than at resting condition (solid line). This result indicates decrease in myocardial blood volume during isometric exercise.

FIG. 4 Actual myocardial contrast echocardiography images, at rest (A) and at peak handgrip (B), in an individual patient from the control group (increase or no change in left ventricular function during handgrip) and the corresponding acoustic video intensity versus time plots (C). The plateau of the time intensity curves is higher during handgrip evaluation (dotted line) compared with resting condition (solid line). This result indicates increase in myocardial blood volume during isometric exercise. Left ventricular function and perfusion changes are considered normal.

FIG. 5 Linear correlation ($r = 0.805$, $p < 0.0001$) between myocardial blood flow reserve and left ventricular ejection fraction (LVEF, %) at peak handgrip (HG) in type 1 diabetes mellitus (black triangles = Group 1, $n = 14$; white squares = Group 2, $n = 8$).

atherosclerotic coronary arteries.^{22, 23} In diabetic patients, the cold pressure test has been shown to induce coronary artery constriction,²⁴ which may reflect endothelial dysfunction. Increased myocardial metabolism leads to an enhanced dilation and flow of epicardial coronary arteries, which is mainly determined by endothelium-derived nitric oxide.²⁵ The inability of some patients with T1DM to increase myocardial perfusion and LV performance under the condition of acute overload and increased sympathetic stimulation indicates that a paradoxical mismatched response between epicardial and microvascular vessels of coronary circulation occurs. The incapacity to increase myocardial perfusion significantly during exercise in diabetic patients with exercise-induced LV dysfunction is determined by the absent increase in MBV. This index assessed by myocardial contrast echocardiography is significantly related to microvascular indices (total microvascular density, capillary density, and capillary area) in biopsied myocardial segments.²⁶ Moreover, myocardial contrast echocardiography may offer an approach to the noninvasive detection of endothelial function.^{27, 28} Thus, the present data suggest that myocardial perfusion defects induced by exercise may be related to deterioration in coronary microvascular circulation and endothelial dysfunction in type 1 diabetic patients with angiographically normal coronary arteries.

Whatever the mechanism, in clinical setting it will be important to identify those patients with T1DM who will present decreased coronary perfusion and ischemia leading to LV dysfunction under conditions of increased oxygen demand (i.e., exercise), even in the absence of overt coronary atherosclerosis. These alterations may lead to a mismatch between myocardial oxygen demand and supply and participate in progressive LV dilation and dysfunction.

Conclusion

This study shows that patients with T1DM and without major micro- or macrovascular complications exhibit abnormali-

ties of myocardial perfusion recruitment during exercise in the early phase of the disease characterized by a normal resting LV function. In these patients, a limited ventricular adaptable capacity to match an acute overload is demonstrable. This exercise-induced LV dysfunction is related to an absent or inadequate increase in myocardial perfusion.

Acknowledgment

The authors thank Mr. Claudio Bellini for his technical assistance.

References

- Fein FS, Sonnenblick EH: Diabetic cardiomyopathy. *Prog Cardiovasc Dis* 1985;27:255–270
- Tahiliani AG, McNeill JH: Diabetes-induced abnormalities in the myocardium. *Life Sci* 1986;38:959–974
- Scognamiglio R, Avogaro A, Casara D, Crepaldi C, Marin M, Palisi M, Mingardi R, Erle G, Fasoli G, Dalla Volta S: Myocardial dysfunction and adrenergic cardiac innervation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 1998;31:404–412
- Ganguly PK, Pierce GN, Dhalla KS, Dhalla NS: Defective sarcoplasmic reticular calcium transport in diabetic cardiomyopathy. *Am J Physiol* 1983; 244:E528–535
- Penpargkul S, Schaible T, Yipintsoi T, Scheuer J: The effect of diabetes on performance and metabolism of rat hearts. *Circ Res* 1980;47:911–921
- Factor SM, Okun EM, Minase T: Capillary microaneurysms in the human diabetic heart. *N Engl J Med* 1980;302:384–388
- Nitenberg A, Ledoux S, Valensi P, Sachs R, Attali JR, Antony I: Impairment of coronary microvascular dilation in response to cold pressor-induced sympathetic stimulation in type 2 diabetic patients with abnormal stress thallium imaging. *Diabetes* 2001;50:1180–1185
- Nahser PJ Jr, Brown RE, Oskarsson H, Winniford MD, Rossen JD: Maximal coronary flow reserve and metabolic coronary vasodilation in patients with diabetes mellitus. *Circulation* 1995;91:635–640
- Pitkanen OP, Nuutila P, Raitakari OT, Ronnemaa T, Koskinen PJ, Iida H, Lehtimäki TJ, Laine HK, Takala T, Viikari JS, Knuuti J: Coronary flow reserve is reduced in young men with IDDM. *Diabetes* 1998;47:248–254
- Nitenberg A, Valensi P, Sachs R, Dali M, Aptekar E, Attali JR: Impairment of coronary vascular reserve and ACh-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. *Diabetes* 1993;42:1017–1025
- Gueret P, Meerbaum S, Wyatt HL, Uchiyama T, Lang TW, Corday E: Two-dimensional echocardiographic quantitation of left ventricular volumes and ejection fraction. Importance of accounting for dyssynergy in short-axis reconstruction models. *Circulation* 1980;62:1308–1318
- Wei K, Jayaweera AR, Firoozan S, Linka A, Skyba DM, Kaul S: Basis for detection of stenosis using venous administration of microbubbles during myocardial contrast echocardiography: Bolus or continuous infusion? *J Am Coll Cardiol* 1998;32:252–260
- Wei K, Ragosta M, Thorpe J, Coggins M, Moos S, Kaul S: Noninvasive quantification of coronary blood flow reserve in humans using myocardial contrast echocardiography. *Circulation* 2001;103:2560–2565
- Wray RA, Quinones MA, Zoghbi WA, Cheirif J: Relation of mean pixel intensity to concentration of sonicated albumin microspheres: Effects of ultrasound system settings. *Clin Cardiol* 1991;14:V23–28
- Porter TR, D'Sa A, Turner C, Jones LA, Minisi AJ, Mohanty PK, Vetrovec GW, Nixon JV: Myocardial contrast echocardiography for the assessment of coronary blood flow reserve: Validation in humans. *J Am Coll Cardiol* 1993;21:349–355
- van Hoven KH, Factor SM: Diabetic heart disease—Part II: The clinical and pathological spectrum. *Clin Cardiol* 1989;12:667–671
- Carugo S, Giannattasio C, Calchera I, Paleari F, Gorgoglione MG, Grapio A, Gamba P, Rovaris G, Failla M, Mancina G: Progression of functional and structural cardiac alterations in young normotensive uncomplicated patients with type 1 diabetes mellitus. *J Hypertens* 2001;19:1675–1680
- Strauer BE, Motz W, Vogt M, Schwartzkopff B: Evidence for reduced coronary flow reserve in patients with insulin-dependent diabetes. A possible cause for diabetic heart disease in man. *Exp Clin Endocrinol Diabetes* 1997; 105:15–20

19. Pitkanen OP, Nuutila P, Raitakari OT, Ronnema T, Koskinen PJ, Iida H, Lehtimäki TJ, Laine HK, Takala T, Viikari JS, Knuuti J: Coronary flow reserve is reduced in young men with IDDM. *Diabetes* 1998;47:248–254
20. Fisher BM, Gillen G, Lindop GB, Dargie HJ, Frier BM: Cardiac function and coronary arteriography in asymptomatic type 1 (insulin-dependent) diabetic patients: Evidence for a specific diabetic heart disease. *Diabetologia* 1986;29:706–712
21. Shepherd JT, Blomqvist CG, Lind AR, Mitchell JH, Saltin B: Static (isometric) exercise. Retrospection and introspection. *Circ Res* 1981;48:1179–1188
22. Nabel EG, Ganz P, Gordon JB, Alexander RW, Selwyn AP: Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold pressor test. *Circulation* 1988;77:43–52
23. Antony I, Aptekar E, Lerebours G, Nitenberg A: Coronary artery constriction caused by the cold pressor test in human hypertension. *Hypertension* 1994;24:212–219
24. Nitenberg A, Paycha F, Ledoux S, Sachs R, Attali JR, Valensi P: Coronary artery responses to physiological stimuli are improved by deferoxamine but not by L-arginine in non-insulin-dependent diabetic patients with angiographically normal coronary arteries and no other risk factors. *Circulation* 1998;97:736–743
25. Shiode N, Morishima N, Nakayama K, Yamagata T, Matsuura H, Kajiyama G: Flow-mediated vasodilation of human epicardial coronary arteries: Effect of inhibition of nitric oxide synthesis. *J Am Coll Cardiol* 1996;27:304–310
26. Shimoni S, Frangogiannis NG, Aggeli CJ, Shan K, Quinones MA, Espada R, Letsou GV, Lawrie GM, Winters WL, Reardon MJ, Zoghbi WA: Microvascular structural correlates of myocardial contrast echocardiography in patients with coronary artery disease and left ventricular dysfunction. Implications for the assessment of myocardial hibernation. *Circulation* 2002;106:950–956
27. Villanueva FS, Klibanov A, Wagner WR: Microbubble-endothelial cell interactions as a basis for assessing endothelial function. *Echocardiography* 2002;19:427–438
28. Villanueva FS, Jankowski RJ, Manaugh C, Wagner WR: Albumin microbubble adherence to human coronary endothelium: Implications for assessment of endothelial function using myocardial contrast echocardiography. *J Am Coll Cardiol* 1997;30:689–693