C-Peptide Exerts Beneficial Effects on Myocardial Blood Flow and Function in Patients With Type 1 Diabetes

Alexander Hansen,[1] Bo-Lennart Johansson,[2] John Wahren,[2] and Helene von Bibra[1]

Myocardial dysfunction, perfusion abnormalities, and the extent to which these abnormalities may be reversed by C-peptide administration was assessed in type 1 diabetic patients. Eight patients were studied before and during a 0.84-mg/kg dipyridamole administration using a randomized double-blind crossover protocol with infusion of C-peptide (6 pmol · kg^{-1} · min^{-1}) or saline during 60 min on two different days. Myocardial function was measured as peak myocardial velocity during systole (Vs) and early diastole (Vd) by pulsed tissue Doppler imaging. Myocardial contrast echocardiography was used for assessment of myocardial blood volume (SI_{max}) and myocardial blood flow index (MBFI) calculated from the relation between trigger interval and signal intensity. Eight age-matched healthy volunteers served as control subjects. In the basal state, Vd (13.8 ± 0.6 vs. 15.6 ± 0.5 cm/s, $P < 0.04$) and SI_{max} (6.6 ± 0.6 vs. 8.2 ± 0.6 a.u. $P < 0.04$) were reduced in patients compared with control subjects. Dipyridamole administration significantly increased indexes of myocardial function and blood flow to a similar extent in patients and control subjects. During C-peptide administration, Vs and Vd increased by 12% ($P = 0.03$), SI_{max} increased from 6.6 ± 0.6 to 8.1 ± 0.7 a.u. ($P < 0.02$), and MBFI increased from 3.3 ± 0.4 to 5.3 ± 0.9 ($P < 0.05$). The results demonstrate that type 1 diabetic patients have impaired myocardial function and perfusion in the basal state that can be improved by short-term replacement of C-peptide. *Diabetes* 51:3077–3082, 2002

Administration of proinsulin C-peptide in the physiological concentration range (0–1.5 nmol/l) to patients with type 1 diabetes is accompanied by improved renal function (1), amelioration of nerve dysfunction (2), and increased blood flow in several tissues, notably in skeletal muscle (3,4) at rest and during exercise (5), whereas its effects on myocardial blood flow and function have not been studied. Although myocardial perfusion abnormalities (6) and diastolic dysfunction are early subclinical phenomena in patients with diabetes (7,8), the lack of noninvasive or nonionizing methods with sufficient sensitivity to evaluate both cardiac dysfunction and perfusion abnormalities in these patients has hindered sequential studies.

Recently, myocardial contrast echocardiography has emerged as a promising method for the repetitive assessment of myocardial perfusion (9,10) after intravenous injection of microbubbles and subsequent myocardial opacification. This enhancement can be measured as an increase in ultrasound signal intensity that is primarily proportional to the myocardial blood volume (9). Myocardial contrast echocardiography has been used for the assessment of myocardial perfusion at rest and stress, of ischemia and myocardial viability (10–12).

Tissue Doppler imaging is a new ultrasonographic technique that measures regional systolic and diastolic myocardial velocities. This technique has been used for the assessment of both global and regional myocardial function and is reported to be more sensitive to functional changes than traditional echocardiographic and Doppler methods (13,14).

The present placebo-controlled randomized crossover study was designed to assess myocardial function and perfusion abnormalities in type 1 diabetic patients using tissue Doppler imaging and myocardial contrast echocardiography and to evaluate the extent to which these abnormalities may be reversed by C-peptide infusion.

RESEARCH DESIGN AND METHODS

Study groups. Eight male type 1 diabetic patients were studied during normoglycemia (mean age 28 ± 2 years and BMI 23.9 ± 0.7 kg/m^2). The mean duration of diabetes was 18 ± 2 years, mean insulin dosage was 0.72 ± 0.12 units · kg^{-1} · day^{-1}, and mean HbA_{1c} was 7.1 ± 0.5%. There were no diabetic complications apart from microalbuminuria and retinopathy in one patient. All patients had a normal resting electrocardiogram and normal echocardiography data for mitral valve movement, left atrial size and left ventricular size, global function, and wall motion at rest and during dipyridamole stress. Exclusion criteria were cardiac autonomic neuropathy, peripheral neuropathy, hypertension (>150/85 mmHg), and other medical treatment except insulin, angina, dyspnoea, or other evidence of significant heart disease. Coronary artery stenosis was to be excluded by means of a perfusion-imaging stress test (10,11) according to the study protocol. Eight age-matched healthy volunteers (aged 30 ± 2 years, BMI 23.6 ± 1.3 kg/m^2) served as a control group. All subjects were informed of the nature, purpose, and possible risks of the study before giving their voluntary consent to participate. The institutional ethics committee reviewed and approved the study protocol.

Study protocol. The patients came to the laboratory early in the morning in the fasting state without having taken their morning insulin dose. An intravenous infusion of insulin was given to regulate blood glucose levels, and the study began when normoglycemia was achieved, usually within 2–3 h. Ultrasound measurements for the assessment of myocardial perfusion and function were performed before and after maximal vasodilatation with an

From the [1]Section of Cardiology, Department of Medicine, Karolinska Hospital, Stockholm, Sweden; and the [2]Section of Clinical Physiology, Department of Surgical Sciences, Karolinska Hospital, Stockholm, Sweden.

Address correspondence and reprint requests to Professor Helene von Bibra, Section of Cardiology N3:05, Karolinska Hospital, SE-17176 Stockholm, Sweden. E-mail: helene.vonbibra@ks.se.

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CV, coefficient of variation; MBFI, myocardial blood flow index; PET, positron emission tomography; SI, signal intensity; SI_{max}, myocardial blood volume; Va, late diastolic velocity; Vd, velocity during early diastole; Vs, velocity during systole.

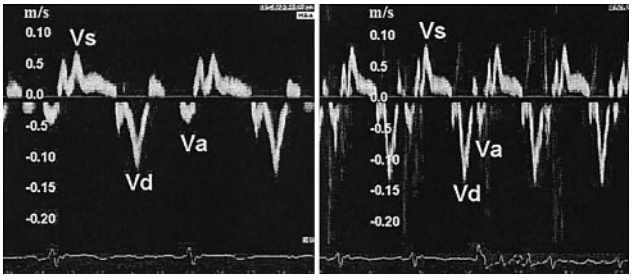

FIG. 1. Representative pulsed Doppler myocardial recordings obtained from the basal anterior wall in a patient with type 1 diabetes at rest (left panel) displaying systolic (Vs), early diastolic (Vd), and late diastolic (Va) peak myocardial velocities. All velocities increase during dipyridamole stress (right panel).

intravenous infusion of dipyridamole (0.84 mg/kg over 6 min, Persantin; Boehringer Ingelheim). This protocol was repeated 60 min later after randomization to an intravenous infusion of either C-peptide (6 pmol · kg^{-1} · min^{-1}; Schwarz Pharma, Monheim, Germany) or vehicle (placebo). The entire study procedure was repeated on another day with the alternative treatment. Blood samples were taken for analysis of glucose (every 30 min), plasma C-peptide, and insulin. All patients had measurements of blood pressure and heart rate and a 12-lead electrocardiogram before and after the pharmacological tests.

Myocardial contrast echocardiography. Myocardial contrast echocardiography, performed in apical 4 and 2 chamber views, was carried out with a commercially available ultrasound system (HDI 5000; ATL, Bothell, WA) using a broadband transducer at 1.77 MHz and pulse inversion gray-scale imaging. Instrument setting was used as recently described (10). The contrast agent Levovist (Schering, Berlin, Germany) was administered as an intrevenous infusion of 600 mg/min for 7 min. Triggered two-dimensional images were recorded at incremental microbubble replenishment times using the trigger intervals 1, 2, 3, 4, 6, and 8 cardiac cycles. Five end-systolic images were acquired at each trigger interval. At the pulsing interval of one cardiac cycle, three consecutive frames were acquired at 40-ms intervals in order to use the respective third image as a measure of background signal intensity, assuming that the two preceding ultrasound exposures had effectively destroyed the contrast bubbles in the myocardium (15).

Quantitative analysis of the digital images was performed with a dedicated image analysis program (HDI Lab v 1.89; ATL) as recently described (9,10). Signal intensity was measured by densitometry in myocardial regions of interest placed in the basal, mid, and apical segments of the left ventricular walls and in the cavity. Myocardial segments with artifacts such as attenuation and rib shadowing were excluded from analysis. Background-subtracted myocardial signal intensity (SI) was plotted over the increasing trigger intervals for the ultrasound determination of the two primary components of myocardial flow—SI_{max}, which correlates to capillary crossectional area and to myocardial blood volume, and the rate-constant β, which provides a measure of flow velocity— their product representing a dimensionless index of myocardial blood flow (9,12). To compensate for differences in cardiac output and attenuation between rest and stress studies, an index of myocardial blood volume (MBVI) was calculated by normalizing SI_{max} for the simultaneous signal intensity in the left ventricular cavity (10) and expressed in percent. The regional data were averaged for assessing the different perfusion parameters for the individual patient. The intra- and interobserver coefficients of variation (CVs) for signal intensity SI (6.7 and 9.0%, respectively) have been recently reported (10).

Tissue Doppler imaging. Pulsed wave tissue Doppler images were recorded on a commercially available ultrasound system (Vingmed System FiVe; GE Medical Systems, Milwaukee, WI) with a 2.5 MHz transducer. The technique of using tissue Doppler imaging has been recently described (14). In short, the Doppler sampling gate (8 mm) was consecutively placed in the basal septal, anteroseptal, anterior, lateral, posterior, and inferior wall for the assessment of longitudinal left ventricular function. From the respective velocity curves, the following peak velocities were determined and averaged from three consecutive cardiac cycles: systolic velocity (Vs), early diastolic velocity (Vd), and late diastolic velocity (Va) (Fig. 1). Myocardial function for individual patients was calculated as an average value based on the regional data.

Analyses. Plasma glucose was analyzed with a glucose oxidase method using a glucose analyzer (Glucostat; Beckman). HbA_{1c} was determined with a liquid-chromatographic assay (16), normal reference values 3.5–5.5%. Plasma immunoreactive free insulin was analyzed after immediate polyethylene glycol precipitation (17). Plasma C-peptide was assessed by a radioimmunoassay technique using a commercial kit (C-peptide RD 315, MILAB; Malmö, Sweden).

Statistical analysis. Data were expressed as means ± SEM. For two-tailed test, a P value <0.05 was considered statistically significant. A Student's t test, paired or unpaired as appropriate, with assumption of homogenous variance, was used for group-to-group comparisons. To determine the variability of signal intensity and myocardial velocity measurements, the CV was calculated for repeated measurements performed in the basal state within 2 h (intraday variability).

RESULTS

C-peptide, insulin, and glucose concentrations. Blood glucose levels were not significantly different before and after infusion of C-peptide (6.0 ± 0.4 vs. 5.9 ± 0.4 mmol/l) or saline. Free serum insulin levels were 14.3 ± 3.7 mU/l before and 7.4 ± 1.6 mU/l (NS) after infusion of C-peptide and 11.1 ± 1.5 and 8.3 ± 1.1 mU/l (NS) before and after saline infusion. C-peptide plasma concentrations rose from 0.12 ± 0.01 to 1.52 ± 0.09 nmol/l after 60 min of

TABLE 1
Hemodynamic and echocardiographic characteristics of type 1 diabetic patients and healthy control subjects in basal state and during dipyridamole stress before and during C-peptide infusion

	Patients (before)			Patients (C-peptide)			Control subjects	
	Basal	P*	Dipyridamole	Basal	P†	Dipyridamole	Basal	Dipyridamole
Hemodynamics								
Heart rate (bpm)	54 ± 2	NS	79 ± 3‖	58 ± 3	NS	80 ± 3‖	61 ± 3	80 ± 5‖
Blood pressure systole (mmHg)	115 ± 3	NS	121 ± 5	112 ± 5	NS	117 ± 5‡	118 ± 5	107 ± 3‡
Blood pressure diastole (mmHg)	68 ± 4	NS	66 ± 4	62 ± 4	NS	62 ± 4	71 ± 2	60 ± 4‡
Rate pressure product (mmhg/min)	6,193 ± 109	NS	9,626 ± 609‖	6,438 ± 412	NS	9,443 ± 641‖	7,233 ± 522	8,689 ± 650‡
Tissue Doppler velocities								
Systole (cm/s)	80 ± 3	0.06	9.9 ± 0.4‖	8.9 ± 0.5	<0.06	9.8 ± 0.5‡	8.8 ± 0.2	10.0 ± 0.3§
Early diastole (cm/s)	13.8 ± 0.6	<0.04	15.5 ± 0.5‖	15.4 ± 0.6	<0.04	16.3 ± 0.7§	15.6 ± 0.5	17.3 ± 0.4‖
Atrial contraction (cm/s)	7.0 ± 0.7	NS	9.0 ± 0.5‖	7.2 ± 0.6	NS	8.9 ± 0.6§	7.8 ± 0.4	8.8 ± 0.6‡
Perfusion								
SI_{max} (a.u.)	6.6 ± 0.6	<0.04	11.4 ± 1.5‖	8.1 ± 0.7	<0.02	11.5 ± 0.8§	8.2 ± 0.6	10.5 ± 0.4‖
MBVI (%)	23 ± 2	<0.05	38 ± 3‖	28 ± 2	NS	42 ± 4§	29 ± 2	43 ± 4‖
β	0.52 ± 0.06	NS	0.74 ± 0.2	0.67 ± 0.11	NS	0.81 ± 0.20	0.55 ± 0.06	0.68 ± 0.09
MBFI (SI_{max} × β)	3.3 ± 0.4	<0.07	6.2 ± 1.2‡	5.3 ± 0.9	<0.05	9.7 ± 2.4‡	4.5 ± 0.4	5.6 ± 0.2§

Data are means ± SEM. *P values between the patients' basal values before C-peptide infusion and the control subjects' basal values; †P values between patients' basal values before and during C-peptide infusion; ‡$P < 0.05$, §$P < 0.01$, ‖$P < 0.001$ between dipyridamole stress values versus corresponding basal values. β, rate constant as measure of flow rate.

infusion of 6 pmol · kg^{-1} · min^{-1} i.v. but remained unchanged (0.13 ± 0.02 nmol/l) during the placebo study.

Hemodynamic parameters. Heart rate and blood pressure were similar in the control group and type 1 diabetic patients at rest (Table 1). In the healthy individuals, the heart rate increased during dipyridamole stress ($P < 0.001$) to a similar magnitude as observed in the patients. In response to dipyridamole, both systolic and diastolic blood pressure decreased in the control group ($P < 0.04$). In the diabetic group, the diastolic blood pressure remained unchanged and the systolic blood pressure increased from 115 ± 3 to 121 ± 5 mmHg ($P < 0.03$, Table 1). Thus, the increase of the rate pressure product during dipyridamole stress was greater in the diabetic group (3,433 ± 756) compared with the control subjects (1,455 ± 576, $P < 0.02$). No significant differences in the hemodynamic parameters were observed after C-peptide or placebo administration.

Myocardial velocities. Myocardial velocities (Fig. 1) in the basal state were lower in the patients compared with the control group (Fig. 2; Table 1) during early diastole (Vd 13.8 ± 0.6 vs. 15.6 ± 0.5 cm/s, $P < 0.04$).

The same tendency was present during systole (Vs 8.0 ± 0.3 vs. 8.8 ± 0.2 cm/s, $P < 0.06$). Diastolic myocardial function in the basal state showed a significant inverse relationship to the duration of diabetes ($r = 0.75$, $P < 0.03$, Fig. 3), whereas systolic function did not.

Systolic and diastolic peak velocities increased significantly during dipyridamole stress in both patients and control subjects (Table 1).

After C-peptide infusion myocardial velocities in the basal state increased during both early diastole (Vd 12 ± 3%, $P < 0.004$) and systole (Vs by 12 ± 4%, $P < 0.03$) but not after placebo administration (Fig. 4).

However, C-peptide had no significant effect during maximal vasodilatation by dipyridamole. Changes in myocardial velocities were not related to the simultaneous changes in blood pressure or heart rate. The CV for intraday variability was 4.9% for Vs and 4.6% for Vd.

Myocardial perfusion. The results of the perfusion imaging stress test excluded the presence of subepicardial coronary artery disease in all patients with type 1 diabetes. Figure 5 demonstrates the increased myocardial opacification during both C-peptide and dipyridamole infusion in a diabetic patient.

In the basal state, SI_{max} and myocardial blood volume index were reduced in the diabetic patients compared with the control subjects ($P < 0.05$, Table 1 and Fig. 2). After maximal vasodilatation by dipyridamole, all perfusion variables rose to similar levels in the two groups. After C-peptide infusion, SI_{max} increased by 26% (Fig. 4) in type 1 diabetic patients ($P < 0.02$) to virtually the same level as that of the control group (Table 1). Likewise, basal myocardial blood flow index was also improved during C-peptide infusion; it increased from 3.3 ± 0.4 to 5.3 ± 0.9, $P < 0.05$ (Table 1). This increase tended to correlate positively to the corresponding value during dipyridamole infusion ($r = 0.68$, $P < 0.06$) in type 1 diabetic patients. Saline infusion did not alter basal perfusion data. The CV for SI_{max} was 9.6% for intraday variability.

DISCUSSION

This placebo-controlled study was undertaken with the dual purpose to examine myocardial function and perfusion in type 1 diabetes and to assess the possible effects of short-term administration of C-peptide on systolic and diastolic myocardial velocities and perfusion using the novel ultrasound techniques tissue Doppler and myocardial contrast echocardiography. Compared with an age-matched control group, diastolic velocities and myocardial blood volume were reduced in type 1 diabetic patients, and they subsequently increased during C-peptide administration to levels corresponding to those in the control group, suggesting that short-term proinsulin C-peptide may exert a beneficial effect on myocardial function and perfusion in type 1 diabetic patients.

Functional abnormalities in the diabetic heart. Impairment of diastolic myocardial function has been observed in patients with type 1 diabetes with normal systolic ventricular function and independently of the presence of coronary artery disease (7,8). Traditional Doppler imaging had become an accepted method for assessment of left ventricular diastolic function by determining mitral valve inflow patterns also in type 1 diabetic patients (8,18), although it cannot detect moderate diastolic impairment (19). We have therefore used the novel technique tissue Doppler echocardiography, which measures myocardial velocities directly and relatively independent of preload changes (20,21). The normal E/A ratio (Table 1) of these velocities is in line with the normal results obtained by the traditional indexes of diastolic function in the present patient group.

The present study confirms, however, that the myocardial velocity during early diastole is reduced in type 1 diabetic patients compared with age-matched control subjects (Table 1) and that there is also a trend toward systolic dysfunction. This observation is in line with recent studies demonstrating that sensitive indexes of systolic function are abnormal when diastolic function is impaired (21,22) and confirms the concept that diastolic function is linked to systolic function via elastic recoil (23). The magnitude of the diastolic impairment is correlated to the duration of the disease (Fig. 3), emphasizing the progressive nature of the myocardial disorder. The current findings thus provide evidence of subclinical cardiac morbidity in type 1 diabetic patients possibly related to abnormalities in myocardial perfusion. This was suggested by the reduction of myocardial blood volume in the diabetes group while the hemodynamic state was similar in both groups (Table 1) and, furthermore, by the increase of myocardial function parameters in both groups during maximal vasodilatation (dipyridamole).

Myocardial perfusion in patients with diabetes. The traditional techniques for assessment of myocardial blood flow have been either invasive (24) or based on the administration of ionizing agents (6) and do not readily allow repeated measurements of dynamic changes. However, myocardial blood flow is modulated in keeping with metabolic demands also in diabetes with its functional and structural modifications of the myocardium (25,26). Changes in myocardial blood flow are mainly mediated by alterations in flow velocity at the level of subepicardial coronary arteries but less so at the level of the microcir-

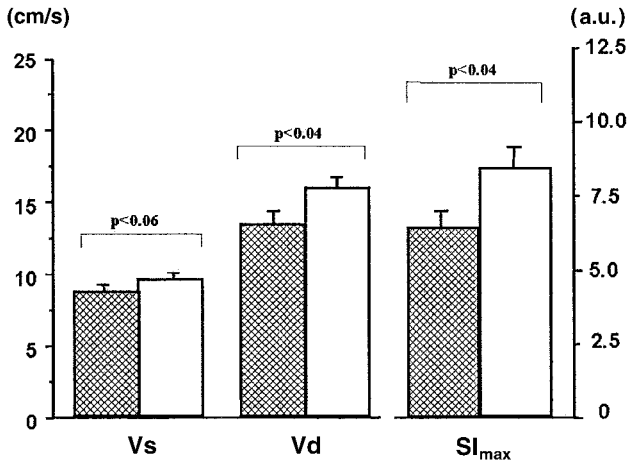

FIG. 2. In the basal state, myocardial systolic (Vs) and early diastolic (Vd) velocities and SI_{max}, the equivalent of myocardial blood volume, are reduced in patients with type 1 diabetes (cross-hatched bars) compared with healthy control subjects (open bars).

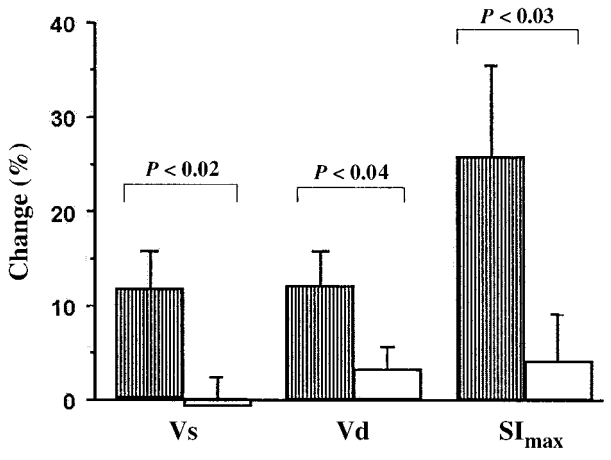

FIG. 4. Percent changes in systolic (Vs) and diastolic (Vd) myocardial velocities and SI_{max}, the equivalent of myocardial blood volume, in patients with type 1 diabetes during infusion of 6 pmol · kg^{-1} · min^{-1} C-peptide (striped bars) significantly exceed those measured during saline infusion (open bars).

culation because coronary autoregulation stabilizes perfusion pressure, which controls flow velocity (27). This concept is supported by an unchanged β value during pharmacological interventions in the present ultrasound study (Table 1). It appears more likely, therefore, that the capillary cross-sectional area is modified, as suggested also by the change in myocardial blood volume in the present data. Obviously, an increase in the number of perfused capillaries reduces diffusion distances for metabolic and gas exchange, while the opposite is true for a reduction in the number of open capillaries. Thus, changes in capillary blood volume, which makes up 90% of myocardial blood volume (28), are of major clinical importance and may even precede the respective changes in blood flow (27,29). In keeping with these considerations, the patients with diabetes had signs of both reduced myocardial function and blood volume (Fig. 2 and Table 1), which has not been shown before. The intraday variability (9%) confirms that the observed changes in blood volume (28%) were meaningful.

Notably, myocardial contrast echocardiography is the only method available for differentiating myocardial blood volume changes from flow modifications serially in patients (12,30), since the microbubbles remain strictly intravascular. Positron emission tomography (PET) and angiography studies have shown various results suggesting that myocardial blood flow in patients with type 1 diabetes at rest is not different (6,24) or increased (31) compared with that of an age-matched control group. Using the new technique of myocardial contrast echocardiography, we observed a tendency toward impaired flow in the diabetic individuals. But the difference in results is likely to be a consequence of different patient selection (24), techniques, and protocols in the previous reports. PET technique for the evaluation of myocardial blood flow after administration of ^{15}O-labeled water (6) uses a freely diffusible indicator with high tissue extraction that provides an indirect measure of tissue perfusion and, thus, cannot differentiate myocardial volume effects from flow modifications. In addition, in some of the PET studies, myocardial blood flow in diabetic patients is assessed during hyperinsulinemic euglycemia (6,31) and compared with data obtained during normoinsulinemia in control individuals. This is likely to influence the results due to the well-known vasoactive effects of insulin (32). Further studies will be needed to allow more insight into the complex regulation of myocardial microcirculation in diabetes.

Effects of C-peptide. Previous experimental and clinical reports have demonstrated an effect of C-peptide on blood flow regulation in several tissues. Thus, C-peptide has been shown to increase muscle blood flow at rest (5,33) and during exercise (3), to affect a redistribution of skin

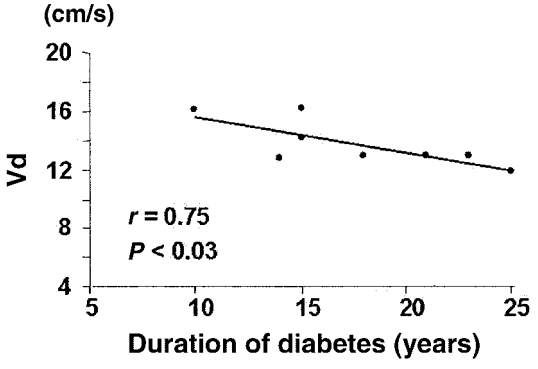

FIG. 3. Inverse correlation between the diastolic myocardial function at rest, taken as myocardial velocity during early diastole (Vd), and the duration of type 1 diabetes.

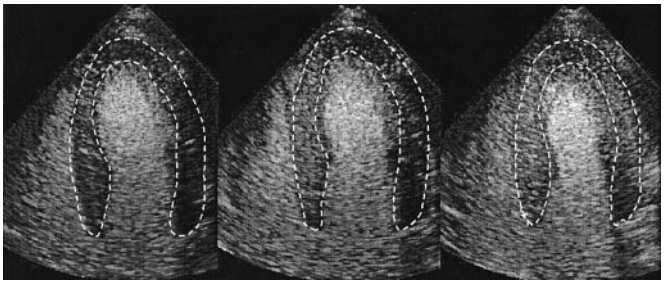

FIG. 5. Example of an end-systolic contrast image (apical 4-chamber view) in a patient with type 1 diabetes at rest (left panel). Myocardial (dotted line) contrast enhancement increased during C-peptide (middle) and dipyridamole infusion (right panel).

blood flow from shunt channels to nutritive blood flow (34), to increase renal blood flow (35), and to augment nerve blood flow (36). C-peptide effects in this regard are mediated via a stimulatory effect on endothelial nitric oxide synthase (4,37). In addition, it is possible that C-peptide's ability to stimulate Na^+-K^+-ATPase contributes to tissue capillary recruitment and subsequent augmentation of blood flow (38).

The present data demonstrate that indexes of both myocardial blood flow and blood volume in the type 1 diabetic patients increase in response to short-term C-peptide infusion, sufficient to raise the C-peptide concentrations into the physiological range. The patients' myocardial blood volume in the basal state was 22% lower than that in the healthy control subjects, but during C-peptide infusion blood volume and flow rose to the same levels as seen in the control subjects. It is likely that C-peptide elicited the myocardial changes in flow and vascular volume by capillary recruitment and/or relaxation of resistance vessels, which is in keeping with previous findings for skeletal muscle (3). It may be speculated that the increase in number of perfused capillaries per cross-sectional area of the myocardium may have augmented myocyte energy availability and, as a consequence, contributed to the observed improvement in both systolic and diastolic myocardial function in the type 1 diabetic patients. It is noteworthy that the C-peptide–induced changes in myocardial function and flow were not associated with increased oxygen requirements, at least not as reflected by the rate-pressure product (Table 1).

The blood flow response to C-peptide was ~50% of that seen after dipyridamole administration (Table 1). The responses to these two agents tended to be correlated, suggesting that similar mechanisms of flow regulation may be involved. It may be seen as an encouraging prognostic result that the reduction in myocardial perfusion and function in the present young type 1 diabetic patients is, in part, reversible. Further studies will be required to assess more specifically the influence of C-peptide on myocardial endothelial function and blood flow regulation.

C-peptide effects were not examined in the healthy control subjects, since it has been a consistent finding that no effects of C-peptide have been observed in subjects with normal levels of circulating C-peptide (7). As a consequence, C-peptide effects are likely to be observable primarily in type 1 diabetic patients who lack C-peptide secretion. We conclude that the present findings of increased myocardial perfusion and improved myocardial function in type 1 diabetic patients are in line with previous findings for C-peptide effects in other tissues, primarily skeletal muscle. The present results provide additional support for the hypothesis that C-peptide replacement in type 1 diabetic patients exerts beneficial effects on microvascular function and that C-peptide administration together with insulin (39) may serve to retard or prevent the development of long-term complications.

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