C-peptide improves adenosine-induced myocardial vasodilation in type 1 diabetes patients

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Sections of ¹Clinical Physiology and Oncology/Pathology and of ²Nuclear Medicine, Department of Surgical Sciences, Karolinska Institutet, SE-171 76 Stockholm, Sweden; ³Turku Positron Emission Tomography Centre and ⁴Division of Clinical Physiology, Turku University, Turku, FIN-20014 Finland

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Johansson, Bo-Lennart, Jan Sundell, Karin Ekberg, Cathrine Jonsson, Marko Seppänen, Olli Raitakari, Matti Luotolahti, Pirjo Nuutila, John Wahren, and Juhani Knuutti. C-peptide improves adenosine-induced myocardial vasodilation in type 1 diabetes patients. Am J Physiol Endocrinol Metab 286: E14–E19, 2004. First published September 3, 2003; 10.1152/ajpendo.00236.2003.—Patients with type 1 (insulin-dependent) diabetes show reduced skeletal muscle blood flow and coronary vasodilatory function despite intensive insulin therapy and good metabolic control. Administration of proinsulin C-peptide increases skeletal muscle blood flow in these patients, but a possible influence of C-peptide on myocardial vasodilatory function in type 1 diabetes has not been investigated. Ten otherwise healthy young male type 1 diabetic patients (Hb A1c 6.6%, range 5.7–7.9%) were studied on two consecutive days during normoinsulinemia and euglycemia in a double-blind, randomized, cross-over design, receiving intravenous infusion of C-peptide (5 pmol·kg⁻¹·min⁻¹) for 120 min on one day and saline infusion on the other day. Myocardial blood flow (MBF) was measured at rest and during adenosine administration (140 μg·kg⁻¹·min⁻¹) both before and during the C-peptide or saline infusions by use of positron emission tomography and [¹⁵O]H₂O administration. Basal MBF was not significantly different in the patients compared with an age-matched control group, but adenosine-induced myocardial vasodilation was 30% lower (P < 0.05) in the patients. During C-peptide administration, adenosine-stimulated MBF increased on average 35% more than during saline infusion (P < 0.02) and reached values similar to those for the healthy controls. Moreover, as evaluated from transthoracal echocardiographic measurements, C-peptide infusion resulted in significant increases in both left ventricular ejection fraction (+5%, P < 0.05) and stroke volume (+7%, P < 0.05). It is concluded that short-term C-peptide infusion in physiological amounts increases the hyperemic MBF and left-ventricular function in type 1 diabetic patients.

echocardiography; myocardial blood flow; positron emission tomography; rate-pressure product

Type 1 diabetes is a major risk factor for atherosclerotic vascular diseases. Subjects with diabetes have a two- to fourfold increased risk of developing coronary artery disease (26). An early event in the progression toward atherosclerosis in diabetes is the development of endothelial dysfunction (35). Several studies have shown that young subjects with type 1 diabetes have a reduced myocardial blood flow (MBF) response to pharmacologically induced vasodilation (4, 24, 31), this abnormality being present even before the appearance of other diabetic long-term complications. Recently, it was demonstrated that short-term insulin infusion induces coronary vasodilation in type 1 diabetic patients (31); this finding might contribute to the observed beneficial effects of intensive insulin therapy on myocardial ischemia (22). Patients with type 1 diabetes lack not only insulin but also C-peptide. This peptide is cleaved from proinsulin and released from the pancreas into the circulation in amounts equimolar with insulin. Recent studies have demonstrated important cellular effects and clinical findings for C-peptide (19, 34). Administration of C-peptide in replacement doses to patients with type 1 diabetes is accompanied by improved renal function (14, 18) and amelioration of nerve dysfunction (14, 15). C-peptide infusion also results in increased blood flow in several tissues, notably in skeletal muscle at rest and during exercise (8, 12, 16, 21).

Positron emission tomography (PET) has been used to detect early alterations in myocardial vasodilatory capacity in type 1 diabetic patients (24) and other patients with risk factors for coronary artery disease (25). The present study was primarily designed to examine the effects of C-peptide on MBF at rest and during adenosine-induced hyperemia in patients with type 1 diabetes by use of PET and oxygen-15-labeled water ([¹⁵O]H₂O). In addition, the possible effects of C-peptide on left ventricular function during basal conditions were evaluated by using standardized echocardiographic technique.

SUBJECTS AND METHODS

Subjects. Ten nonsmoking, male, type 1 diabetic patients [age 32 ± 2 yr, body mass index (BMI) 24.0 ± 0.7 kg/m², duration of diabetes 11 ± 1 yr, insulin dose 0.75 ± 0.06 U·kg⁻¹·24 h⁻¹, Hb A1c 6.6% (range 5.7–7.9)] without clinical signs of cardiovascular disease participated in the study. Except for insulin, the patients did not use any medication. They showed no clinical signs or symptoms of retinopathy, neuropathy, or nephropathy. Eye examinations revealed no more than simple background retinopathy in three patients, and urinary and blood analyses for albumin excretion and serum creatinine were all within normal reference values. Neurological examination showed no signs of autonomic or peripheral neuropathy. Ten nonsmoking, age- and weight-matched healthy men (age 32 ± 2 yr, BMI 24.1 ± 0.6 kg/m²) served as control subjects.

The study was conducted in accord with the guidelines of the Declaration of Helsinki. The study protocol was reviewed and approved by the Finnish Medical Product Agency and by the local Ethics and Radiation Protection Committees at the Karolinska Hospital (Stockholm, Sweden) and the Turku University Central Hospital.


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assessed by PET with [15 O]H₂O. Blood samples for glucose determinations were drawn every 30 min, and samples were also collected before and at the end of the C-peptide or saline infusion period for analyses of insulin and C-peptide. The healthy control subjects were studied only once before and during adenosine infusion. ECG (heart rate) and blood pressure were recorded at rest and during each adenosine infusion period. Blood pressure was monitored with an automatic oscillometric blood pressure monitor (Omron HEM-705C; Omron Healthcare, Hamburg, Germany). Transthoracic echocardiographic examinations were performed in the diabetic patients before and during the C-peptide or saline infusion period on both study days (Fig. 1) and on one occasion in the healthy control subjects in basal state before PET.

Study design. The study protocol is presented in Fig. 1. Patients were studied twice within 2 days. They received intravenous C-peptide infusion on one day and intravenous saline infusion on the other day in randomized order. PET, positron emission tomography; ECHO, echocardiography.

Image acquisition, processing, and corrections. The production of [15 O]H₂O was performed as described earlier (29). The subjects were positioned supine in a 15-slice ECAT 931/08–12 tomograph (Siemens/CTI, Knoxville, TN). After the transmission scan, myocardial perfusion was measured after an intravenous injection of [15O]H₂O (~1.5 GBq) at rest and at 60 s after the beginning of each intravenous administration of adenosine. Each dynamic scan lasted for 6 min (6 × 5 s, 6 × 15 s, 8 × 30 s). All data were corrected for dead time, radioactive decay, and photon attenuation and were reconstructed into a 128 × 128 matrix. Data were reconstructed with a filter back projection method, in which the final inplane resolution in the reconstructed and Hann-filtered (0.3 cycles/s) 9.5-mm images (full-width half-maximum) was used with a recently developed median root prior reconstruction method (1). Calculation of regional blood flow and coronary flow reserve. Four regions of interest (ROIs) were drawn, the lateral, anterior, septal, and whole wall of the left ventricle, in four representative transaxial slices. The baseline ROIs were copied to the images obtained after each consecutive study sequence. Values for regional MBF (expressed in ml/g of tissue−1·min−1) were calculated as previously described, with a single-compartment model (11). The arterial input function was obtained from the left-ventricular (LV) time-activity curve by a validated method (10). The MBF, calculated as the average of the whole wall of the LV ROIs, showed the lowest intra-individual day-to-day coefficient of variation (15%, both at rest and during adenosine) and was used in the further analysis. Coronary vasodilatory function, i.e., adenosine-induced myocardial vasodilatation, was calculated as the difference between the adenosine-stimulated flow and basal flow in absolute terms.

Echocardiographic examination. All recordings and analyses were performed with an ultrasound scanner (Acuson 128XP/10, Acuson, Mountain View, CA) with 2.5/3.5 MHz scanning frequency (phased array transducer). LV dimensions and wall thickness were obtained from two-dimensionally guided M-mode tracings. LV systolic function was assessed by the calculation of the ejection fraction [in %; (LV diastolic volume − LV systolic volume)/LV diastolic volume] and the stroke volume [in ml; (LV diastolic volume − LV systolic volume)] (7). Analytical methods. Glucose was analyzed on test strips using an Accutrend sensor (Roche). Hb A₁c was determined by a liquid-chromatographic assay (13) with normal reference values 3.5–5.5%. Plasma samples for immunoreactive free insulin were immediately precipitated with polyethylene glycol (2). Insulin and plasma C-peptide were assessed by radioimmunoassay technique using commercial kits (Pharmacia Insulin RIA; Pharmacia Diagnostica, Uppsala, Sweden, and Euria-C-peptide, Eurodiagnostica, Malmö, Sweden).

![Fig. 1. Study design. Patients were studied twice within 2 days. They received intravenous C-peptide infusion on one day and intravenous saline infusion on the other day in randomized order. PET, positron emission tomography; ECHO, echocardiography.](http://ajpendo.physiology.org/DownloadedFrom/10.1152/ajpendo.00292.2003)
Statistics. Results are expressed as means ± SE. Student’s paired and unpaired t-tests, as well as Wilcoxon paired and unpaired tests, were used when appropriate. *P < 0.05 was considered statistically significant.

RESULTS

Metabolic and hormonal findings. The fasting serum lipid profile was within the normal range for both the diabetic patients and the healthy control subjects (patients: 4.2 ± 0.2, 0.98 ± 0.09, 1.57 ± 0.11, and 2.19 ± 0.18 mM, and control subjects: 5.0 ± 0.2, 1.10 ± 0.16, 1.50 ± 0.09, and 3.10 ± 0.19 mM for cholesterol, triglycerides, and high- and low-density lipoproteins, respectively). In the morning after the intravenous insulin infusion of the preceding night and during the study, the average blood glucose level was 6.6 ± 0.1 mM on the C-peptide infusion day and 6.3 ± 0.2 mM on the saline infusion day. Likewise, plasma insulin concentrations were within the physiological concentration range on both study days (40 ± 4 and 39 ± 7 pM, respectively). Neither glucose nor insulin concentrations changed significantly during the C-peptide or saline infusion periods. During C-peptide infusion, its concentration rose from <0.12 nM (detection limit 0.12 nM) to 1.48 ± 0.05 nM and, as expected, remained unchanged at 0.12 nM during the saline infusion day. In the healthy subjects, basal plasma glucose and insulin concentrations were 5.3 ± 0.2 mM and 42 ± 4 pM, respectively.

Echocardiographic examination. Echocardiographic measurements before and during C-peptide or saline infusion showed that all LV dimensional and functional variables in the patients were within the normal range, according to standard reference values (7), and the basal measurements did not differ significantly between the patients and healthy control subjects (Table 1). In the patients, LV ejection fraction and stroke volume both increased significantly by 5 and 7%, respectively (P < 0.05), during the C-peptide infusion period, whereas both variables were unchanged during saline infusion (Table 1). End-diastolic LV volume was within the normal range and did not change during the infusion periods; systolic LV volume tended to decrease during the C-peptide infusion period (−14%, P = 0.06) but was unchanged during saline infusion (Table 1).

Hemodynamic measurements during PET. Data for blood pressure, heart rate, and rate-pressure product (RPP) are presented in Table 2. Adenosine administration elicited a marked increase in heart rate (P < 0.001 vs. basal), and the heart rate responses were similar during C-peptide and saline infusions. Diastolic and systolic blood pressures did not change signifi-

Table 2. Hemodynamic data for diabetic patients and healthy subjects measured in the basal state and during adenosine stimulation before and during C-peptide or saline infusion

<table>
<thead>
<tr>
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<th>Patients</th>
<th>Control Subjects</th>
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<tr>
<td></td>
<td>Before</td>
<td>Adenosine</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>58 ± 4</td>
<td>101 ± 3*</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>115 ± 3</td>
<td>116 ± 2</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>66 ± 3</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>RPP, beats-min⁻¹×mmHg</td>
<td>6,710 ± 520</td>
<td>11,660 ± 490*</td>
</tr>
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</table>

Values are means ± SE. RPP, rate-pressure product. *Statistically significant differences (P < 0.001) between measurements during adenosine stimulation and the corresponding basal value.
cantly during adenosine infusion or during C-peptide or saline infusion. RPP after adenosine infusion increased similarly during C-peptide and saline infusion (Table 2). No differences were found in any of the above hemodynamic variables between diabetic patients and healthy control subjects.

**MBF.** Basal MBF was 0.74 ± 0.05 ml·g⁻¹·min⁻¹ on the C-peptide study day and 0.77 ± 0.05 ml·g⁻¹·min⁻¹ on the saline infusion day (Table 3). These values tend to be lower but not significantly different from those found in healthy control subjects (0.86 ± 0.06 ml·g⁻¹·min⁻¹). MBF rose in response to adenosine infusion but less so in the patients than in the control subjects. The adenosine-stimulated MBF was ~25% lower in diabetes patients than in healthy subjects (P < 0.03, Table 3), and the adenosine-induced increase in MBF was 2.7 ± 0.2 and 3.8 ± 0.4 ml·g⁻¹·min⁻¹ in patients and control subjects, respectively, P < 0.04, Fig. 2. A marked increase in adenosine-stimulated blood flow was recorded during infusion of C-peptide compared with saline infusion (P < 0.02; Figs. 2 and 3). Thus C-peptide infusion resulted on average in a 35 ± 10% increase in the adenosine-induced rise in MBF, reaching a level similar to that of the control subjects during adenosine stimulation (Table 3). As expected, no change in adenosine-stimulated MBF was observed during saline infusion.

No significant variation was found in MBF between the lateral, anterior, and septal parts of the left ventricle before and during adenosine stimulation. Therefore, the global MBF (lateral + anterior + septal wall) showing the lowest interindividual coefficient of variation was used in the presentation of MBF.

**DISCUSSION**

Proinsulin C-peptide, previously thought to be biologically inert, has recently been found to show the characteristics of a cell membrane protein, and it activates intracellular Ca²⁺-dependent signaling pathways, resulting in stimulation of Na⁺-K⁺-ATPase and endothelial nitric oxide synthase (eNOS) activities (19, 34). In patients with type 1 diabetes, C-peptide administration elicits effects on renal and nerve function (14, 38, 39). In patients with type 1 diabetes, C-peptide administration elicits effects on renal and nerve function (14, 15, 18) and results in an increase in blood flow of skeletal muscle both under basal conditions (8) and during rhythmic forearm exercise (16). In the present study, the observed effect of C-peptide on skeletal muscle blood flow is extended to basal and pharmacologically stimulated myocardial perfusion by use of the PET technique.

The present results demonstrate that C-peptide in physiological concentrations augments the capacity for myocardial vasodilation, as measured by the PET technique in young type 1 diabetic patients without symptoms of long-term diabetic complications or cardiac dysfunction. It can be difficult to exclude silent myocardial ischemia in patients with diabetes, but because the patients in this study presented a normal exercise test, normal echocardiographic results, and absence of regional variations in MBF during PET, we considered it unlikely that they were afflicted with significant coronary artery disease. Thus the present group of type 1 diabetes patients without signs of cardiac disease showed an increased adenosine-stimulated MBF compared with healthy control subjects. Short-term C-peptide infusion in replacement dose was found to substantially improve this subclinical dysfunction. The present results are in line with those of a previous report in which MBF and LV functions in type 1 diabetic patients were evaluated before and during C-peptide administration by use of myocardial contrast tissue Doppler imaging techniques (9). In that study, indexes of MBF showed lower values in the patients than in the control subjects in the basal state and a rise in MBF to normal levels during C-peptide infusion. In the present study, basal MBF also tended to be lower in the patient group (Table 3), but not significantly so, and it did not increase in response to C-peptide. These differences may be accounted for in part by the

Table 3. **MBF in diabetes patients and healthy control subjects in the basal state and during adenosine stimulation before and during infusion of C-peptide or saline**

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>Infusion</th>
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<tr>
<td></td>
<td>Basal</td>
<td>Adenosine</td>
</tr>
<tr>
<td>Patients, C-peptide study day</td>
<td>0.74±0.05</td>
<td>3.31±0.29*</td>
</tr>
<tr>
<td>Patients, saline study day</td>
<td>0.77±0.05</td>
<td>3.69±0.19*</td>
</tr>
<tr>
<td>Healthy control subjects</td>
<td>0.86±0.06</td>
<td>4.63±0.45*</td>
</tr>
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</table>

Myocardial blood flow (MBF) values are means ± SE expressed in ml·g⁻¹·min⁻¹. *Significant differences (P < 0.03) in adenosine-stimulated MBF between diabetic patients and healthy subjects before infusions; †significant difference (P < 0.02) in adenosine-stimulated rise in MBF between C-peptide and saline infusion.

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**Fig. 2.** Adenosine-induced myocardial vasodilation, i.e., the difference between basal and adenosine-stimulated myocardial blood flow (MBF) in diabetic patients before and during C-peptide infusion and in healthy control subjects. Values are means ± SE. *P < 0.05; N.S., not significant.

**Fig. 3.** Percent change in adenosine-stimulated MBF during infusion of C-peptide or saline in the diabetic patients. Values are means ± SE. *P < 0.05.
different methods used for MBF determination and stimulation, the considerable variability of blood flow in the basal state, and also by the fact that patients with longer duration of diabetes participated in the study by Hansen et al. (9). In both studies, an increase in myocardial workload was induced pharmacologically, but the stimulation by adenosine in the present study was more marked. This is indicated by the greater increase in heart rate (+33%) and RPP (+23%) after adenosine than after dipyridamole in the earlier study (9). Dipyridamole stimulation was accompanied by a similar rise in MBF in patients and in control subjects, and no further increase was seen during C-peptide (9). On the other hand, adenosine administration in the present study resulted in a greater increase in MBF than that evoked by dipyridamole, and it elicited a more marked response in the healthy subjects than in the patients. This difference was almost fully corrected after C-peptide. Thus the different responses to C-peptide after adenosine and dipyridamole administration may be related to the difference in myocardial stimulation, which was more robust in the present study than in the study by Hansen et al.

The adenosine-induced coronary flow response reflects the combined effect of endothelium-mediated vasodilatory function and vascular smooth muscle relaxation and has been used as an integrated measure of coronary reactivity (33). In contrast to the situation under resting conditions, when flow and myocardial work (oxygen consumption) are tightly coupled, the metabolic control of MBF is lost during adenosine stimulation. However, endothelial and neurogenic controls are still functional (23), and it has been found that approximately one-half of the adenosine-induced response is endothelium dependent (3). In addition, MBF is directly dependent on blood pressure and modulated by mechanical forces within the myocardial wall (23). In this context, it is noteworthy that the patients in the present study showed no measurable difference in blood pressure during C-peptide or saline infusion. The adenosine-induced increase in MBF during C-peptide administration is thus not explainable on the basis of a rise in blood pressure. The mechanism underlying C-peptide’s ability to increase adenosine-stimulated MBF is not immediately apparent. However, C-peptide is known to induce vasodilation in skeletal muscle via an endothelium-dependent mechanism by stimulation of L-arginine transport and eNOS activity (20). In addition to an improved endothelial function of the myocardial vasculature, a C-peptide-elicited stimulation of Na\(^{+}\)-K\(^{-}\)-ATPase activity of capillary smooth muscle, resulting in augmented capillary recruitment, may also have contributed to the results, as has been suggested for skeletal muscle (21).

Recent studies demonstrate that insulin also may act as a vasoactive hormone in cardiac vasculature, both in healthy subjects and in type 1 diabetic patients (31, 32), but only at high physiological concentrations. Thus an insulin concentration of \(\sim500\) pM for 1 h increases the adenosine-stimulated MBF by \(\sim20\)% in type 1 diabetes patients (31). The mechanism of insulin-induced coronary vasodilation is not known, but in peripheral arteries, hyperinsulinemia induces vasodilation mainly via an endothelium-dependent mechanism, including the L-arginine-nitric oxide pathway (30). The present study demonstrates that C-peptide infusion enhances adenosine-stimulated MBF at basal insulin concentrations (\(\sim40\) pM). Thus physiological C-peptide concentrations appear to exert a vasodilatory effect in the presence of low physiological insulin concentrations in patients with type 1 diabetes. Further studies will be required to determine whether a permissive insulin concentration is required for the C-peptide effect, as suggested from in vitro studies of vascular smooth muscle (12) and/or whether a synergistic interaction between C-peptide and insulin can be observed. However, the metabolic conditions of the present study resemble those during normal daily life, when circulating insulin concentrations are in a range that does not influence myocardial vasodilation. Consequently, the present findings emphasize the possible physiological significance of C-peptide’s effect on MBF.

Mild LV diastolic and systolic dysfunction is an early subclinical finding in otherwise healthy type 1 diabetic patients (27). In this study we found, as reported earlier (8), a small but significant increase in both LV ejection fraction and stroke volume during the C-peptide infusion period, indicating an improved systolic function. By evaluating the basal end-diastolic and systolic LV volume before and during infusion of C-peptide or saline, it was found that the end-diastolic volume was unchanged, whereas the systolic volume tended to decrease during the C-peptide but not the saline infusion period. These results are also in agreement with findings in a previous report (9), in which improved LV function was demonstrated by increases in both contraction and relaxation velocities during C-peptide infusion. This suggests that the effects on ejection fraction and stroke volume are related to the systolic rather than the diastolic function. These inotropic effects of C-peptide may be related to a stimulation by C-peptide of myocardial Na\(^{+}\)-K\(^{-}\)-ATPase activity and/or to an effect on myocardial circulation. The findings may be of clinical significance and warrant further studies in patients with overt diabetic cardiomyopathy or heart failure.

Blunted myocardial vasodilatory capacity and coronary endothelial dysfunction are common and early findings in type 1 diabetic patients (4, 24, 31). Several of the classical risk factors for coronary artery disease are often present in these patients. Among these, autonomic neuropathy, hyperglycemia, and hyperinsulinemia have been suggested to contribute to the coronary dysfunction (6). Chronic hyperglycemia may increase the risk for endothelial dysfunction and coronary artery disease via mechanisms such as irreversible glycation of proteins in the arterial wall, formation of free radicals, abnormalities in lipoprotein particle composition, and oxidation of lipoproteins (6). In addition, chronic hyperglycemia in type 1 diabetic patients is associated with insulin resistance. Hyperinsulinemia has harmful effects on endothelial function and may also promote arterial smooth muscle cell proliferation, cause cholesterol ester accumulation in the arterial wall, and increase sympathetic activity (5). Thus a variety of different mechanisms may be involved in the development of the myocardial vasodilatory dysfunction in patients with type 1 diabetes. On the basis of the present results, the possibility should be considered that C-peptide deficiency may be an additional risk factor for the development of reduced myocardial endothelial function in type 1 diabetes.

Finally, the question may be raised as to why C-peptide was not administered to the healthy control subjects. This would seem an obvious step in obtaining adequate control data, but a number of studies have now demonstrated that C-peptide exerts no measurable physiological effects in healthy individuals (16, 17). The background to this finding rests with the fact
that C-peptide binding to cell membranes saturates at an already low physiological concentration (28). Further increases in C-peptide plasma concentrations do not result in additional binding. Consequently, no physiological effect over and above that elicited by the ambient C-peptide level in healthy subjects is to be expected. With this background, we chose not to undertake control studies with C-peptide infusion in healthy subjects.

In summary, the results of the present study demonstrate that C-peptide acts as a vasodilating agent in the cardiac vascular bed and that it exerts an inotropic effect on the left ventricular systolic function in type 1 diabetic patients.

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GRANTS

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