ISSN 0735-1097/03/$30.00
doi:10.1016/S0735-1097(03)00625-9

Diastolic Dysfunction Is Associated With Altered Myocardial Metabolism in Asymptomatic Normotensive Patients With Well-Controlled Type 2 Diabetes Mellitus

Michaela Diamant, MD, PHD,*‖ Hildo J. Lamb, PHD,† Ymte Groeneveld, MD, PHD,‡ Edwin L. Endert,† Jan W. A. Smit, MD, PHD,* Jeroen J. Bax, MD, PHD,§ Johannes A. Romijn, MD, PHD,* Albert de Roos, MD, PHD,† Jasper K. Radder, MD, PHD*

Leiden and Amsterdam, the Netherlands

OBJECTIVES This study evaluated myocardial function in relation to high-energy phosphate (HEP) metabolism in asymptomatic patients with uncomplicated type 2 diabetes mellitus using magnetic resonance (MR) techniques.

BACKGROUND Myocardial dysfunction may occur in patients with type 2 diabetes mellitus in the absence of coronary artery disease or left ventricular (LV) hypertrophy. The mechanisms underlying this diabetic cardiomyopathy are largely unknown, but may involve altered myocardial energy metabolism.

METHODS We assessed myocardial systolic and diastolic function and HEP metabolism in 12 asymptomatic normotensive male patients with recently diagnosed, well-controlled type 2 diabetes and 12 controls, using MR imaging and phosphorus-31-nuclear MR spectroscopy (^{31}P-MRS) on a 1.5 T clinical scanner; ^{31}P-MR spectra were quantified, and myocardial HEP metabolism was expressed as phosphocreatine to adenosine-triphosphate (PCr/ATP) ratio.

RESULTS No differences were found in LV mass and systolic function between patients and controls. However, early (E) acceleration peak, deceleration peak, peak filling rate, and transmitral early-to-late diastolic peak flow (E/A) ratio, all indexes of diastolic function, were significantly decreased in patients compared with controls ($p < 0.02$). In addition, myocardial PCr/ATP in patients was significantly lower than in controls (1.47 vs. 1.88, $p < 0.01$). Inverse associations were found between myocardial PCr/ATP and E acceleration peak, E deceleration peak, and E peak filling rate (all, $p < 0.05$).

CONCLUSIONS These results indicate that altered myocardial energy metabolism may contribute to LV diastolic functional changes in patients with recently diagnosed, well-controlled and uncomplicated type 2 diabetes. (J Am Coll Cardiol 2003;42:328–35) © 2003 by the American College of Cardiology Foundation

Type 2 diabetes mellitus is associated with an increased risk of cardiovascular disease (CVD) (1). In particular, diabetic patients have a higher incidence of congestive heart failure as compared with age-matched nondiabetic subjects (2). Higher left ventricular (LV) mass and LV dysfunction was observed in newly diagnosed type 2 diabetic patients without clinically manifest heart disease (3,4). Myocardial functional abnormalities in diabetes have been related to hyperglycemia by some authors (5,6), but were found to be independent of glycemic control by others (7). In the absence of coronary artery disease (CAD) and hypertension, myocardial structural and functional changes in patients with diabetes have been ascribed to diabetic cardiomyopathy (DCM) (8,9).

Several causative mechanisms for DCM have been postulated, including microangiopathy, autonomic nervous dysfunction, defective cellular calcium transport, as well as structural changes in myocardial contractile proteins and accumulation of collagen, leading to increased stiffening of the ventricular wall. However, the exact pathogenesis of this condition has as yet not been elucidated (8). Recent evidence suggests that alterations in myocardial energy metabolism, resulting from altered substrate supply and utilization by cardiac myocytes, may be the primary injury in the pathogenesis of DCM (9–11). In the diabetic heart, myocardial energy status may be reduced as a result of inappropriate use of elevated free fatty acids (FFA) as a metabolic substrate, which may by cycled through intramyocardial lipolysis and re-esterified, leading to accumulation of potentially toxic intermediates and suppression of glucose

From the *Departments of Endocrinology, †Radiology, ‡General Practice, and §Cardiology, Leiden University Medical Center, Leiden, the Netherlands; and ‖Department of Endocrinology/Diabetes Center, VU University Medical Center, Amsterdam, the Netherlands. Supported by grant 97.703 from the Dutch Diabetes Research Foundation. Drs. Diamant and Lamb contributed equally to this work.

Manuscript received December 16, 2002; revised manuscript received April 4, 2003, accepted April 17, 2003.

Abbreviations and Acronyms

ACR	= albumin/creatinine ratio
ATP	= adenosine-triphosphate
CAD	= coronary artery disease
CK	= creatine kinase
CVD	= cardiovascular disease
DCM	= diabetic cardiomyopathy
E/A	= early-to-late diastolic
EDV	= end-diastolic volume
ESV	= end-systolic volume
FFA	= free fatty acids
HEP	= high-energy phosphate
LV	= left ventricle/ventricular
MR	= magnetic resonance
MRI	= magnetic resonance imaging
PCr	= phosphocreatine
SV	= stroke volume
^{31}P-MRS	= phosphorus-31 magnetic resonance spectroscopy

metabolism (9–12). The altered myocardial energy metabolism may adversely affect myocardial function.

Using magnetic resonance imaging (MRI) and phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P-MRS), the simultaneous existence of diastolic dysfunction and altered myocardial high-energy phosphate (HEP) metabolism was previously demonstrated in hypertensive patients with increased LV mass (13). The purpose of the present study was to study LV systolic and diastolic function in relation to myocardial energy metabolism, using these magnetic resonance (MR) techniques, in normotensive patients with well-controlled type 2 diabetes mellitus of short duration who had no clinical evidence of CAD.

METHODS

Subjects. Twelve male patients with uncomplicated type 2 diabetes and 12 age- and gender-matched healthy controls were studied. Patients (aged 50 to 65 years) were selected from general practices after approval of their physicians. Selection criteria were type 2 diabetes of short duration (<5 years, diagnosed by WHO criteria) (14); no signs or symptoms or history of CVD; and a normal electrocardiogram (ECG), body mass index (BMI) < 28 kg/m^2, seated office blood pressure (BP) < 150/90 mm Hg, good metabolic control (glycated hemoglobin [HbA1c] < 7.8%), no use of drugs other than sulfonylureas and/or metformin; and no diabetic complications including albuminuria, retinopathy, and neuropathy. Healthy controls who had no history or clinical evidence of CVD were included through advertisements in local newspapers. During a screening visit, a medical history (including a short questionnaire regarding physical fitness in which the participants estimated the time spent performing moderate exercise such as walking, biking, and swimming), physical examination (including standardized measurements of the vibration sense and Ewing's cardiovascular tests [15]), an ECG, and screening laboratory tests were obtained from all participants; HbA1c was determined by high performance liquid chromatography after hemolysis (reference range, 4.3 to 6.3% in nondiabetic subjects) (Bio Rad, Richmond, California). Microalbuminuria was determined in a 24-h urine collection and measured by immunonephelometry (Array Protein System, Beckman, Fullerton, California) (normoalbuminuria was defined as albumin/creatinine ratio [ACR] < 2.5 mg/mmol). Mean ACR in patients was 0.5 ± 0.5 mg/mmol and 0.4 ± 0.3 mg/mmol in controls ($p = 0.454$). In patients only, fundus photography was performed, unless a recent (<6 months) written report from the patient's ophthalmologist was available. Laboratory determinations were performed according to standard procedures. Subjects with metallic implants were excluded. Patients were asked to stop their medication one week before the MR studies. The protocol was approved by the local ethics committee, and all subjects gave informed consent.

MRI. All MR studies were performed using a 1.5-T whole-body MR scanner (Gyroscan ACS/NT15; Philips, Best, the Netherlands) equipped with multinuclei hardware for ^{31}P-MRS; MRI and MR spectroscopy were performed at a single occasion, at rest and in the supine position. The entire heart was imaged in the short-axis orientation using breath-hold multishot echo-planar imaging as described earlier (16); MRI velocity mapping with retrospective ECG-gating was performed to measure flow (expressed as ml/s) across the mitral valve and through the ascending aorta (13,17). During the entire MR examination, BP and heart rate (HR) were recorded every 2 min with a semiautomated device (Dinamap, Critikon, Tampa, Florida). Image analyses were performed by two blinded trained observers; LV functional parameters were calculated as described previously (18). The surface areas of the endocardial tracings in end diastole and end systole were summed up and multiplied by section thickness and section factor to produce the end-diastolic chamber volume (EDV) and end-systolic chamber volume (ESV). Stroke volume (SV) was the difference between EDV and ESV. Cardiac output was SV multiplied by the average HR, and cardiac index was cardiac output divided by body surface area. Ejection fraction was SV divided by the EDV. The difference between the summed end-systolic epicardial and endocardial borders multiplied by section thickness and section factor served as an estimate of wall volume (18). Wall volume was multiplied by the specific density of cardiac muscle (1.05 g/cm^3) to obtain wall mass. Acceleration and deceleration peak values were calculated as the maximal change in ml/s (expressed as ml/s^2) obtained from the velocity encoded MRI acquisitions (13).

^{31}P-MRS. A 100-mm-diameter surface coil was used to acquire ^{31}P-MR spectra of the LV anterior wall. The coil was placed over the precordium and secured with a Velcro strap to minimize respiratory artifacts. Volumes of interest were selected by image-guided spectroscopy using image-selected in vivo spectroscopy (ISIS) based on transverse and

Table 1. Clinical Characteristics of Diabetic and Control Subjects

	Patients (n = 12)	Controls (n = 12)
Age (yrs)	56 ± 6	54 ± 7
Duration of disease (months)	14 (6–60)	NA
BMI (kg/m^2)	25.4 ± 1.6	24.2 ± 1.6
Systolic BP (mm Hg)	135 ± 8	134 ± 9
Diastolic BP (mm Hg)	83 ± 6	79 ± 7
Heart rate (beats/min)	69 ± 10	66 ± 12
Rate pressure product (mm Hg × beats/min × 10^{-2})	93 ± 16	83 ± 19
HbA1c (%)	6.1 ± 1.1†	5.0 ± 0.6
Fasting plasma glucose (mmol/l)	8.2 ± 1.6†	5.5 ± 0.4
Fasting plasma insulin (MU/l)	17.8 ± 9.9*	10.0 ± 3.2
C-peptide (nmol/l)	1.1 ± 0.4†	0.7 ± 0.2
Total cholesterol (mmol/l)	5.4 ± 0.8	5.3 ± 0.9
HDL cholesterol (mmol/l)	1.0 ± 0.3†	1.5 ± 0.3
Triglycerides (mmol/l)	1.8 ± 0.9	1.5 ± 1.2
LDL cholesterol (mmol/l)	3.9 ± 0.7	3.5 ± 0.9

Values are mean ± SD or median (range). *$p < 0.05$; †$p < 0.02$.
BMI = body mass index; BP = blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NA = not applicable.

sagittal spin-echo MR scout images. The entered volume size was typically 6 (caudo-cranial) × 7 × 7 cm^3. Because the predicted maximal effective depth of the surface coil was approximately 9 cm below the coil center, the exact distance in the posterior direction was determined by coil sensitivity. In practice, ^{31}P-MR spectra are mainly obtained from the LV anterior wall. Acquisitions were based on 192 averaged free induction decays, and total acquisition time was 10 min; ^{31}P-MR spectra were transferred to a remote SUN-SPARC work station for analysis according to previously described methods (19–22). Briefly, ^{31}P-MR spectra were quantified automatically in the time domain and were corrected for partial saturation effects and for the adenosine-triphosphate (ATP) contribution from blood in the cardiac chambers as described earlier (21,23). Myocardial HEP metabolism was quantified as phosphocreatine to ATP (PCr/ATP) ratio. The ratio of ATP to 2,3-diphosphoglycerate (2,3-DPG) was 0.36 and was used in the present study to calculate the contribution of blood ATP to the observed ATP signal in cardiac ^{31}P-MR spectra. In addition, depending on the repetition time (TR), PCr/ATP ratios also had to be corrected for partial saturation effects; T1 values of a recent study were used, obtained from inversion recovery experiments on the human left ventricle, and were 4.43 s for PCr, 2.61 s for γ-ATP, 2.51 s for α-ATP, and 2.67 s for β-ATP. Based on these data and a TR of 3.6 s, a saturation correction factor of 1.35 was obtained and applied to all "blood corrected" myocardial PCr/ATP ratios acquired in the present study (19,22,23). Other tissues, such as liver and diaphragm/skeletal muscle were carefully excluded from the volume of interest, and, therefore, did not contribute to the final spectrum. Other technical details were similar as described earlier (19,20).

Statistical analysis. Data are mean ± SD or median (range) if not normally distributed. The normal distribution was tested using the Shapiro-Wilk test. Differences between patients and controls were assessed by unpaired two-tailed Student *t* test. Because only minor differences in clinically relevant variables were found between the two groups, data of patients and controls were pooled to assess correlations between variables by Pearson's correlation test or by univariate regression analyses. A p value < 0.05 was considered statistically significant.

RESULTS

Subject characteristics. Table 1 lists the characteristics of patients and controls. Patients and controls differed with respect to HbA1c, fasting plasma glucose, insulin, C-peptide, and high-density lipoprotein cholesterol concentrations; otherwise they had similar age, BMI, and BP. Three of 12 patients were treated by diet only, three used sulfonylureas, two metformin, and four were treated by sulfonylureas and metformin (data not shown). Median self-reported estimated time spent performing moderate exercise (walking, biking, or swimming) was 60 min (range 30 to 180) per week, and this was comparable in patients and controls (data not shown). No deterioration of glycemic control was observed after one week of discontinuation of antidiabetic medication (mean fasting glucose levels, 8.1 ± 1.7 [before] vs. 8.2 ± 1.6 [after]; p = 0.722).

Myocardial function and metabolism. Left ventricular mass was similar in both groups, as well as indexes of

Table 2. Left Ventricular Dimensions and Systolic Function

Parameters	Patients	Controls	*r* to PCr/ATP
LV mass (g)	120 ± 25	125 ± 27	0.030
LV mass index (g/m^2)	59 ± 11	64 ± 13	0.128
End-diastolic volume (ml)	143 ± 19	149 ± 18	0.125
End-systolic volume (ml)	58 ± 14	58 ± 6	0.040
Stroke volume (ml)	85 ± 15	92 ± 15	0.121
Stroke index (ml/m^2)	42 ± 8	47 ± 7	0.239
Ejection fraction (%)	59 ± 8	61 ± 4	0.024
Cardiac output (l/min)	5.8 ± 1.2	6.0 ± 1.1	−0.254
Cardiac index (l/min/m^2)	2.8 ± 0.6	3.0 ± 0.5	−0.174
End-systolic wall stress (kN/m^2)	46 ± 8	43 ± 7	−0.164

Values are mean ± SD. Statistical analysis showed no differences between patients and controls, no significant correlations.
ATP = adenosine-triphosphate; LV = left ventricular; PCR = phosphocreatine; *r* = Pearson's correlation coefficient.

Table 3. Left Ventricular Diastolic Function

Parameters	Patients	Controls	*r* to PCr/ATP
E/A peak flow	1.07 ± 0.3†	1.40 ± 0.4	0.199
E peak filling rate (ml/s)	394 ± 79†	492 ± 102	+0.504†
E peak filling rate/EDV (s^{-1})	2.7 ± 0.5	3.2 ± 0.6	0.464*
E acceleration peak (ml/s^2) × 10^{-3}	6.4 ± 1.6†	8.2 ± 1.7	+0.497†
E deceleration peak (ml/s^2) × 10^{-3}	−3.1 ± 0.9*	−4.0 ± 1.3	−0.420*
A peak filling rate (ml/s)	382 ± 73	363 ± 76	0.248
A peak filling rate/EDV (s^{-1})	2.7 ± 0.5	2.4 ± 0.5	0.193
A acceleration peak (ml/s^2) × 10^{-3}	7.2 ± 1.6	7.4 ± 2.0	−0.026
A deceleration peak (ml/s^2) × 10^{-3}	−7.3 ± 2.1	−7.1 ± 3.4	−0.045

Values are mean ± SD. Statistical significance is indicated for comparisons between groups or correlations: *$p < 0.05$; †$p < 0.02$.
A = atrial; E = early; EDV = end-diastolic volume. Other abbreviations as in Table 2.

systolic function (Table 2). However, parameters of diastolic function, including early to late diastolic (E/A) peak flow ratio ($p = 0.019$), early peak filling rate ($p = 0.016$), early acceleration peak ($p = 0.010$), and early deceleration peak ($p = 0.05$) were lower in patients (Table 3). On average, in diabetic patients E/A peak flow ratio was 22%, early peak filling rate 24%, early acceleration peak 23%, and early deceleration peak 24% lower than in controls.

In patients, mean PCr/ATP was significantly lower than in controls (Fig. 1, A) (1.47 ± 0.28 vs. 1.88 ± 0.34, $p < 0.01$). Figure 1, B, shows representative ^{31}P-MR spectra of a patient (upper panel) and nondiabetic subject (lower panel). When data from patients and controls were pooled, significant correlations were found between myocardial PCr/ATP and LV diastolic functional parameters (Table 3): early peak filling rate ($r = +0.504$, $p = 0.014$), early acceleration peak ($r = +0.497$, $p = 0.016$), and early deceleration peak ($r = -0.420$, $p = 0.046$). Figure 2 shows the association between myocardial PCr/ATP and indexes of diastolic function in patients and controls. No associations were found between myocardial HEP metabolism and indexes of systolic function or LV mass (Table 2).

To evaluate the mathematical determinants of the observed decrease in myocardial PCr/ATP in patients, PCr and ATP values were calculated separately in arbitrary units. In patients, PCr was 5.8 ± 1.9, as compared with 8.1 ± 2.3 in healthy subjects ($p = 0.02$); ATP in patients was 4.0 ± 1.1, as compared with 4.4 ± 1.3 in healthy subjects ($p >$

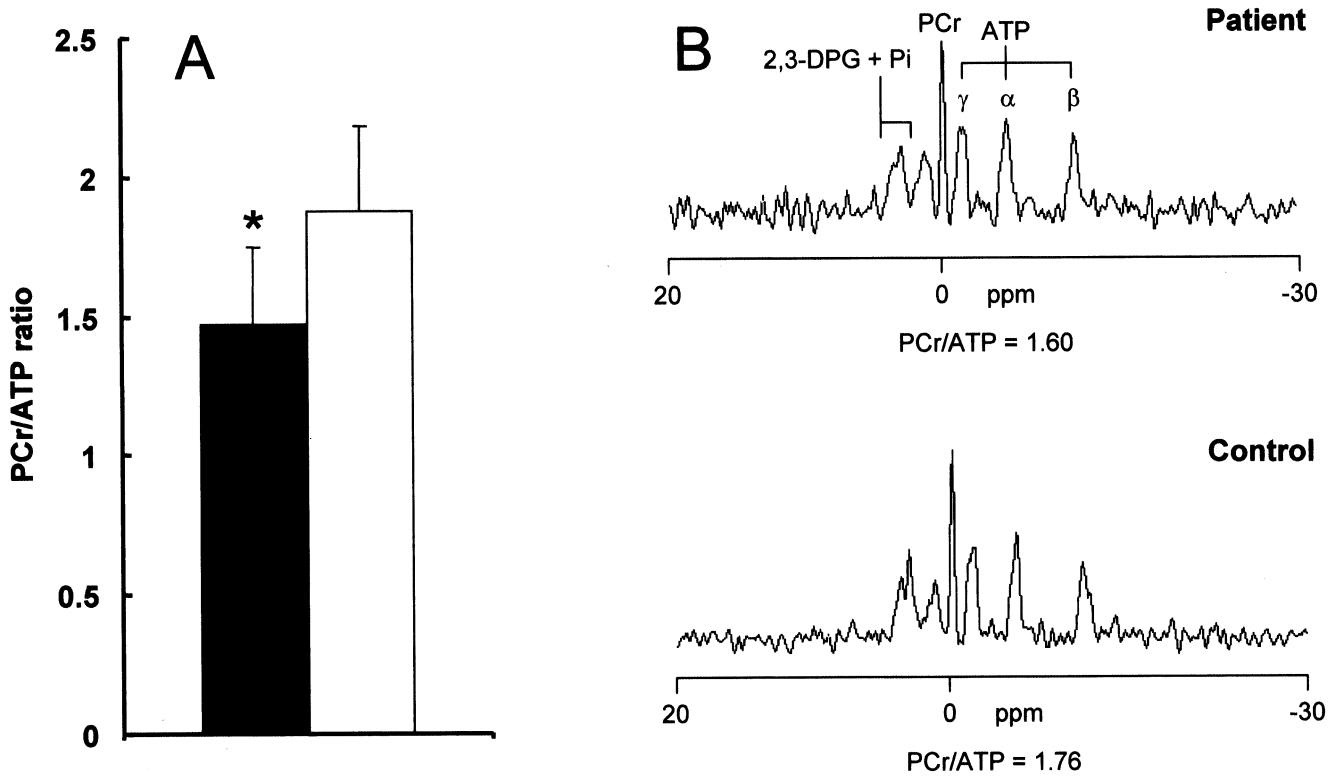

Figure 1. (A) Mean ± SD myocardial phosphocreatine to adenosine-triphosphate (PCr/ATP) ratios in type 2 diabetic patients **(solid bar)** and controls **(open bar)**. *$p < 0.01$. **(B)** Representative phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) obtained at rest from the anterior left ventricular wall of a patient **(upper panel)** and healthy subject **(lower panel).** The peaks of PCr, ATP, and inorganic phosphate (Pi) plus 2,3-diphospho-glycerate (2,3-DPG) are identified in the **upper panel**. Myocardial PCr/ATP ratios, as presented below the ^{31}P-MRS, were corrected for partial saturation effects and blood-ATP contamination, and line broadening of 15 Hz was applied.

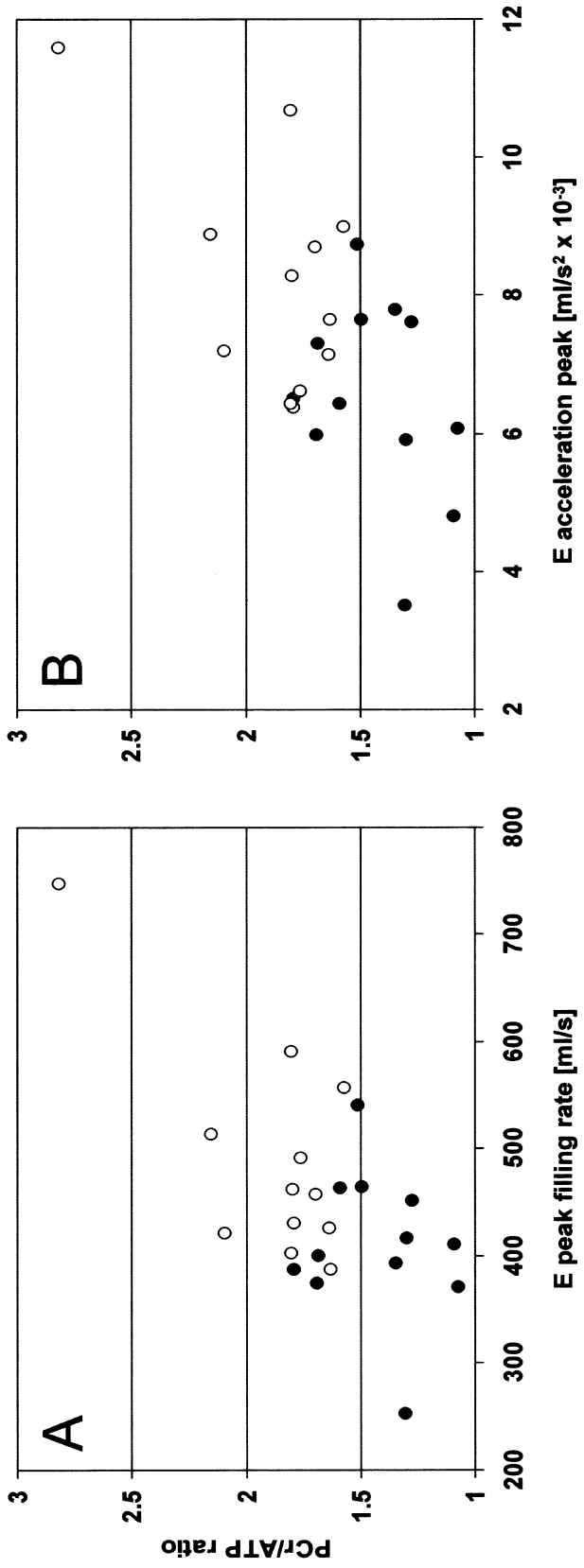

Figure 2. The association between myocardial phosphocreatine/adenosine-triphosphate (PCr/ATP) and E peak filling rate **(A)** and E acceleration peak **(B),** representing indexes of diastolic function, in type 2 diabetic patients **(solid dots)** and healthy controls **(open dots)**. See also Table 3 and text.

0.05). Therefore, the observed decrease in myocardial PCr/ATP in patients may be explained by a decrease in the derived signal for PCr.

When data from patients and controls were pooled, E/A peak flow was negatively associated with age ($r = -0.605$, $p = 0.002$), BMI ($r = -0.468$, $p = 0.021$), and fasting plasma glucose ($r = -0.396$, $p = 0.05$) and positively with high-density lipoprotein cholesterol ($r = +0.419$, $p = 0.041$). Myocardial PCr/ATP was inversely associated with HbA1c ($r = -0.631$, $p = 0.001$), and there was a trend for an association with fasting plasma glucose ($r = -0.399$, $p = 0.06$) and fasting plasma insulin ($r = -0.406$, $p = 0.054$; Table 4). A significant inverse association was found between the level of cardiac workload (rate pressure product) and myocardial PCr/ATP ($r = -0.413$, $p = 0.049$; not shown in Table 4).

DISCUSSION

The present data support earlier findings (4,7) indicating that patients with type 2 diabetes, free of clinical CAD, have (subclinical) LV diastolic dysfunction with normal systolic function. This study indicates that myocardial PCr/ATP is reduced and associated with altered diastolic function in patients with type 2 diabetes, despite good metabolic control and normal BP.

The existence of myocardial dysfunction, both systolic (3) and diastolic (4,6,7), in patients with type 2 diabetes in the absence of CAD has been ascribed to DCM. The pathogenesis of DCM, however, remains unclear, although several mechanisms, including microvascular and metabolic alterations of the diabetic myocardium have been proposed (8). Hypertension is present in up to 70% of type 2 diabetic patients, and poor metabolic control (3,8) may play an important role in the development of myocardial dysfunction. However, LV diastolic dysfunction was also described in normotensive patients with well-controlled type 2 diabetes in the absence of CAD (24). In that study, however, no control group was included, and the echocardiographic measurements were compared with historical reference values. Using MRI, we found altered LV diastolic function in asymptomatic, non-obese and normotensive patients with well-controlled, recently diagnosed type 2 diabetes, as compared with controls who were matched for age, gender, BMI, and BP. In our study, patients had no LV hypertrophy in contrast with previous findings in type 2 diabetic subjects (3,5). Using MRI we found lower LV mass values than those measured by echocardiography, which seems to overestimate LV geometry (25).

The clinical relevance of diastolic dysfunction in asymptomatic patients in terms of prognosis and treatment still needs to be determined. Recently, Poirier et al. (26) found that asymptomatic type 2 diabetic patients with impaired LV diastolic function had lower maximal treadmill performance than patients with normal diastolic function. Because interventions such as aerobic exercise and angiotensin-

Table 4. Univariate Coefficients for Correlation Between Diastolic Function, Myocardial Metabolism, and Metabolic and Cardiovascular Risk Factors in Patients With Type 2 Diabetes and Controls

	E/A Peak Flow	PCr/ATP Ratio
Age	−0.605†	0.063
BMI	−0.468*	−0.158
Systolic BP	0.173	0.048
HbA1c	0.001	−0.631†
Fasting plasma glucose	−0.396*	−0.399
Fasting plasma insulin	−0.182	−0.406
HDL cholesterol	+0.419*	0.398

*$p < 0.05$; †$p < 0.02$.
A = atrial; ATP = adenosine-triphosphate; BMI = body mass index; BP = blood pressure; E = early; HDL = high-density lipoprotein; PCR = phosphocreatine.

converting enzyme inhibition may improve diastolic function (27,28), early detection may have therapeutic implications.

We found significantly lower resting myocardial PCr/ATP in type 2 diabetic patients as compared with controls. Previously, we reported similar findings in nondiabetic patients with hypertension and LV hypertrophy (13). We then suggested that reduced PCr/ATP may be explained by a decrease in creatine kinase (CK) activity and lower total creatine content, accompanied by a switch in myocardial substrate utilization from FFA to glucose, which occurs during exercise-stress, in the hypertrophied myocardium and in ischemic heart disease (13,29). However, myocardial substrate availability and utilization may be also altered due to type 2 diabetes, even in the absence of LV hypertrophy (9,10). In perfused hearts from type 2 diabetic db/db mice, the rate of glycolysis and glucose oxidation was impaired due to reduced content of insulin-sensitive glucose (GLUT4) transporters, whereas palmitate oxidation was increased (30). In obese Zucker Diabetic Fatty (ZDF fa/fa) rats, triglyceride accumulation in the heart was observed (11). These changes were associated with increases of ceramide content, a mediator of apoptosis, and inducible nitric oxide (NO) synthase expression. Clinically, these rats exhibited LV diastolic dysfunction (11); NO was found to inhibit CK and impair contractile reserve in rat hearts (31). Thus, lipotoxicity may be an important mechanism underlying myocardial dysfunction in obesity and diabetes (11).

In humans, the impact of type 2 diabetes on myocardial metabolism is difficult to study, not only because it requires the use of expensive and experimental methods such as positron emission, single-photon emission tomography and MR spectrometry (13,32–36), but also mainly because of the heterogeneity of this patient group with a high prevalence of comorbid conditions such as CAD, hypertension, and the use of multiple drugs, including insulin. As a consequence, conflicting data on myocardial metabolism in type 2 diabetic patients have been reported, including decreased (32) and normal (33) myocardial glucose utilization, impaired (34,35) and normal (36) FFA metabolism, and increased triglyceride synthesis (34). Although ^{31}P-MRS measurements do not link the CK system to specific metabolic pathways in the heart, in theory, three factors may explain the lower PCr/ATP observed in our patients as compared with controls. First, the difference in prevailing metabolic substrates as well as insulin levels may have adversely affected myocardial PCr/ATP. Secondly, triglyceride accumulation in cardiomyocytes may not only impair substrate oxidation but also induce nonoxidative metabolic pathways with subsequent formation of toxic intermediates, thus compromising myocardial energy metabolism. In addition to the mechanisms described in rats (11), another possible mechanisms underlying "lipotoxicity" linking metabolic substrate use to the CK system may be the proposed inhibition of mitochondrial adenine nucleotide translocators by increased intracellular long-chain acyl-CoA esters, resulting in a decreased cytosolic ATP/ADP ratio (37). Finally, subclinical ischemia may decrease PCr/ATP, resulting in reduction of both glucose and FFA oxidation, with glycolysis becoming the dominant source of energy production. This uncoupling of glycolysis from glucose oxidation causes anaerobic hydrolysis of ATP and excessive production of cytosolic protons, resulting in intracellular acidosis (9). Subsequently, these protons need to be exchanged for other cations, leading to intracellular calcium overload. Moreover, ATP is needed to re-establish cation homeostasis, which contributes to a decrease in cardiac energy efficiency during ischemia (38). All these mechanisms, however, need to be confirmed in humans in vivo.

We found an association between myocardial diastolic dysfunction and abnormal HEP metabolism in subjects with type 2 diabetes and normal LV mass. Although altered myocardial metabolism may contribute to the observed functional changes, no causative relation was established in this study. From previous studies in nondiabetic humans with LV hypertrophy, it was suggested that the lower PCr content and the switch in substrate preference from fatty acids to glucose may lead to lower levels of ATP at the sarcomeres, which is not compensated for by increased mitochondrial ATP production (13). Lower cytosolic ATP concentration is associated with impaired calcium sequestration by the sarcoplasmatic reticulum and impaired relaxation of cardiomyocytes and may be responsible for the diastolic dysfunction observed. Similarly, the lower myocardial PCr/ATP in our diabetic patients could mathematically be explained by a decrease in the derived signal for PCr, while ATP levels were not different from controls. Although our patients had no LV hypertrophy, the metabolic changes in diabetic patients leading to altered substrate availability for myocardial energy metabolism may converge on similar cellular mechanisms resulting in diastolic dysfunction. Indeed, in lean patients with type 2 diabetes, a correlation between reduced FFA uptake and LV wall motion abnormalities was observed (35). Also, several reports described the relation between systemic hyperglycemia and LV diastolic dysfunction (6,7). In our study, diastolic

dysfunction was associated with BMI and fasting plasma glucose and low high-density lipoprotein cholesterol levels, that is, components of the insulin resistance syndrome. Clearly, the cellular mechanisms underlying the observed association between altered myocardial HEP metabolism and diastolic functional changes in type 2 diabetic subjects need further research.

Study limitations. The number of subjects evaluated in the present study was limited, mainly due to the demanding combined MR imaging and ^{31}P-MR spectroscopy examinations. However, the sample size was sufficient to show statistically significant differences between healthy controls and patients, and significant correlations between myocardial function and metabolism.

Because we did not perform angiography, the possibility of the presence of CAD in our patients cannot be entirely excluded. However, none of the subjects had evidence of CAD based on clinical history, examination, ECG, or wall motion analysis by MRI (data not shown). Thus, although subclinical atherosclerosis may have been present, CAD is unlikely to be an important confounding variable in explaining the observed alterations in LV diastolic function and metabolism.

Although long-term effects of sulfonylureas on cardiac function and metabolism cannot be ruled out entirely, it is unlikely that these agents have influenced the results. First, patients stopped taking these agents one week before the MR studies, and, second, no differences in functional and metabolic parameters were found between subjects taking drugs and those treated by diet only (data not shown).

Only baseline assessment of circulating metabolic substrates was performed. Therefore, our future studies will assess the impact of prevailing concentrations of metabolic substrates on myocardial energy metabolism and function by performing cardiac MR examinations before and after manipulation of systemic glucose, insulin and/or fatty acids levels.

Finally, diastolic functional parameters are sensitive for loading conditions (i.e., preload and afterload), which were not evaluated in detail, and should be incorporated in future research.

Conclusions. Impaired LV diastolic function in association with reduced myocardial HEP metabolism was observed in patients with well-controlled and uncomplicated type 2 diabetes. These results may contribute to the understanding of the pathophysiology and natural course of diabetic cardiomyopathy. Future studies are needed to further explore the determinants of myocardial function and metabolism and to establish the implications of these findings in this high-risk population.

Reprint requests and correspondence: Dr. Michaela Diamant, Department of Endocrinology/Diabetes Center, VU University Medical Center, De Boelelaan 1117, P.O. Box 7057, 1007 MB Amsterdam, the Netherlands. E-mail: m.diamant@vumc.nl.

REFERENCES

1. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors and 12-year CVD mortality for men screened in the Multiple Risk Factor Intervention trial. Diabetes Care 1993;16:434–44.
2. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. Am J Cardiol 1974;34:29–34.
3. Vanninen E, Mustonen J, Vainio P, Lansimies E, Uusitupa M. LV function and dimensions in newly diagnosed NIDDM. Am J Cardiol 1992;70:371–8.
4. Di Bonito P, Cuomo S, Moio N, et al. Diastolic dysfunction in patients with NIDDM of short duration. Diabet Med 1996;13:321–4.
5. Felicio JS, Ferreira SRG, Plavnik FL, et al. Effect of blood glucose on LV mass in patients with hypertension and type 2 diabetes mellitus. Am J Hypertens 2000;13:1149–54.
6. Sánchez-Barriga JJ, Rangel A, Castañeda R, et al. LV diastolic dysfunction secondary to hyperglycemia in patients with type 2 diabetes. Arch Med Res 2001;32:44–7.
7. Beljic T, Miric M. Improved metabolic control does not reverse LV filling abnormalities in newly diagnosed NIDDM patients. Acta Diabetol 1994;31:147–50.
8. Bell DSH. Diabetic cardiomyopathy: a unique entity or a complication of coronary artery disease. Diabetes Care 1995;18:708–14.
9. Stanley WC, Lopaschuk GD, McCormack JG. Regulation of the energy substrate metabolism in the diabetic heart. Cardiovasc Res 1997;34:25–34.
10. Rodrigues B, Cam MC, McNeill JH. Metabolic disturbances in diabetic cardiomyopathy. Mol Cell Biochem 1998;180:53–7.
11. Zhou YT, Grayburn P, Karim A, et al. Lipotoxic heart disease in obese rats: implications for human obesity. Proc Natl Acad Sci USA 2000;97:1784–9.
12. Lopaschuk GD, Belke DD, Gamble J, Itoi T, Schonekess BO. Regulation of FFA oxidation in the mammalian heart in health and disease. Biochim Biophys Acta 1994;1213:263–76.
13. Lamb HJ, Beyerbracht HP, Van der Laarse A, et al. Diastolic dysfunction in hypertensive heart disease is associated with altered myocardial metabolism. Circulation 1999;99:2261–7.
14. WHO Expert Committee on Diabetes Mellitus. Second report. WHO Tech Rep Ser 1980;646:1–80.
15. Ewing D, Clarke B. Diagnosis and management of diabetic autonomic neuropathy. BMJ 1982;285:916.
16. Lamb HJ, Doornbos J, Vander Velde EA, Kruit MC, Reiber JH, De Roos A. Echo planar MRI of the heart on a standard system: validation of measurements of LV function and mass. J Comput Assist Tomogr 1996;20:942–9.
17. Hartiala JJ, Mostbeck GH, Foster E, et al. Velocity-encoded cine MRI in the evaluation of LV diastolic function: measurement of mitral valve and pulmonary vein flow velocities and flow volume across the mitral valve. Am Heart J 1993;125:1054–66.
18. Pattynama PMT, Lamb HJ, Van der Velde EA, Van der Geest RJ, Van der Wall EE, De Roos A. LV measurements with cine and spin-echo MR imaging: a study of reproducibility with variance component analysis. Radiology 1993;187:261–8.
19. Lamb HJ, Doornbos J, Den Hollander JA, et al. Reproducibility of human cardiac ^{31}P-NMR spectroscopy. NMR Biomed 1996;9:217–27.
20. Lamb HJ, Beyerbracht HP, Ouwerkerk R, et al. Metabolic response of normal human myocardium to high-dose atropine-dobutamine stress studied by ^{31}P-MRS. Circulation 1997;96:2969–77.
21. Van der Veen JWC, de Beer R, Luyten PR, Van Ormondt D. Accurate quantification of in vivo 31-P NMR signals using the variable projection method and prior knowledge. Magn Reson Med 1988;6:92–8.
22. De Roos A, Doornbos J, Luyten PR, Oosterwaal LJ, Van der Wall EE, Den Hollander JA. Cardiac metabolism in patients with dilated and hypertrophic cardiomyopathy: assessment with proton-decoupled P-31 MR spectroscopy. J Magn Reson Imaging 1992;2:711–9.
23. Bottomley PA, Hardy CJ, Weiss RG. Correcting human heart 31P-NMR spectra for partial saturation: evidence that saturation factors for PCr/ATP are homogeneous in normal and disease states. J Magn Reson 1991;95:341–55.
24. Poirier P, Bogaty P, Garneau C, Marois L, Dumesnil JG. Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes:

importance of maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. Diabetes Care 2001;24:5–10.
25. Missouris CG, Forbat SM, Singer DRJ, Markandu ND, Underwood R, MacGregor GA. Echocardiography overestimates LV mass: a comparative study with MRI in patients with hypertension. J Hypertens 1996;14:1005–10.
26. Poirier P, Garneau C, Bogaty P, et al. Impact of LV diastolic dysfunction on maximal treadmill performance in normotensive subjects with well-controlled type 2 DM. Am J Cardiol 2000;85:473–7.
27. Forman DE, Manning WJ, Hauser R, Gervino GV, Evans WJ, Wei JY. Enhanced LV diastolic filling associated with long-term endurance training. J Gerontol 1992;47:M56–8.
28. Hoffmann U, Globits S, Stefenelli T, Loewe C, Kostner K, Frank H. The effects of ACE inhibitor therapy on LV myocardial mass and diastolic filling in previously untreated hypertensive patients: a cine MRI study. J Magn Reson Imaging 2001;14:16–22.
29. Ingwall S, Kramer MF, Fifer MA, et al. The creatine kinase system in normal and diseased human myocardium. N Engl J Med 1985;313: 1050–4.
30. Belke DD, Larsen TS, Gibbs EM, Severson DL. Altered metabolism causes cardiac dysfunction in perfused hearts from diabetic (db/db) mice. Am J Physiol Endocrinol Metab 2000;279:E1104–13.
31. Gross WL, Bak MI, Ingwall JS, et al. NO inhibits creatine kinase and regulates rat heart contractile reserve. Proc Natl Acad Sci USA 1996;93:5604–9.
32. Ohtake T, Yokoyama I, Watanabe T, et al. Myocardial glucose metabolism in NIDDM patients evaluated by FDG-PET. J Nucl Med 1995;36:456–63.
33. Utriainen T, Takala T, Luotolahti M, et al. Insulin resistance characterizes glucose uptake in skeletal muscle but not in the heart in NIDDM. Diabetologia 1998;41:555–9.
34. Kuikka JT, Mustonen JN, Uusitupa MIJ, et al. Demonstration of disturbed FFA metabolism of myocardium in patients with NIDDM as measured with iodine-123-heptadecanoic acid. Eur J Nucl Med 1991;18:475–81.
35. Sakamoto K, Yamasaki Y, Nanto S, et al. Mechanism of impaired LV wall motion in the diabetic heart without coronary artery disease. Diabetes Care 1998;21:2123–8.
36. Knuuti J, Takala TO, Nagren K, et al. Myocardial fatty acid oxidation in patients with impaired glucose tolerance. Diabetologia 2001;44: 184–7.
37. Bakker SJL, Ijzerman RG, Teerlink T, Westerhoff HV, Gans RO, Heine RJ. Cytosolic triglycerides and oxidative stress in central obesity: the missing link between excessive atherosclerosis, endothelial dysfunction, and β-cell failure? Atherosclerosis 2000;148:17–21.
38. Liu B, Clanachan AS, Schulz R, Lopaschuk GD. Cardiac efficiency is improved after ischemia by altering both the source and fate of protons. Circ Res 1996;79:940–8.