Creatine kinase kinetics in diabetic cardiomyopathy

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Matsumoto, Yuji, Masanori Kaneko, Akira Kobayashi, Yutaka Fujise, and Noboru Yamazaki. Creatine kinase kinetics in diabetic cardiomyopathy. *Am. J. Physiol.* 268 (*Endocrinol. Metab.* 31): E1070–E1076, 1995.—One feature of the diabetic cardiomyopathy is the appearance of contractile dysfunction as the workload increases. We hypothesized that this resulted from an impaired creatine kinase/phosphocreatine system and therefore examined the creatine kinase kinetics at both low and high workloads. Creatine kinase flux (by ^{31}P nuclear magnetic resonance saturation transfer method), cardiac performance, and oxygen consumption were measured in control and streptozotocin-induced diabetic rat hearts. Creatine kinase flux was inhibited by iodoacetamide in control hearts to confirm the role of the creatine kinase/phosphocreatine system in cardiac performance. In diabetic hearts, *1*) the contractile dysfunction became apparent only at high workloads, *2*) the ATP synthesis rate was not significantly different from control hearts, *3*) the creatine kinase flux was reduced by 30.8% (257.5 ± 7.7 μmol·g wet $wt^{-1}\cdot min^{-1}$ in control vs. 178.3 ± 9.4 in diabetes, $P < 0.001$), and *4*) the creatine kinase flux did not increase as the workload increased. In control hearts, *5*) iodoacetamide inhibited the creatine kinase flux to the same degree as that in diabetic hearts, and *6*) the contractile dysfunction was not as severe as that observed in diabetic hearts. These results suggest that the impaired creatine kinase/phosphocreatine system is, at least in part, responsible for the contractile dysfunction in the diabetic cardiomyopathy.

phosphorus nuclear magnetic resonance; saturation transfer; iodoacetamide; diabetes mellitus; phosphocreatine

CARDIAC DYSFUNCTION DURING diabetes is characterized by diminished shortening speed and relaxation rate (4, 5, 20). Cardiac output is also decreased at high filling pressures, whereas it remains normal at lower filling pressures in isolated perfused hearts (5, 20). Many previous reports have attributed this contractile dysfunction to defects in the performance of several subcellular organelles in the streptozotocin (STZ)-induced diabetic rat heart. These include: *1*) mitochondrial dysfunction (17, 22, 27), *2*) a decrease in the V_1 isoenzyme content and Ca^{2+}-adenosinetriphosphatase (ATPase) activity of cardiac myosin (3), *3*) an inhibition of sarcoplasmic reticular (SR) Ca^{2+}-ATPase (19), and *4*) a reduction in the activity of sarcolemmal membrane-bound enzymes and exchangers such as Na^+-K^+-ATPase (21), Ca^{2+}-ATPase (7), Na^+-Ca^{2+} exchange (16), and Na^+-H^+ exchange (23). Although the diminished myosin V_1 isoenzyme and SR Ca^{2+}-ATPase activity could result in a decreased shortening speed and relaxation rate, respectively (3, 19), little is known about the mechanism by which the cardiac output is reduced only at high filling pressures.

In mammalian hearts, creatine kinase (CK) is functionally coupled with adenine nucleotide translocase and various ATPases (29) in mitochondria, myofibrils, SR, and sarcolemmal membrane. The CK/phosphocreatine (PCr) system accelerates the transport of high-energy phosphates between sites of ATP production and utilization. Mitochondrial CK (CK_m) isoenzyme catalyzes the transfer of γ-phosphate of ATP, synthesized by mitochondrial oxidative phosphorylation, to creatine (Cr) in the mitochondrial intermembrane space (reverse CK reaction). Subsequently, PCr diffuses out of mitochondria to the energy utilization sites such as myofibrils, SR, and sarcolemma, and then ATP is regenerated via CK at each utilization site (forward CK reaction). Diffusion of Cr back to the mitochondria closes the cycle (29). This pathway is known as the PCr shuttle. Thus the CK/PCr system plays an important role in energy supply and utilization in cardiac contraction. The inhibition of CK activity by iodoacetamide (IAAm) has been shown to depress peak systolic pressure in rat hearts, especially at higher workloads (6). Furthermore, rat heart depleted of Cr by a diet containing a Cr analogue resulted in more pronounced reductions of cardiac output and peak systolic pressure as the afterload increased (12). Therefore, because CK activity appears to play an important role in cardiac function at higher workloads, and the diabetic heart exhibits dysfunction at higher workloads, it was reasonable to hypothesize that defects in CK activity may be involved in the contractile dysfunction during diabetes. Savabi and Kirsch (26) have demonstrated that total CK activity, CK-MM, and CK_m isoenzyme activities were decreased in diabetic rat hearts. Popovich et al. (24) also showed that mRNA levels of CK were reduced consistently with the decrease in CK activity. However, a direct relationship between CK kinetics and cardiac performance was never proven in these experiments. Saturation transfer method with phosphorus nuclear magnetic resonance (^{31}P NMR) has now made it possible to measure CK flux (unidirectional CK reaction rate) and cardiac function simultaneously in the same heart. This study was therefore undertaken to clarify the role of the CK/PCr system in cardiac performance of diabetic hearts using the ^{31}P NMR saturation transfer method.

MATERIALS AND METHODS

Animal preparation and heart perfusion. Male Sprague-Dawley rats weighing ~350 g were used in this experiment. Diabetes was induced by a single intravenous injection of 0.1 mM citrate-buffered STZ (pH 4.5) at a dosage of 45 mg/kg body wt, as reported previously (18). Nondiabetic normal rats that matched in heart size to diabetic rats were used as the control group so that each heart could occupy a constant position inside the sample tube. This precaution has made it

possible to quantitate the content of phosphate metabolites more accurately by ^{31}P NMR.

After STZ injection (8 wk), rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and then were injected intravenously with 400 units of sodium heparin. The hearts were removed and perfused by the Langendorff method. The perfusion medium was a modified Krebs-Henseleit buffer at pH 7.4 supplemented with glucose and pyruvate (in mM): 108 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 0.5 EDTA, 25 $NaHCO_3$, 10 glucose, and 10 sodium pyruvate. The hearts from both control and diabetic animals were perfused with the same perfusion medium. The buffer was continuously bubbled with 95% O_2-5% CO_2. The heart was perfused at a constant temperature of 37°C and a constant pressure of 100 cmH_2O.

Measurement of cardiac performance. A water-filled latex balloon was inserted into the left ventricle through the left atrium via the mitral valve. The balloon was connected to a pressure transducer (Nihon Koden) via a small-bore polyethylene tube for the continuous measurement of the left ventricular pressure. The heart was electrically paced with a definite frequency through a 3 M KCl-agar salt bridge. We evaluated cardiac performance in terms of rate of pressure rise (contraction; $+dP/dt$), rate of pressure decline (relaxation; $-dP/dt$) and left ventricular developed pressure (LVDP). The rate-pressure product (RPP) was calculated as a product of LVDP and heart rate.

Determination of ATP synthesis rate. The oxygen tension in the perfusate before the heart (at the aortic cannula; Pa_{O_2}) and that in the coronary effluent (at the cannula inserted into the pulmonary artery; Pv_{O_2}) were measured polarographically with two pairs of monopolar needle-shaped electrodes (Intermedical) fixed in the perfusion line. Oxygen consumption rate ($M\dot{V}O_2$) was calculated from these oxygen tensions and the coronary flow ($\dot{Q}$) according to the following formula: $M\dot{V}O_2 = (Pa_{O_2} - Pv_{O_2}) \cdot \dot{Q} \cdot K/HW$, where K is a oxygen solubility constant at 37°C (10^3 K = 1.416), and HW is the heart wet weight. ATP synthesis rate (V_{ATP}) was calculated from $M\dot{V}O_2$ by assuming that the ADP-to-oxygen ratio was 3. We determined the V_{ATP} of diabetic heart with the same calculation procedure as that of control heart. The validity of the V_{ATP} value is supported by the following findings. *1*) In normoxic perfusion, >93% of ATP synthesis depends on the oxidative phosphorylation in mitochondria, and the remainder depends on cytosolic glycolysis (14). Using a perfusion medium that contains pyruvate in large quantities, we further reduced the contribution of glycolysis to ATP synthesis because, in contrast to glucose, pyruvate can bypass the regulatory steps of glycolysis and enter the tricarboxylic acid cycle (30). Thus the glycolytically synthesized ATP is minimal not only in diabetic hearts but also in control hearts. *2*) The ADP-to-oxygen ratio was unchanged in the isolated mitochondria from diabetic hearts (15, 17, 22). *3*) The adenine nucleotide translocase activity, which catalyzes the translocation between ATP in a mitochondrial matrix and ADP in a mitochondrial intermembrane space, was maintained in diabetic myocardium (17).

^{31}P NMR measurement. The heart was placed in a sample tube (17 mm in diameter) that was mounted by a single-turn Helmholz coil. We used a JEOL GSX270 wide-bore NMR spectrometer (6.35 tesla; Nihon Denshi) and operated at 109.16 MHz. The intracellular contents of PCr, ATP, P_i, and sugar phosphate were determined from their respective peak areas compared with the external standard peak area of 1 M hexamethylphosphoric triamide, which was enclosed in a capillary tube and fixed inside the sample tube. To simplify the analysis and obtain truly quantitative data, we acquired the fully relaxed spectra (8). The intracellular pH was calculated from the difference of chemical shifts between P_i and PCr peaks.

The ^{31}P NMR saturation transfer technique was utilized to measure the unidirectional CK forward flux (from PCr to ATP). The theoretical principles underlying this method have been described previously (1, 2, 28). Briefly, the following equations were used to determine the CK flux

$$M^+/M^0 = (1 + \kappa T_1)^{-1} \quad (1)$$

$$1/\tau_1 = 1/T_1 + \kappa \quad (2)$$

$$\text{CK flux} = \kappa \cdot [\text{PCr}] \quad (3)$$

where M^+ is the magnetization intensity of PCr resonance in the presence of a selective irradiation of the γ-phosphate of ATP and M^0 is that in the absence of a selective irradiation. T_1 is the intrinsic longitudinal relaxation time (in the absence of chemical change), and τ_1 is the apparent longitudinal relaxation time which can be determined with an inversion recovery experiment. From *Eqs. 1* and *2*, we could obtain the pseudo-first-order rate constant (κ). Subsequently, we could determine the unidirectional forward CK flux from *Eq. 3*. If the CK reaction is in equilibrium, the forward and reverse fluxes of CK should be equal. However, the reverse flux may be underestimated when the conventional saturation transfer technique is utilized (28). Therefore, we decided to measure only forward flux as an index of CK kinetics.

Experimental protocol. We divided the animals into the following four groups: *1*) lower workload/control group (n = 7), *2*) lower workload/diabetic group (n = 7), *3*) higher workload/control group (n = 6), and *4*) higher workload/diabetic group (n = 6). Each workload was defined in terms of pacing frequency and left ventricular end-diastolic pressure (LVEDP) as follows: lower workload was defined as 300 beats/min pacing and 0–4 mmHg LVEDP; higher workload, 390 beats/min pacing and 8–12 mmHg LVEDP. We cauterized the sinus node and destroyed its automaticity to perform the optimal pacing. The experimental schedule was as follows. After an equilibrium period (20 min), we initially acquired the fully relaxed control spectrum using a 90° pulse with a 12-s repetition time (64 transients). Subsequently, we accumulated the spectrum with a selective irradiation of the γ-phosphate of ATP in an identical manner. In the middle of this data collection, we determined cardiac function (LVDP and $\pm dP/dt$) and $M\dot{V}O_2$ simultaneously with the predictable CK flux. To obtain the τ_1 value, we further performed an inversion recovery experiment in the presence of a selective irradiation of γ-phosphate of ATP, using a 90° observation pulse with a variable time delay (100, 250, 400, 550, and 6,000 ms; see Ref. 2). At the end of the experiment, we obtained the fully relaxed spectrum again to evaluate the metabolic deterioration and excluded the hearts in which the PCr or ATP concentration was decreased by >10%. The hearts that had a >10% decline in LVDP during the experiment were also excluded from this study. Two hearts (13.3%) in the control group and three hearts (18.8%) in the diabetic group were excluded for the metabolic reason. One heart was excluded for the hemodynamic reason in each group.

Biochemical analysis of total CK activity. According to methods reported previously (24), the biochemical assay of total CK activity was performed in a separate experiment using diabetic rats (n = 5), size-matched control rats (n = 5), and age-matched control rats (n = 5). CK activity was expressed as international units per gram wet weight (IU/g wet wt).

Inhibition of CK flux with IAAm. It is well known that IAAm inhibits sulfhydryl enzymes irreversibly. We exposed a heart to

Table 1. *General features of experimental animals*

	Control	Diabetes
Body wt, g	348 ± 10	319 ± 8*
Heart wt, mg	1,167 ± 23	1,124 ± 30
Heart wt/body wt, mg/g	3.37 ± 0.07	3.53 ± 0.06*
Blood glucose, mM	5.6 ± 0.4	19.8 ± 0.5*
Total CK activity, IU/g wet wt	1,313 ± 24	917 ± 87*

Values are means ± SE; n = 13 rat hearts in each group except data of total creatine kinase (CK) activity in separate experiment (n = 5 in each group). *$P < 0.05$ compared with control group.

the IAAm-containing perfusate at a defined concentration for a predetermined duration, as described elsewhere (6). Ultimately, the treatment with IAAm was performed in the following two ways: lower-dosage IAAm treatment (0.05 mM IAAm, 5 min exposure, n = 5) and higher-dosage IAAm treatment (0.1 mM IAAm, 10 min exposure, n = 5). The control hearts were used in this experiment and were perfused at higher workloads. Before and after the treatment (washout time was 20 min), we determined the intracellular concentration of phosphate metabolites, pH, CK flux, and cardiac performance.

Statistical analysis. All data are presented as means ± SE. Student's t-test was used for statistical comparison. The relation between two factors was tested using linear regression analysis. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

General features of the experimental animals. General characteristics of the experimental rats are summarized in Table 1. Diabetes was confirmed in the experimental animals by the presence of elevated blood glucose, reported previously (18). Because we adjusted the heart size in both control and diabetic animals, the age of the animals was different in both groups. In the control group, the age of the animals was 9–10 wk after birth, whereas the age of the animals was 18 wk in the diabetic group. Although the heart weight was similar in these two groups, the body weight was significantly smaller, and the heart weight-to-body weight ratio was significantly larger in the diabetic group. Myocardial total CK activity measured by a biochemical assay was significantly decreased in diabetic rats as expected. We examined the total CK activity of age-matched control rats as well as size-matched control rats to exclude the possibility that the aging influenced the CK activity. The total CK activity of age-matched control rats (1,500 ± 32 IU/g wet wt) was not significantly different from size-matched control rats. Therefore, the decrease in total CK activity in diabetic animals could not be due to the aging.

Effects of diabetes on cardiac performance. Cardiac performance of control and diabetic hearts was assessed in terms of LVDP and ±dP/dt (Table 2). At lower workloads, LVDP and +dP/dt were statistically similar between both groups, but −dP/dt was significantly decreased in diabetic hearts. On the other hand, not only −dP/dt but also LVDP and +dP/dt were strikingly decreased in diabetic hearts at higher workloads. These changes in cardiac performance at higher workloads were in agreement with previous reports (5, 20).

Effects of diabetes on the phosphate metabolites and intracellular pH. The concentrations of ATP, PCr, and P_i were not significantly different between control and diabetic groups at either workload (Table 2). Intracellular pH was also similar in both groups.

Effects of diabetes on the relationships among ATP synthesis, CK kinetics, and cardiac work. At lower workloads, the V_{ATP} in diabetic hearts was similar to that in control hearts [37.5 ± 1.3 μmol·g wet wt^{-1}·min^{-1} in control vs. 36.4 ± 1.4 in diabetes, P = not significant (NS); Fig. 1*A*]. The CK flux was significantly decreased by 22.8% in diabetic hearts (205.8 ± 9.6 μmol·g wet wt^{-1}·min^{-1} in control vs. 158.8 ± 5.8 in diabetes, $P < 0.01$; Fig. 1*B*), whereas the RPP was preserved in diabetic hearts (29.0 ± 1.2 × 10^3 mmHg/min in control vs. 26.4 ± 0.7 in diabetes, P = NS; Fig. 1*C*). At higher workloads, the CK flux was further decreased by 30.8% in diabetic hearts (257.5 ± 7.7 μmol·g wet wt^{-1}·min^{-1} in control vs. 178.3 ± 9.4 in diabetes, $P < 0.001$), and the RPP in diabetic hearts was much lower than that in control hearts (29.3 ± 1.1 × 10^3 mmHg/min in control vs. 17.8 ± 0.3 in diabetes, $P < 0.001$). However, the V_{ATP} in diabetic hearts was not significantly different from that in control hearts at this time (41.0 ± 1.1 μmol·g wet wt^{-1}·min^{-1} in control vs. 39.6 ± 2.4 in diabetes, P = NS).

The CK flux of control hearts was significantly increased at higher workloads (205.8 ± 9.6 μmol·g wet wt^{-1}·min^{-1} at lower workloads vs. 257.5 ± 7.7 at higher

Table 2. *Effects of diabetes on cardiac performance, phosphate metabolites, and intracellular pH at two different workloads*

	Lower Workload		Higher Workload	
	Control (n = 7)	Diabetes (n = 7)	Control (n = 6)	Diabetes (n = 6)
Cardiac performance				
LVDP, mmHg	96.7 ± 4.2	87.9 ± 2.4	75.3 ± 2.4	46.2 ± 0.7*
+dP/dt, mmHg/s	2,057 ± 69	1,914 ± 46	1,917 ± 40	1,100 ± 45*
−dP/dt, mmHg/s	−1,614 ± 94	−1,300 ± 44*	−1,400 ± 73	−733 ± 33*
Phosphate metabolites				
[PCr], μmol/g wet wt	8.81 ± 0.27	8.67 ± 0.26	9.47 ± 0.34	8.72 ± 0.27
[ATP], μmol/g wet wt	4.87 ± 0.24	4.96 ± 0.25	4.78 ± 0.25	4.37 ± 0.27
[P_i], μmol/g wet wt	2.73 ± 0.26	2.65 ± 0.34	2.26 ± 0.29	2.05 ± 0.29
Intracellular pH	7.22 ± 0.04	7.19 ± 0.02	7.21 ± 0.03	7.19 ± 0.03

Values are means ± SE; n, no. of rat hearts. LVDP, left ventricular developed pressure; +dP/dt, contraction rate; −dP/dt, relaxation rate; PCr, phosphocreatine. Brackets denote concentration. *$P < 0.05$ compared with control group.

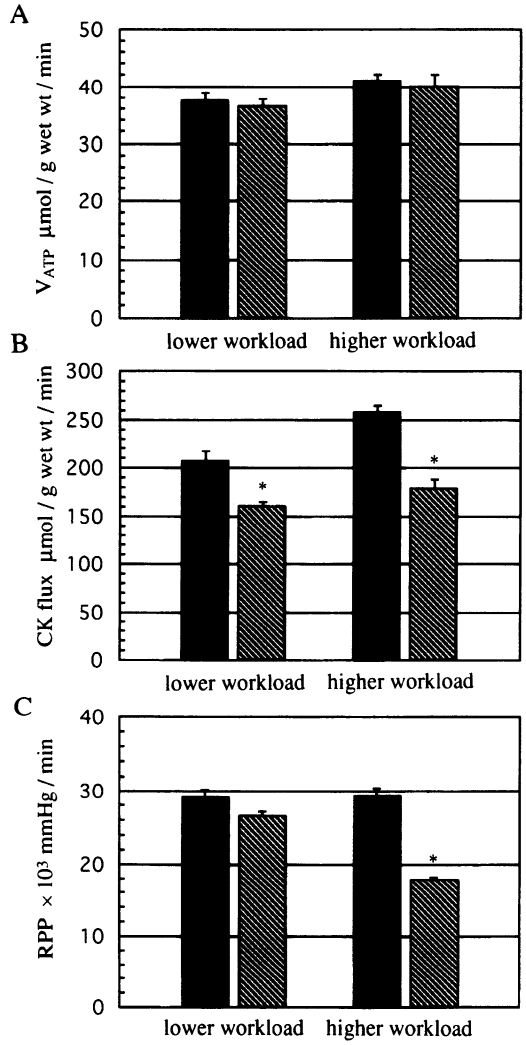

Fig. 1. Bar graph showing effects of diabetes on ATP synthesis rate (V_{ATP}; *A*), creatine kinase (CK) flux (*B*), and rate-pressure product (RPP; *C*). Filled bar, control group (n = 7 rat hearts at lower workload, n = 6 at higher workload); hatched bar, diabetic group (n = 7 at lower workload, n = 6 at higher workload). *$P < 0.05$ compared with data of control group.

workloads, $P < 0.01$), whereas the CK flux was unchanged in diabetic hearts (158.8 ± 5.8 μmol·g wet $wt^{-1}\cdot min^{-1}$ at lower workloads vs. 178.3 ± 9.4 at higher workloads, P = NS; Fig. 1*B*). We also evaluated the relationship between V_{ATP} and CK flux in control and diabetic hearts (Fig. 2). In control hearts, the CK flux showed a good linear correlation to V_{ATP} ($r = 0.68$, $P < 0.05$; Fig. 2*A*). However, there was no significant correlation between V_{ATP} and CK flux in diabetic hearts ($r = 0.04$, P = NS; Fig. 2*B*).

Effects of IAAm on CK kinetics and cardiac performance. To estimate the role of CK kinetics in cardiac performance, we examined the effects of a CK inhibitor (IAAm) on cardiac performance in control hearts. The concentration of ATP was not significantly changed after the lower-dosage IAAm treatment; however, it was significantly decreased after the higher-dosage IAAm treatment (Table 3). The concentrations of PCr, P_i, and sugar phosphate and intracellular pH were not significantly changed after either IAAm treatment.

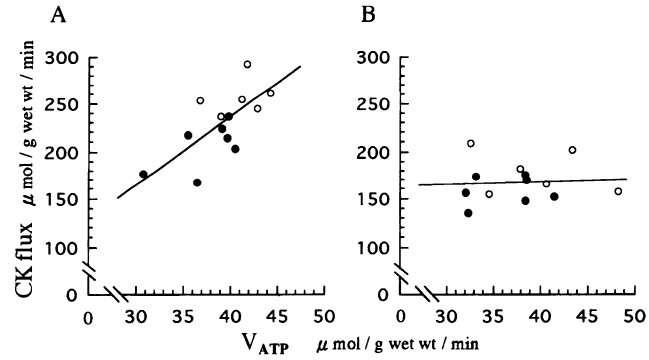

Fig. 2. Scatter diagram showing correlation between V_{ATP} and CK flux in control group (*A*) and diabetic group (*B*). Open circles, higher workload group; closed circles, lower workload group. Straight line was calculated by linear regression analysis (*A*: $r = 0.68$, $P < 0.05$; *B*: $r = 0.04$, P = not significant).

The lower-dosage IAAm treatment inhibited CK flux from 236.1 ± 9.6 μmol·g wet $wt^{-1}\cdot min^{-1}$ to 171.2 ± 9.0 ($P < 0.01$; 27.4% decrease of CK flux). The CK flux was further inhibited by the higher-dosage IAAm treatment (224.3 ± 11.4 μmol·g wet $wt^{-1}\cdot min^{-1}$ before treatment vs. 94.5 ± 10.4 after treatment, $P < 0.001$; 58.2% decrease of CK flux). The percent decreases of RPP after the lower- and higher-dosage IAAm treatments were 7.0% (27.6 ± 0.5 × 10^3 mmHg/min before treatment vs. 25.6 ± 0.8 after treatment, $P < 0.01$) and 13.9% (30.7 ± 1.0 × 10^3 mmHg/min before treatment vs. 26.4 ± 0.7 after treatment, $P < 0.01$), respectively. Figure 3 depicts the relationship between the percent decreases of CK flux and RPP. There was a good linear correlation between these two factors ($r = 0.73$, $P < 0.05$).

DISCUSSION

It has been reported that the CK/PCr system plays an important role in cardiac contraction and relaxation (29). Kapelko et al. (12, 13) and Zweier and Jacobus (31) have reported that the CK flux was inhibited by a reduction of intracellular Cr content using β-guanidinopropionic acid (β-GPA) and β-guanidinobutyric acid (β-GBA). They also showed that the contractile function of the myocardium was depressed by β-GPA and β-GBA at higher workloads as well. Furthermore, Fossel and Hoefeler (6) showed that the complete inhibition of CK activity by IAAm in rat hearts failed to maintain the control values of peak systolic pressure and LVEDP as

Table 3. *Effects of IAAm on phosphate metabolites and intracellular pH at two different dosage treatments*

	Lower-Dosage IAAm		Higher-Dosage IAAm	
	Before (n = 5)	After (n = 5)	Before (n = 5)	After (n = 5)
Phosphate metabolites, μmol/g wet wt				
[PCr]	9.94 ± 0.18	9.57 ± 0.26	9.45 ± 0.28	8.81 ± 0.29
[ATP]	5.21 ± 0.25	5.18 ± 0.22	5.04 ± 0.29	4.02 ± 0.13*
[P_i]	2.84 ± 0.29	2.60 ± 0.49	2.82 ± 0.18	2.56 ± 0.25
[SP]	2.58 ± 0.21	2.77 ± 0.21	2.58 ± 0.17	3.09 ± 0.28
Intracellular pH	7.19 ± 0.02	7.19 ± 0.03	7.19 ± 0.02	7.23 ± 0.03

Values are means ± SE; n, no. of rat hearts; IAAm, iodoacetamide; SP, sugar phosphate. *$P < 0.05$ compared with data before treatment.

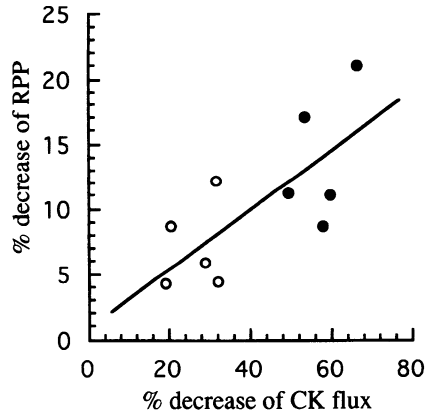

Fig. 3. Scatter diagram showing correlation between percent decrease of CK flux and that of RPP. Open circles, data from control rats treated by lower dosage of iodoacetamide (IAAm); closed circles, data from control rats treated by higher dosage of IAAm. Straight line was calculated by linear regression analysis ($r = 0.73$, $P < 0.05$).

the workload increased. Ingwall et al. (9) have also observed a positive correlation between the total CK activity and the ejection fraction in human diseased hearts. These findings suggested that a suppression of the CK/PCr system might lead to cardiac dysfunction, especially at higher workloads.

One feature of the cardiac dysfunction exhibited by the diabetic cardiomyopathy is the appearance of contractile dysfunction as the workload increases (5, 20). This change in contractile function is very similar to that in a heart which has had its CK/PCr system inhibited (6, 12, 13, 31). Therefore, we studied the CK/PCr system in the diabetic cardiomyopathy at both low and high workloads. We observed that the contractile dysfunction was apparent only at higher workloads in diabetic hearts. This finding was in agreement with previous reports (5, 20). The CK flux was significantly decreased in diabetic hearts at both workloads. Although the CK flux in diabetic hearts was significantly smaller than that in control hearts at lower workloads, the RPP was not different between these two groups. These findings may be as a result of the lesser importance of the CK/PCr system at lower workloads (6, 12, 13, 31). At higher workloads, the CK flux was significantly increased in control hearts, whereas it was unchanged in diabetic hearts. This may have been due to the absence of an increase in CK flux that should have occurred as the workload increased. Bittl and Ingwall (1) showed that CK flux was closely coupled to the rate of ATP synthesis in isolated perfused rat hearts. According to these investigators, the CK/PCr system can be simultaneously stimulated as the energy production increases. In fact, we observed a good correlation between V_{ATP} and CK flux in control hearts. However, there was no significant correlation between these two factors in diabetic hearts. Therefore, the diminished functional capacity of the CK/PCr system may induce contractile dysfunction (at higher workloads) in diabetic hearts. It has been reported that oxidative phosphorylation rate was depressed in mitochondria isolated from STZ-induced diabetic rat hearts (17, 22). Others have shown that oxygen consumption was reduced in cardiomyocytes isolated from STZ-induced diabetic rat hearts (27). In contrast, Penpargkul et al. (20) reported that oxygen consumption in diabetic hearts was the same as in control hearts when studied in an isolated working rat heart apparatus. In the present study, V_{ATP} was not significantly different between control and diabetic hearts at either workload condition. Therefore, it was likely that ATP synthesis was not responsible for the functional defects of diabetic hearts.

Another feature of the cardiac dysfunction in diabetes is the impairment of the relaxation process. Fein et al. (4) studied the performance of isolated left ventricular papillary muscle in STZ-induced diabetic rat hearts and showed that the most prominent abnormality was the process of relaxation. Although most of the myocardial energy is consumed by myosin ATPase activity during systole to optimize contraction, relaxation is also an energy-requiring process. The cytosolic Ca^{2+} level that is elevated during contraction must be reduced to the basal level during relaxation primarily by the SR Ca^{2+}-ATPase (25). The efficiency of various ATPases depends on the free energy of ATP hydrolysis (11). It has been reported that the SR Ca^{2+}-ATPase required the highest free energy to maintain Ca^{2+} transport efficiency (10, 11, 30). The free energy of ATP hydrolysis becomes smaller after the inhibition of the CK/PCr system because the cytosolic ADP concentration cannot be maintained at a lower level (10, 11, 29). Because the SR Ca^{2+}-ATPase is very sensitive to the decrease in the free energy of ATP hydrolysis, the relaxation process may be impaired quite easily by the inhibition of the CK/PCr system. In this study, we observed that the relaxation rate ($-dP/dt$) was reduced in diabetic hearts even at lower workloads. This feature also may be due to the decreased CK kinetics in the diabetic cardiomyopathy.

To confirm a role of CK kinetics in cardiac performance, we also examined the effects of a CK inhibitor (IAAm) on cardiac function in control hearts. The relationship between CK flux and cardiac function in these CK-inhibited hearts was also compared with that in diabetic hearts. We observed that both CK flux and RPP were decreased after treatment with IAAm. There was a good correlation between the reduction of CK flux and the depression of RPP. It has been reported that IAAm can affect not only CK but also key glycolytic enzymes like glyceraldehyde-3-phosphate dehydrogenase (6). However, according to the ^{31}P NMR spectra in this study, the intracellular content of sugar phosphates, such as glucose 6-phosphate and fructose 1,6-bisphosphate, was not changed throughout the experiment. Therefore, this suggests that the effect of IAAm on cardiac performance could be attributed to the reduction of CK kinetics but not to the inhibition of glycolysis. At the higher-dosage IAAm treatment, the intracellular ATP concentration was decreased without a significant change in PCr. It has been reported that ATP was decreased without a noticeable change in PCr content in a completely CK-inhibited heart (6). Therefore, these data suggest that the CK inhibition induced by the higher-dosage of IAAm might have been so severe that the ATP level could not be preserved. The reduction

of CK flux in diabetic hearts was less than that induced by the higher-dosage IAAm treatment in control hearts. Conversely, the decrease of RPP in diabetic hearts was much larger than that in the CK-inhibited control hearts. These results indicate that the inhibition of the CK/PCr system is unlikely to be the sole factor for the cardiac dysfunction in the diabetic cardiomyopathy. Dillmann (3) has previously reported that a marked change in the predominance of V_1 and V_3 myosin components occurred in diabetic rat hearts. He suggested that the decreased myosin Ca^{2+}-ATPase activity, due to the predominance of V_3 isoenzyme, mediated in part the diminished contractility of diabetic hearts. Penpargkul et al. (19) also showed that Ca^{2+} uptake was depressed and Ca^{2+}-Mg^{2+}-ATPase activity was reduced in SR isolated from STZ-induced diabetic rat hearts. They indicated that these defects were at least partially responsible for the relaxation abnormality in diabetic myocardium. Moreover, the reduced activities of sarcolemmal enzymes, such as the Na^+-K^+-ATPase (21), Ca^{2+}-ATPase (7), and Na^+-Ca^{2+} exchanger (16), can lead to the alteration in Ca^{2+} homeostasis and may contribute to the cardiac dysfunction observed in the diabetic cardiomyopathy.

In conclusion, *1*) contractile dysfunction became apparent only at higher workloads in diabetic hearts; *2*) the relaxation rate was decreased in diabetic hearts; *3*) the ATP synthesis rate of diabetic hearts was not significantly different from control hearts; *4*) the CK flux was significantly reduced in diabetic hearts; *5*) CK flux did not increase as the workload increased in diabetic hearts; *6*) in control hearts, the administration of IAAm inhibited CK flux to the same degree as that in diabetic hearts and provoked the contractile dysfunction; and *7*) cardiac performance was not depressed to the same degree in the CK-inhibited control hearts as it was in diabetic hearts. These results indicate that the impaired CK/PCr system is, at least in part, responsible for the cardiac dysfunction in the diabetic cardiomyopathy.

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REFERENCES

1. **Bittl, J. A., and J. S. Ingwall.** Reaction rates of creatine kinase and ATP synthesis in the isolated rat heart. *J. Biol. Chem.* 260: 3512–3517, 1985.
2. **Dagani, H., M. Laughlin, S. Campbell, and R. G. Shulman.** Kinetics of creatine kinase in heart: a 31P NMR saturation- and inversion-transfer study. *Biochemistry* 24: 5510–5516, 1985.
3. **Dillmann, W. H.** Diabetes mellitus induces changes in cardiac myosin of the rat. *Diabetes* 28: 579–582, 1980.
4. **Fein, F. S., L. B. Kornstein, J. E. Strobeck, J. M. Cappaso, and E. H. Sonnenblick.** Altered myocardial mechanics in diabetic rats. *Circ. Res.* 47: 922–933, 1980.
5. **Fein, F. S., and E. H. Sonnenblick.** Diabetic cardiomyopathy. *Prog. Cardiovasc. Dis.* 27: 255–270, 1985.
6. **Fossel, E. T., and H. Hoefeler.** Complete inhibition of creatine kinase in isolated perfused rat hearts. *Am. J. Physiol.* 252 (*Endocrinol. Metab.* 15): E124–E130, 1987.
7. **Heyliger, C. E., A. Prakash, and J. McNeill.** Alteration in cardiac sarcolemmal Ca^{2+} pump activity during diabetes mellitus. *Am. J. Physiol.* 252 (*Heart Circ. Physiol.* 21): H540–H544, 1987.
8. **Humphrey, S. M., and P. B. Garlic.** NMR-visible ATP and Pi in normoxic and reperfused rat hearts: a quantitative study. *Am. J. Physiol.* 260 (*Heart Circ. Physiol.* 29): H6–H12, 1991.
9. **Ingwall, J. S., D. E. Atkinson, K. Clarke, and J. K. Fetters.** Energetic correlates of cardiac failure: changes in the creatine kinase system in the failing myocardium. *Eur. Heart J.* 11: 108–115, 1990.
10. **Kammermeier, H.** Why do cells need phosphocreatine and a phosphocreatine shuttle. *J. Mol. Cell. Cardiol.* 19: 115–119, 1987.
11. **Kammermeier, H., P. Schmidt, and E. Jüngling.** Free energy change of ATP-hydrolysis: a causal factor of early hypoxic failure of myocardium? *J. Mol. Cell. Cardiol.* 14: 267–277, 1982.
12. **Kapelko, V. I., V. V. Kupriyanov, N. A. Novikova, V. L. Lakomkin, A. Y. Steinschneider, M. Y. Severina, V. I. Verksler, and V. A. Saks.** The cardiac contractile failure induced by chronic creatine and phosphocreatine deficiency. *J. Mol. Cell. Cardiol.* 20: 465–479, 1988.
13. **Kapelko, V. I., V. A. Saks, N. A. Novikova, M. A. Golikov, V. V. Kupriyanov, and M. I. Popovich.** Adaptation of cardiac contractile function to conditions of chronic energy deficiency. *J. Mol. Cell. Cardiol.* 21: 79–83, 1989.
14. **Kobayashi, K., and J. R. Neely.** Control of maximum rates of glycolysis in rat cardiac muscle. *Circ. Res.* 44: 166–175, 1979.
15. **Kuo, T. H., K. H. Moore, F. Giacomelli, and J. Wiener.** Defective oxidative metabolism of heart mitochondria from genetically diabetic mice. *Diabetes* 32: 781–787, 1983.
16. **Makino, N., K. S. Dhalla, V. Elimban, and N. S. Dhalla.** Sarcolemmal Ca^{2+} transport in streptozotocin-induced diabetic cardiomyopathy in rats. *Am. J. Physiol.* 253 (*Endocrinol. Metab.* 16): E202–E207, 1987.
17. **Mokhtar, N., J. P. Lavoie, S. R. Migneron, and A. Nadeau.** Physical training reverses defect in mitochondrial energy production in heart of chronically diabetic rats. *Diabetes* 42: 682–687, 1993.
18. **Noda, N., H. Hayashi, H. Miyata, S. Suzuki, A. Kobayashi, and N. Yamazaki.** Cytosolic Ca^{2+} concentration and pH of diabetic rat myocytes during metabolic inhibition. *J. Mol. Cell. Cardiol.* 24: 435–446, 1992.
19. **Penpargkul, S., F. Fein, E. H. Sonnenblick, and J. Scheuer.** Depressed cardiac sarcoplasmic reticular function from diabetic rats. *J. Mol. Cell. Cardiol.* 13: 303–309, 1981.
20. **Penpargkul, S., T. Schaible, T. Yipintsoi, and J. Scheuer.** The effect of diabetes on performance and metabolism of rat hearts. *Circ. Res.* 47: 911–921, 1980.
21. **Pierce, G. N., and N. S. Dhalla.** Sarcolemmal Na^+-K^+-ATPase activity in diabetic rat heart. *Am. J. Physiol.* 245 (*Cell Physiol.* 14): C241–C247, 1983.
22. **Pierce, G. N., and N. S. Dhalla.** Heart mitochondrial function in chronic experimental diabetes in rats. *Can. J. Cardiol.* 1: 48–54, 1985.
23. **Pierce, G. N., B. Ramjiawan, N. S. Dhalla, and R. Ferrari.** Na^+-H^+ exchange in cardiac sarcolemmal vesicles isolated from diabetic rats. *Am. J. Physiol.* 258 (*Heart Circ. Physiol.* 27): H255–H261, 1990.
24. **Popovich, B. K., K. R. Boheler, and W. H. Dillmann.** Diabetes decreases creatine kinase enzyme activity and mRNA level in the rat heart. *Am. J. Physiol.* 257 (*Endocrinol. Metab.* 20): E573–E577, 1989.
25. **Pouleur, H.** Diastolic dysfunction and myocardial energetics. *Eur. Heart J.* 11, *Suppl.* C: 30–34, 1990.
26. **Savabi, F., and A. Kirsch.** Alteration of the phosphocreatine energy shuttle components in diabetic rat heart. *J. Mol. Cell. Cardiol.* 23: 1323–1333, 1991.
27. **Tanaka, Y., N. Konno, and K. J. Kako.** Mitochondrial dysfunction observed in situ in cardiomyocytes of rats in experimental diabetes. *Cardiovasc. Res.* 26: 409–414, 1992.
28. **Ugurbil, K., M. Petein, R. Maidan, S. Michurski, and A. H. L. From.** Measurement of an individual rate constant in the presence of multiple exchanges: application to myocardial creatine kinase reaction. *Biochemistry* 25: 100–107, 1986.

29. **Wyss, M., J. Smeitink, R. A. Wevers, and T. Wallimann.** Mitochondrial creatine kinase: a key enzyme of aerobic energy metabolism. *Biochim. Biophys. Acta* 1102: 119–166, 1992.
30. **Zweier, J. L., and W. E. Jacobus.** Substrate-induced alterations of high energy phosphate metabolism and contractile function in the perfused heart. *J. Biol. Chem.* 262: 8015–8021, 1987.
31. **Zweier, J. L., and W. E. Jacobus.** Bioenergetic consequences of cardiac phosphocreatine depletion induced by creatine analogue feeding. *J. Biol. Chem.* 266: 20296–20304, 1991.