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PRELIMINARY REPORT

Cardiac Dysfunction in the Euglycemic Diabetic-Prone BB Wor Rat

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The purpose of this study was to examine cardiac function in the diabetic-prone BB Wor rat. The study involved 2 groups: diabetic resistant control littermates of BB rats and diabetic-prone BB rats that had yet to demonstrate overt signs of diabetes. Hearts from these animals were isolated and cardiac function examined in response to incremental increases in left atrial filling pressure. Hearts were also perfused at an increased aortic afterload resistance with buffer consisting of glucose alone or glucose in the presence of palmitate. Hearts from diabetic-prone rats exhibited depressed contractility and ventricular relaxation at high filling pressures. Ventricular function, expressed as cardiac output, was also depressed in diabetic-prone rats perfused at increased afterload resistance, but only in the presence of palmitate. Our results indicate that hearts from diabetic-prone BB Wor rats demonstrate abnormalities in contractile performance and thus may be a useful model for the study of cardiac function in the prediabetic state.

INSULIN-DEPENDENT (type 1) diabetes is a prevalent disease that increases the incidence and severity of cardiovascular complications.[1] The complications associated with type 1 diabetes are typically due to coronary artery disease and hypertension as well as a specific cardiomyopathy resulting in left ventricular dysfunction.[2,3]

The genetically diabetic-prone BB Wor rat is an animal model of type 1 diabetes, characterized by insulin deficiency, hyperglycemia, and ketoacidosis.[4] Most reports examining the diabetes-induced deterioration in cardiac performance in this model have been limited to chronic forms of diabetes[5]; few studies on cardiac function have been performed in the acute diabetic state.

Recently, we have reported that hearts from diabetic BB Wor rats perfused 48 hours following the onset of diabetes exhibited a decreased contractile performance to high workload and ischemia.[6] With the observation of ventricular dysfunction occurring in the prediabetic OLEFT rat, a model resembling type 2 diabetes,[7] this prompted us to determine whether a cardiomyopathy also exists before the onset of diabetes in the BB Wor rat.

MATERIALS AND METHODS

Animals

Diabetic-prone BB Wor rats, aged 21 to 30 days, were purchased from a colony bred and maintained at the Biomedical Research Models (Worcester, MA). The diabetic-prone BB Wor rat, representative of human type 1 diabetes, is characterized by spontaneous autoimmune pancreatic islet cell destruction and insulitis, which occurs when rats reach the age of approximately 70 days.[4] A group of diabetic-resistant BB Wor rats served as a control group. Although diabetic-resistant rats are derived from diabetic forbearers, this strain is genetically engineered to not develop type 1 diabetes during their lifespan. They were derived from BB diabetic-prone forbearers at the 5th generation of inbreeding by selection for the absence of disease.[8,9] These rats share the $RT1^U$ major histocompatibility complex haplotype of the BB diabetic-prone rat and are not lymphopenic, because the breeding selected against the recessive *Ian4l1* mutation. BB diabetic-resistant rats circulate normal numbers of $CD4^+$, $CD8^+$, and $ART2^+$ T cells and never become spontaneously diabetic.[10] Diabetic-prone rats were examined daily for the signs of diabetes, indicated by increased urine output or by the positive measurement of glucose and ketone bodies using urine test strips (Keto-Diastix, Bayer, Elkhart, IN). Rats demonstrating such signs of diabetes were excluded from this study. When animals reached the aged of 50 to 55 days and had yet to show signs of diabetes, heart perfusions were performed as described below. All animals used in this study were cared for in accordance with the recommendations in *The Guide for the Care and Use of Laboratory Animals* (National Institute of Health, Publ. No. 85-23, 1986).

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Table 1. Physical Characteristics of Diabetic-Resistant and Pre–Insulin-Dependent Diabetic BB Wor Rats

Group	Body Weight (g)	Heart Weight (g)	Plasma Glucose (mmol/L)	Plasma Free Fatty Acids (mmol/L)
Diabetic-resistant	303 ± 11	0.20 ± 0.02	6.45 ± 0.39	0.37 ± 0.05
Prediabetic	257 ± 9*	0.19 ± 0.01	7.04 ± 0.45	0.37 ± 0.03

NOTE. Values are reported as mean ± SEM for 7 or 8 animals in each group.
*$P < .05$ by unpaired t test compared to the diabetic-resistant group.

Heart Perfusions

Experiments were performed in 3- to 4-hour postprandial rats. Rats were euthanized with CO_2 gas, followed by decapitation. Thereafter, hearts were rapidly excised and cannulated as working hearts. The working heart buffer consisted of Krebs-Henseleit buffer containing in (mmol/L): NaCl (118), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $CaCl_2$ (2.5), $NaHCO_3$ (25), and glucose (5.5), as well as 100 μU/mL insulin and 1.2 mmol/L palmitate prebound to 3% bovine serum albumin (pH 7.4, oxygenated with 95% O_2/5% CO_2). After a 15-minute baseline equilibration period at 15 cm H_2O filling pressure and 100 cm H_2O aortic resistance, the left atrial filling pressure was reduced to 5 cm H_2O and then increased by 5-cm increments to 25 cm H_2O. Throughout this procedure, hearts were electrically paced (Grass-Telefactor, model SD9, West Warwick, RI) at a rate of 260 beats per minute. Ventricular pressure development, rate of ventricular contraction ($+dP/dt$) and rate of ventricular relaxation ($-dP/dt$) by the heart were measured using a transducer (Digi-Med Heart Performance Analyzer, Louisville, KT) inserted in the aortic line. In a second series of perfusions, cardiac output was measured in hearts subjected to an aortic afterload resistance of 140 cm H_2O. The working heart buffer consisted of 11 mmol/L glucose alone or 11 mmol/L glucose and 1.2 mmol/L palmitate. Cardiac output was measured using flow probes inserted into the left atrial and aortic afterload lines (Transonic Systems, Ithaca, NY). The temperature of the perfusate was maintained at 37°C.

Measurement of Plasma Metabolites

Mixed blood draining from the neck following decapitation was rapidly collected in chilled heparinized tubes. The blood was spun at 8,000 rpm and the plasma separated and frozen at −80°C. The plasma concentrations of glucose and nonesterified free fatty acids were measured using commercially available kits (Wako Chemicals USA, Richmond, VA).

Statistical Analysis

All values are reported as the mean ± SEM. For heart function in response to left atrial filling pressure, a repeated-measures analysis of variance (ANOVA) followed by a Student-Newman-Keuls was used. Student's t test was applied to determined differences between group means of hearts subjected to an increased in afterload resistance. A P value less than .05 was considered statistically significant.

RESULTS

The physical characteristics of diabetic-resistant and diabetic-prone BB rats are summarized in Table 1. At 50 days of age, diabetic-prone rats exhibited no signs of diabetes. Plasma glucose and free fatty acids measured at the time of death were similar between diabetic-prone and diabetic-resistant littermates. Although dry heart weight was also similar in these groups, body weight was lower in diabetic-prone rats.

As shown in Table 2, baseline mechanical function of spontaneously beating hearts of diabetic-resistant and diabetic-prone rats was similar. Figure 1 shows that cardiac output (Fig 1A) and systolic pressure (Fig 1B) increased to the same extent with the varying filling pressures in both groups of hearts. However, as illustrated in Fig 2A, hearts from diabetic-prone rats, unlike their nondiabetic littermates, exhibited a depression in $+dP/dt$ at filling pressures higher than 10 cm H_2O. A depression in $-dP/dt$ was also seen in the diabetic prone heart at higher filling pressures (Fig 2B).

Differences in pump function, expressed as cardiac output, were also observed when hearts were perfused at increased aortic afterload resistance. Figures 3 and 4 show that when hearts from diabetic-resistant and diabetic-prone rats were perfused with glucose only, cardiac output remained unchanged when the afterload resistance was increased to 140 cm H_2O. At this resistance in the presence of palmitate, cardiac output remained unchanged in hearts from diabetic-resistant rats, whereas it was further decreased in hearts from diabetic-prone rats (Fig 4).

DISCUSSION

This study compared the response of isolating working hearts from diabetic-resistant and diabetic-prone rats under different workload conditions. We found that in perfusions in which hearts were beating spontaneously, there were no differences in cardiac function between hearts from diabetic-prone and diabetic-resistant BB rats. However, with the increased metabolic demand of pacing, and elevations in left atrial filling pressure and aortic afterload resistance, hearts from diabetic-prone rats displayed a contractile dysfunction. Unlike in diabetic-resistant hearts in which the increases in positive and negative dP/dt were significant with increases in left atrial filling pressure, hearts from diabetic-prone rats exhibited a blunted response in both ventricular contraction and relaxation at high filling pressures. Further, when the aortic afterload resistance was increased, cardiac output was depressed in hearts from diabetic-prone rats perfused with fatty acids. Our results suggest that the presence of a distinct cardiomyopathy

Table 2. Mechanical Function in Spontaneously Beating Hearts During the Equilibration Period Before Left Atrial Filling Pressure Challenge

Group	Heart Rate (beats/min)	Systolic Pressure (mm Hg)	Diastolic Pressure (mm Hg)	Cardiac Output (mL/min)	Positive dP/dt (mm Hg/s)	Negative dP/dt (mm Hg/s)
Diabetic-resistant	250 ± 14	109 ± 3	53 ± 1	58 ± 5	2,738 ± 96	1,255 ± 104
Prediabetic	237 ± 10	102 ± 1	54 ± 1	55 ± 4	2,461 ± 126	1,291 ± 113

NOTE. Values are reported as mean ± SEM for 7 or 8 hearts in each group.

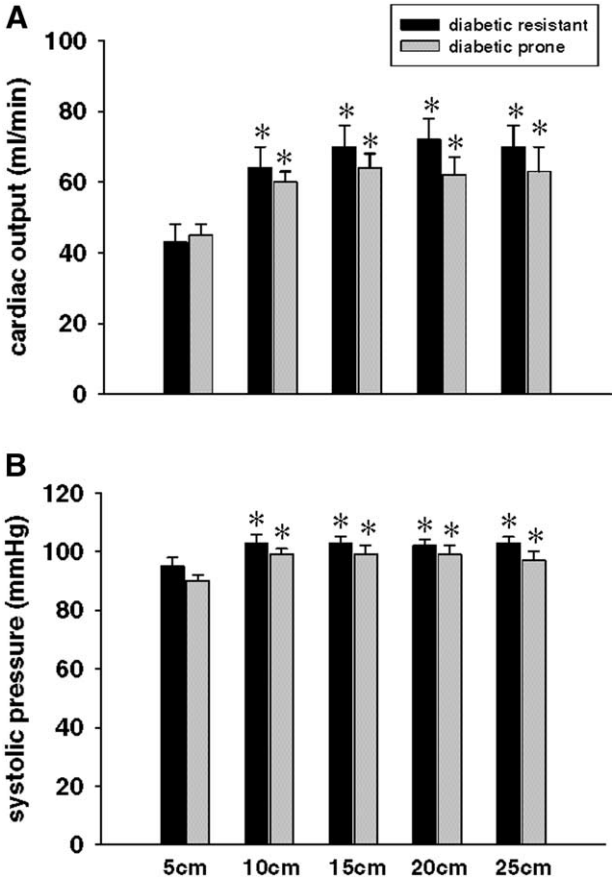

Fig 1. (A) Cardiac output and (B) peak systolic pressure in diabetic-resistant and diabetic-prone rats during incremental increases in left atrial filling pressure. Values are reported as means ± SEM for 7 or 8 hearts in each group. *Significantly different compared to 5 cm H_2O left atrial filling pressure.

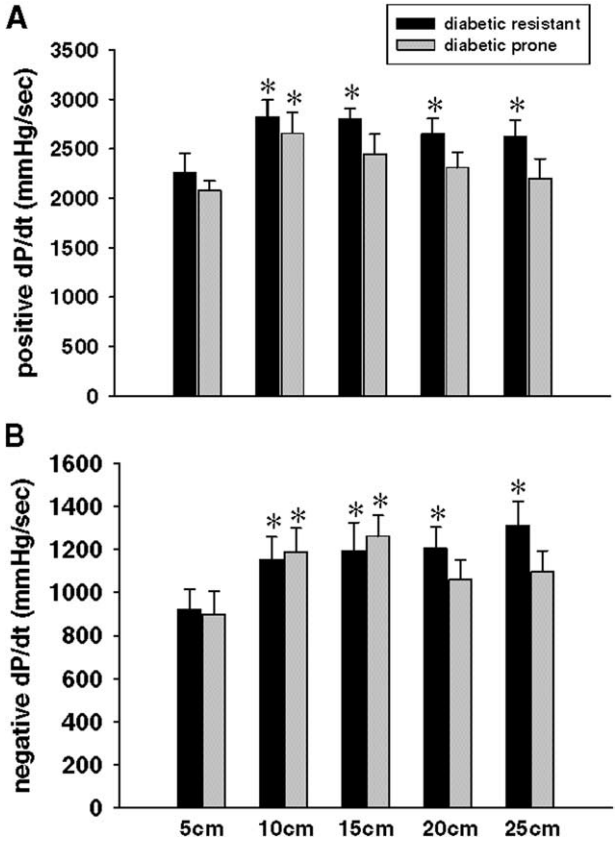

Fig 2. (A) Positive d*P*/d*t* and (B) negative d*P*/d*t* in diabetic-resistant and diabetic-prone rats during incremental increases in left atrial filling pressure. Values are reported as means ± SEM for 7 or 8 hearts in each group. *Significantly different compared to 5 cm H_2O left atrial filling pressure.

exists in the diabetic-prone rat, occurring independently of left ventricular heart mass and metabolic control.

Earlier work by Rodrigues et al[11] also reported differences in cardiac function, with functional assessment determined in hearts from 6-week diabetic BB rats treated with low-dose insulin. In contrast to our results, however, significant differences in cardiac function between diabetic-resistant and diabetic BB rats were observed. At high filling pressures, diminished contractility and left ventricular pressure development were seen in the diabetic rat. These differences in function are likely due to the biochemical and metabolic changes that are associated with the chronic diabetic state, including depressed Ca^{2+}-stimulated myosin adenosine triphosphatase (ATPase) activity,[11,12] increased cardiac lipid metabolism,[13] and impaired glucose metabolism.[14]

In hearts of diabetic-prone rats, the alterations in left ventricular function could be a result of the cardiac cells inability to handle intracellular Ca^{2+}. Alterations in ventricular relaxation, the earliest form of cardiac dysfunction and manifested before systolic dysfunction, reflect defects within the sarcoplasmic reticulum, such as depressed Ca^{2+}, ATPase, and Na^{+}/Ca^{2+} exchanger activities.[11,12] While many studies have demonstrated this to be the case in hearts of chronic diabetic BB animals, as well as in isolated

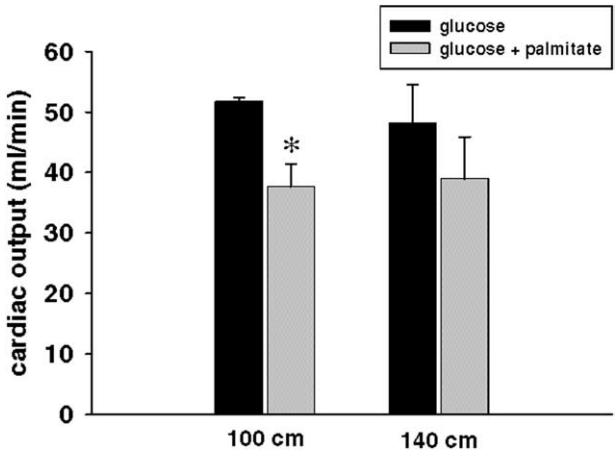

Fig 3. Cardiac output of hearts from diabetic-resistant rats at 100 cm H_2O and 140 cm H_20 afterload resistance perfused with 11 mmol/L glucose alone or 11 mmol/L glucose and 1.2 mmol/L palmitate. Values are reported as means ± SEM for 4 hearts in each group. *Significantly different compared to hearts perfused with glucose.

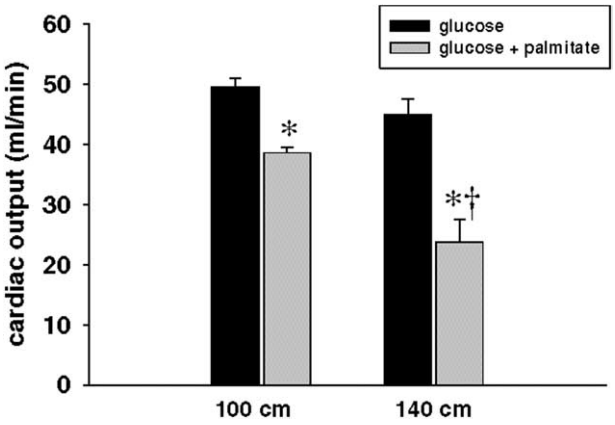

Fig 4. Cardiac output of hearts from diabetic prone rats at 100 cm H_2O and 140 cm H_2O afterload resistance perfused with 11 mmol/L glucose alone or 11 mmol/L glucose and 1.2 mmol/L palmitate. Values are reported as means ± SEM for 4 hearts in each group. *Significantly different compared to hearts perfused with glucose. †Significantly different compared to hearts perfused with glucose and palmitate at 100 cm H_2O aortic resistance.

ventricular myocytes acutely exposed to high glucose,[15] further studies are clearly warranted to investigate whether these disturbances are present in the pre–insulin-dependent diabetic stage. If Ca^{2+} handling is defective prior to frank diabetes, it is likely that ventricular relaxation would be impaired during conditions of limited energy supply and at high workloads.

While many experimental groups have studied the effect of chronic diabetes on ventricular function,[16,17] very few studies have determined the ventricular outcome in the acute diabetic state. Hearts obtained from BB rats 48 following the onset of diabetes have pronounced post-ischemic dysfunction following global ischemia.[6] Hearts from acute chemically induced diabetes are more vulnerable to anoxia[18] and exhibit accelerated failure rates when subjected severe ischemia.[19] Although ventricular dysfunction generally occurs with the progression of diabetes, a recent study by Mizushige et al[7] reported subtle defects in ventricular function prior to overt signs of diabetes. Indeed, in hearts from 15-week-old euglycemic prediabetic OLEFT rats, early diastolic transmitral inflow exhibited a prolonged deceleration time.[7] Histology also revealed an abnormal accumulation of myocardial collagen and increased content of transforming growth factor β_1 receptors in the left ventricle, suggesting that histopathologic changes clearly precede and contribute to the development of the alterations in cardiac function in this model. Although the etiologies differ between prediabetic type 1 and type 2 cardiomyopathies, and are likely multifactorial in nature, the preclincial entity is similar and encompasses impaired contractility and slowed ventricular relaxation.[20,21]

In conclusion, the results of this study demonstrate that hearts from euglycemic diabetic-prone BB Wor rats exhibit ventricular abnormalities when subjected to increased workload. The diabetic-prone BB Wor rat may thus be useful to study the mechanisms contributing to the alterations in ventricular contractility and relaxation prior to the overt signs of diabetes.

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