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Cardiac expression of adiponectin and its receptors in streptozotocin-induced diabetic rats

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Abstract

Adiponectin can improve both glucose metabolism and insulin resistance via the adenosine monophosphate–activated protein kinase (AMPK) signaling pathway. Activated AMPK phosphorylates a variety of intracellular proteins, including acetyl coenzyme A carboxylase (ACC) that is involved in fatty acid oxidation. Adenosine monophosphate–activated protein kinase increases glucose transport by stimulating the translocation of glucose transporter 4 (GLUT4) to the sarcolemma in the heart. Adiponectin exerts its effect through adiponectin receptors, which are predominantly expressed in the liver and skeletal muscle. It is unknown whether the cardiac expression of adiponectin and its receptors is changed in diabetic rats. In the present study, we investigated the protein expression of adiponectin and its receptors in streptozotocin (STZ)–induced diabetic rat hearts. We also explored whether the levels of AMPK, ACC, and GLUT4 will be altered with the changed adiponectin and its receptors in STZ diabetic rat hearts. Plasma and cardiac adiponectin levels were measured by radioimmunoassay. Plasma and cardiac interleukin 6 and plasma tumor necrosis factor α (TNF-α) were assayed by enzyme-linked immunosorbent assay. Cardiac adiponectin receptors, AMPK-α, ACC, GLUT4, and TNF-α were analyzed by Western blot in control and STZ diabetic rats. The plasma adiponectin level was decreased, but the cardiac protein expression of adiponectin receptor 1 was increased in diabetic rats. There was no difference in the cardiac adiponectin level and the cardiac adiponectin receptor 2 protein expression between control and diabetic rats. The phosphorylation of AMPK-α and protein expression of GLUT4 were decreased, but the phosphorylation of ACC was unchanged in diabetic rat hearts. Plasma and cardiac levels of interleukin 6 and TNF-α were increased in diabetic rats. In conclusion, STZ-induced diabetes up-regulates adiponectin receptors in the heart. Despite an increase in cardiac adiponectin receptor 1 expression, there is an increased cardiac inflammatory response and a decreased GLUT4 protein expression associated with a reduction in circulating adiponectin.

1. Introduction

Cardiovascular disease is one of the major complications of diabetes, resulting in a high percentage of morbidity and mortality and producing significant costs for the health care system [1]. Diabetes and its cardiovascular complications are related to multiple pathogenic factors, including oxidative stress, hyperglycemia, hyperlipidemia, and inflammatory responses [2-4]. Alterations in cardiac energy metabolism may also be associated with the development of cardiomyopathy in the diabetic heart [5].

Adiponectin is an adipocyte-derived protein with anti-inflammatory, antidiabetic, and anitiatherogenic properties [6]. Circulating adiponectin levels are decreased in insulin resistance [7], obesity [7], type 2 diabetes mellitus [8,9], and coronary artery disease [9], and are increased in chronic renal failure [10] and in type 1 diabetic patients [11]. Adiponectin is also synthesized and secreted by human and murine cardiomyocytes. Local production of adiponectin by cardiomyocytes may have important functions in the regulation of the cardiac function and/or metabolism by autocrine and/or paracrine glands [12].

Adiponectin can improve both glucose metabolism and insulin resistance via the adenosine monophosphate–activated protein kinase (AMPK) signaling pathway [13]. AMPK is a fuel-sensing enzyme present in most mammalian

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tissues. AMPK is phosphorylated and activated by a still uncharacterized upstream AMPK kinase in response to an increase in the adenosine monophosphate–adenosine triphosphate ratio. Activated AMPK phosphorylates a variety of intracellular proteins, including acetyl coenzyme A carboxylase (ACC; Ser79) that is involved in fatty acid oxidation [14]. Adiponectin stimulates glucose utilization and fatty acid oxidation [13] and inhibits hypertrophic signaling in the myocardium [15].

Two novel adiponectin receptors (adipoR1 and adipoR2) have been cloned. Adiponectin receptor 1 is abundantly expressed in skeletal muscle, whereas adipoR2 is predominantly expressed in the liver [16]. They serve as receptors for globular and full-length adiponectin and mediate increased AMP kinase and proliferator-activated receptor α ligand activities, as well as fatty acid oxidation and glucose uptake by adiponectin [16]. The expression of adipoR1/R2 was increased in skeletal muscle of streptozotocin (STZ)-diabetic mice but decreased in skeletal muscle of *ob*/*ob* mice. Insulin replenishment reduced the expression of adipoR1/R2 in the muscle and liver of STZ-diabetic mice [17,18]. Adiponectin receptor 2 was also up-regulated in the heart during neonatal development [19]. Regulation of adipoR1, but not of aipoR2, may be involved in glucose and lipid metabolism in diabetic states [17]. However, it is unknown whether the expression of adiponectin and its receptors is changed in diabetic rat hearts.

Heart failure, diabetes, and obesity are recognized as states of chronic inflammation; and inflammatory cytokines may play a role in all 3 of these conditions [20]. Interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) have been shown to be secreted by cardiomyocytes and to be increased in the failing heart [21,22]. Tumor necrosis factor α is expressed at a very low level in the normal heart but is significantly increased in response to various stresses including diabetes [23]. Increased circulating concentrations of TNF-α and IL-6 have been described in type 1 [24] and type 2 diabetic patients [25-27] and in STZ-diabetic mice [23]. Cardiac expression of TNF-α was also increased in STZ-diabetic mice [23]. The increased release of T helper 1 cytokines, such as TNF-α, and T helper 2 cytokines, such as IL-6, are involved in diabetic deterioration [28,29]. Tumor necrosis factor α can directly cause contractile dysfunction in the heart and has been implicated in pathologic remodeling in heart failure [30]. Tumor necrosis factor α also suppresses expression and secretion of adiponectin in 3T3-L1 adipocytes [31].

Therefore, we hypothesized that the expression of adiponectin and its receptors would also be changed in STZ-diabetic rat hearts. In the present study, we investigated the plasma and cardiac adiponectin levels and cardiac protein expression of adiponectin receptors in STZ-diabetic rats. In addition, we measured the plasma and cardiac levels of IL-6 and TNF-α, as well as the cardiac protein levels of AMPK-α, ACC, and glucose transporter 4 (GLUT4) in STZ-diabetic rats.

2. Materials and methods

2.1. Induction of diabetes

Male Wistar rats weighing 220 to 240 g, purchased from Charles River Laboratories (Laval, Quebec, Canada), were used in the study. All rats were housed in a temperature-controlled room (22°C-24°C) and kept on a 12-hour light-dark cycle. Animals had free access to standard rat chow and water. All animals received humane care in accordance with the principles of the Canadian Council on Animal Care. Rats were randomly divided into 2 groups: control (n = 10) and diabetic (n = 10). Diabetes was induced with a single intravenous tail vein injection of streptozotocin (60 mg/kg body weight, Sigma, St Louis, MO) under halothane anesthesia. Three days post-STZ injection, blood glucose was measured using Accusoft glucose test strips read on a glucometer (Roche Diagnostics, Laval, Quebec, Canada). A blood glucose level greater than 15 mmol/L was considered to be diabetic. Subsequently, blood samples were collected every 2 weeks. Blood was centrifuged at 10 000*g* for 45 minutes, plasma was collected, and plasma glucose levels were measured. At termination (9 weeks after the onset of diabetes), animals were weighed and then anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg/kg). A blood sample was withdrawn from the inferior vena cava and then centrifuged, and plasma aliquots were stored at −80°C until assays were done. After completion of function perfusion experiments, the hearts were washed, blotted dry, weighed, snap-frozen in liquid nitrogen, and then stored at −80°C until analyses were carried out.

2.2. Isolated working heart function

At termination, the hearts were excised and mounted on a working heart apparatus. Isolated working heart function was performed as described previously [32]. Briefly, the hearts were perfused with Chenoweth-Koelle buffer (composition [mmol/L]: NaCl 120, KCl 5.6, $CaCl_2$ 2.18, $MgCl_2$ 2.1, glucose 10, and $NaHCO_3$ 19.2), aerated with 95% O_2 and 5% CO_2, and maintained at 37°C. Perfusion with the Chenoweth-Koelle buffer was initiated in a retrograde manner through the aorta. Cardiac work was initiated by switching the perfusion system from the retrograde mode to the working heart mode. The perfusion buffer was pumped into the left atrium and through to the left ventricle before being ejected out the aorta. The aortic outflow was subjected to an afterload of 19 cm H_2O. The heart was paced at a constant rate of 300 beats per minute. A 20-gauge needle, inserted through the apex of the heart into the left ventricle, was used to measure left ventricular pressure via a Stratham P23A pressure transducer (Gould Statham Instruments, Cleveland, OH). Left ventricular performance was assessed in terms of the rate of contraction (+dP/dT), rate of relaxation (−dP/dT), and left ventricular developed pressure (LVDP) in response to increasing left atrial filling pressure (preload).

2.3. Plasma analytical procedures

Plasma glucose levels were measured using a Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma cholesterol, triglyceride, and free fatty acid were measured using colorimetric assays from kits commercially available from Roche Diagnostics (Laval, Quebec, Canada). Plasma adiponectin level was measured using a commercially available radioimmunoassay kit (Linco Research, St Charles, MO). Plasma TNF-α levels were determined using a rat TNF-α enzyme-linked immunosorbent assay (ELISA) kit (Biosource International, Burlington, Ontario, Canada). Plasma IL-6 levels were measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN).

2.4. Determination of cardiac adiponectin level and IL-6

Frozen heart tissue was pulverized and homogenized at 4°C in cold buffer (20 mmol/L Tris-HCl, pH 7.5, 50 mmol/L 2-mercaptoethanol, 5 mmol/L EGTA, 2 mmol/L EDTA, 1 mmol/L PMSF, 10 mmol/L NaF, 25 μg/mL leupeptin, 2 μg/mL aprotinin) and then centrifuged at 1500*g* for 5 minutes at 4°C. The supernatant was collected and stored at −80°C until analyses were conducted. The protein content of the samples was measured using the Bradford [33] protein assay with the use of bovine serum albumin as a standard. Cardiac adiponectin level was measured using a commercially available radioimmunoassay kit (Linco Research). Interleukin 6 level was measured using a commercially available ELISA kit (R&D Systems).

2.5. Determination of cardiac triglyceride

Total lipid was extracted from 50 mg of tissue using the method of Folch et al [34]. Cardiac triglycerides were measured by colorimetric assay using a commercially available kit from Roche Diagnostics.

2.6. Western blotting

Heart tissue (150 mg) was pulverized and homogenized using a Polytron homogenizer in 1.5 mL cold lysis buffer as described in section 2.4. The homogenate was centrifuged at 1000*g* for 10 minutes at 4°C, and the supernatant was collected and labeled as total preparation. The homogenate was further centrifuged at 100 000*g* for 60 minutes at 4°C, and the supernatant was collected and labeled as cytosol preparation. The pellet was resuspended in buffer containing 1% nonylphenyl-polyethylene glycol (NP-40), 0.1% sodium dodecyl sulfate, and 0.5% deoxycholic acid; homogenized; incubated on ice for 30 minutes; and then centrifuged at 100 000*g* for 60 minutes at 4°C. The supernatant was collected and labeled as membrane preparation. The protein content of each fraction was measured using the Bradford [33] protein assay. Aliquot samples were stored at −80°C until use.

After boiling at 95°C for 5 minutes, samples (50 μg protein per lane) were subjected to 10% to 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel and then transferred to nitrocellulose membranes. The membranes were blocked in 5% nonfat milk and 0.05% Tween 20 in 1× tris-buffered saline (TBS), and then incubated with primary antibodies against TNF-α (1:500, Santa Cruz Biotechnology, Santa Cruz, CA); adiponectin receptor type 1 (1:400) and type 2 (1:200) (Phoenix Pharmaceuticals, Belmont, CA); AMPK-α (1:1000), phosphor-AMPK-α (1:1000) and ACC (1:500) (Cell Signaling Technology, Beverly, MA); phosphor-ACC (Ser79) (1:1000, Upstate Biotechnology, Lake Placid, NY) and GLUT4 (1:2500, Abcam, Cambridge, MA). The membranes were washed and then incubated with a secondary horseradish peroxidase–conjugated antirabbit immunoglobulin G antibody (Santa Cruz Biotechnology). Proteins were visualized by enhanced chemiluminescence reagent (Amersham Pharmacia Biotech, Piscataway, NJ) and quantified using an image analysis software. In all instances, the membranes were stained with ponceau stain and reblotted with antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:1000, Santa Cruz Biotechnology) after stripping to verify the uniformity of protein load and the transfer efficiency across the test samples.

2.7. Statistical analysis

Data are expressed as mean ± SEM. Cardiac functional data were analyzed using a repeated-measures analysis of variance followed by Newman-Keuls post hoc test. Other parameters were analyzed by 2-tailed unpaired Student *t* test. Statistical significance was defined as $P < .05$.

3. Results

3.1. General characteristics in control and diabetic rats

Plasma glucose and lipids, cardiac triglycerides, and the ratio of heart weight to body weight were measured in control and diabetic rats at termination. As shown in Table 1, plasma glucose, cholesterol, triglyceride, and free fatty acid

Table 1
General characteristics and inflammatory factors in control and diabetic rats

Parameter	Control (n = 10)	Diabetic (n = 10)
Body weight (g)	526 ± 11	424 ± 9[a]
Heart weight (g)	1.7 ± 0.1	1.6 ± 0.1
Heart weight–body weight ratio (g/kg)	3.2 ± 0.2	3.9 ± 0.1[a]
Plasma glucose (mmol/L)	7.8 ± 0.2	27.7 ± 0.7[a]
Plasma cholesterol (mmol/L)	1.1 ± 0.1	2.1 ± 0.3[a]
Plasma triglyceride (mmol/L)	0.4 ± 0.1	3.1 ± 0.8[a]
Plasma free fatty acid (mmol/L)	0.5 ± 0.04	0.8 ± 0.1[a]
Cardiac triglyceride (μmol/mg wet weight)	1.3 ± 0.1	2.0 ± 0.2[a]
Plasma IL-6 (pg/mL)	37.3 ± 5.0	88.1 ± 6.8[a]
Cardiac IL-6 (pg/mg protein)	19.7 ± 2.1	34.4 ± 3.8[a]
Plasma TNF-α (pg/mL)	8.0 ± 1.2	16.8 ± 3.2[a]

Samples were collected 9 weeks after the onset of diabetes. Values are expressed as mean ± SEM. Statistical analysis was done using 2-tailed unpaired Student *t* test, $P < .05$.

[a] Different from control.

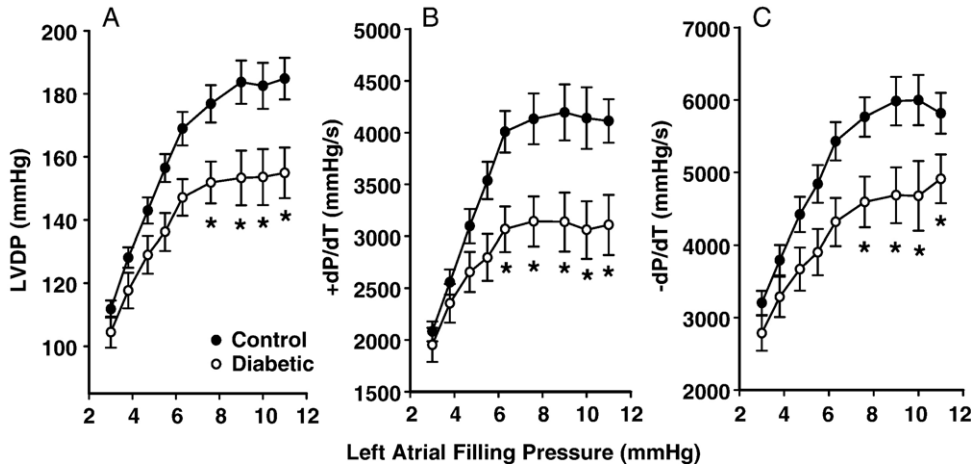

Fig. 1. Heart function was determined in control and diabetic rats. The indices used were (A) LVDP, (B) +dP/dT, and (C) −dP/dT. Data were expressed as mean ± SEM (n = 10 per group). *$P < .05$, different from control.

were increased in diabetic rats compared with control rats. Cardiac triglycerides were also increased in diabetic rats. The ratio of heart weight to body weight, an index of cardiac hypertrophy, was increased in diabetic rats compared with controls. These data show that the STZ-induced diabetic rats used in the present study manifested hyperglycemia, hyperlipidemia, and cardiac hypertrophy.

3.2. Heart function

Functional cardiac performance was assessed by measuring the left ventricular response to changes in left atrial filling pressure in terms of LVDP, +dP/dT, and −dP/dT. In control hearts, there was a progressive increase in these indices in response to increases in filling pressure. Cardiac dysfunction was apparent in diabetic rat hearts, which exhibited an inability to respond to increases in preload (Fig. 1).

3.3. Plasma and cardiac adiponectin and its receptors in control and diabetic rats

Adiponectin has been shown to be produced and secreted primarily by adipose tissue [35] as well as in human and murine cardiomyocytes [12]; therefore, we determined both plasma and cardiac adiponectin levels in control and diabetic rats. As shown in Fig. 2, plasma adiponectin level was decreased in diabetic rats compared with controls. In contrast to what we expected, cardiac adiponectin level was unchanged in diabetic rats. The data indicate that the plasma adiponectin level does not parallel with the cardiac adiponectin level and that diabetes does not produce an effect on the latter.

To test if diabetes produced an effect on the cardiac adiponectin receptors in rats, we determined the protein expression of adipoR1 and adipoR2 in the hearts of control and diabetic rats using Western blot technique. As shown in Fig. 3, the expression of cardiac adipoR1 was increased in diabetic rats compared with control rats. However, there was no difference in cardiac adipoR2 protein expression between control and diabetic rats. Our results are similar to the reports of Tsuchida et al [18] and Inukai et al [17] that showed that the level of adipoR1 messenger RNA was increased in the muscle of STZ-diabetic mice.

3.4. Cardiac phosphorylation of AMPK-α

Adenosine monophosphate–activated protein kinase consists of 1 catalytic subunit (α) and 2 noncatalytic subunits

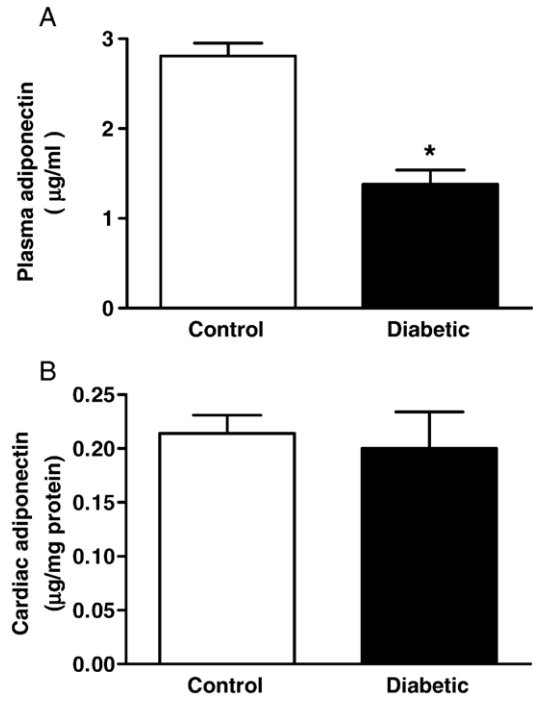

Fig. 2. Plasma and cardiac adiponectin levels assessed by radioimmunoassay in control and diabetic rats. Plasma adiponectin levels (A) and cardiac adiponectin levels (B) in control and diabetic rats. Data are expressed as mean ± SEM (n = 8 per group), *$P < .05$, different from control.

(β and γ). It was reported that AMPK-α was activated by adiponectin [13]; therefore, we examined the phosphor-AMPK-α (Thr172) and AMPK-α protein expression in the hearts of control and diabetic rats. As shown in Fig. 4, the cardiac phosphorylation of AMPK-α was significantly decreased in diabetic rats compared with control rats.

3.5. Cardiac phosphorylation of ACC

To determine the functional significance of decreased AMPK activation, we measured the protein expression of cardiac phosphor-ACC (Ser79) and total ACC in control and diabetic rats because activated AMPK phosphorylates a variety of intracellular proteins, including ACC that is involved in fatty acid oxidation [14]. As shown in Fig. 5, the cardiac phosphorylation of ACC was unchanged in diabetic rats compared with controls.

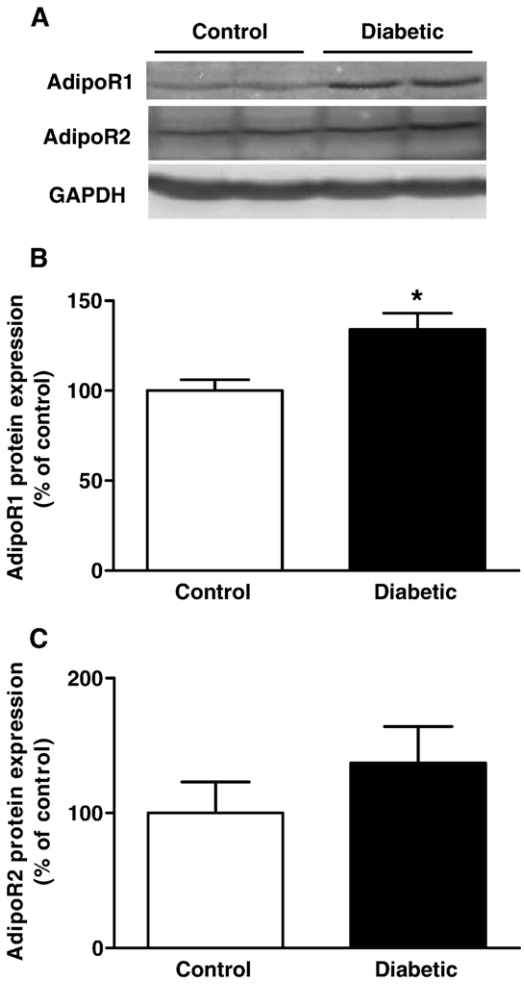

Fig. 3. Western blot analysis of cardiac protein expression of adipoR1 and adipoR2 in control and diabetic rats. Equal protein loading was confirmed with GAPDH. Mean band density was normalized relative to GAPDH. Representative blot (A), densitometry analysis of adipoR1 protein (B), and densitometry analysis of adipoR2 protein (C) in control and diabetic rats. Data are expressed as mean ± SEM (n = 6 per group), *$P < .05$, different from control.

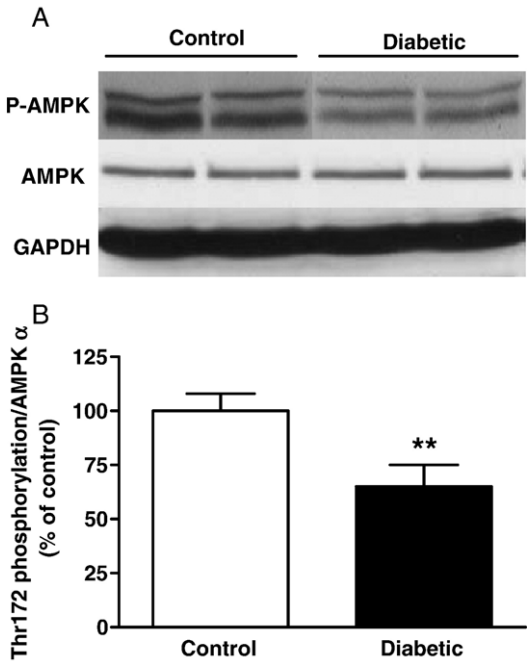

Fig. 4. Western blot analysis of cardiac protein expression of phosphor-ACC (Ser79) and total ACC in control and diabetic rats. Equal protein loading was confirmed with GAPDH. Mean band density was normalized relative to GAPDH. Representative blot (A) and densitometry analysis (B) of protein expression of AMPK (Thr172) phosphorylation corrected to total AMPK-α in control and diabetic rats. Data are expressed as mean ± SEM (n = 6 per group), **$P < .01$, different from control.

3.6. Cardiac total crude membrane GLUT4 expression

As shown in Fig. 6, the cardiac protein expression of total crude membrane was decreased in diabetic rats compared with controls, indicating that glucose metabolism was reduced in the hearts of diabetic rats.

3.7. Plasma and cardiac IL-6 and TNF-α

Because adiponectin is a protein with anti-inflammatory properties [6], we determined both plasma and cardiac IL-6 and TNF-α levels in control and diabetic rats. As shown in Table 1 and Fig. 7, plasma and cardiac IL-6 and TNF-α levels were increased in the hearts of diabetic rats. These data indicate that the increased plasma inflammatory factors may contribute to the decreased plasma adiponectin level in the diabetic rats. The increased cardiac adipoR1 may be a compensatory response to the increased inflammatory factors in the diabetic rat hearts.

4. Discussion

We show in this study that plasma adiponectin level is significantly decreased, but cardiac adiponectin level is unchanged in diabetic rats. Cardiac adipoR1 was increased in diabetic rats, but cardiac adipoR2 was not significantly changed. To our knowledge, this is the first study

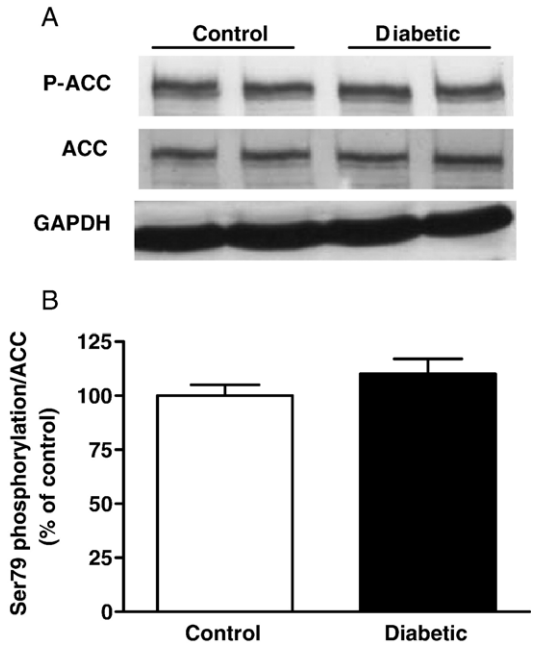

Fig. 5. Western blot analysis of cardiac protein expression of phosphor-ACC (Ser79) and total ACC in control and diabetic rats. Equal protein loading was confirmed with GAPDH. Mean band density was normalized relative to GAPDH. Representative blot (A) and densitometry analysis (B) of protein expression of ACC (Ser79) phosphorylation corrected to total AMPK-α in control and diabetic rats. Data are expressed as mean ± SEM (n = 6 per group).

investigating the expression of cardiac adiponectin and adiponectin receptors in diabetic rats.

It has been reported that plasma adiponectin level was increased in type 1 diabetic patients in some studies [11,36-38]. In contrast, our study and the results of Thule et al [39] showed that plasma adiponectin level was reduced in STZ-induced diabetic rats. The reasons for this discrepancy remain unclear and merit further study. One possible reason may be that the STZ-diabetic rat is insulin resistant [40]. An increase in plasma levels of adiponectin in type 1 diabetes mellitus has been shown to be associated with higher insulin sensitivity; however, a decrease in adiponectin levels has been reported in insulin-resistant states, such as in obesity and type 2 diabetes mellitus and in patients with coronary artery disease [41]. Another possible reason may be attributed to the decreased body weight and whole-body fat content in the STZ-diabetic rat. The decreased plasma adiponectin level in diabetic rats in this study could have contributed to the development of myocardial hypertrophy as shown by the increased heart-to-body weight ratio and the deterioration in cardiac function. It was reported that pressure overload in adiponectin-deficient mice resulted in enhanced concentric cardiac hypertrophy, which was attenuated by adiponectin supplementation [15].

Adiponectin is mainly produced and secreted by adipose tissues [35]. However, it has also been shown to be synthesized and secreted by human and murine cardiomyocytes [12]. Our study results show that adiponectin is expressed in rat hearts and that STZ-induced diabetes did not significantly change its expression in the heart. The reason why changes in plasma adiponectin level were not in parallel with changes in cardiac adiponectin level is not clear and requires further study. It is possible that tissue and systemic adiponectin levels are regulated by separate mechanisms.

Adiponectin exerts its effect through adipoR1 and adipoR2, which are both expressed in the heart [16] and in the cardiomyocyte [12]. Our study showed that cardiac adipoR1 was increased, but there was no change in cardiac

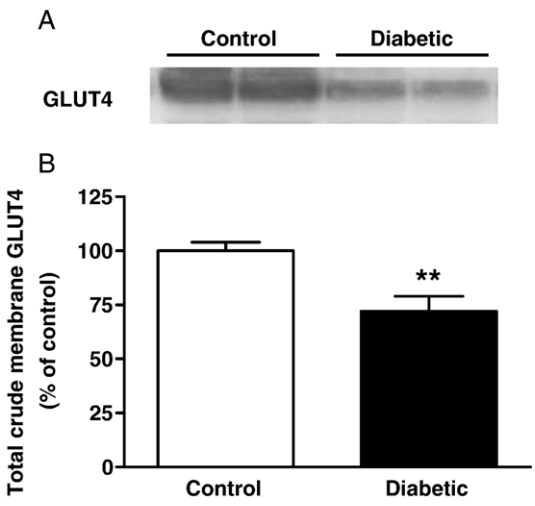

Fig. 6. Western blot analysis of cardiac total crude membrane GLUT4 in control and diabetic rats. Representative blot (A) and densitometry analysis (B) of GLUT4 protein. Data are expressed as mean ± SEM (n = 6 per group), $^{**}P < .01$, different from control.

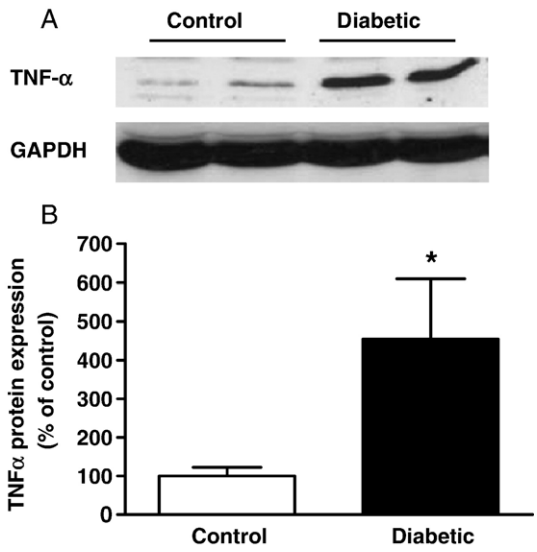

Fig. 7. Western blot analysis of cardiac protein expression of TNF-α in control and diabetic rats. Equal protein loading was confirmed with GAPDH. Mean band density was normalized relative to GAPDH. Representative blot (A) and densitometry analysis (B) of TNF-α protein in control and diabetic rats. Data are expressed as mean ± SEM (n = 6 per group), $^{*}P < .05$, different from control.

adipoR2 in diabetic rats. The increased cardiac protein expression of adipoR1 in diabetic rats may be due to (1) insulin deficiency because it has been shown that insulin deficiency increased but insulin replenishment decreased the expression of adipoR1/2 in animals in vivo [18]; (2) a decrease in plasma adiponectin level, which may function as a negative regulator of the expression of adiponectin receptors [42]; and (3) a compensatory mechanism that attempts to protect the heart against the harmful actions of the increased inflammatory cytokines. Adiponectin and adiponectin receptors detected in rat hearts in this study may function in a paracrine manner in the heart. Although adiponectin is produced in the heart, its expression in the myocardium is extremely low compared with that in adipose tissue [43]. Therefore, circulating adiponectin, rather than adiponectin produced locally in the myocardium, may act as the predominant ligand for myocardial adiponectin receptors [43].

Interleukin 6 and TNF-α have been shown to be secreted by cardiomyocytes and have been reported to be increased in failing hearts [21,22]. Tumor necrosis factor α is expressed at a very low level in the normal heart but is significantly increased in response to various stresses including diabetes [23]. Tumor necrosis factor α and adiponectin have been shown to both suppress and antagonize the action of each other [44]. In this study, both plasma and cardiac TNF-α and IL-6 levels were significantly increased in diabetic rats. Therefore, the elevated systemic and cardiac levels of TNF-α and IL-6 may be one of the mechanisms responsible for the decreased plasma adiponectin level and the increased cardiac adipoR1 and may be involved in the observed cardiac dysfunction [28,29].

Adenosine monophosphate–activated protein kinase was initially found to be an important regulator of fatty acid oxidation in the heart [45]. Our study showed that cardiac phosphorylation of AMPK-α (Thr172) was decreased in diabetic rats. There was no difference in cardiac phosphorylation of ACC (Ser79) in control and diabetic rats. The decreased cardiac phosphorylation of AMPK may contribute to the depressed cardiac function and myocardial hypertrophy in diabetic rats because AMPK deficiency has been reported to be associated with depressed cardiac function under stress conditions [46].

Glucose enters the heart via the facilitative glucose transporters GLUT1 and GLUT4 [47]. Adenosine monophosphate–activated protein kinase increases glucose transport by stimulating the translocation of GLUT4 to the sarcolemma in the heart [48]. Glucose transporter expression in the heart is altered in various pathologic states. In untreated diabetes, there is profound down-regulation of GLUT4 [49]. Our study showed that the protein expression of total crude membrane GLUT4 was decreased, indicating that glucose metabolism was reduced in diabetic rat hearts. The decreased GLUT4 may be due to the decreased circulating adiponectin level in diabetic rats. It was reported that changes in glucose transporter expression contributed to myocardial dysfunction in diabetes [50]. In addition, GLUT4-deficient mice developed striking cardiac hypertrophy [51-53]. Normalization of glucose homeostasis by transgenic reexpression of GLUT4 in the skeletal muscle resulted in a reversal of the cardiac pathology in mice heterozygous for GLUT4 ablation [54]. Hence, we propose that the decreased cardiac GLUT4 observed in this study may contribute to the deterioration in heart function and to the cardiac hypertrophy seen in STZ-diabetic rats.

Some limitations of this study need to be considered. The hearts used in this study were not rapidly frozen but were rather stressed by ex vivo perfusion. This could affect AMPK and ACC activity and might affect cardiac adiponectin content and receptor density. The lengthy perfusion with a fatty acid–free buffer would likely alter AMPK activity [55,56]. Hence, further experiments designed to investigate the potential effects of ex vivo perfusion vs rapid freezing on cardiac AMPK and ACC activity and cardiac adiponectin and its receptor levels are needed.

In summary, this study demonstrates that the plasma adiponectin level is decreased, but the cardiac protein expression of adipoR1 is increased in diabetic rats. Despite an increase in cardiac adipoR1 expression, there is an increased cardiac inflammatory response and a decreased GLUT4 protein expression due to reduced circulating adiponectin. The dysfunction of energy metabolism and the increased inflammatory factors may contribute to the deterioration in cardiac function observed in STZ-diabetic rats.

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