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Research Paper

Effect of telmisartan on the expression of cardiac adiponectin and its receptor 1 in type 2 diabetic rats

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Abstract

Objectives This study investigated the effect of telmisartan on the expression of cardiac adiponectin and its receptor 1 in type 2 diabetic rats.

Methods Thirty-six male Wistar rats were randomly divided into control (C, n = 10) and diabetic (n = 26) groups. Type 2 diabetes was induced by high-fat and high-sugar diet and intraperitoneal injection of a low dose of streptozotocin (STZ). After induction of diabetes, diabetic rats were again randomly divided into diabetic (D, n = 10) and diabetic treated (DT, n = 10) groups. Telmisartan (5 mg/kg/day) was administrated to diabetic treated rats by gavage for 12 weeks. Twelve weeks later, the heart function was investigated. Plasma and myocardial adiponectin levels were detected by enzyme-linked immunosorbent assay (ELISA). The cardiac mRNA expression of adiponectin receptor 1 (adipiR1) was assayed by reverse transcript–polymerase chain reaction (RT-PCR). The cardiac protein expression of adipiR1, AMP-activated protein kinase (AMPK)-α, phospho-AMPK-α (Thr172) and glucose transporter 4(GLUT4) was determined by Western blotting.

Key findings The ratio of heart weight to body weight was significantly increased in diabetic rats compared with control. The decreased levels of plasma and myocardial adiponectin and the decreased protein and mRNA expression of myocardial adipoR1 led to the decreased myocardial phosphorylation of AMPK-α (Thr172) and the decreased protein expression of myocardial GLUT4 in diabetic rats. Consequently, the heart function was decreased in diabetic rats. Telmisartan treatment significantly attenuated the increased ratio of heart weight to body weight in diabetic rats. The levels of plasma and myocardial adiponectin and the expression of myocardial adipoR1 in diabetic rats were upregulated by telmisartan. Subsequently, the levels of myocardial phospho-AMPK-α (Thr172) and the expression of myocardial GLUT4 in diabetic rats were increased by telmisartan. Consequently, the heart function was improved in diabetic rats treated with telmisartan.

Conclusions These results suggest that the levels of myocardial adiponectin and its receptor 1 are decreased in type 2 diabetic rats. Telmisartan treatment up-regulates the levels of myocardial adiponectin and its receptor 1, resulting in the increase in myocardial phospho-AMPK-α (Thr172) and GLUT4 expression, which may contribute to the improvement of heart function and the decrease in cardiac hypertrophy in diabetic rats.

Keywords adiponectin receptor; cardiomyopathy; telmisartan; type 2 diabetes

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 healthcare system.[1] Increased fatty acid oxidation and decreased glucose metabolism contribute to the development of diabetic cardiomyopathy and can decrease the ability of the heart to withstand an ischaemic insult.[2]

Adiponectin is an adipocyte-derived protein with anti-inflammatory, anti-diabetic and anti-atherogenic properties.[3] Adiponectin is also synthesized and secreted by human and murine cardiomyocytes. Local production of adiponectin by cardiomyocytes might play an important role in the regulation of the cardiac function or metabolism by autocrine or paracrine mechanisms.[4]

There are two types of adiponectin receptor–adiponectin receptor type 1 and adiponectin receptor type 2. They serve as receptors for globular and full-length adiponectin, and mediate increased AMP kinase and PPAR-alpha ligand activity, as well as fatty-acid oxidation and glucose uptake by adiponectin. Adiponectin receptor type 1 and adiponectin

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receptor type 2 are not only expressed in skeletal muscle and liver, but also in heart and kidney.[5] It was reported that the expression of adiponectin receptor type 1 was increased in the heart in STZ-induced type 1 diabetic rats.[6] It is unknown whether the expression of adiponectin and its receptors is also changed in type 2 diabetic rat hearts.

Telmisartan, a unique angiotensin II receptor antagonist with selective peroxisome proliferator-activated receptor gamma(PPARgamma)-modulating activity, functions as a partial agonist of PPARgamma and achieves 25–30% of maximal receptor activation attained with conventional PPARgamma ligands.[7,8] Telmisartan produces a significant reduction in left ventricular mass index that is accompanied by an improvement in the characteristics of diastolic function in patients with essential hypertension and left ventricular hypertrophy.[9] Telmisartan increases plasma adiponectin level in hypertensive patients with type 2 diabetes[10] and also stimulates adiponectin protein expression in murine 3T3-L1 adipocytes.[11] It is unknown whether the expression of myocardial adiponectin and its receptors are influenced by telmisartan in type 2 diabetic rat hearts.

This study aimed: (1) to explore the expression of cardiac adiponectin and its receptors in type 2 diabetic rats induced by feeding with high-fat and high-sugar diet and then injecting a low dose of streptozotocin (STZ) by abdominal cavity; (2) to investigate the effect of telmisartan on the plasma and myocardial adiponectin levels and the protein expression of cardiac adiponectin receptors in type 2 diabetic rats.

Materials and Methods

Induction of diabetes

Thirty-six male Wistar rats, 140–180 g, purchased from Physiological Laboratory of Shan Xi Medical University (Taiyuan, Shanxi, China), were used in the study. All rats were housed in a temperature-controlled room (22–24°C) and kept on a 12-h light–dark cycle. All rats received humane care in accordance with the principles of the Chinese Council on Animal Care. After two week's adaptation, all rats were randomly divided into two groups: control (C, n = 10) and diabetic (n = 26). Control rats were fed with standard rat chow. Diabetic rats were fed with high-fat chow (ingredients: 10% refined lard, 20% sucrose, 2% cholesterol, 1% sodium cholate and 67% common food), which was provided by Animal Experimental Centre of Shanxi Medical University. Four weeks later, diabetic rats were given a peritoneal injection of a low dose of streptozotocin (30 mg/kg body weight; Sigma, St Louis, USA),[11] while the control group was given an equivalent volume of citric acid buffer. One week after STZ injection, fasting plasma glucose (FPG) was tested and the rats with FPG $\geq$ 7.8 mmol/l and insulin resistance were considered to be diabetic (n = 20).[12] Diabetic rats were again randomly divided into diabetic (D, n = 10) and diabetic treated (DT, n = 10) groups. Telmisartan (5 mg/kg/day; Boehringer Ingelheim, Germany)[13] was administered to diabetic treated rats by gavage for 12 weeks. The equivalent volume of normal sodium was administered to control and diabetic rats by lavage for 12 weeks. Subsequently, blood samples were collected every two weeks. Blood was centrifuged at 10 000*g* for 45 min, plasma was collected and plasma glucose levels were measured. At termination, one day before the experiments were finished, all rats were fasted for 12–14 h and then were anaesthetized with an intraperitoneal injection of 10% chloral hydrate (0.3 ml/100 g body weight). The maximum descent-speed of pressure in isovolumetric relaxation period in the left ventricle (−dp/dtmax) and the maximum ascendant-speed of pressure in isovolumetric contraction period in the left ventricle (+dp/dtmax) were measured by carotid artery cannula. The rats were sacrificed after blood sample had been withdrawn from the abdominal cardinal vein. The blood sample was centrifuged and plasma was stored at −80°C until assays were done. The heart was immediately taken out of the thoracic cavity after the rat was sacrificed. The heart was rinsed with normal sodium and dried by filter-paper and then weighed. The ratio of heart to body weight was calculated. The apex of heart was fixed in 10% neutral buffered formalin and processed for histological analysis. The remaining part of the cardiac ventricle was immediately thrown into the liquid nitrogen and stored at −70°C until analyses were carried out.

Measurement of cardiac function

Following anaesthesia of rats with an intraperitoneal injection of 10% chloral hydrate (0.3 ml/100 g), the neck skin was cut open and the right common carotid artery was fully exposed. A micromanometer-tipped catheter was inserted into the left ventricle through the right common carotid artery for measurement of left ventricular pressure. Left ventricular end-diastolic pressure (LVEDP) and the maximal rate of rise and decline of ventricular pressure ($\pm$dp/dt [max]) were obtained by BL-410 Bio-signal analysis system (Chengdu TME Technology Co., Ltd, Sichuan, China).

Plasma analytical procedures

The plasma glucose level was measured by the glucose oxidase method using an autoanalyser (Beckman Instruments, Miami, USA). Plasma cholesterol, triglyceride and free fatty acid were measured colorimetrically by using commercially available kit (SiRuiKe Biotechnology Co., Ltd, Shanghai, China). Plasma adiponectin was measured by using a commercially available ELISA kit (Westang Biotechnology Co., Ltd, Shanghai, China). Plasma insulin was measured by using a commercially available radioimmunoassay kit (China Institute of Atomic Energy, Beijing, China). Insulin sensitivity index (ISI) was calculated as the following formula: $ISI = In[1/(FPG \times FINS)]$.

Determination of cardiac adiponectin

Frozen heart tissue was pulverized and homogenized at 4°C in cold buffer (20 mM Tris-HCl, pH 7.5, 50 mM 2-mercaptoethanol, 5 mM ethylene glycol tetraacetic acid, 2 mM ethylenediaminetetraacetic acid, 1 mM phenylmethanesulfonyl fluoride, 10 mM sodium fluoride, 25 μg/ml leupeptin, 2 μg/ml aprotinin) and then centrifuged at 1500*g* for 5 min 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 protein assay[14] with the use of bovine serum albumin as a standard. Cardiac adiponectin was measured using a commercially available ELISA kit (Westang Biotechnology Co., Ltd, Shanghai, China).

Morphologic study

Tissues fixed in 10% buffered formalin were embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin (HE) for light microscopic morphologic study.

Immunohistochemical analysis of adiponectin receptor type 1

Ventricular samples were immediately fixed in 10% neutral buffered formalin overnight and embedded in paraffin. Paraffin-embedded tissue blocks were sectioned at 3 μm and sections were mounted on positively charged slides. The slides were de-paraffinized, rehydrated, blocked with 3% hydrogen peroxide (to block endogenous peroxidase activity, washed with phosphate-buffered saline (PBS)), and blocked with 5% normal goat serum in PBS for 30 min. The slides were subsequently incubated with primary rabbit polyclonal adiponectin receptor type 1 antibody (1 : 200; Beijing Biosynthesis Biotechnology Co., Ltd, Beijing, China) in PBS containing 1% normal goat serum overnight at 4°C. The primary antibody was rinsed off with PBS, and the sections were incubated with biotin-labelled goat anti-rabbit secondary antibodies (1 : 100; Zhongshan Goldenbridge Biotechnology Co., Ltd, Beijing, China) for 30 min. After three washing steps in PBS were completed, the sections were stained using horseradish enzyme-labelled strepto-avidin solution (1 : 100; Zhongshan Goldenbridge Biotechnology Co., Ltd, Beijing, China) for 10 min, washed with PBS, coloured with 3,3′-diaminobenzidine (DAB), and washed with distilled water. The sections were counterstained using hematoxylin, washed in running water, dehydrated in increasing grades of alcohol, and cleared in xylene before being mounted in resinous mounting medium with cover-slips. Some sections incubated with nonspecific rabbit immunoglobulins (IgG) served as negative controls. Quantification was performed with the observer blinded to details. With the use of high power microscope, all slides were observed and photographed. The average of five fields under microscope (×400) in each slice was randomly selected, and the positive stained intensity was quantified by using BI-2000 color image processing system (Chengdu TME Technology Co., Ltd, Sichuan, China).

Semi-quantitative reverse transcription–PCR (RT-PCR)

Total RNA extraction was performed using Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's recommendations. The primers of adiponectin receptor type 1 were: sense: 5′-AACTGGACTATTCAGGGA-3′; antisense: 5′-TGGTTCCAGTCTCATCAG-3′. The length of PCR product was 398 bp.

GAPDH gene was an internal control, its primers were: sense: 5′-ATGGTGAAGGTCGGTGTG-3′; antisense: 5′-AACTTGCCGTGGGTAGAG-3′. The length of PCR product was 161 bp. The PCR reaction was initiated by incubation at 94°C for 5 min, 30 cycles for denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s, then terminated after a 10-min extension at 72°C. Ten microlitres of each PCR reaction mixture were electrophoresed in a 1.5% agarose gel and bands were visualized by ethidium bromide staining. The density of the DNA bands of the PCR products was analysed by using the software (Tianjin DiDe Technology Co., Ltd, Tianjin, China).

Western blotting

Heart tissue (100 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 15 000*g* for 20 min at 4°C and the supernatant was collected and labelled as total preparation. The homogenate was further centrifuged at 100 000*g* for 60 min at 4°C and the supernatant was collected and labelled as cytosol preparation. The pellet was re-suspended in buffer containing 1% NP-40, 0.1% SDS and 0.5% deoxycholic acid, homogenized, incubated on ice for 30 min and then centrifuged at 100 000*g* for 60 min at 4°C. The supernatant was collected and labelled as membrane preparation. The protein content of each fraction was measured using the Bradford protein assay.[14] Samples were stored at −80°C until use.

After boiling at 95°C for 5 min, samples (50 μg protein/lane) were subjected to 10–15% SDS-PAGE gel and then transferred to nitrocellulose membranes. The membranes were blocked in 5% non-fat milk in TBST (0.05% Tween-20 in 1 × Tris-buffered saline) and then incubated with primary antibodies against: adiponectin receptor type 1 (1 : 400) (Abcam Inc., Cambridge, USA); AMPK-α (1 : 1000), phospho-AMPK-α (1 : 1000) (Cell Signalling Technology, Beverly, USA); and glucose transporter 4 (1 : 2500; Abcam Inc., Cambridge, USA). The membranes were washed and then incubated with a secondary horseradish peroxidase-conjugated anti-rabbit IgG antibody (Santa Cruz Biotechnology Inc., Santa Cruz, USA). Proteins were visualized by DAB (Zhongshan Goldenbridge Biotechnology Co., Ltd, Beijing, China) and quantified by using image analysis software. In all instances, the membranes were stained with Ponceau stain and reblotted with antibody against GAPDH (1 : 1000; Santa Cruz Biotechnology Inc., Santa Cruz, USA) after stripping to verify the uniformity of protein load and the transfer efficiency across the test samples.

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance followed by Tukey's post-hoc test. $P < 0.05$ was considered statistically significant.

Results

Animal characteristics

Plasma glucose levels were significantly increased in diabetic rats compared with controls. Telmisartan treatment slightly but significantly reduced the plasma glucose level in diabetic rats. The levels of plasma insulin, total cholesterol, triglycerides and free fatty acid were significantly increased in diabetic rats compared with controls and telmisartan treatment prevented these changes in diabetic rats. Insulin sensitivity index (ISI) was significantly reduced in diabetic rats compared with controls and telmisartan treatment significantly increased the ISI in diabetic rats. The ratio of heart to body weight, an index

Table 1 General characteristics in control and diabetic rats with or without telmisartan treatment

	C	D	DT
Plasma glucose (mM)	4.02 ± 0.44	17.55 ± 0.76*	13.62 ± 0.77*#
Plasma insulin (IU/ml)	23.44 ± 1.47	43.06 ± 1.24*	30.78 ± 0.72*#
Plasma total cholesterol (mM)	1.41 ± 0.06	2.80 ± 0.12*	2.04 ± 0.05#
Plasma triglycerides (mM)	0.86 ± 0.10	1.49 ± 0.74*	1.04 ± 0.52#
Plasma free fatty acid (mM)	1.48 ± 0.23	3.16 ± 0.28*	2.17 ± 0.15#
Insulin resistance index	−4.55 ± 0.14	−6.59 ± 0.17*	−5.79 ± 0.08*#
Body weight (g)	605.6 ± 11.08	455.3 ± 11.78*	562.7 ± 8.46#
Heart weight (g)	1.93 ± 0.66	2.00 ± 0.45*	1.97 ± 0.21
Heart/body weight (g/kg)	2.99 ± 0.6	4.38 ± 0.07*	3.49 ± 0.39#

Values were obtained at sacrifice, after administration with or without 12 weeks of telmisartan treatment. Control (C), diabetic (D), diabetic treated (DT). All values were expressed as mean ± SEM, n = 10 per group. *$P < 0.05$ vs C; #$P < 0.05$ vs D.

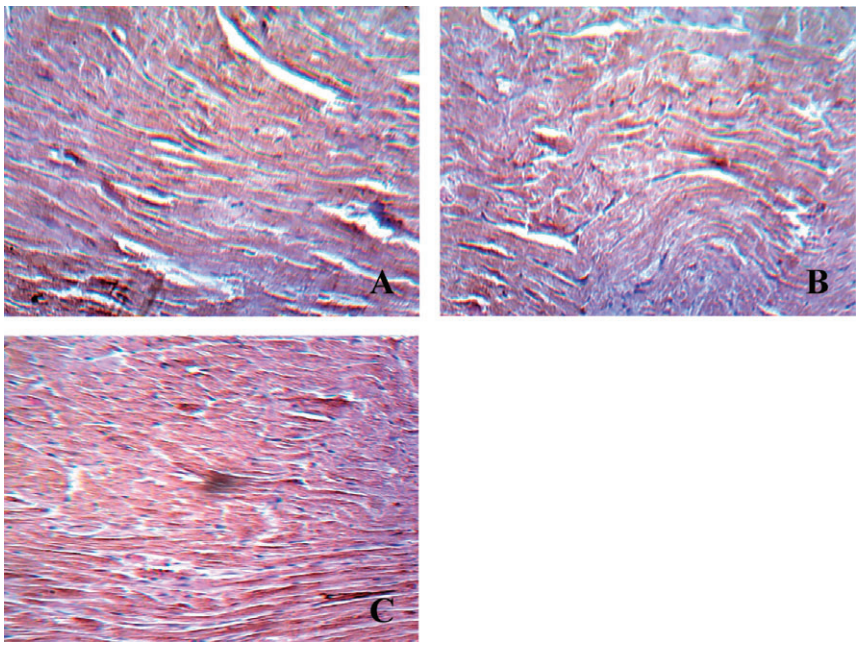

Figure 1 Representative slides showing HE staining in the myocardium of rats. Slides A, B, C represent control, diabetic and diabetic treated with telmisartan, respectively. Amplifications ×200.

of cardiac hypertrophy, was significantly increased in diabetic rats compared with controls and telmisartan treatment significantly decreased the ratio of heart to body weight in diabetic rats (Table 1).

Histological changes

Observation of the myocardium, stained by hematoxylin and eosin (HE), through the light microscope in diabetic rats revealed the following: the myocardial cells lined up in order, the size of cellular nucleus was the same and the cytoplasm was stained in uniformity in control rats (group C); the myocardial cells lined up in disorder, the size of cellular nucleus was irregular, and the myocardial fibre was broken and lined up in disorder in diabetic rats (group D); the pathological changes were obviously improved in diabetic rats treated with telmisartan (group DT) compared with diabetic rats untreated (group D); the myocardial cells comparatively lined up in order, the size of cellular nucleus was comparatively regular and the obviously broken myocardial fibre was unseen in diabetic rats treated with telmisartan (group DT) compared with diabetic rats untreated (group D) (Figure 1).

Heart function

Compared with controls, +dp/dtmax and −dp/dtmax were significantly reduced and LVEDP was significantly increased in diabetic rats, indicating that the heart function was significantly decreased in diabetic rats. Telmisartan treatment attenuated these changes in diabetic rats (Table 2).

Immunohistochemical assay

The yellow positive staining of adiponectin receptor 1 was mainly located in myocardial cellular membrane and cytoplasm. The integrated optical density (IOD) of positive staining of adiponectin receptor 1 was significantly decreased in diabetic rats compared with controls. Telmisartan treatment

Table 2 Heart function, plasma and myocardial adiponectin levels in control and diabetic rats with or without telmisartan treatment

	C	D	DT
LVEDP (kPa)	0.09 ± 0.01	0.22 ± 0.01*	0.13 ± 0.16#
+dP/dtmax (kPa/s)	611.9 ± 5.34	471.1 ± 6.81*	547.4 ± 2.86#
−dP/dtmax (kPa/s)	416.8 ± 2.96	289.5 ± 5.15*	354.5 ± 4.95#
Plasma adiponectin(μg/ml)	1.87 ± 0.05	1.09 ± 0.03*	1.41 ± 0.02*#
Myocardial adiponectin(μg/mg protein)	0.21 ± 0.02	0.12 ± 0.02*	0.16 ± 0.01*#

Values were obtained at sacrifice, after administration with or without 12 weeks of telmisartan treatment. Control (C), diabetic (D), diabetic treated (DT). All values were expressed as mean ± SEM, n = 10 per group. *$P < 0.05$ vs C; #$P < 0.05$ vs D.

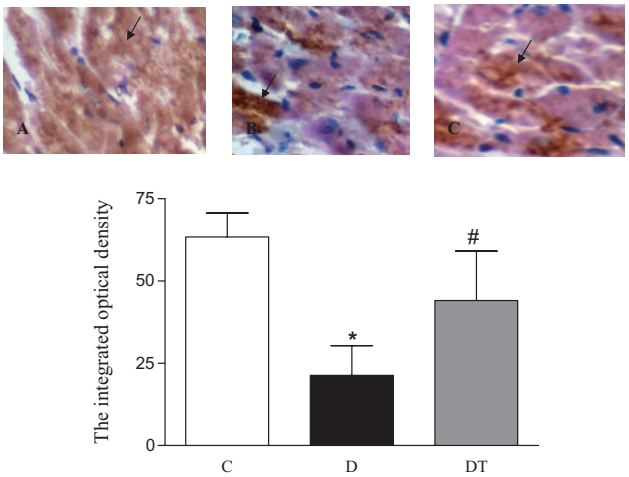

Figure 2 Top panel: representative slides showing immunohistochemical staining of AdipoR1 (as shown by arrow) in the rat myocardium. Slides A, B, C represent control, diabetic and diabetic treated with telmisartan, respectively. Amplifications ×400.Bottom: bar graph shows quantitative analysis of myocardial adipoR1 expression in control and diabetic rats treated with or without telmisartan. Control (C), diabetic (D), diabetic treated (DT). Data are expressed as mean ± SEM, n = 10 per group. *$P < 0.05$ compared with control, #$P < 0.05$ compared with diabetic.

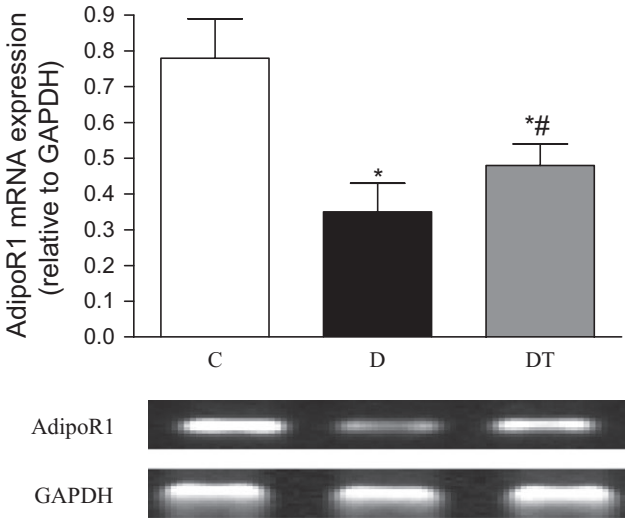

Figure 3 RT-PCR analysis of myocardial mRNA expression of adipoR1 in control and diabetic rats treated with or without telmisartan. Mean band density was normalized relative to GAPDH. Control (C), diabetic (D), diabetic treated (DT). Data are expressed as mean ± SEM, n = 10 per group. *$P < 0.05$ compared with control, #$P < 0.05$ compared with diabetic.

significantly increased the IOD of positive staining of adiponectin receptor 1 in diabetic rats (Figure 2).

Plasma and myocardial adiponectin levels

Plasma and myocardial adiponectin levels were significantly decreased in diabetic rats compared with controls. Telmisartan treatment significantly increased plasma and myocardial adiponectin levels in diabetic rats (Table 2).

Myocardial mRNA and protein expression of adiponectin receptor 1

The mRNA and protein expression of myocardial adiponectin receptor 1 was significantly reduced in diabetic rats compared with controls. Telmisartan treatment significantly increased the mRNA and protein expression of myocardial adiponectin receptor in diabetic rats (Figures 3 and 4).

Myocardial phospho-AMPK-alpha (Thr172) and GLUT4 protein expression

The myocardial phosphorylation of AMPK-alpha (Thr172) and the myocardial protein expression of GLUT4 were significantly decreased in diabetic rats compared with controls. Telmisartan treatment significantly increased the myocardial phosphorylation of AMPK-alpha (Thr172) and the myocardial protein expression of GLUT4 in diabetic rats (Figures 5 and 6).

Discussion

This study showed that plasma and myocardial adiponectin levels and the myocardial protein and mRNA expression of adipoR1 were decreased in type 2 diabetic rats compared with controls. Telmisartan treatment prevented these changes in diabetic rats. To our knowledge, this is the first report on the effect of telmisartan on the expression of myocardial adiponectin receptor in diabetic rats.

Adiponectin is mainly produced and secreted by adipose tissues.[15] However, it has also been shown to be synthesized and secreted by human and murine cardiomyocytes.[16] Our results show that the levels of plasma and myocardial adiponectin are significantly decreased in type 2 diabetic rats induced by feeding with high-fat and high-sugar diet and then injecting a low dose of streptozotocin (STZ) by abdominal

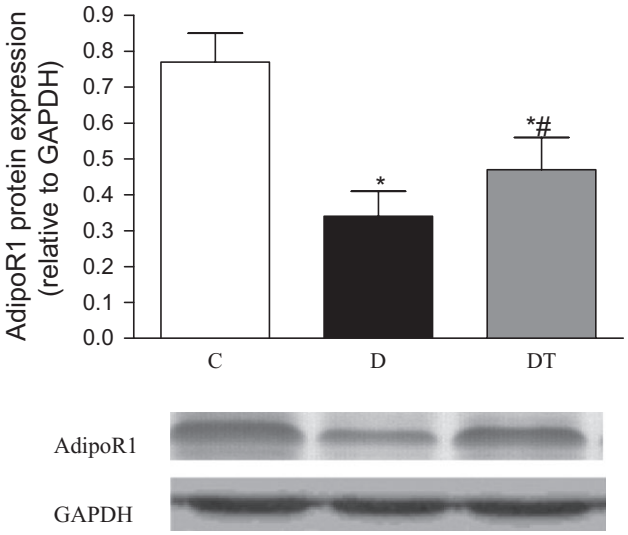

Figure 4 Western blot analysis of myocardial protein expression of adipoR1 in control and diabetic rats treated with or without telmisartan. Equal protein loading was confirmed with GAPDH. Mean band density was normalized relative to GAPDH. Control (C), diabetic (D), diabetic treated (DT). Data are expressed as mean ± SEM, n = 10 per group. *$P < 0.05$ compared with control, #$P < 0.05$ compared with diabetic.

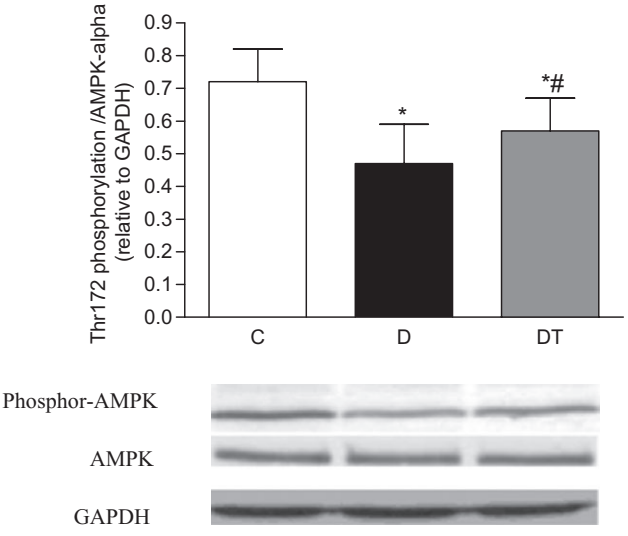

Figure 5 Western blot analysis of cardiac protein expression of phospho-AMPK-alpha (Thr172) and AMPK-alpha in control and diabetic rats treated with or without telmisartan. Equal protein loading was confirmed with GAPDH. Mean band density was normalized relative to GAPDH. Control (C), diabetic (D), diabetic treated (DT). Data are expressed as mean ± SEM, n = 10 per group. *$P < 0.05$ compared with control, #$P < 0.05$ compared with diabetic.

cavity. It has been reported that insulin, oxidative stress and angiotensin II down-regulates adiponectin expression.[16,17] Therefore, the decreased plasma and myocardial adiponectin in diabetic rats in this study may be attributable to insulin resistance, increased oxidative stress and angiotensin II levels. Adiponectin secreted by cardiomyocytes is reported to regulate heart function and myocardial metabolism by autocrine

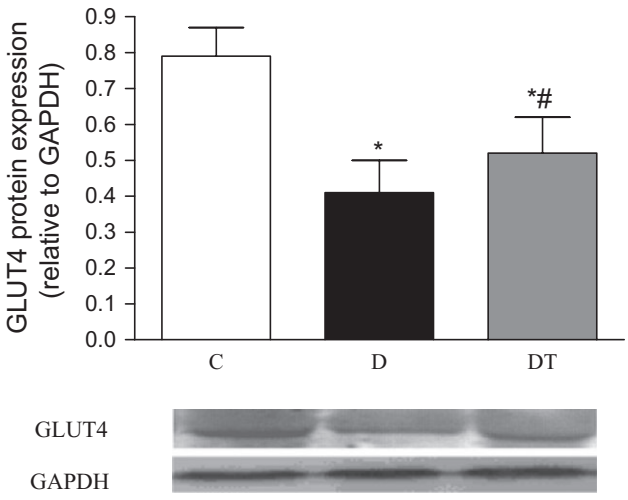

Figure 6 Western blot analysis of myocardial protein expression of GLUT4 in control and diabetic rats treated with or without telmisartan. Equal protein loading was confirmed with GAPDH. Mean band density was normalized relative to GAPDH. Control (C), diabetic (D), diabetic treated (DT). Data are expressed as mean ± SEM, n = 10 per group. *$P < 0.05$ compared with control, #$P < 0.05$ compared with diabetic.

and paracrine means.[4] Pressure overload in adiponectin-deficient mice results in enhanced concentric cardiac hypertrophy, which is attenuated by adiponectin supplementation.[18] Adiponectin improves cardiomyocyte contractile function in *db/db* diabetic obese mice.[19] Therefore, the decreased plasma and myocardial adiponectin levels may contribute to the cardiac hypertrophy and the decreased heart function in diabetic rats in this study.

Adiponectin exerts its effect through adiponectin receptors 1 and 2, which are both expressed in the heart *in vivo*[5] and in the cardiomyocyte *in vitro*.[4] Our study shows that the mRNA and protein expression of myocardial adipoR1 is decreased in diabetic rats. The decreased adipoR1 in the heart of diabetic rats may be due to hyperinsulinaemia since it has been reported that insulin deficiency increased, but insulin replenishment decreased the expression of adipoR1/2 in animals *in vivo*.[20] The decreased adiponectin and its receptor 1 in the heart of diabetic rats may lead to adiponectin resistance,[21] which may limit adiponectin's biological effects, especially its protective effect on diabetic heart. It was reported that the decreased levels of adiponectin and AdipoR1 in obesity may have a causal role in mitochondrial dysfunction and insulin resistance seen in diabetes.[22] Therefore, the decreased adiponectin and its receptor may together play a role in the occurrence and progression of cardiomyopathy in type 2 diabetic rats.

Adiponectin can improve both glucose metabolism and insulin resistance via the AMP-activated protein kinase (AMPK) signalling pathway.[23] Targeted disruption of AdipoR1 results in the abrogation of adiponectin-induced AMPK activation.[21,22] Our study shows that myocardial phosphorylation of AMPK-α (thr172) is decreased in diabetic rats. The decreased cardiac phosphorylation of AMPK may contribute to the depressed cardiac function and myocardial hypertrophy in diabetic rats since AMPK deficiency has been

reported to be associated with depressed cardiac function under stress conditions.[24] The decreased plasma and myocardial adiponectin levels and the decreased myocardial AdipoR1 may partly explain the decreased cardiac phosphorylation of AMPK-α (Thr172) in diabetic rats.

AMPK is reported to be an important mediator of glucose metabolism.[25] Glucose enters the heart via the facilitative glucose transporters GLUT1 and GLUT4.[26] AMPK increases glucose transport by stimulating the translocation of GLUT4 to the sarcolemma in the heart.[27] Glucose transporter expression in the heart is altered in various pathological states. Our study shows that the protein expression of total crude membrane GLUT4 is decreased, indicating that glucose metabolism would be reduced in diabetic rat hearts. The decreased GLUT4 may be due to the reduced phosphorylation of AMPK in the hearts of diabetic rats. It was reported that changes in glucose transporter expression contributed to myocardial dysfunction in diabetes.[28] In addition, GLUT4-deficient mice developed striking cardiac hypertrophy.[29] Normalization of glucose homoeostasis by transgenic re-expression of GLUT4 in the skeletal muscle resulted in a reversal of the cardiac pathology in mice heterozygous for GLUT4 ablation.[30] 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 diabetic rats.

Telmisartan, a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity, functions as a partial agonist of PPARgamma.[7,8] We showed in this study that telmisartan treatment reduces plasma cholesterol and triglyceride levels, decreases the cardiac hypertrophy index, improves the heart function and prevents the myocardial pathological changes in diabetic rats. The results of our study are in agreement with the results of a recent study[13] showing that telmisartan treatment produces beneficial effect on heart by reducing plasma lipids and preventing cardiac hypertrophy in STZ-induced diabetic rats.

It has been reported that PPARgamma ligands increase expression and plasma concentrations of adiponectin in humans and rodents *in vivo* and *in vitro*.[31] Adiponectin receptors were expressed in human atherosclerotic lesions and macrophages and could be modulated by agonists of the nuclear receptors PPARalpha, PPARgamma and LXR.[32] Telmisartan augments glucose uptake and GLUT4 protein expression in 3T3-L1 adipocytes.[33] Our study shows that telmisartan treatment increases the levels of plasma and myocardial adiponectin, myocardial phosphorylation of AMPK-α (thr172) and the protein expression of myocardial AdipoR1 and GLUT4 in diabetic rats, indicating that angiotensin II could down-regulate, while PPARgamma could up-regulate, the expression of adiponectin and its receptors. The results of our study are in agreement with the results of Ding *et al*.,[34] who reported that adiponectin and its receptors were expressed in adult ventricular cardiomyocytes and upregulated by activation of peroxisome proliferator-activated receptor gamma.

Conclusions

In summary, this study demonstrates that cardiac hypertrophy index and the plasma lipid levels are increased and that the plasma and myocardial adiponectin levels, the cardiac protein expression of adipoR1 and the heart function are decreased in diabetic rats. The decreased adiponectin and its receptors lead to the decreased cardiac phosphorylation of AMPK-α (Thr172) and the subsequent decrease in cardiac GLUT 4 protein expression, which may contribute to the deterioration of heart function and the cardiac hypertrophy in diabetic rats. Telmisartan treatment up-regulates the levels of myocardial adiponectin and its receptor 1, resulting in the increase in myocardial phosphor-AMPK-α (Thr172) and GLUT4 expression, which may contribute to the improvement of heart function and the decrease in cardiac hypertrophy in diabetic rats.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. American Diabetes Association. Economic consequences of diabetes mellitus in the U.S. in 1997. *Diabetes Care* 1998; 21: 296–309.
2. Lopaschuk GD. Metabolic abnormalities in the diabetic heart. *Heart Fail Rev* 2002; 7: 149–159.
3. Trujillo ME, Scherer PE. Adiponectin – journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *J Intern Med* 2005; 257: 167–175.
4. Piñeiro R *et al*. Adiponectin is synthesized and secreted by human and murine cardiomyocytes. *FEBS Lett* 2005; 579: 5163–5169.
5. Yamauchi T *et al*. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; 423: 762–769.
6. Guo Z *et al*. Cardiac expression of adiponectin and its receptors in streptozotocin-induced diabetic rats. *Metabolism* 2007; 56: 1363–1371.
7. Kurtz TW. Treating the metabolic syndrome: telmisartan as a peroxisome proliferator-activated receptor-gamma activator. *Acta Diabetol* 2005; 42(Suppl. 1): S9–16.
8. Benson SC *et al*. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. *Hypertension* 2004; 43: 993–1002.
9. Conrady AO *et al*. Effect of 24-week treatment with telmisartan on myocardial structure and function: relationship to insertion/deletion polymorphism of the angiotensin-converting enzyme gene. *J Int Med Res* 2005; 33(Suppl. 1): 30A–38A.
10. Mori Y *et al*. Telmisartan improves lipid metabolism and adiponectin production but does not affect glycemic control in hypertensive patients with type 2 diabetes. *Adv Ther* 2007; 24: 146–153.
11. Clasen R *et al*. PPARgamma-activating angiotensin type-1 receptor blockers induce adiponectin. *Hypertension* 2005; 46: 137–143.
12. Zhang M *et al*. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. *Exp Diabetes Res* 2008; 2008: 704045.

13. Goyal BR *et al*. Effect of telmisartan on cardiovascular complications associated with streptozotocin diabetic rats. *Mol Cell Biochem* 2008; 314: 123–131.
14. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248–254.
15. Maeda K *et al*. cDNA cloning and expression of a novel adipose specific collage-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* 1996; 221: 286–289.
16. Hattori Y *et al*. Angiotensin-II-induced oxidative stress elicits hypoadiponectinaemia in rats. *Diabetologia* 2005; 48: 1066–1074.
17. Sun X *et al*. Negative regulation of adiponectin receptor 1 promoter by insulin via a repressive nuclear inhibitory protein element. *FEBS Lett* 2008; 582: 3401–3407.
18. Shibata R *et al*. Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat Med* 2004; 10: 1384–1389.
19. Dong F, Ren J. Adiponectin improve cardiomyocyte contractile function in db/db diabetic obese mice. *Obesity (Silver Spring)* 2009; 17: 262–268.
20. Tsuchida A *et al*. Insulin / Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. *J Biol Chem* 2004; 279: 30817–30822.
21. Yamauchi T *et al*. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med* 2007; 13: 332–339.
22. Iwabu M *et al*. Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1. *Nature* 2010; 464: 1313–1319.
23. Yamauchi T *et al*. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; 8: 1288–1295.
24. Liao Y *et al*. Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. *Cardiovasc Res* 2005; 67: 705–713.
25. Rutter GA *et al*. Roles of 5′-AMP-activated protein kinase (AMPK) in mammalian glucose homeostasis. *Biochem J* 2003; 375: 1–16.
26. Mueckler M. Family of glucose transporter genes. Implications for glucose homeostasis and diabetes. *Diabetes* 1990; 39: 6–11.
27. Russell RR *et al*. Translocation of myocardial GLUT4 and increased glucose uptake through activation of AMPK by AICAR. *Am J Physiol Heart Circ Physiol* 1999; 277: H643–H649.
28. Garvey WT *et al*. Effects of diabetes on myocardial glucose transport system in rats: implications for diabetic cardiomyopathy. *Am J Physiol* 1993; 264: H837–H844.
29. Abel ED *et al*. Cardiac hypertrophy with preserved contractile function after selective deletion of GLUT4 from the heart. *J Clin Invest* 1999; 104: 1703–1714.
30. Tsao TS *et al*. Prevention of insulin resistance and diabetes in mince heterozygous for GLUT4 ablation by transgenic complementation of GLUT4 in skeletal muscle. *Diabetes* 1999; 48: 775–782.
31. Maeda N *et al*. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 2001; 50: 2094–2099.
32. Chinetti G *et al*. Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors PPARalpha, PPARgamma, and LXR. *Biochem Biophys Res Commun* 2004; 314: 151–158.
33. Fujimoto M *et al*. An angiotensin II AT1 receptor antagonist, telmisartan augments glucose uptake and GLUT4 protein expression in 3T3-L1 adipocytes. *FEBS Lett* 2004; 576: 492–497.
34. Ding G *et al*. Adiponectin and its receptors are expressed in adult ventricular cardiomyocytes and upregulated by activation of peroxisome proliferator-activated receptor gamma. *J Mol Cell Cardiol* 2007; 43: 73–84.