

Original Article

Fibrosis and cardiac dysfunction in insulin-resistant rats: changes in myocardial type I collagen and telmisartan's effects

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Abstract: Myocardial fibrosis may be an important contributor to the association of insulin-resistance with cardiac dysfunction. This study aimed to investigate the effects of telmisartan on cardiac function and myocardial type I collagen of insulin-resistant rats. Wistar rats were divided into control, control + telmisartan, insulin-resistant, and insulin-resistant + telmisartan groups. After 34 weeks of intervention, carotid catheterizations were performed to measure left ventricular end diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), maximum rise rates of left ventricular pressure (+dP/dtmax), and maximum drop rates of left ventricular pressure (-dP/dtmax). Masson's staining was used for analysis of myocardial collagen. RT-PCR was used to analyze expression of myocardial type I collagen mRNA. Western blot was employed to analyze expression of myocardial type I collagen, AT1R, ERK1/2, TGF- β 1, and p-Smad2 and p-Smad3 proteins. The insulin-resistant group had increased LVEDP, decreased -dP/dtmax ($P < 0.01$), and increased collagen volume fraction of the left ventricle ($P < 0.01$). Expression of myocardial type I collagen mRNA and proteins in the insulin-resistant group increased significantly ($P < 0.05$), while expression of AT1R, ERK1/2, TGF β 1, and pSmad2/3 in myocardium increased significantly ($P < 0.05$). No corresponding alterations were found in control groups. The insulin-resistant + telmisartan group had decreased LVSP and LVEDP ($P < 0.01$) and increased dP/dtmax ($P < 0.05$), along with decreased left ventricle collagen volume fraction ($P < 0.01$), compared to the insulin-resistant group and control + telmisartan group. Expression of myocardial type I collagen mRNA and proteins decreased significantly ($P < 0.05$), while expression of AT1R, ERK1/2, TGF β 1, and pSmad2/3 in myocardium decreased significantly ($P < 0.05$). Insulin resistance may reduce heart diastolic function by increasing myocardial collagen deposition. TGF- β 1/ERK1/2/pSmad2/3 signaling pathways play an important role on this. Telmisartan can improve myocardial fibrosis in insulin-resistant rats. The effects are independent of temisartan's depressurization function.

Keywords: Insulin resistance, cardiac diastolic function, collagen, remodeling, telmisartan

Introduction

Insulin resistance is a pathological condition in which the cells fail to respond correctly to physiological levels of insulin. In the case of insulin resistance, the cells fail to respond to insulin, leading to high blood glucose levels [1]. Insulin resistance is a major metabolic abnormality, driving the risk of cardiovascular diseases independently of type 2 diabetes [2, 3]. The eventual development of type 2 diabetes will further increase cardiovascular risks. About 70-80% of

diabetic patients will die of cardiovascular diseases [4, 5], including patients in China [6].

Insulin resistance can cause abnormal heart function, mainly manifesting as diastolic dysfunction [7, 8]. Angiotensin II not only promotes the development and progression of cardiovascular disease, but also plays a significant role during the development of insulin resistance [9]. Indeed, animal studies of insulin-resistance have shown that elevated angiotensin II in local tissues increases the reactive oxygen free radi-

cals, resulting in vascular inflammation, insulin resistance, myocardial apoptosis, and ventricular remodeling [10]. However, this process can be reverted by selective AT1 receptor antagonists, such as telmisartan [11] and PPAR- γ partial agonists [12-15]. Previous studies have shown that telmisartan reduces unfavorable cardiac remodeling through reduced cardiac hypertrophy and fibrosis [16]. These effects are achieved through anti-inflammation, activation of PPAR- γ , inhibition of matrix metalloproteinases, and suppression of angiotensin II activity [16, 17]. Along with a deepened understanding of the pathological mechanisms of heart failure and insulin-resistant cardiomyopathy, the roles of interstitial remodeling in insulin-resistant cardiomyopathy have attracted increasing attention. However, the exact mechanisms remain to be elucidated. Abnormal myocardial collagen metabolism is an important part of myocardial interstitial remodeling [18, 19].

Therefore, the aim of the present study was to examine the effects of telmisartan on interstitial remodeling in rats with diet-induced insulin-resistant cardiomyopathy. Present results may provide a theoretical basis for management of myocardial remodeling in insulin resistance, improving patient prognoses.

Materials and methods

Animals

Thirty-six specific pathogen-free Wistar rats (males, 100-120 g, Beijing Vital River Laboratory Animals, Beijing, China) were fed at the Laboratory Animal Center of the Department of Medicine, Xi'an Jiao Tong University, at $22\pm 2^\circ\text{C}$. There was a relative humidity of 50%, with free access to water and food and a 12/12 hour-light-dark cycle. All procedures and animal experiments were approved by the Animal Care and Use Committee of Xi'an Jiao Tong University.

Grouping and intervention

The animals were randomized to two groups: one given a normal diet ($n=18$, total calories 13.4 kJ/g, of which fat represented 10.2%, proteins 23.3%, and carbohydrates 66.5%) and the other was given a high-fat and high-glucose diet ($n=18$, total calorie 36.8 kJ/g, aside to basic feed, the rats were given additional lard, egg yolk, glucose and pig bile, with glucose

accounting for 40.2% of the total calorie, fat 29.0%, proteins 7.0%, and carbohydrates 23.8%). Rats with a normal diet were taken as controls. After 12 weeks of feeding, glucose tolerance tests were performed to examine whether the insulin-resistant rat model was established. Insulin resistance (IR) was determined as HOMA-IR >2.5 and ISI <-4.48 [20, 21]. The HOMA insulin resistance index was calculated as fasting blood glucose level (mmol/L) \times fasting insulin level ($\mu\text{IU/ml}$)/22.5). After modeling, insulin-resistant rats in the high-fat and high-glucose groups were further randomized to the insulin-resistant and telmisartan groups. Controls were randomized to the control and control + telmisartan groups. The insulin-resistant + telmisartan group and the control + telmisartan group were given telmisartan (Dawnrays Pharmaceuticals, Suzhou, China) at 8 mg/kg/d (the dose for rats weighing 200 g could be calculated as the adult dosage \times the conversion coefficient of 0.02). Control and insulin-resistant groups were administered with an equal amount of PBS with 5 g/L carboxymethyl cellulose (Sunhere Pharmaceutical Excipients, Anhui, China).

Rat hemodynamics

After 34 weeks of dietary intervention, carotid catheterizations were performed to measure the hemodynamics of the rats, including left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), maximum rates of rise of left ventricular pressure (+dp/dtmax), and maximum rates of drop of left ventricular pressure (-dp/dtmax).

Left ventricular mass index (LVMI) of the rats

Chests of the rats were opened immediately after measuring hemodynamics. Blood samples were taken from the thoracic aorta. The hearts were isolated, washed by PBS (pH 7.4, 4°C , 0.1 mol/L) three times, dried with filter paper, and weighed. The atriums and ventricles were separated along the mitral annular plane. The atriums and right ventricles were separated along the interventricular septum, while the left ventricles and interventricular septum were retained. The weight was the left ventricular weight (LVW). The left ventricular mass index (LVMI = LVW/body weight \times 100%) was used to reflect the degree of myocardial hypertrophy. For morphological observation of cardiomyocytes and

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interstitial fibers, myocardial tissues sampled from the left ventricles were fixed in 10% polyoxymethylene at 4°C for 12 hours. This was followed by routine paraffin embedding and sectioning. Left tissue samples were preserved in liquid nitrogen for future use.

Morphological observation and quantitative analysis of myocardial collagen in rat left ventricles

The myocardial tissues were fixed, paraffin-embedded, and sectioned to 5 µm. After Masson's staining, the cardiomyocytes showed red. The collagen showed blue. Using an automatic image analyzer (Olympus, Tokyo, Japan), the collagen volume fraction (CVF, the area of collagen/the area of measured field), as well as the vessel-surrounding collagen volume fraction (VSCVF, the area of perivascular collagen/the area of measured field), was measured. For each sample, one section was chosen. The average values of four random fields were used.

Detection of serum/tissue Ang II/ALD

Plasma and myocardial tissue ANGII and ALD were determined by radioimmunoassay. The ¹²⁵I Angiotensin II RIA Kit was purchased from Beijing North Institute of Biological Technology (Beijing, China).

RT-PCR

Total RNA was extracted with TRIzol Reagent (Invitrogen Inc., Carlsbad, CA, USA), according to manufacturer instructions. Reverse transcription was conducted as recommended by the manufacturer (TaKaRa, Otsu, Japan). Primer sequences were: type I collagen gene forward 5'-GTT CGT GGT TCT CAG GGT AG-3' and reverse 5'-TTG TCG TAG CAG GGT TCT TT-3' (SBS Genetech Co., Ltd., Beijing, China); and GAPDH forward 5'-TCC CTC AAG ATT GTC AGC AAT G-3' and reverse 5'-AGA TCC ACA ACG GAT ACA TTG G-3' (Biological Engineering Technology, Shanghai, China). The PCR reaction was carried out in the presence of reverse transcribed product, upstream and downstream primers, dNTPs, 1 × PCR buffer, MgCl₂, and Taq DNA polymerase. The total volume of the reaction was 20 µl. PCR was conducted according to the following parameters: pre-denaturation at 92°C for 1 minute, denaturation at 92°C for 30 seconds, annealing at 56°C for 30 seconds, and extension

at 68°C for 2 minutes. A total of 25 cycles were performed and a final extension was carried out at 68°C for 7 minutes. PCR products (8 µl) were separated by 2% agarose gel electrophoresis. The gray value for each band was measured using the gel image analysis system (Bio-Rad, Hercules, CA, USA). Type I collagen/GAPDH ratios indicated relative levels of type I collagen mRNA.

Western blotting

Proteins were extracted from left ventricular muscles by lysis buffer and phosphatase inhibitors (Beyotime Biotechnology, Shanghai, China). Protein concentrations were measured using a BCA assay kit (Beyotime Institute of Biotechnology, Shanghai, China). For Western blotting, equal amounts of protein (50 µg/lane) were separated by SDS-PAGE. After polyvinylidenedifluoride (PVDF) membranes (Millipore corp., Billerica, MA, USA) were blotted with proteins, they were blocked and incubated with the corresponding primary antibodies overnight at 4°C. Antibodies were: AT1R, ERK1/2, and TGF-β1 antibodies (all 1:500; R&D Systems, Minneapolis, MN, USA); p-Smad2 and p-Smad3 antibodies (all 1:500; Cell Signaling, Danvers, MA, USA); and rat type I collagen antibody (1:500; Abcam, Cambridge, MA, USA). After the PVDF membranes were incubated with appropriate secondary goat anti-mouse IgG antibodies (all 1:8000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), protein bands were visualized by enhanced chemiluminescence (ECL Plus) (Thermo Fisher Scientific, Waltham, MA, USA). Intensities of the digitally detected bands were evaluated by densitometry using Image-Pro Plus software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Continuous data are presented as mean ± standard deviation. Multi-group comparisons were made using ANOVA, with Tukey's post hoc test (normally distributed) or the Kruskal-Wallis test (non-parametric). If the data met normal distribution, Pearson's correlation was performed for correlation analysis. Otherwise, Spearman's correlation was used. Two-sided *P*-values <0.05 indicate statistical significance.

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Table 1. Metabolic parameters of the three groups

	Body mass (g)	FBG (mM)	FINS (ng/mL)	HOMA-IR	TC (mM)	TG (mM)	LDL-C (mM)	HDL-C (mM)
Con	672±19	2.49±0.34	19.7±2.4	2.16±0.19	3.43±0.67	0.87±0.17	0.84±0.27	1.89±0.51
ConT	667±21	2.17±0.25	21.4±2.0	2.36±0.27	3.01±0.45	0.96±0.24	0.82±0.21	1.74±0.43
IR	761±35 ^{a,c}	6.35±0.45 ^{a,c}	80.0±6.8 ^{a,c}	23.03±1.24 ^{a,c}	4.66±1.47 ^{a,c}	0.92±0.30	1.79±0.25 ^{a,c}	2.08±0.22
IRT	685±19 ^b	3.06±0.35 ^b	20.3±1.9 ^b	5.73±0.73 ^{a,b,c}	4.50±0.55 ^{a,c}	0.89±0.23	1.63±0.17 ^{a,c}	1.94±0.49

^aP<0.05 vs. Con, ^bP<0.05 vs. IR, ^cP<0.05 vs. ConT. FBG: fasting blood glucose; FINS: fasting insulin; HOMA: homeostasis model assessment; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; Con, control group; ConT, control, and telmisartan group; IR, insulin resistance group; IRT, insulin resistance, and telmisartan group; n=9.

Results

High-fat diet-induced insulin resistance rat model

Body mass, fasting blood glucose, fasting insulin, HOMA-IR, total cholesterol, and low-density lipoprotein cholesterol were significantly higher in insulin-resistant rats than in control rats (all P<0.05), but there were no significant differences in triglycerides and high-density lipoprotein cholesterol (all P>0.05). In the IRT group, the HOMA-IR was lower than in IR rats (P<0.05). In the ConT group, the HOMA-IR was not significantly different from the control group. Fasting blood glucose and fasting insulin were significantly reduced, close to normal levels (P<0.05 vs. the control group). Present data supports a role for telmisartan in insulin resistance (**Table 1**).

Cardiac function changes in high-fat and high-glucose diet-induced insulin-resistant rats

A significant increase in heart rates was observed in rats fed with the high-fat and high-glucose diet, compared to rats fed with a normal diet (P<0.05). Left ventricular mass, left ventricular mass index, systolic blood pressure, and diastolic blood pressure showed no significant changes among groups (P>0.05). Present results suggest that the basic cardiovascular features of insulin-resistant rats had not yet been affected. Moreover, compared with the control group, the LVDEP of rats in the IR group was increased (P<0.05), while diastolic function abnormalities started to appear (decreased -dp/dtmax; P<0.05). These results suggest that the diastolic function of the hearts was impaired in the state of insulin resistance. Additionally, non-statistically significant compensatory increases appeared in the myocar-

dial contractile function of rats in the IR group (P>0.05).

Compared with the control group, systolic blood pressure and diastolic blood pressure were decreased by telmisartan (P<0.05). Heart rates and left ventricular mass showed a downward trend, compared to those of the IR group, but there were no significant differences (P>0.05) in left ventricular mass index. It was found that, in the IRT group, telmisartan slightly reduced the LVSP and LVDEP was significantly improved (compared with the IR group; P<0.05). These appearances did not exist in the ConT group. Telmisartan significantly corrected diastolic function abnormalities in the insulin-resistant group (P<0.05). This means that there is another way telmisartan could improve insulin resistant rat cardiac function that is not dependent on antihypertensive effects. There were no significant differences in LVSP, LVDEP, maximum rising rates of LVSP, and -dp/dtmax, compared with the control group (P>0.05) (**Table 2**).

Telmisartan attenuated myocardial fibrosis in high-fat and high-glucose diet-induced insulin-resistant rats

The rat model of continuous high-fat and high-glucose diet showed a greater degree of cardiac fibrosis than controls, according to Masson's staining (**Figure 1A-C**). Normal myocardial tissue was red, while the blue collagen component appeared in the myocardial interstitium in IR rats. Interstitial fibrosis was partly alleviated by telmisartan. In the ConT group, telmisartan has no significant effects on myocardial interstitial fibrosis. Myocardial interstitial fibrosis could limit myocardial diastolic function. The CVF of the IR group was higher, compared with the control group (13.55±1.54 vs. 5.95±0.98, P<0.05). Telmisartan significantly reduced the

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Table 2. Cardiac function testing in the three groups

	HR (b.p.m)	LVM (mg)	LVMI (mg/g)	SBP (mmHg)	DBP (mmHg)	LVSP (mmHg)	LVDEP (mmHg)	+dp/dtmax (mmHg)	-dp/dtmax (mmHg/s)
Con	292.20±30.12	1288.01±162.50	1.92±0.23	106.30±4.28	62.11±2.18	114.91±9.41	-7.23±1.16	6145.01±510.17	5530.01±520.53
ConT	301.40±27.12	1347.01±149.30	1.98±0.41	89.42±3.64 ^a	55.24±3.48 ^a	117.51±7.59	-6.98±0.87	6041.41±494.37	5498.75±504.37
IR	355.60±30.80 ^{a,c}	1304.12±198.07	1.89±0.22	108.67±3.71	65.77±2.89	115.24±9.01	0.72±0.50 ^{a,c}	5940.02±575.75	4517.04±663.48 ^{a,c}
IRT	302.06±27.41 ^b	1101.42±129.43 ^b	1.85±0.31	92.70±5.39ab ^{a,b}	49.37±3.72 ^{a,b}	105.25±8.47 ^b	-6.56±0.52 ^b	6193.33±478.82	5153.32±422.41 ^b

^a*P*<0.05 vs. Con, ^b*P*<0.05. vs. IR, ^c*P*<0.05. vs. ConT. HR: heart rate; LVM: left ventricular mass; LVMI: left ventricular mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LVSP: left ventricular systolic pressure; LVDEP: left ventricular end-diastolic pressure; +dp/dtmax: maximum rising rate of LVSP; -dp/dtmax: maximum descent rate of LVSP; Con, control group; ConT, control, and telmisartan group; IR, insulin resistance group; IRT, insulin resistance and telmisartan group; n=9.

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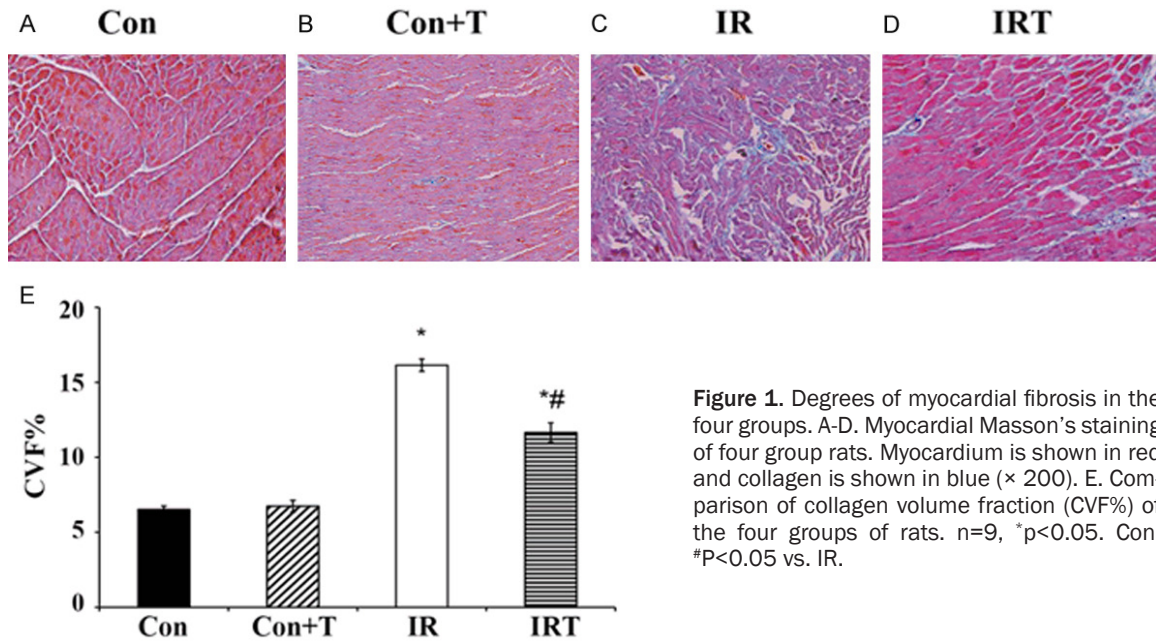


Figure 1. Degrees of myocardial fibrosis in the four groups. A-D. Myocardial Masson's staining of four group rats. Myocardium is shown in red and collagen is shown in blue ($\times 200$). E. Comparison of collagen volume fraction (CVF%) of the four groups of rats. $n=9$, $*p<0.05$. Con, $\#P<0.05$ vs. IR.

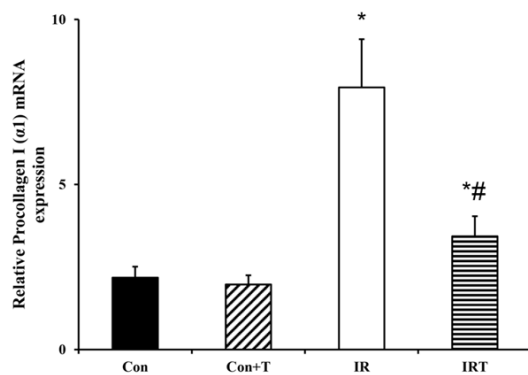


Figure 2. Real-time PCR analysis of mRNA levels of procollagen type I ($\alpha 1$) in the four groups. Telmisartan reduced the mRNA of procollagen type I ($\alpha 1$) at the transcriptional level in the insulin-resistant group, but not in the control group. $*p<0.05$. Con, $\#P<0.05$ vs. IR.

degree of myocardial fibrosis in IR rats (11.31 ± 2.21 vs. 13.55 ± 1.54 , $P<0.05$) (**Figure 1D**). The ConT group showed no significant differences in CVF%, compared with the control group (5.95 ± 0.98 vs. 5.14 ± 0.86 , $P<0.05$).

Changes in mRNA levels of procollagen type I in high-fat and high-glucose diet-induced insulin-resistant rats

This study detected mRNA levels of procollagen type I ($\alpha 1$ chain) at the transcriptional level. Compared with the control group, mRNA levels of procollagen type I in the IR group were increased significantly ($P<0.05$), whereas pro-

collagen type I mRNA in the IRT group was decreased significantly, compared with the IR group ($P<0.05$) (**Figure 2**).

Relevant parameters of RAAS in high-fat diet-induced insulin-resistant rats

Ang II and ALD levels were measured in rat myocardium and plasma. Levels of Ang II and ALD in the IR group rats myocardium and plasma were elevated, compared with the control group ($P<0.05$). Telmisartan, a blocker of the renin-angiotensin-aldosterone system (RAAS), led to decreased ALD expression levels in the myocardium and plasma, compared with the IR group. However, compared with the IR group, Ang II levels in myocardium showed a downward trend ($P<0.05$), but not in plasma ($P>0.05$). This phenomenon suggests that telmisartan, locally, played a crucial role in the myocardium (**Table 3**). Telmisartan downregulated expression of AT1R proteins (**Figure 3**).

Effects of telmisartan on myocardial fibrosis via ERK1/2/TGF- $\beta 1$ signaling pathways in high-fat diet-induced insulin-resistant rats

The phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) in the IR group was significantly increased, compared with the control group ($P<0.05$). Transforming growth factor- $\beta 1$ (TGF- $\beta 1$) and its downstream signaling proteins p-Smad2/3 were increased significantly ($P<0.05$). Moreover, compared with the control group, expression of myocardial colla-

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Table 3. Angiotensin II and ALD levels in the three groups

	Serum		Tissue	
	Ang II (pg ml ⁻¹)	ALD (pg ml ⁻¹)	Ang II (pg ml ⁻¹)	ALD (pg ml ⁻¹)
Con	133.4±21.5	556.1±25.1	68.8±6.2	51.8±5.2
ConT	189.4±29.5	497.1±35.1	70.5±4.2	48.9±6.4
IR	439.3±30.4 ^a	962.6±68.3 ^a	132.7±9.5 ^a	96.2±8.8 ^a
IRT	476.9±27.2 ^a	593.4±30.2 ^b	74.1±6.1 ^b	56.2±7.9 ^b

^aP<0.05 vs. Con, ^bP<0.05 vs. IR, ^cP<0.05 vs. ConT, Ang II: angiotensin II; ALD: aldosterone; Con, control group; ConT, control and telmisartan group; IR, insulin resistance group; IRT, insulin resistance and telmisartan group; n=9.

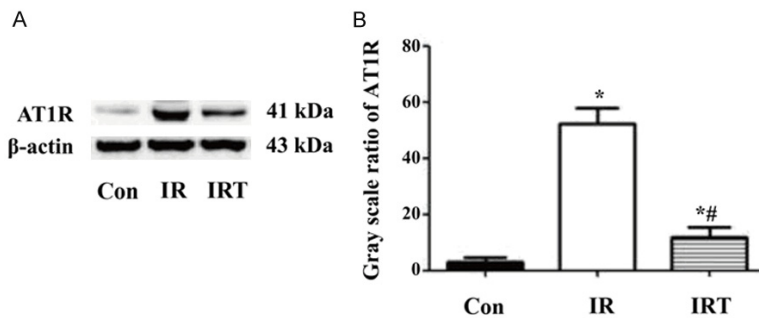


Figure 3. AT1R protein expression changes in the three groups. Telmisartan (TEL) could significantly reduce myocardial AT1R protein expression of IR rats. (n=9, *P<0.05 vs. Con, #P<0.05 vs. IR).

gen I proteins showed a significant increase in IR groups (P<0.05). It was found that expression of myocardial TGF-β1 and collagen I proteins in the telmisartan group was significantly reduced, compared with the IR group (P<0.05). Phosphorylation of ERK1/2 and Smad2/3 was also decreased (P<0.05), but still higher than in controls (P<0.05) (Figure 4).

Discussion

Abnormal myocardial collagen metabolism is an important part of myocardial interstitial remodeling [12, 15, 19, 22]. However, with insulin resistance, researchers have no idea how the pathways act on remodeling. Therefore, this study aimed to examine the effects of the TGF-β1/ERK1/2/pSmad2/3 signaling pathways on interstitial remodeling in rats with diet-induced insulin-resistant cardiomyopathy. Present results suggest that insulin resistance could activate AT1 receptors, promote myocardial type I collagen synthesis, increase myocardial collagen deposition, and reduce heart diastolic function. Telmisartan may improve insulin resistance, decrease collagen deposition, and improve cardiac diastolic function by TGF-β1/ERK1/2/pSmad2/3 signaling pathways.

Long-term high-energy intake and hyper-insulinemia can lead to insulin resistance [1, 22]. High fat diets can lead to increases in expression of IRS-1, AKT, and GLUT4 [1, 22]. Long-term high-fat diets not only cause obesity-related insulin resistance, but activate the RAAS [23]. Angiotensin II activates AT1 receptors to regulate the activation of insulin receptors, thus increasing the degree of insulin resistance [24]. In a follow-up study of patients with type 2 diabetes, the AT1 receptor antagonist telmisartan not only improved heart function, but also regulated blood glucose and blood lipids, while improving insulin resistance in patients [25].

Cardiomyocyte extracellular matrix is a stable three-dimensional network structure regulated by collagen synthesis and degradation in a dynamic balance under mechanical or chemical stress stimuli. If the dynamic balance is broken, this can lead to myocardial interstitial collagen deposition, resulting in myocardial interstitial remodeling [26, 27]. Myocardial collagen is mainly composed of type I and type III collagen fibers. In the high-fat and high-glucose diet-induced insulin-resistant rats, the volume fraction of myocardial collagen and the volume fraction of collagen around the myocardium were significantly increased, suggesting an increase in collagen synthesis and collagen deposition. Additionally, the cardiac function of the high fat and high glucose groups showed that the diastolic function of the heart was decreased. The main reason was an increase of left ventricular end diastolic pressure, along with a decrease of left ventricular pressure. Correlation studies have found that the maximum rates of left ventricular pressure, CVF, and VSCVF were negatively correlated, indicating that increased myocardial collagen content will reduce cardiac diastolic function. Insulin resistance index has been positively correlated with CVF and VSCVF, indicating that insulin resistance may promote the deposition of myocar-

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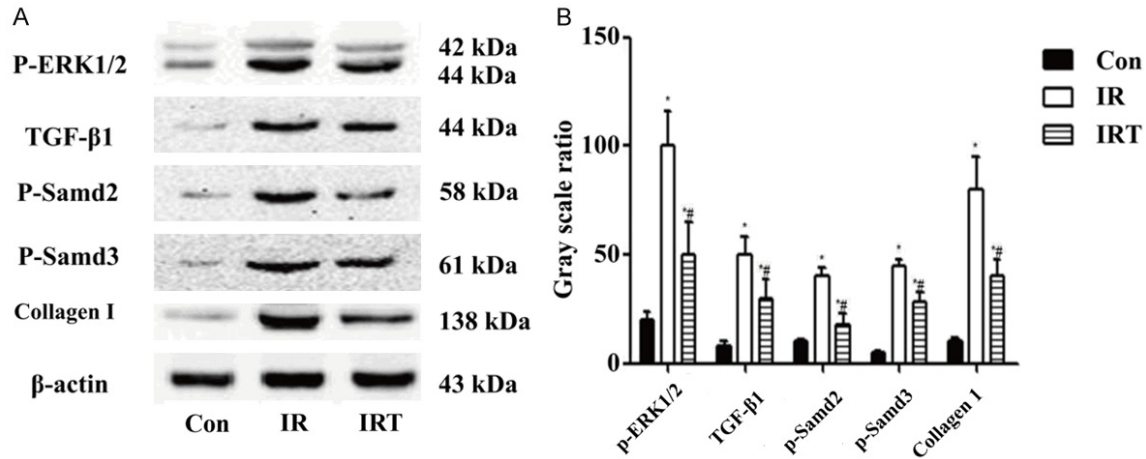


Figure 4. ERK1/2/TGF-β1 signaling pathway protein expression changes in the three groups. Telmisartan (TEL) could significantly reduce myocardial p-ERK1/2, TGF-β1, p-Smad2/3, and collagen I protein expression of HF rats. (n=9, *P<0.05 vs. Con, #P<0.05. vs. IR).

dial collagen. A previous study showed that insulin-resistant cardiomyopathy heart diastolic dysfunction is due to abnormal insulin pathways caused by myocardial interstitial collagen deposition [28]. In addition, the fibronectin fragment is involved in myocardial remodeling [29].

Insulin resistance has been associated with the RAAS in the development and progression of insulin-resistant cardiomyopathy. RAAS plays an important role in the pathogenesis of insulin resistance and insulin resistance-induced cardiomyopathy [24]. AT1 receptor blockers can improve dietary induction of insulin resistance caused by ventricular remodeling and insulin resistance [30], in which prolonged activation of AT1 receptors can cause myocardial interstitial fibrosis and collagen deposition, leading to ventricular hypertrophy [31]. In the present study, myocardial tissue Masson's staining showed that telmisartan significantly decreased the myocardial deposition in the high-fat group and decreased CVF in insulin-resistant rats. Moreover, levels of angiotensin II and aldosterone in myocardial tissues of rats with insulin resistance were significantly increased, while expression of AT1 receptor proteins was also significantly increased. Moreover, mRNA transcription and protein expression of type I collagen was increased. These pathological changes were improved by telmisartan. Telmisartan can reduce the high-fat diet-induced insulin-resistant rat myocardial interstitial collagen deposition and improve cardiac diastolic function.

TGF-β1 is involved in collagen fiber metabolism. In the present study, AT1R, TGFβ1, ERK1/2, and pSmad2/3 protein expression in myocardial tissues of insulin-resistant rats were significantly increased. Expression of its downstream signaling proteins p-Smad2/3 and pERK1/2 was decreased after telmisartan treatment, indicating that telmisartan affects the heart, partly through the TGF-β1 and MAPK pathways, to regulate collagen metabolism. Additionally, the Tp-Te interval had a significant correlation with left ventricular mass index and left ventricular end diastolic diameter, while the Tp-Te interval had some clinical significance for predicting left ventricular remodeling in patients with dilated cardiomyopathy [32]. Of course, additional studies are necessary to determine the exact mechanisms through which telmisartan improves cardiac function in insulin resistance.

In conclusion, insulin resistance may promote myocardial type I collagen synthesis, increase myocardial collagen deposition, and reduce heart diastolic function. TGF-β1/ERK1/2/pSmad2/3 signaling pathways play essential role in this. Telmisartan, as a AT1 receptor antagonist, may improve insulin resistance and cardiac diastolic function and reverse myocardial fibrosis in insulin resistance.

Disclosure of conflict of interest

None.

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References

- [1] Chiu HK, Tsai EC, Juneja R, Stoeber J, Brooks-Worrell B, Goel A and Palmer JP. Equivalent insulin resistance in latent autoimmune diabetes in adults (LADA) and type 2 diabetic patients. *Diabetes Res Clin Pract* 2007; 77: 237-244.
- [2] Ginsberg HN. Insulin resistance and cardiovascular disease. *J Clin Invest* 2000; 106: 453-458.
- [3] Reaven G. Insulin resistance and coronary heart disease in nondiabetic individuals. *Arterioscler Thromb Vasc Biol* 2012; 32: 1754-1759.
- [4] Rao Kondapally Seshasai S, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I, Njølstad I, Fletcher A, Nilsson P, Lewington S, Collins R, Gudnason V, Thompson SG, Sattar N, Selvin E, Hu FB, Danesh J; Emerging Risk Factors Collaboration. Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med* 2011; 364: 829-841.
- [5] Zoungas S, Woodward M, Li Q, Cooper ME, Hamet P, Harrap S, Heller S, Marre M, Patel A, Poulter N, Williams B, Chalmers J and group AC. Impact of age, age at diagnosis and duration of diabetes on the risk of macrovascular and microvascular complications and death in type 2 diabetes. *Diabetologia* 2014; 57: 2465-2474.
- [6] Bragg F, Holmes MV, Iona A, Guo Y, Du H, Chen Y, Bian Z, Yang L, Herrington W, Bennett D, Turnbull I, Liu Y, Feng S, Chen J, Clarke R, Collins R, Peto R, Li L, Chen Z and China Kadoorie Biobank Collaborative Group. Association between diabetes and cause-specific mortality in rural and urban areas of China. *JAMA* 2017; 317: 280-289.
- [7] Fontes-Carvalho R, Ladeiras-Lopes R, Bettencourt P, Leite-Moreira A and Azevedo A. Diastolic dysfunction in the diabetic continuum: association with insulin resistance, metabolic syndrome and type 2 diabetes. *Cardiovasc Diabetol* 2015; 14: 4.
- [8] Bajraktari G, Koltai MS, Ademaj F, Rexhepaj N, Qirko S, Ndrepepa G and Elezi S. Relationship between insulin resistance and left ventricular diastolic dysfunction in patients with impaired glucose tolerance and type 2 diabetes. *Int J Cardiol* 2006; 110: 206-211.
- [9] Bertrand L, Horman S, Beauloye C and Vanoverschelde JL. Insulin signalling in the heart. *Cardiovasc Res* 2008; 79: 238-248.
- [10] Wei Y, Whaley-Connell AT, Chen K, Habibi J, Uptergrove GM, Clark SE, Stump CS, Ferrario CM and Sowers JR. NADPH oxidase contributes to vascular inflammation, insulin resistance, and remodeling in the transgenic (mRen2) rat. *Hypertension* 2007; 50: 384-391.
- [11] Alfarano C, Sartiani L, Nediani C, Mannucci E, Mugelli A, Cerbai E and Raimondi L. Functional coupling of angiotensin II type 1 receptor with insulin resistance of energy substrate uptakes in immortalized cardiomyocytes (HL-1 cells). *Br J Pharmacol* 2008; 153: 907-914.
- [12] Frantz S, Hu K, Widder J, Bayer B, Witzel CC, Schmidt I, Galuppo P, Strotmann J, Ertl G and Bauersachs J. Peroxisome proliferator-activated-receptor agonism and left ventricular remodeling in mice with chronic myocardial infarction. *Br J Pharmacol* 2004; 141: 9-14.
- [13] Liu HJ, Liao HH, Yang Z and Tang QZ. Peroxisome proliferator-activated receptor-gamma is critical to cardiac fibrosis. *PPAR Res* 2016; 2016: 2198645.
- [14] Ehara N, Ono K, Morimoto T, Kawamura T, Abe M and Hasegawa K. The possible role of peroxisome proliferator-activated receptor gamma in heart failure. *Exp Clin Cardiol* 2004; 9: 169-173.
- [15] Kamimura D, Uchino K, Ishigami T, Hall ME and Umemura S. Activation of peroxisome proliferator-activated receptor gamma prevents development of heart failure with preserved ejection fraction; inhibition of wnt-beta-catenin signaling as a possible mechanism. *J Cardiovasc Pharmacol* 2016; 68: 155-161.
- [16] Maejima Y, Okada H, Haraguchi G, Onai Y, Kosuge H, Suzuki J and Isobe M. Telmisartan, a unique ARB, improves left ventricular remodeling of infarcted heart by activating PPAR gamma. *Lab Invest* 2011; 91: 932-944.
- [17] Okada M, Harada T, Kikuzuki R, Yamawaki H and Hara Y. Effects of telmisartan on right ventricular remodeling induced by monocrotaline in rats. *J Pharmacol Sci* 2009; 111: 193-200.
- [18] Quilliot D, Alla F, Bohme P, Bruntz JF, Hammadi M, Dousset B, Ziegler O and Zannad F. Myocardial collagen turnover in normotensive obese patients: relation to insulin resistance. *Int J Obes (Lond)* 2005; 29: 1321-1328.
- [19] Mizushige K, Yao L, Noma T, Kiyomoto H, Yu Y, Hosomi N, Ohmori K and Matsuo H. Alteration in left ventricular diastolic filling and accumulation of myocardial collagen at insulin-resistant prediabetic stage of a type II diabetic rat model. *Circulation* 2000; 101: 899-907.
- [20] Mather K. Surrogate measures of insulin resistance: of rats, mice, and men. *Am J Physiol Endocrinol Metab* 2009; 296: E398-399.

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- [21] Cacho J, Sevillano J, de Castro J, Herrera E and Ramos MP. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *Am J Physiol Endocrinol Metab* 2008; 295: E1269-1276.
- [22] Oliveira Junior SA, Padovani CR, Rodrigues SA, Silva NR, Martinez PF, Campos DH, Okoshi MP, Okoshi K, Dal-Pai M and Cicogna AC. Extensive impact of saturated fatty acids on metabolic and cardiovascular profile in rats with diet-induced obesity: a canonical analysis. *Cardiovasc Diabetol* 2013; 12: 65.
- [23] Chen X, Zhai S and Li C. The influence of insulin resistance on blood glucose in patients with Hyperthyroidism. *J Zhengzhou Univ (Med Ed)* 2015; 50: 563-565.
- [24] Olivares-Reyes JA, Arellano-Plancarte A and Castillo-Hernandez JR. Angiotensin II and the development of insulin resistance: implications for diabetes. *Mol Cell Endocrinol* 2009; 302: 128-139.
- [25] Derosa G, Fogari E, D'Angelo A, Cicero AF, Salvadeo SA, Ragonesi PD, Ferrari I, Gravina A, Fassi R and Fogari R. Metabolic effects of telmisartan and irbesartan in type 2 diabetic patients with metabolic syndrome treated with rosiglitazone. *J Clin Pharm Ther* 2007; 32: 261-268.
- [26] Witteles RM and Fowler MB. Insulin-resistant cardiomyopathy clinical evidence, mechanisms, and treatment options. *J Am Coll Cardiol* 2008; 51: 93-102.
- [27] Jia G, DeMarco VG and Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nat Rev Endocrinol* 2016; 12: 144-153.
- [28] Gray S and Kim JK. New insights into insulin resistance in the diabetic heart. *Trends Endocrinol Metab* 2011; 22: 394-403.
- [29] Wei S, Ma S and Yang D. The effect of fibronectin degradation fragments on the expression of tenascin-C in cardiac myocytes. *J North Sichuan Med Coll* 2015; 30: 135-139.
- [30] Oliveira-Junior SA, Martinez PF, Guizoni DM, Campos DH, Fernandes T, Oliveira EM, Okoshi MP, Okoshi K, Padovani CR and Cicogna AC. AT1 receptor blockade attenuates insulin resistance and myocardial remodeling in rats with diet-induced obesity. *PLoS One* 2014; 9: e86447.
- [31] Barry SP, Davidson SM and Townsend PA. Molecular regulation of cardiac hypertrophy. *Int J Biochem Cell Biol* 2008; 40: 2023-2039.
- [32] Zhang M, Ren L and Lv Z. The study of Tp-Te interval and ventricular remodeling in dilated cardiomyopathy. *J North Sichuan Med Coll* 2015; 30.