

# Effect of pioglitazone on expression of hypoxia-inducible factor 1 $\alpha$ and vascular endothelial growth factor in ischemic hindlimb of diabetic rats

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**Abstract. – OBJECTIVE:** To observe effects of the drug pioglitazone on expression of hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) and vascular endothelial growth factor (VEGF) in diabetic rats with hindlimb ischemia, and explore the role of pioglitazone in angiogenesis after ischemia and its possible mechanism.

**MATERIALS AND METHODS:** The diabetic rat model was established by high-fat and high-sugar diet and intraperitoneal injection of streptozotocin. The diabetic rats with the unilateral hindlimb ischemia were randomly divided into diabetic model group and pioglitazone treated group, and the normal rats with unilateral hindlimb ischemia were selected as the control group. RT-PCR and Western blotting techniques were employed for analysis and detection of HIF-1 $\alpha$  and VEGF expression, as well as detection of capillary density by immunohistochemical staining and ischemic hindlimb perfusion by Doppler ultrasonography were measured.

**RESULTS:** Compared with the control group, the fasting glucose, fasting insulin, insulin resistance index, total cholesterol, triglycerides and low-density lipoprotein cholesterol in diabetic rats were significantly increased. This was accompanied by increased mRNA and protein expression of HIF-1 $\alpha$  and VEGF, and decreased microvessel density (MVD) of the ischemic limb ( $p < 0.05$ ). The above indicators in pioglitazone-treated diabetic rats were significantly decreased ( $p < 0.01$ ) with decreased expression of HIF-1 $\alpha$  and VEGF ( $p < 0.01$ ), while the microvessel density (MVD) of the ischemic limb was increased ( $p < 0.01$ ) and blood perfusion was also increased ( $p < 0.01$ ). The expression of HIF-1 $\alpha$  and VEGF were positively correlated ( $p < 0.05$ ) in diabetic rats with hind limb angiopathy, while HIF-1 $\alpha$  and VEGF were all negatively correlated with the microvessel density (MVD).

**CONCLUSIONS:** HIF-1 $\alpha$  and VEGF expression in diabetic rats with hind limb angiopathy were increased. Pioglitazone has a promoting effect

on ischemic limb angiogenesis in diabetic rats. It suggested that pioglitazone may improve ischemic limb angiogenesis mechanisms correlated with regulating the HIF-1 $\alpha$ /VEGF hypoxia response pathway.

*Key Words:*

Pioglitazone, Limb ischemia, Diabetes mellitus, Hypoxia-inducible factor-1 $\alpha$ , Vascular endothelial growth factor.

## Introduction

Angiopathy in patients with type 2 diabetes is one of the principal chronic complications, and the current pathogenesis is not fully understood. Studies have shown that hypoxia-inducible factor (HIF)-1 is an important transcription factor in a hypoxic environment, which may mediate the onset and development of diabetic angiopathy by inducing gene expression of downstream vascular endothelial growth factor (VEGF)<sup>1-2</sup>. The aim of this study is to explore the role and possible mechanisms of the drug pioglitazone in angiogenesis after hypoxia-ischemia, by determining the effect of pioglitazone on expression of hypoxia-inducible factor-1 (HIF-1 $\alpha$ ) and vascular endothelial growth factor (VEGF) in type 2 diabetic rats with ischemic hindlimb and diabetic angiopathy.

## Materials and Methods

### *Grouping and Modeling of Experimental Animals*

40 healthy male Wistar rats at 8 weeks of age (Experimental Animal Center of Zhejiang Uni-

versity) were randomly divided into two groups after adaptive feeding for one week: (1) normal control group with 12 rats; (2) modeling group with 28 rats. Normal group was given basal feeding; The rats in modeling group were given high-sugar and high-fat diet for 4 weeks by weighing to 260-310 g (body weight 260-310 g) with an intraperitoneal injection of streptozotocin (STZ, 30 mg/kg), and the rats with blood glucose greater than 16.7 mmol/L collected from tail vein after 72h were accepted as a standard model. 25 diabetic rats and 12 normal Wistar rats were anesthetized, and the femoral artery of the right hind limb was cut, including its branches, to provide an acute hindlimb ischemia model. 25 successful model rats were randomly divided into diabetic model group (12 rats) and the pioglitazone treated group (13 rats). The pioglitazone intervention group received daily gavage at a dose of 4 mg/kg, and the control group and diabetic model group were administered by gavage the equivalent volume of distilled water for a period of 28 days.

#### ***Comparison of General Characteristics in Each Group***

To determine the weight, HbA1C levels, fasting glucose, fasting insulin, insulin resistance index (HOMA-IR), total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C) and other indicators.

#### ***Immunohistochemical Detection of Capillary Density***

The hindlimb muscle, cut from the surgical site was fixed and then sliced. Using immunohistochemical staining (with rabbit anti-mouse CD31 polyclonal antibody, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The number of anti-CD31 monoclonal antibody-stained microvessels were counted from 10 random fields of  $\times 200$  magnification, and the microvessel density (MVD) per unit area ( $1 \text{ mm}^2$ ) was calculated.

#### ***Hemodynamic Detection***

Laser Doppler ultrasound scanning was applied to detect hindlimb hemodynamic changes in each group, with the non-operated side hindlimb as a benchmark, to calculate the percentage of the operated side blood perfusion accounted for the non-operated side blood perfusion (blood perfusion ratio = ischemic hind limb/non-ischemic hind limb), to evaluate recovery of ischemic limb blood perfusion.

#### ***Analysis of HIF-1 $\alpha$ mRNA Expression by RT-PCR***

Trizol was used to extract the total RNA of ischemic muscular tissue in each group, 2  $\mu\text{g}$  of this RNA was then used for reverse transcription. HIF-1 $\alpha$  forward primer: 5'-ACAGGATTCCAGCAGAC-3', reverse primer: 5'-TTCCAAGAAAGCGACAT-3', gave a product that was 461bp;  $\beta$ -actin forward primer: 5'-AGCCATGTACGTAGCCATCC-3', reverse primer: 5'-TCTCAGCTGTGGTGGTGAAG-3', to give a product that was 227bp, with an annealing temperature of 49.5°C and 30 cycles. After PCR amplification of HIF-1 $\alpha$  and  $\beta$ -actin, the products were run on a 2% agarose gel and visualized by Goldview staining. Using  $\beta$ -actin as an internal reference, the relative expression levels of HIF-1 $\alpha$  mRNA were calculated RT-PCR reactions for each sample were repeated at least three times.

#### ***Protein Expression of HIF-1 $\alpha$ and VEGF Analysis***

The hind limb muscle was rinsed with phosphate buffered saline (PBS), and total protein extracted in cell lysis buffer [20 mM Tris (pH 7.5)], 150 mM NaCl, 1% Triton X-100, sodium pyrophosphate,  $\beta$ -glycerophosphate, EDTA,  $\text{Na}_3\text{VO}_4$ , leupeptin. The proteins were separated by polyacrylamide agarose gel electrophoresis (PAGE) and were transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked and exposed to primary antibodies [anti HIF-1 $\alpha$  antibody (1:1000), VEGF antibody (1:1000) or  $\beta$ -actin (1:5000)] overnight at 40°C. Membranes were then washed in TBST, and then exposed to horseradish peroxidase (HRP) conjugated secondary antibody, and incubated at room temperature for 2h. After Tris-buffered saline with twin (TBST) rinsing, membranes were exposed to electrochemiluminescence (ECL) and the Fluor-s S gel imaging system was used to analyze the protein bands, the relative content of protein was expressed as a ratio to  $\beta$ -actin expression Western blot analysis of each sample was repeated at least three times.

#### ***Statistical Analysis***

SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for *t* test, the single factor analysis of variance (ANOVA) and univariate correlation analysis in each group. *p* < 0.05 was defined as statistical significance.

**Results**

**Comparison of General Characteristics of Each Group of Rats**

Before injection of STZ, the weight of rats fed with the high sugar and high fat diet significantly increased compared with control-fed rats. The body weight of rats gradually decreased after modeling, and the body weight of rats in diabetic model group and pioglitazone treated group was significantly lower than that of the control group at the end of the experiment. However, the weight of the pioglitazone intervention group increased compared with the diabetic model group without pharmaceutical intervention. In the diabetic model group and pioglitazone treated group, feeding, drinking and urine output were significantly increased (data not shown), with higher fasting glucose, fasting insulin and HOMA-IR than those in the control group ( $p < 0.01$ ). HbA1C, fasting glucose, fasting insulin, HOMA-IR, blood lipids and the other indicators in the pioglitazone intervention group were lower than those of the diabetic model group ( $p < 0.01$ , Table I).

**Analysis of Angiogenesis**

After 4 weeks of treatment, microvascular density (MVD) of non-ischemic muscle in each group were similar ( $p > 0.05$ , data not shown). In the ischemic side, MVD of the diabetic model group decreased to 17.82% compared with that in the control group. Pioglitazone treatment caused MVD of ischemic muscle in diabetic rats to increase to 3.11 times that of the diabetic model group, which is still lower than that of the normal control group (Figure 3 and Table II).

**Hemodynamic Testing**

With the immediate perfusion before and after surgery, the difference among three groups of rats was not statistically significant ( $p > 0.05$ , data not shown). However, after seven days, the hind limb perfusion of diabetic rats significantly decreased compared with that of non-diabetic group, and this extended to the end of experiment. After treatment for 7 days in the pioglitazone intervention group, ischemic hind limb perfusion was not statistically significant compared with that in diabetic group, while on day 14th blood perfusion showed a recovery trend, which was statistically significant compared with that in diabetic group, but still significantly lower than that of non-diabetic group ( $p < 0.01$ , Table III).

Table I. Comparison of characteristics in each group of rats ( $\bar{x} \pm s$ ).

| Group                                      | n  | Weight (mmol/L)               | HbA1c (mmol/L)             | insulin (%)                 | Fasting glucose (mmol/L)    | Fasting IR (%)              | HOMA-TG (mmol/L)           | TC (mmol/L)                | LDL-C (mmol/L)             |
|--|----|-------------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|
| Control group (NC)                         | 12 | 421.20 $\pm$ 20.17            | 5.09 $\pm$ 0.76            | 9.02 $\pm$ 1.28             | 5.12 $\pm$ 0.39             | 2.05 $\pm$ 0.59             | 1.92 $\pm$ 0.17            | 2.01 $\pm$ 0.31            | 0.42 $\pm$ 0.15            |
| Diabetic group with hindlimb ischemia (DM) | 12 | 366.62 $\pm$ 14.51**          | 13.22 $\pm$ 3.67**         | 19.66 $\pm$ 3.54**          | 21.56 $\pm$ 4.17            | 18.85 $\pm$ 3.07**          | 4.22 $\pm$ 0.39**          | 5.07 $\pm$ 0.42**          | 1.69 $\pm$ 0.33**          |
| Pioglitazone treated group (DM+P)          | 13 | 391.70 $\pm$ 24.43** $\Delta$ | 9.04 $\pm$ 1.41** $\Delta$ | 14.02 $\pm$ 2.13** $\Delta$ | 16.84 $\pm$ 2.68** $\Delta$ | 10.49 $\pm$ 2.36** $\Delta$ | 3.56 $\pm$ 0.11** $\Delta$ | 3.69 $\pm$ 0.29** $\Delta$ | 1.21 $\pm$ 0.20** $\Delta$ |

Note: Compared with NC group, \* $p < 0.05$ , \*\* $p < 0.01$ ; Compared with DM group,  $\Delta p < 0.05$ ,  $\Delta p < 0.01$ .

**Table II.** Expression of angiogenesis related factors in ischemic hindlimb side of each group ( $\bar{x} \pm s$ ).

| Group                                      | n  | HIF-1 $\alpha$ (mmol/L)    | VEGF (mmol/L)              | MVD (%)                  |
|--|----|----------------------------|----------------------------|--------------------------|
| Control group (NC)                         | 12 | 0.85 $\pm$ 0.11            | 0.71 $\pm$ 0.16            | 10.1 $\pm$ 2.1           |
| Diabetic group with hindlimb ischemia (DM) | 12 | 3.04 $\pm$ 0.28**          | 4.02 $\pm$ 0.37**          | 1.8 $\pm$ 0.6**          |
| Pioglitazone treated group (DM+P)          | 13 | 1.92 $\pm$ 0.23** $\Delta$ | 1.41 $\pm$ 0.17** $\Delta$ | 5.6 $\pm$ 0.8** $\Delta$ |

Note: Compared with NC group, \* $p < 0.05$ , \*\* $p < 0.01$ ; Compared with DM group,  $\Delta p < 0.05$ ,  $\Delta p < 0.01$ .

**mRNA and Protein Expression of HIF-1 $\alpha$  and VEGF in Each Group of Rats**

Compared with the normal control group, after 28 days, mRNA and protein levels of HIF-1 $\alpha$  and VEGF in the ischemic hindlimb in the diabetic model group were significantly increased ( $p < 0.01$ ). Compared with the diabetic model group, after 4 weeks of treatment in the pioglitazone treated group, expression of HIF-1 $\alpha$ , and VEGF were significantly decreased ( $p < 0.01$ ), but still higher than that of the normal control group (Figures 1, 2 and Table II).

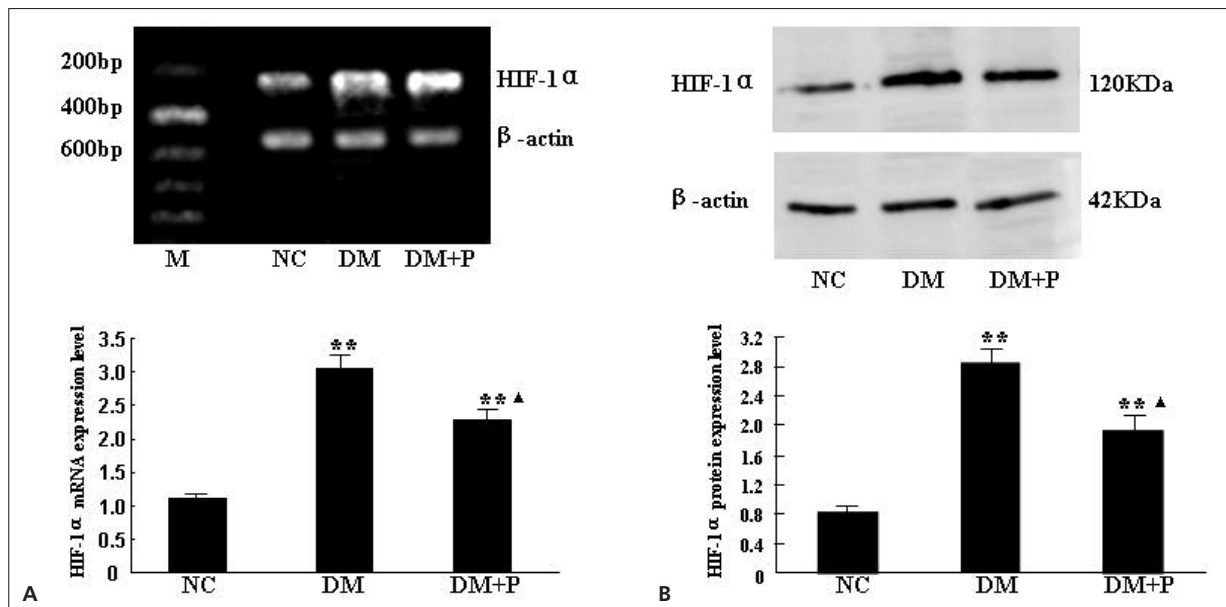
**Correlation Analysis**

Univariate correlation analysis showed that HIF-1 $\alpha$  expression was positively correlated with serum glucose, insulin level, cholesterol, triglycerides, low-density lipoprotein cholesterol, and

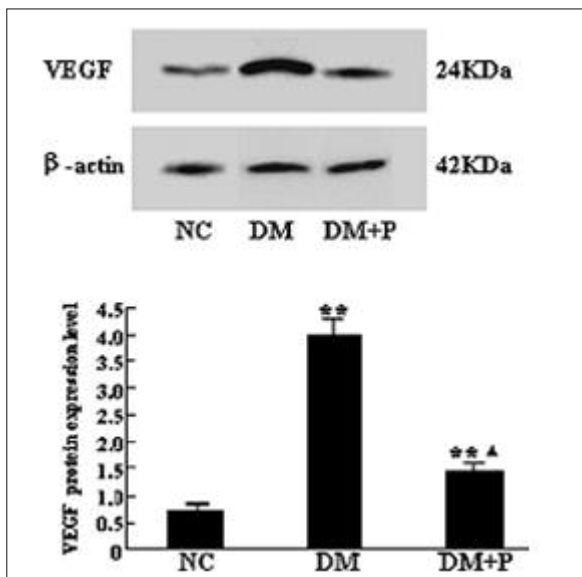
HOMA-IR (r values were 0.839, 0.846, 0.899, 0.857, 0.919, 0.906,  $p < 0.01$ ). VEGF and HIF-1 $\alpha$  expression in the diabetic ischemic hind limb also showed a positive correlation ( $r = 0.912$ ,  $p < 0.01$ ).

**Discussion**

The diabetic lower extremity angiopathy dominated by arteriosclerosis obliterans is a major complication of diabetes. Angiogenesis refers to the pathophysiological process development of existing endothelial cells into new blood vessels. A large number of studies have proved that the angiogenesis of the ischemic limb under diabetic state is defective, which may be an important reason why the consequence of blood vessel oc-



**Figure 1.** Expression of HIF-1 $\alpha$  mRNA and protein in each group of rats with hindlimb ischemia. A, Analysis of HIF-1 $\alpha$  mRNA expression by RT-PCR; B, Protein expression of HIF-1 $\alpha$  analysis by Western blot. M: Marker; NC: Control group; DM: Diabetic group; DM+P: Pioglitazone treated group (Note: Compared with NC group, \* $p < 0.05$ , \*\* $p < 0.01$ ; Compared with DM group,  $\Delta p < 0.05$ ,  $\Delta p < 0.01$ )



**Figure 2.** Expression of VEGF protein in each group of rats with hindlimb ischemia. NC: Control group; DM: Diabetic group; DM+P: Pioglitazone treated group (Note: Compared with NC group, \* $p < 0.05$ , \*\* $p < 0.01$ ; Compared with DM group,  $\Delta p < 0.05$ ,  $\blacktriangle p < 0.01$ ).

clusion in diabetes is more severe than that of non-diabetic patients<sup>3</sup>. The pathogenesis of diabetic angiogenesis defects is not yet clear, and chronic hypoxia may be an important factor resulting in defects in diabetic angiogenesis<sup>4</sup>. So far, HIF-1 is the only transcription factor found with activity in the absence of oxygen. It is located on chromosome 14q21-q24, and contains two subunits,  $\alpha$  and  $\beta$ , of which HIF-1 $\alpha$  plays a major biological role. In a normoxic environment, HIF-1 $\alpha$  is degraded via the prolyl hydroxylation and ubiquitination of proteases. Under hypoxic conditions, however, the decreased ubiquitination increases the expression of HIF-1 $\alpha$ . HIF-1 $\alpha$  proteins translocate to the nucleus and bind to HIF-1 $\beta$  to form heterodimers, which can bind the

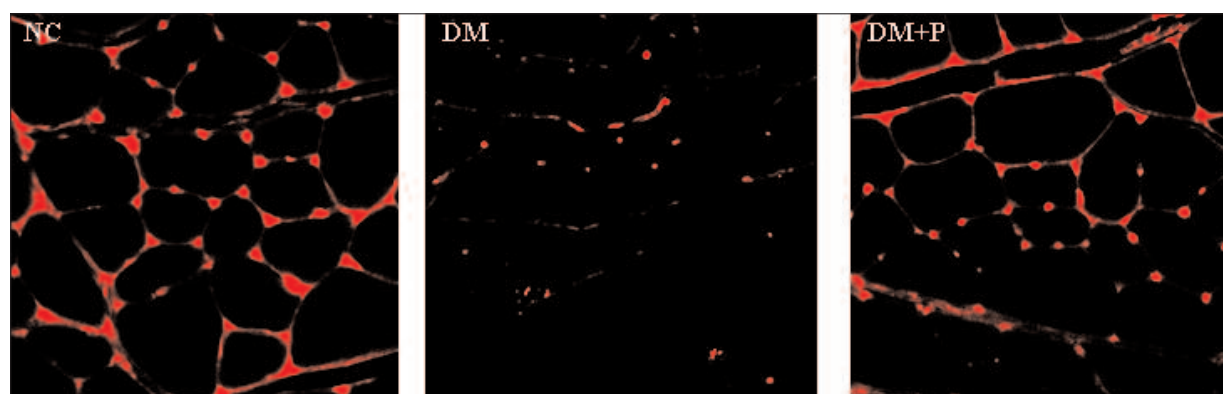
hypoxia response element (HRE) to initiate transcription. HIF-1 $\alpha$  is a functional subunit of HIF-1 and its protein expressions and transcription activities are mainly regulated by the intracellular oxygen concentrations. Research has demonstrated that the HIF-1 $\alpha$  levels are low in a normoxic environment. In contrast, hypoxic conditions provoke massive HIF-1 $\alpha$  accumulation, and the regulation on its expression profile occurs after the protein translation; in other words, HIF-1  $\alpha$  expression is regulated by regulating the stability of HIF-1 $\alpha$  proteins. HIF-1 $\alpha$  regulates the expression profiles of a series of hypoxia-related genes including transforming growth factor  $\beta$  (TGF- $\beta$ ), VEGF, nitric oxide synthase, and connective tissue growth factors; also, it is closely related with the proliferation and apoptosis of cells, the glycolysis, the angiogenesis, the constriction and dilation of blood vessels, and the growth and metastasis of tumors<sup>5</sup>. Thus, it plays a key role in the physiopathological processes of human body. HIF-1 $\alpha$  expression increases under hypoxic conditions<sup>5-6</sup>.

Under conditions of tissue ischemia-hypoxia in the diabetic state, the up- or down-regulation of HIF-1 $\alpha$  currently remains controversial. Some studies show that chronic hyperglycemia causes increased reactive oxygen species (ROS) and reduced anti-oxidation enzyme with lower activities. Hypoxia leads to a series of gene expressions in the body as it changes to adapt to low oxygen environments, such as stimulation of a variety of angiogenic factors, the synthesis of integrin and matrix metalloproteinases, inducing dissolution and extinction of extracellular matrix and basement membranes, and thus leading to cell migration and proliferation, finally leading to diabetic vascular structural and functional changes<sup>6-8</sup>. By use of Western blots in this study, it was found that HIF-1 $\alpha$  expression in hind limb ischemic tissue of diabetic rats was

**Table III.** Changes of blood perfusion ratio in each group of rats ( $\bar{x} \pm s$ ).

| Group                                      | n  | Preoperation    | Immediate postoperation | 7 days after operation | 14 days after operation   | 28 days after operation           |
|--|----|-----------------|-------------------------|------------------------|---------------------------|-----------------------------------|
| Control group (NC)                         | 12 | 0.98 $\pm$ 0.03 | 0.38 $\pm$ 0.07         | 0.45 $\pm$ 0.05        | 0.55 $\pm$ 0.08           | 0.68 $\pm$ 0.06                   |
| Diabetic group with hindlimb ischemia (DM) | 12 | 1.02 $\pm$ 0.06 | 0.36 $\pm$ 0.07         | 0.42 $\pm$ 0.07        | 0.44 $\pm$ 0.05**         | 0.49 $\pm$ 0.10**                 |
| Pioglitazone treated group (DM+P)          | 13 | 0.99 $\pm$ 0.06 | 0.35 $\pm$ 0.05         | 0.41 $\pm$ 0.05*       | 0.49 $\pm$ 0.04* $\Delta$ | 0.58 $\pm$ 0.12* $\blacktriangle$ |

Note: Compared with NC group, \* $p < 0.05$ , \*\* $p < 0.01$ ; Compared with DM group,  $\Delta p < 0.05$ ,  $\blacktriangle p < 0.01$ .



**Figure 3.** MVD changes in each group of rats with hindlimb ischemia. Fluorescence immunohistochemical staining of ischemic hindlimb muscle tissue CD31 in each group of rats. NC: Control group; DM: Diabetic group; DM+P: Pioglitazone treated group.

significantly increased compared with that of normal rats. The above results confirm that ischemia and hypoxia plays an important role in the pathogenesis of diabetic angiopathy, which suggests the possible role of HIF-1 $\alpha$  performed in the occurrence and development of diabetic angiopathy. Correlation analysis shows that HIF-1 $\alpha$  expression was positively correlated with serum glucose, insulin level, cholesterol, triglycerides, low density lipoprotein (LDL) and HOMA-IR index.

VEGF is one of the most effective pro-angiogenic growth factor, and closely related to the neovascularization after tissue hypoxia with ischemia and hypoxia as its strongest inducing factors<sup>9</sup>. In the HIF-1/VEGF hypoxia response pathway, while adaptation of HIF-1 $\alpha$  enhanced cells to hypoxic environment can protect cells against the damage caused by hypoxia, up-regulation of HIF-1 $\alpha$  expression can also further change transforming growth factor and VEGF, i.e., cytokine expression, which plays an important role in the progression of vascular lesions and angiogenesis<sup>10-12</sup>. VEGF action has two aspects, firstly, it can specifically and directly act on vascular endothelial cells, inducing endothelial cell proliferation and vascular lumen formation, which is an essential element for the formation of new blood vessels. Secondly, it can induce endothelial cell metalloproteinases and interstitial collagenase expression, increasing expression and activity of plasminogen activator, urokinase-type plasminogen activator and tissue-type plasminogen activator, to bring about decomposition of the capillary basement membrane promoting monocyte-macrophage cell migration and extracellular matrix accumulation. Thereby, promoting lower ex-

tremity atherosclerosis, eventually leading to the development of diabetic lower extremity angiopathy<sup>13</sup>. In this study, it was observed by Western blot that the control group showed weak VEGF expression, and VEGF expression in hindlimb ischemic tissue in diabetic rats was significantly increased compared with normal rats. This was positively correlated with HIF-1 $\alpha$  expression. Research of Rivard et al<sup>14</sup> is also consistent with these experimental results, probably because the expression of VEGF receptors is inhibited under the diabetic state and hypoxic conditions. Celletti et al<sup>15</sup> found that, in both a rat knockdown model of apo-E/apo-B100 and a rabbit hypercholesterolemia model, administration of VEGF can promote formation of atherosclerotic plaques. VEGF is also a pro-inflammatory cytokine causing atherosclerosis, which participates in the arterial restenosis process<sup>16</sup> through angiogenesis and induction of monocyte chemotaxis. In this study, HIF-1 $\alpha$  expression and VEGF, known to be downstream gene of HIF-1, have a significant positive correlation, the interacted hypoxia response element between them is located at VEGF RNA 5' end, the combination of HIF-1 and VEGF RNA 5' not only increases the stability of VEGF mRNA, but also enhances the transcriptional activity of VEGF, suggesting that in the presence of the regulator chain of hypoxia, HIF-1 $\alpha$  and VEGF in diabetic rats, the regulation of VEGF expression may be one of the most important mechanisms of action involved in diabetic angiopathy regulated by HIF-1 $\alpha$ .

Pioglitazone is a highly specific agonist of PPAR, which has been confirmed as having a role in the improvement of both glucose and lipid metabolism, while inhibiting proliferation of vas-

cular endothelial cell, reducing atherosclerosis, and having potential vascular protection<sup>17-18</sup>. In the present study, the fasting blood glucose, blood lipids and insulin resistance index in diabetic rats treated by pioglitazone for 4 weeks were significantly decreased. Furthermore, HIF-1 $\alpha$  and VEGF expression in ischemic hindlimb was also significantly reduced, suggesting that pioglitazone, by inhibiting HIF-1 $\alpha$  expression in diabetic ischemic tissue may be one of the mechanisms for protecting lower extremity vessels, which is consistent with the results reported in the literature<sup>19</sup>, i.e., pioglitazone can down-regulate ischemic glomerular HIF-1 $\alpha$  expression in diabetic rats.

Microvessel density (MVD) is the one of best indicators to reflect angiogenesis. In this study, the hindlimb vascular MVD of diabetic rats was significantly different compared with the control group, which is consistent with HIF-1 $\alpha$  and VEGF expression after pioglitazone intervention, directly reflecting the results of HIF-1 $\alpha$  and VEGF expression. HIF-1 $\alpha$  and VEGF protein expression levels in the diabetic rat group are all positively correlated with MVD, suggesting that HIF-1 $\alpha$  and VEGF protein expression levels in vascular endothelial cells are closely related to vascular proliferation. Currently, it is considered that HIF-1 is one of the initiation factors of a series of molecular reactions after limb ischemia. Increased expression of HIF-1 $\alpha$  and VEGF under limb ischemia can promote the formation of ischemic limb angiogenesis leading to compensatory adaptation, but HIF-1 $\alpha$  and VEGF are a double-edged sword on the generation and development process of diabetic angiopathy. The HIF-1 $\alpha$ -VEGF axis plays a role not only in up-regulating neovascularization under diabetic state, but also in upregulation of VEGF, increased vascular permeability, promoting lipoproteins and inflammatory cells into the intima, and endometrial angiogenesis, leading to the occurrence and development of atherosclerosis and plaque formation<sup>20</sup>, as well as coronary atherosclerosis (AS), degradation of neovascularization associated with AS plaque degradation. The above results suggest that angiogenesis plays an important role in the diabetic angiopathy characterized by AS lesions.

### Conclusions

This study shows that there is a significant expression of HIF-1 $\alpha$  and VEGF protein in is-

chemic hindlimbs of diabetic rats, indicating that hypoxic-ischemia is an important pathological mechanism in diabetic angiopathy. We believe that, in the HIF-1 $\alpha$ /VEGF hypoxia-induced signaling pathway, hypoxia/ischemia can induce the expression of HIF-1 $\alpha$ , thereby activating HIF-1 $\alpha$ , which is involved in a series of hypoxia-induced gene expression and regulation, including promoting enhanced expression of VEGF so as to mediate the pathophysiological process of diabetic angiopathy. HIF-1 $\alpha$  and VEGF expression, on the one hand, can promote cell metabolism and enhance the cell's ability to adapt to the hypoxic environment, having a positive effect on protecting cells from hypoxic injury. On the other hand, it also induces abnormal expression of multiple cytokines to change the local normal microenvironment, and then change the structure and function of normal tissue leading to a further deterioration of disease. Therefore, we can speculate that the observation of HIF-1 $\alpha$  and VEGF expression dynamics in diabetic angiopathy, as well as developing drugs aimed at HIF-1 $\alpha$  expression targets, may have some significance on prevention and treatment of diabetic angiopathy.

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### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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