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BENEFICIAL EFFECTS OF LIRAGlutIDE ON PERIPHERAL BLOOD VESSELS

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Abstract

Background/Aim. Macroangiopathy is the major cause of death and disability in type 2 diabetic patients. Studies have shown that liraglutide, a GLP-1 receptor agonist, can protect the cardiovascular system by inhibiting chronic inflammation of diabetes. However, the effects of liraglutide on peripheral blood vessels and peripheral blood leukocytes have not reported at home and abroad.

Objective: To observe and explore vascular protection and mechanism of liraglutide in addition to hypoglycemic effect.

Methods: 60 hospitalized patients with type 2 diabetes were recruited from December 2013 to December 2014 at the First Affiliated Hospital of Dalian Medical University. Before the treatment of liraglutide, height and weight were measure to calculate body mass index (BMI). Blood urea nitrogen (BUN) and so on were detected. Homeostasis model assessment of insulin resistance (HOMA-IR) and islet β cell function (HOMA-β) were computed. After applying liraglutide for three months, all indexes were measured again. The effects of liraglutide on these indexes were analyzed by paired sample t test.

Results: After treatment with liraglutide, HbA1c (8.46±1.62 vs 7.26±1.40%) and 2hPBG (11.95 vs 9.6 mmol/L) decreased significantly (P<0.05). Body weight (87.3 vs 82.5 kg) and BMI (30.37 vs 28.63 kg/m²) decreased by 5.5% and 5.7% (P<0.05). TG (2.57±1.54 vs 1.81±0.70 mmol/L) and LDL-C (2.92±0.78 vs 1.89±0.66 mmol/L) reduced significantly (P<0.05). ABI decreased from 1.24±0.10 to 1.14±0.06 cm/s by 8%, while baPWV decreased from 1442.15±196.26 to 1316.85±146.63 cm/s by 8.7%, and both difference was statistically significant (P < 0.001).

Conclusion: Liraglutide, with a good hypoglycemic effect, can significantly reduce postprandial blood glucose and HbA1c, but can not significantly improve fasting plasma glucose, insulin resistance and islet function. It also significantly decreased body weight, BMI and TG. Liraglutide can significantly lower ba-PWV and ABI to protect peripheral blood vessels.

Key words: type 2 diabetes, liraglutide, peripheral blood vessel, peripheral leukocyte.
INTRODUCTION

The China chronic Diseases and Risk Factors Surveillance in 2013 showed that the prevalence of diabetes in people aged 18 or older was 10.4%. Diabetes is an independent risk factor for cardiovascular and cerebrovascular diseases. The key to the treatment of type 2 diabetes (T2DM) is long-term stable control of blood glucose to prevent or delay the occurrence and development of chronic complications of diabetes. Epidemiological studies have found that type 2 diabetic patients have a 2-4 times higher risk of developing myocardial infarction and stroke than normal people. The prevention and treatment of diabetic macroangiopathy should be based on the control of blood sugar, blood pressure, blood lipids, body weight and other factors. The deposition of advanced glycosylation end products caused by persistent hyperglycemia, insulin resistance, hypertension and hyperlipidemia lead to an increase in oxidative stress, promotion of aggregation of blood mononuclear macrophages, and release of proinflammatory cytokines, that further damage vascular endothelium leading to atherosclerosis.

GLP-1 receptor agonists are a newer generation of hypoglycemic drugs. In addition to controlling blood sugar, they have good effects on reducing body weight, improving insulin resistance, treating fatty liver, and protecting the cardiovascular system. Liraglutide is a GLP-1 analogue produced by yeast using genetic recombination. In the LEADER study, Liraglutide was confirmed to have the effects of lowering blood sugar and glycosylated hemoglobin (HbA1c), improving insulin resistance, protecting islet function, decreasing blood lipids and body weight, and reducing vasoconstriction pressure. Liraglutide has been shown to protect cardiovascular function at both cellular and animal models. Liraglutide has also been shown to reduce cardiovascular events in diabetic patients during clinical trials and to protect the cardiovascular system by improving cardiovascular risk factors.

Atherosclerosis is a systemic disease, which often involves multiple blood vessels such as the carotid artery, lower extremity arteries, and renal arteries, in addition to coronary arteries and cerebral arteries. Because the carotid and lower extremity arteries are superficial and easy to examine, they are often considered to be the windows of systemic atherosclerotic disease. Epidemiological investigations have shown that peripheral
arteriosclerotic diseases seriously affect the prognosis of other cardiovascular and cerebrovascular diseases. With the advancement of inspection techniques, high-sensitivity noninvasive vascular examinations have been used in clinical practice. Pulse wave velocity (PWV) and ankle-brachial index (ABI) are two important evaluation indicators in these examinations. As an indicator of arterial stiffness, PWV can independently predict the risk of cardiovascular and cerebrovascular development and death.\(^2\) ABI can be used for early diagnosis of lower extremity obstructive disease.\(^2\) These two examinations can evaluate vascular arteriosclerosis from both function and structure of the blood vessels. Arteriosclerosis often changes in vascular function first, then changes in vascular structure. Increased PWV can occur in a variety of diseases associated with atherosclerosis, such as diabetes, high blood pressure, dyslipidemia, and severe kidney disease. Depending on the selected arteries, PWV can be divided into carotid-femoral PWV, carotid-radial PWV, carotid-brachial PWV, and brachial-ankle PWV (ba-PWV). The study found that ba-PWV was a better predictor of type 2 diabetic macrovascular complications than carotid PWV.\(^3\) Ba-PWV more than 1400 cm/s usually suggests an increase in vascular stiffness. ABI is the ratio of systolic pressure of the ankle to systolic pressure of the arm, which reflects the degree of openness of peripheral blood vessels. The normal value of ABI is 0.9–1.3. ABI more than 0.9 often indicates peripheral obstructive vascular disease. While ABI less than 1.3 often points out arterial calcification and weakened vasoconstriction. A systematic retrospective study manifested that ABI could be used to predict the occurrence of cardiovascular diseases.\(^4\)

Inflammation and insulin resistance are thought to be the basis of atherosclerosis in type 2 diabetes.\(^5\) Studies have shown that increased white blood cells can worsen insulin sensitivity and predict the development of diabetes.\(^6\) Mononuclear cells in the blood infiltrate into the intima and differentiate into macrophages. Infiltrating macrophages phagocytose oxidatively modified low-density lipoproteins and gradually transform into foam cells, which leads to the development of atherosclerosis. That mononuclear cells adhere to vascular endothelial cells is considered to be one of the earliest events in the complex mechanism of atherosclerosis.\(^7\) Inflammatory cytokines associated with its pathogenesis include interleukin (IL-6), C-reactive protein (CPR), and tumor necrosis factor (TNF)-α. CRP is an inflammatory factor in the development and progression of atherosclerosis. And low concentration of CRP is closely related to the occurrence of
cardiovascular disease. CRP can increase the expression of cell adhesion molecules and monocyte chemoattractant protein (MCP)-1, promote the production of matrix metalloproteinase (MMP)-1, activate the complement system to promote the production of arteriosclerosis, and reduce the expression of nitric oxide synthetase mRNA and the bioactive activity of nitric oxide in the endothelia.

**Objectives and methods**

**Objectives**

Inclusion criteria. Type 2 diabetic patients with poor glycemic control admitted to the Department of Endocrinology, the First Affiliated Hospital of Dalian Medical University from December 2013 to December 2014, aged between 18 and 70 years old, with fasting C-peptide (FCP) >1 ng/ml, HbA1c 7-11%. The T2DM diagnosis meets the diagnostic and classification criteria by WHO in 1999.

Exclusion criteria. Those who met any of the following criteria were excluded from the study. Severe renal dysfunction, creatinine clearance <60ml/min; abnormal liver function, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >120U/L; combined with severe acute complications of diabetes and stress; infection and malignancy; severe history of cardiovascular and cerebrovascular disease in the past 3 months; pregnant, preparing pregnant and lactating women; various diseases with a significant effect on blood sugar; using drugs other than hypoglycemic drugs that affect blood sugar, such as hormones; patients with a history of pancreatitis; patients with a history or family history of medullary thyroid carcinoma.

Exit criteria. Violation of inclusion criteria or compliance with exclusion criteria; serious adverse drug reactions; drug allergic reaction; loss during follow-up.

**Experimental drugs and administration methods**

Liraglutide (6 mg / ml, 18 mg / support), produced by Danish Novo Nordisk, is subcutaneous injected once a day. It can be injected at any time, not according to the meal time. The patient is recommended to be injected at the same time every day and the most convenient time.

**Methods**
Patients who met the inclusion criteria were given diabetic education and informed the role and adverse reactions of liraglutide. They filled in the informed consent form and were adjusted the treatment plan.

General information. The age, sex, and medical history of the enrolled patients were recorded in detail. Height and body weight were measured according to standard protocols to calculate body mass index (BMI).

Detection method and detection index. The patients were taken blood in the early morning fasted for 12 hours overnight. The automated biochemical analyzer measured Urea nitrogen (BUN), creatinine (Cre), glycosylated hemoglobin (HbA1c), fasting venous blood glucose (FPG), FCP, alanine aminotransferase (ALT), aspartate aminotransferase (AST), white blood cells (WBC), monocytes (M), total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and C-reactive protein (CRP) were measured by the automated biochemical anayszer. Fingertip blood glucose (2hPBG) was measured by the Roche blood glucose meter.

Parameter calculation formula. Creatinine clearance (Ccr) = \( \left[ \frac{(140 - \text{Age}) \times \text{Body weight (kg)}}{0.818 \times \text{Cre (μmol/L)}} \right] \), Homeostasis model assessment of insulin resistance (HOMA-IR) = 1.5+FPG (mmol/L) ×FCP (pmol/L) / 2800, Islet function (HOMA-β) = 0.27×FCP (pmol/L) / ( FPG (pmol/L) − 3.5), BMI = body weight (kg) / height² (m²).

Determination of ABI and ba-PWV using the arteriosclerosis detector. The temperature of the examination room was kept at 22-25 °C, and the rest was taken for 5 minutes before the measurement. The patient took off the heavy clothing and removed the socks to reveal the heel. The supine position was lying on the bed, with the upper arm and ankle strap, the ECG clip and heart sound map sensor. Heart sound map, heart rate value, left arm blood pressure, left arm pulse wave, right arm blood pressure value, right arm pulse wave, left ankle blood pressure value, left ankle pulse wave, right ankle blood pressure value, and right ankle pulse wave were collected. During the process, the acquisition was completed and the instrument automatically calculated ba-PWV and ABI after cuffs were filled and deflated twice.

Experiment process. The enrolled patients recorded general information in detail and signed informed consent on the day of admission. They were measured the above indicators and the next day for noninvasive peripheral blood vessel testing. The starting dose of liraglutide was 0.6 mg once daily for subcutaneous injection, and the patient was
observed for adverse drug reactions. If there was no obvious adverse reaction after 3-7 days, the dose was increased to 1.2 mg once daily. If there was a significant gastrointestinal reaction after the drug was added, the dose could be reduced to 0.6 mg/day, and the dose was increased after the low-dose administration time was extended. Patients with poor hypoglycemic effect increased the dose to 1.8 mg/day after 1.2 mg/day a week.

**Statistical method**

The normality of continuous variables was verified by S-K test. Variables that conform to normal distribution are expressed as mean ± SD, and the difference before and after liraglutide treatment was analyzed by paired t test. The non-normal distribution variables was expressed by quartile, and the difference was compared by non-parametric Wilcoxon test. The significant variables screened by univariate analysis were further analyzed by multiple regression analysis to clarify the independent factors. SPSS 25.0 software was used for statistical analysis. A double-tailed P value is less than 0.05 was considered statistically significant.

**Results**

3.1 Baseline data and the follow-up

In this study, 64 patients with type 2 diabetes mellitus were enrolled. Four of them dropped out during the follow-up, and 60 patients were finally enrolled (Table 1). Among them, 31 males and 29 females, with an age of 46.85±7.60 years, and a disease history of 8 (3-10) years, 21 had complications and 10 had chronic complications of diabetes mellitus. Noninvasive angiography was performed in 13 cases. After the use of liraglutide, the patients had adverse reactions such as nausea and loss of appetite to varying degrees, but they were tolerable. After that, the patients' nausea and other discomfort gradually disappeared. No patients were withdrawn because of severe gastrointestinal reactions. There was no allergy at the injection site. One of them had a marked hypoglycemia due to liraglutide, which was improved after prompt symptomatic treatment.

**Table 1: Baseline data for the patients**

| Gender (M/F)     | 31/29 |
|------------------|-------|

| Gender (M/F) | 31/29 |
| Variable                      | Value                          |
|-------------------------------|--------------------------------|
| Age (years)                  | 46.85±7.60 (32, 66)            |
| History (years)              | 8(3~10) (0.038, 17)           |
| ALT (U/L)                    | 39.42±18.93 (7, 95)           |
| AST (U/L)                    | 21(17, 31) (9, 64)            |
| Urea (μmol/l)                | 5.69(5.21~6.51) (3.34, 8.85)  |
| Cre (μmol/l)                 | 60.22±13.26 (32, 98)          |
| CCR (ml/min)                 | 178.46±48.34 (81.11, 319.53)  |
| Comorbidity                  | 21                             |
| Chronic complications        | 10                             |
| Gastric reaction             | 25                             |
| Side effects                 | Hypoglycemia                   |
|                              | allergic reactions             |

△ Variables are shown as $\bar{x}$±s, if the variable was conformed to normal distribution by S-K test.

# Variables are shown as quartile 50%(25%~75%), if the variable was not conformed to normal distribution by S-K test.

3.2 Effects of liraglutide on glucose, islet function and insulin resistance

After 3 months treatment of liraglutide, the levels of HbA1c and 2hPBG decreased significantly (P < 0.05), indicating the significant hypoglycemic effect of liraglutide. Although FPG and FCP decreased in varying degrees with no significant difference. HOMA-IR and HOMA-β showed no significant difference, suggesting the insignificant effects on improving insulin resistance and islet function.

3.3 Effects of liraglutide on body weight and BMI

The body weight decreased by 5.5% from 87.3 (80-101) kg to 82.5 (76-93) kg, while BMI decreased by 5.7% from 30.37 (29.43-31.83) kg/m² to 28.63 (27.78-30.80) kg/m², and both difference was statistically significant (P < 0.001). It can be concluded that liraglutide can significantly reduce body weight and BMI.

3.4 Effect of liraglutide on blood lipid

TG decreased from 2.57±1.54 to 1.81±0.70 mmol/L, with a decrease of 29.6%. LDL-C decreased by 35.3% from 2.92±0.78 to 1.89±0.66 mmol/L, and both difference was
statistically significant (P < 0.001). TC and HDL-C decreased by 12% and 4.8% respectively, whereas without significant difference (P>0.05), suggesting that liraglutide may effectively reduce the levels of TG and LDL-C.

3.5 Effect of liraglutide on inflammation

After liraglutide treatment, M, CRP and WBC all decreased in varying degrees, but the difference was not statistically significant.

3.6 Effect of liraglutide on peripheral vascular stiffness

ABI and baPWV are important indexes reflecting the level of peripheral vascular stiffness. Three months after treatment, ABI decreased from 1.24±0.10 to 1.14±0.06 cm/s by 8%, while baPWV decreased from 1442.15±196.26 to 1316.85±146.63 cm/s by 8.7%, revealing liraglutide may improve the peripheral vascular stiffness effectively.

Table 2: Comparing diabetic, blood lipid, inflammatory and vascular stiffness data in patients before and after Liraglutide administration

|                          | Baseline          | 3rd month        | t     | P       |
|--------------------------|-------------------|------------------|-------|---------|
| HbA1c (%)                | 8.46±1.62         | 7.26±1.40        | 5.234 | <0.001  |
| FPG (mmol/L)             | 8.3(7.24~8.84)    | 7.46(6.52~8.67)  | -1.612| 0.107   |
| 2hPBG (mmol/L)           | 11.95(10.15~13.4) | 9.6(8.425~11.05) | -5.913| <0.001  |
| FCP (ng/ml)              | 3.24±1.37         | 3.05±0.77        | 1.198 | 0.236   |
| HOMA-IR                  | 4.60±1.39         | 4.25±0.87        | 1.813 | 0.075   |
| HOMA-β                   | 62.47±33.02       | 69.78±39.09      | -1.711| 0.092   |
| BW (kg)                  | 87.3(80~101)      | 82.5(76~93)      | -5.974| <0.001  |
| BMI (kg/m²)              | 30.37(29.43~31.83)| 28.63(27.78~30.80)| -5.865| <0.001  |
| TC (mmol/L)              | 5.09(4.03~5.67)   | 4.48(3.74~5.36)  | -1.612| 0.107   |
| TG (mmol/L)              | 2.57±1.54         | 1.81±0.70        | 4.083 | <0.001  |
| LDL-C (mmol/L)           | 2.92±0.78         | 1.89±0.66        | 18.936| <0.001  |
| HDL-C (mmol/L)           | 1.24±0.24         | 1.18±0.24        | 1.614 | 0.112   |
| M (×109/L)               | 0.43(0.38~0.55)   | 0.43(0.34~0.54)  | -1.937| 0.053   |
$\Delta$ Paired t test was carried out, if the variable was conformed to normal distribution by S-K test.

# Paired non-parametric Wilcoxon test was carried out, if the variable was not conformed to normal distribution by S-K test.

* $P<0.05$

** $P<0.001$

### 3.7 Multivariate Regression Analysis for baPWV (Table 3)

Multiple regression analysis showed that in-ABI, in-HbA1c and in-TG were independent risk factors for in-baPWV. The contributions to baPWV are in-ABI, in-HbA1c and in-TG in turn. According to correlation coefficient, the following regression equation is constituted:

\[
\text{inbaPWV}=40.975+524.447\times \text{inABI} + 16.287\times \text{inHbA1c}+18.79\times \text{inTG}
\]

### Table 3 Multivariate Regression Analysis for increment of baPWV(3rd month vs baseline)

|                  | Unstandardized β | SD | Standardized β | t     | P      | Collinearity |
|------------------|------------------|----|----------------|-------|--------|--------------|
| Constant         | 40.975           | 22.014 | 0.437           | 1.861 | 0.068  |              |
| in-ABI (cm/s)    | 524.447          | 141.401 | 0.256           | 3.709 | <0.001 | 0.919        | 1.088        |
| in-HbA1c (%)     | 16.287           | 7.227   | 0.24            | 2.047 | 0.045  | 0.929        | 1.076        |
| in-TG (mmol/L)   | 18.79            | 9.181   |                 |       |        |              |

In-ABI=increment of ABI; in-HbA1c=increment of HbA1c; in-TG=increment of TG.

Increment=difference of a variable (baseline vs 3rd month)
**Discussion**

Macroangiopathy is one of the serious complications of T2DM. Prevention of the occurrence and development of macroangiopathy requires comprehensive control. Therefore, a new hypoglycemic drugs are particularly important for the role of diabetic macroangiopathy. This study observed 60 cases, including 13 cases of non-invasive vascular examination. The comparison before and after use of liraglutide showed that liraglutide could not only effectively reduce postprandial blood glucose, HbA1c, body weight, BMI, triglycerides, but also reduce ba-PWV and ABI.

HbA1c guides diabetes treatment, understands blood sugar control levels, adjusts hypoglycemic regimens, assesses quality of care and predicts diabetes complications. Studies abroad have shown that HbA1c was closely related to diabetes complicated with cardiovascular and cerebrovascular diseases. The UKPDS confirmed that for every 1% decrease in HbA1c, the risk of any endpoint associated with diabetes was reduced by 21% and the risk of developing myocardial infarction was reduced by 18%. In Chinese Type 2 Diabetes Guidelines (2017), HbA1c control targets should be <6.5% for patients with type 2 diabetes who have a shorter course, no complications, and a longer life expectancy without cardiovascular disease. In this research, HbA1c decreased significantly from 8.46 ± 1.62 to 7.26 ± 1.40, which was consistent with the results of the LEAD studies. The LEAD series of studies showed that liraglutide significantly reduced HbA1c in patients with type 2 diabetes and was superior to glimepiride and rosiglitazone. However, the average level of HbA1c in some patients in this study still did not meet the standard (HbA1c < 7%), considering the higher levels of HbA1c before the application of liraglutide, the shorter follow-up time, and the smaller dose of Liraglutide. 2hPBG decreased significantly from 11.94 ± 3.17 mmol/L before treatment to 9.63 ± 1.53 mmol/L (P<0.05), but there was no significant difference between before and after FPG (P>0.05), suggesting that liraglutide can effectively reduce postprandial blood glucose, but control limitedly fasting blood glucose. GLP-1 receptor agonists play a hypoglycemic effect by delaying gastric emptying, promoting insulin secretion, inhibiting glucagon and somatostatin secretion, so this drug has an advantage in controlling postprandial blood glucose.

Progressive failure of islet function and insulin resistance are considered to be the main pathological basis of T2DM. Long-term hyperglycemia and insulin resistance can cause
atherosclerosis caused by damage to the vascular endothelium. The insulin resistance index can be expressed by HOMA-IR. It is generally believed that HOMA-IR increase with the increase of insulin resistance. HOMA-IR is a HOMA-IR formula fitted with C-peptide. Studies have confirmed that this formula could also be used to assess an individual's insulin resistance. HOMA-IR and HOMA-β were calculated using this formula in our study. HOMA-IR and FCP decreased, while islet function HOMA-β increased, but there was no statistical significance (P>0.05). This results were different from other studies. LEADER-2 study showed that islet function was assessed by the ratio of proinsulin to insulin, and liraglutide improved islet beta cell function compared to glimepiride. In LEADER-3 study, HOMA-IR evaluated insulin resistance, and liraglutide significantly improved insulin resistance compared with glimepiride. LEADER-4 studies have shown that liraglutide significantly increased islet function compared with gliclazide, on the basis of HOMA-β and proinsulin-to-insulin ratios evaluate islet beta function. Moreover, liraglutide had the effect of reducing body weight, and the decrease in body weight also indirectly improved insulin resistance. In this study, the negative results of liraglutide for insulin resistance and islet function improvement might be related to fewer cases and shorter observation time.

T2DM is often accompanied by obesity. And diabetes with obesity increases the risk of cardiovascular disease. Current hypoglycemic drugs (such as insulin, sulfonylureas, thiazolidinediones) can cause an increase in body weight, and GLP-1 receptor agonists inhibit food intake by directly acting on the hypothalamus. By inhibiting gastric emptying through the autonomic nervous system, weight loss is achieved. Liraglutide was confirmed not to increase body weight in the phase II clinical trial, and liraglutide showed a weight-reducing effect in the phase III clinical trial. Liraglutide 1.8 mg/day individually or in combination with other hypoglycemic agents had a significant weight-reducing effect in the LEADER-1 to 5 series of studies. It was found that liraglutide could reduce visceral fat by using CT to analyze body composition in the LEADER-2 and LEADER-3 studies. Body weight decreased significantly from 87.3 (80-101) kg to 82.5 (76-93) kg, and BMI decreased significantly from 30.37 (29.43-31.83) kg/m² to 28.63 (27.78-30.80) kg/m² (P<0.05) in this study, which was confirmed again that liraglutide could reduce the body weight and BMI of patients. Liraglutide was confirmed to have effect on reducing visceral fat in another parallel study of this study.
Blood lipids are one of the cardiovascular risk factors, including TC, TG, HDL-C, LDL-C, and Free Fatty Acids (FFAs). GLP-1 receptor agonists can directly act, inhibit gastric emptying, promote insulin secretion, and increase the clearance of chylomicrons, to promote and enhance intestinal lipoprotein catabolism. Liraglutide reduces levels of TC, LDL-C, TG, HDL-C, and FFAs in LEADER-4 study. In this study, TG and LDL-C decreased significantly after treatment of liraglutide, while there was no significant difference between TC and HDL-C, that decreased after using liraglutide.

T2DM is often accompanied by systemic complications that lack early symptoms and are difficult to detect through symptoms. Early diagnosis, early intervention, and prevention of progression of diabetes complications are particularly important in the management of diabetes. Diabetic macroangiopathy is the leading cause of death and disability in T2DM, so it is necessary for early diagnosis of macroangiopathy. Arteriosclerosis is the main pathophysiological basis of diabetic macroangiopathy. Arterial stiffness is related to arteriosclerosis and can be used to predict cardiovascular and cerebrovascular diseases. ABI and ba-PWV are indicators of atherosclerosis. Ba-PWV is a vascular functional test for the detection of early atherosclerosis. ABI is a structural examination of blood vessels that can be used to understand the openness of blood vessels in the lower extremities and whether there is occlusion. A large number of clinical trials have demonstrated that liraglutide can reduce the occurrence of cardiovascular adverse events and protect cardiovascular function. However, the impact of liraglutide on peripheral blood vessels has not been reported yet. In this study, the effects of liraglutide on ba-PWV were statistically significant (P<0.05), indicating that liraglutide reduced peripheral vascular stiffness and protected peripheral blood vessels. ABI>1.3 suggests vascular calcification and reduced vasoconstriction. While ABI <0.9 indicates arterial stenosis. In this group study, ABI declined significantly from 1.24±0.10cm/s to 1.14±0.06cm/s after administration, which suggested the vasoconstriction be better than before treatment. The decrease of ABI value also predicted the improvement of arteriosclerosis by liraglutide treatment.

The mechanism of liraglutide in improving arteriosclerosis in patients with T2DM is not fully understood, and may be the result of a combination of inhibition of inflammation and non-inflammation. Noyan-Ashraf MH et al. fed C57BI6 mice for 32 weeks on a high-fat diet (45% of calories from fat) and a normal diet, and randomly divided the two groups into two sub-segments during the last week of feeding. In the sub-groups, liraglutide (30μg/kg,
2 times/day) and placebo were injected respectively. It was found that high-fat diet induced an increase in serum TNF-α, while treatment with liraglutide for 1 week was effective in reducing high-fat diet-induced elevation of TNF-α. It was detected that liraglutide could reduce intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 in vitro culture of human vascular endothelial cells. In HUVEC cultured in vitro, it was discovered that liraglutide intervention induced the synthesis of nitric oxide (NO), and the concentration of NO increased in a dose-dependent manner with liraglutide. At the same time, it was observed that 1μg/ml liraglutide could enhance the activity of endothelial nitric oxide synthase (eNOS) and restore the expression of eNOS at mRNA level induced by cytokines. Studies in HCAEC have also unearthed that liraglutide could increase eNOS phosphorylation and NO production by activating the AMPK pathway. Torres G et al have confirmed that liraglutide could increase the release of calcium ions from endoplasmic network in vascular smooth muscle cells, and increase mitochondrial calcium ion uptake through mitochondrial fusion protein-2, a key factor in mitochondrial-endoplasmic reticulum coupling, to enhance mitochondrial activity. Liraglutide can reduce the endoplasmic reticulum stress induced by high glucose in HUVEC, and also inhibit the expression of p53 up-regulated modulator of apoptosis (PUMA). Liraglutide can prevent endoplasmic reticulum-dependent apoptosis of vascular endothelial cells through mitochondrial modulation.

In this study, Inflammatory related indicators, such as WBC, M and CRP, were detected in the vascular protection mechanism. Increased peripheral blood leukocytes can be used to predict the development of diabetes. The concept that atherosclerosis is an immune-mediated inflammatory lesion has been widely accepted. Monocytes and macrophages are the prototype cells of the innate immune system and are present in various stages of atherosclerosis. Studies on apoE-deficient mice have invented that liraglutide significantly reduced the area of aortic atherosclerosis by inhibiting monocyte/macrophage aggregation and reducing foam cell formation, to prevent the occurrence of atherosclerosis. The clinical study of liraglutide on peripheral blood leukocytes and monocytes has not been reported yet. This research hopes to discuss the effects of liraglutide on WBC and M in peripheral blood, so as to explore the possible mechanism of liraglutide on vascular protection. However, the analysis of WBC and M before and after treatment showed that WBC dropped from (7.03±1.32)×10^9/L to (6.88±1.39)×10^9/L, and M went down from
0.43(0.38-0.55)×10^9/L to 0.43(0.34-0.54)×10^9/L. But the differences were not statistically significant. Therefore, the impact of liraglutide on WBC and M in peripheral blood may require a larger sample size and longer follow-up studies. CRP is an important marker of inflammation. Studies have shown that GLP-1 receptor agonists can reduce the inflammatory response in diabetic patients and reduce the level of CRP in the body. This study found that there was no significant difference in CRP before and after treatment with liraglutide, which was also inconsistent with the results of other studies. Van Raalte et al divided sixty-nine type 2 diabetic patients with poor glycemic control treated by metformin into two groups, each of those receiving exenatide and insulin glargine respectively for 1 and 3 year. The results of the study showed that exenatide reduced significantly serum high-sensitivity CRP compared with glargine. 38 Meta analyses also suggested that liraglutide significantly reduce serum CRP levels. 39 The negative results of CRP in this study may be related to less cases and large dispersion before treatment. Therefore, the effect of liraglutide on chronic inflammation in type 2 diabetic patients still requires further large-scale studies.

There were three deficiencies in this study. Firstly, the sample size included in this study was smaller. Secondly, no control group was used for comparative study results. Thirdly, follow-up time as short.

In summary, this study provides a new clinical basis for the beneficial effects of liraglutide on peripheral blood vessels. Liraglutide protects peripheral blood vessels in addition to lowering blood sugar, reducing body weight, and lowering blood lipids. Therefore, this study may be most beneficial to the treatment of patients with diabetic macroangiopathy or risk factors for diabetic macroangiopathy.

Conclusion
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