Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

# Novel Curcumin Analogue L6H4 in Treating Liver Fibrosis and Type 2 Diabetes

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**Purpose:** The objective of this study was to evaluate the therapeutic efficacy of the curcumin analogue L6H4 in attenuating liver fibrosis and alleviating insulin resistance in streptozotocin-induced diabetic rats.

**Methods:** Male Sprague-Dawley rats were fed a high-fat diet to induce insulin resistance, followed by streptozotocin injection to induce diabetes. The rats were then treated with L6H4 for eight weeks. Body weight, metabolic parameters, liver function, and liver histopathology were evaluated. Immunohistochemistry was performed to assess the expression of TGF-β1, TIMP-2, and MMP-2 in liver tissues. Statistical analysis was conducted using one-way ANOVA and Spearman rank correlation test.

**Results:** L6H4 treatment effectively reversed the weight gain associated with a high-fat diet and improved metabolic parameters in diabetic rats. Liver function markers, such as ALT and AST, were reduced after L6H4 treatment. Histological analysis showed improved liver morphology and reduced fibrosis in L6H4-treated rats. Electron microscopy revealed improved ultrastructural features of hepatocytes. Immunohistochemistry demonstrated downregulation of TGF-β1 and TIMP-2 expression and restoration of MMP-2 expression in the liver tissue of L6H4-treated rats. Correlation analysis showed a significant positive correlation between TGF-β1 and TIMP-2 expression.

**Conclusion:** The findings suggest that L6H4 has therapeutic potential in attenuating liver fibrosis and alleviating insulin resistance in streptozotocin-induced diabetic rats. The hepatoprotective effect of L6H4 may be attributed to its anti-inflammatory properties and its ability to target molecules involved in fibrosis. Further research is warranted to explore the potential of L6H4 as a treatment option for nonalcoholic fatty liver disease and type 2 diabetes.

**Plain Language Summary:** The versatile curcumin, derived from turmeric, has shown potential in treating type 2 diabetes (T2D) and its complications. However, its bioavailability is limited. This study evaluated the efficacy of L6H4, a curcumin analogue, in treating T2D-induced hepatic fibrosis in rats. The rats were fed a high-fat diet and injected with streptozotocin to induce T2D. L6H4 treatment for eight weeks reversed weight gain, abnormal liver function, and histological changes. The analogue reduced markers of T2D severity, including blood glucose, insulin levels, and insulin resistance. L6H4 also decreased liver fibrosis by reducing the expression of TGF-β1 and TIMP-2 and increasing MMP-2 expression. These changes in protein expression were consistent with previous studies on liver fibrosis. The effects of L6H4 on MMP-2 and TIMP-2 expression in the liver of diabetic rats had not been observed before. Additionally, L6H4 exhibited anti-inflammatory properties and regulated the expression of key fibrosis-related proteins. The study suggests that L6H4 has the potential as a therapeutic candidate for treating diabetic hepatopathy. Further research is needed to elucidate the molecular mechanisms of action of L6H4 and other curcumin analogues in T2D and liver fibrosis. In conclusion, L6H4 shows promise as a curcumin analogue with hepatoprotective effects and the ability to modulate protein expression involved in liver fibrosis in T2D.

**Keywords:** type 2 diabetic rats, curcumin analogue, L6H4, TIMP–2, MMP–2, TGF-β1



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## Introduction

Insulin signaling plays a crucial role in regulating glucose levels by inhibiting glucose production and promoting fatty acid synthesis in a healthy liver.[1] The development of type 2 diabetes (T2D), a multifactorial disease, is often attributed to a combination of genetic predisposition and environmental factors that lead to obesity and insulin resistance. Both overnutrition and nonalcoholic fatty liver disease (NAFLD) can contribute to and result from insulin resistance.[1] NAFLD is primarily characterized by the accumulation of triacylglycerol in the liver.[2] Dysregulation of lipid metabolism and hepatic lipid accumulation are part of its etiology. One study showed that liver steatosis, but not fibrosis or inflammation, is independently and significantly associated with insulin resistance in NAFLD patients.[3] Intrahepatic fat, rather than visceral fat, is associated with insulin resistance in the liver, skeletal muscle, and adipose tissue.[4] Adipose tissue inflammation contributes to insulin resistance, and hepatic insulin resistance is the primary event leading to diabetes, followed by peripheral tissue insulin resistance. Lifestyle changes and therapeutic interventions can aid in managing T2D by reducing hyperglycemia and modulating insulin levels and signaling. However, there is currently no approved drug specifically for NAFLD treatment, highlighting the ongoing need for safer alternative drugs with fewer adverse effects.

Curcumin, a versatile compound found in turmeric (Curcuma longa), exhibits a wide range of properties, including anticancer, anti-inflammatory, antioxidant, and antiviral activities.[5,6] Some studies have demonstrated that curcumin can prevent obesity-induced insulin resistance and its associated complications.[7–10] Extensive efforts have been made to modify the structure of curcumin to enhance its bioavailability[11] and efficacy. Various research labs have synthesized several curcumin analogues with improved stability in vitro and pharmacokinetic profiles in vivo, making them potential replacements for curcumin.[12–15] One such analogue, L6H4 (compound 23 in reference 16, Figure 1) exhibits more potent anti-inflammatory properties.[16]

In this study, rats were fed a high-fat diet for four weeks to induce T2D, followed by a single intraperitoneal injection of streptozotocin (STZ) (30 mg/kg) to develop the diabetic condition further. The rats were then orally administered L6H4 at a 0.2 mg/kg/day dose for 56 days. We evaluated the therapeutic efficacy of L6H4 by assessing its ability to attenuate the progression of liver fibrosis and alleviate STZ-induced diabetes in rats. Our findings indicate that L6H4 improved the morphological and histopathological changes observed in the livers of diabetic rats. The hepatoprotective effect of L6H4 can be attributed, at least in part, to its anti-inflammatory properties and ability to target molecules such as TGF-β1, MMP-2, and TIMP-2, along with their corresponding signaling pathways.

## Materials and Methods

### Chemical Reagents

The School of Pharmaceutical Science of Wenzhou Medical University provided the curcumin analogue L6H4, also known as compound 23, in reference 16.[16] It was dissolved in a 1% sodium carboxyl methyl cellulose (CMC-Na) solution for the in vivo experiments. Streptozotocin (STZ) was obtained from Xiamen Xinglongda Chemical Reagent Co., Ltd. (Xiamen, China) and dissolved in citrate buffer for intraperitoneal injection. The high-fat diet used in the study was prepared by the Wenzhou Medical University Animal Center and consisted of 10.0% adipose, 20.0% caramel, 2.5% cholesterol, 1.0% bile salt, and 66.5% regular diet.

### Animal Experiments

Six-week-old male Sprague-Dawley (SD) rats weighing 160–200 g were obtained from the Animal Center of Wenzhou Medical University (Wenzhou, China). They were housed in a controlled environment with a 12:12-hour light-dark cycle, provided with a standard rodent diet and water, and acclimatized for at least three days before the experiment. The rats were randomly divided into five groups, each consisting of eight rats: 1) normal control group (NC); 2) high-fat (HF); 3) high-fat treated with L6H4 (FT); 4) diabetes mellitus (DM); and 5) diabetes mellitus treated with L6H4 (DT). The normal control rats were fed a regular diet, while the other groups were fed a high-fat diet for four weeks to induce insulin resistance. Type 2 diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) (30 mg/kg dissolved in citrate buffer) on the last day of high-fat diet feeding. Rats with serum glucose levels ≥ 16.7 mmol/L three days after STZ injection were considered diabetic and further grouped for treatment. The FT group and DT

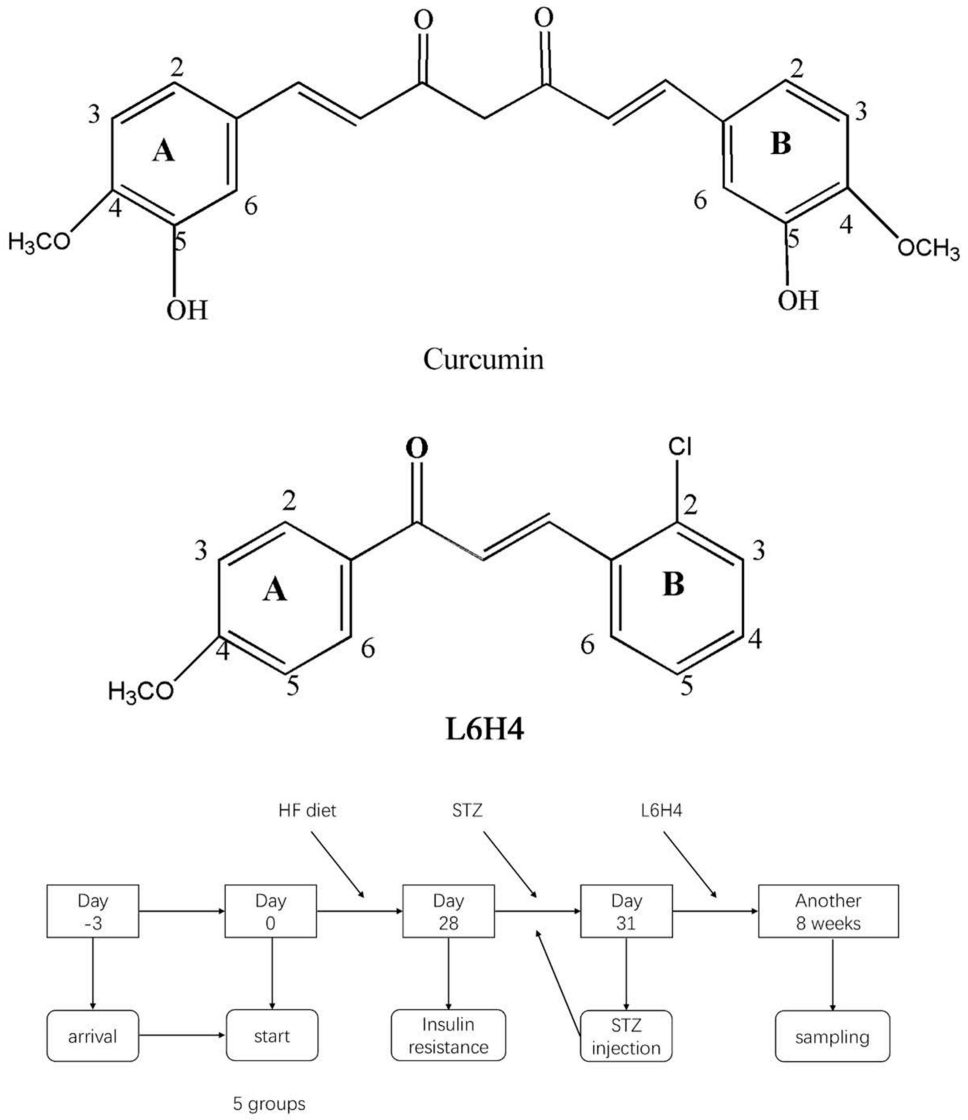


**Figure 1** The structure of curcumin, L6H4, and the timeline of the experiment.
**Abbreviations**: HF, High fat; FT, STZ, Streptozotocin.

group were administered L6H4 orally by gavage at a dosage of 0.2 mg/kg/day for eight weeks, while the remaining groups received an equal volume of 1% sodium carboxyl methyl cellulose (CMC-Na) solution by gavage. After eight weeks of treatment, the rats were euthanized by femoral artery bloodletting. The experimental timeline is depicted in Figure 1. All animal procedures complied with the guidelines and approval of the Ethics Committee of Wenzhou Medical University Animal Policy and Welfare Committee (Approval number wydw2014–0052).

## Body Weight and Biochemical Indicator Analysis

Rats were weighed once a week throughout the experiment. Blood samples were allowed to stand at room temperature for 25 minutes, then centrifuged at 1500 g for 20 minutes to separate the serum, which was collected and stored in a freezer. Fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-c), high-density lipoprotein-cholesterol (HDL-c), LDL/HDL ratio, as well as serum liver function indices including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin (ALB), were measured using a Hitachi 7600 biochemical analyzer (Hitachi, Japan). Free blood insulin (FIN) levels were determined using radioimmunoassay. Insulin resistance homeostasis model assessment-estimated insulin resistance (HOMA-IR) was

calculated using the following formula: HOMA-IR = (FBG × insulin) / 405, where FBG is given in mg/dl and insulin in μIU/mL.

## Microscopic Pathological Analysis

Rat liver samples were fixed in paraformaldehyde and then embedded in paraffin. Sections were prepared and stained for histopathological examination using hematoxylin and eosin (H&E) staining to observe tissue morphology. Adipose analysis was performed using red Oil O staining, and collagen deposition was detected using Masson's Trichrome staining. Five random fields were selected under low magnification (×100) for each tissue section using a light microscope (Olympus, Tokyo, Japan). The sections were examined by a trained pathologist and reviewed by an independent examiner. Liver fibrosis was scored on a scale of 0 to 4 based on severity according to the criteria by Farrell et al.[17] Electron microscopy was conducted following the method described by Hamonic et al.[18] The liver was rapidly excised and immersed in liquid nitrogen buffer, fixed in glutaraldehyde for 2–4 hours at 4 °C, washed, dehydrated, embedded in resin, and sliced using an ultramicrotome (Leica, Germany). Longitudinal sections were placed on copper grids, stained with heavy metals (uranyl acetate and lead nitrate), and visualized using an H-7500 electron microscope (Hitachi, Tokyo, Japan).

## Immunohistochemistry

Formalin-fixed and paraffin-embedded tissues were cut into 4-mm thick sections and subjected to immunohistochemistry using En Vision staining, following the manufacturer's instructions. The sections were initially blocked with 5% BSA and then incubated with specific primary antibodies, including anti-TIMP-2 antibody (1:50, Santa Cruz, CA, USA), anti-MMP-2 antibody (1:200, Abcam Inc, MA, UK), or anti-TGF-β1 antibody (1:150, Boshide Biotech, Wuhan, China). The incubation was performed overnight at 4°C. Subsequently, the sections were incubated with a secondary antibody, goat anti-rabbit immunoglobulin G (Santa Cruz, CA, USA). As a negative control for staining, some tissue sections were incubated with phosphate-buffered saline (PBS) without primary antibodies. For image analysis, the Optical Density (OD) was calculated using Image-Pro Plus 6.0 image processing software based on five randomly chosen microscopic fields.

## Statistical Analysis

The data were presented as means ± standard deviations (SDs) and analyzed using SPSS Version 19.0 statistical software. The statistical significance was assessed using a one-way analysis of variance (ANOVA) and Spearman rank correlation test. A probability value (P value) of less than 0.05 was considered statistically significant.

# Results

## Body Weight and Metabolism Before and After L6H4 Treatment

The rats fed a high-fat diet exhibited significantly higher body weight than those fed with normal chow, while the diabetic rats showed significant weight loss, consistent with previous studies (Figure 2A). [7,9,18] Treatment with the curcumin analogue L6H4 (groups FT & DT) effectively reversed the trend of body weight in these two groups (FT & DT) (Figure 2A). Total cholesterol and triglycerides were significantly elevated in the HF and DM groups compared to the NC group (Figure 2B and C), whereas the albumin level showed the opposite trend (Figure 2F). Furthermore, L6H4 treatment significantly reduced other metabolic parameters in the DT group, including total cholesterol, triglycerides, and LDL/HDL ratio (Figure 2B–D), indicating its potential to correct metabolic disorders.

## Functional and Pathological Evaluation of Livers Before and After L6H4 Treatment

Liver function was assessed by measuring the levels of ALT and AST enzymes in the plasma of rats. The HF group showed a significant increase in AST levels compared to the NC group (Figure 2E), and the DM group had even higher levels of ALT and AST enzymes. After the 8-week treatment with L6H4, the ALT and AST levels in the FT and DT groups were reduced to levels similar to those of the control group (Figure 2E).

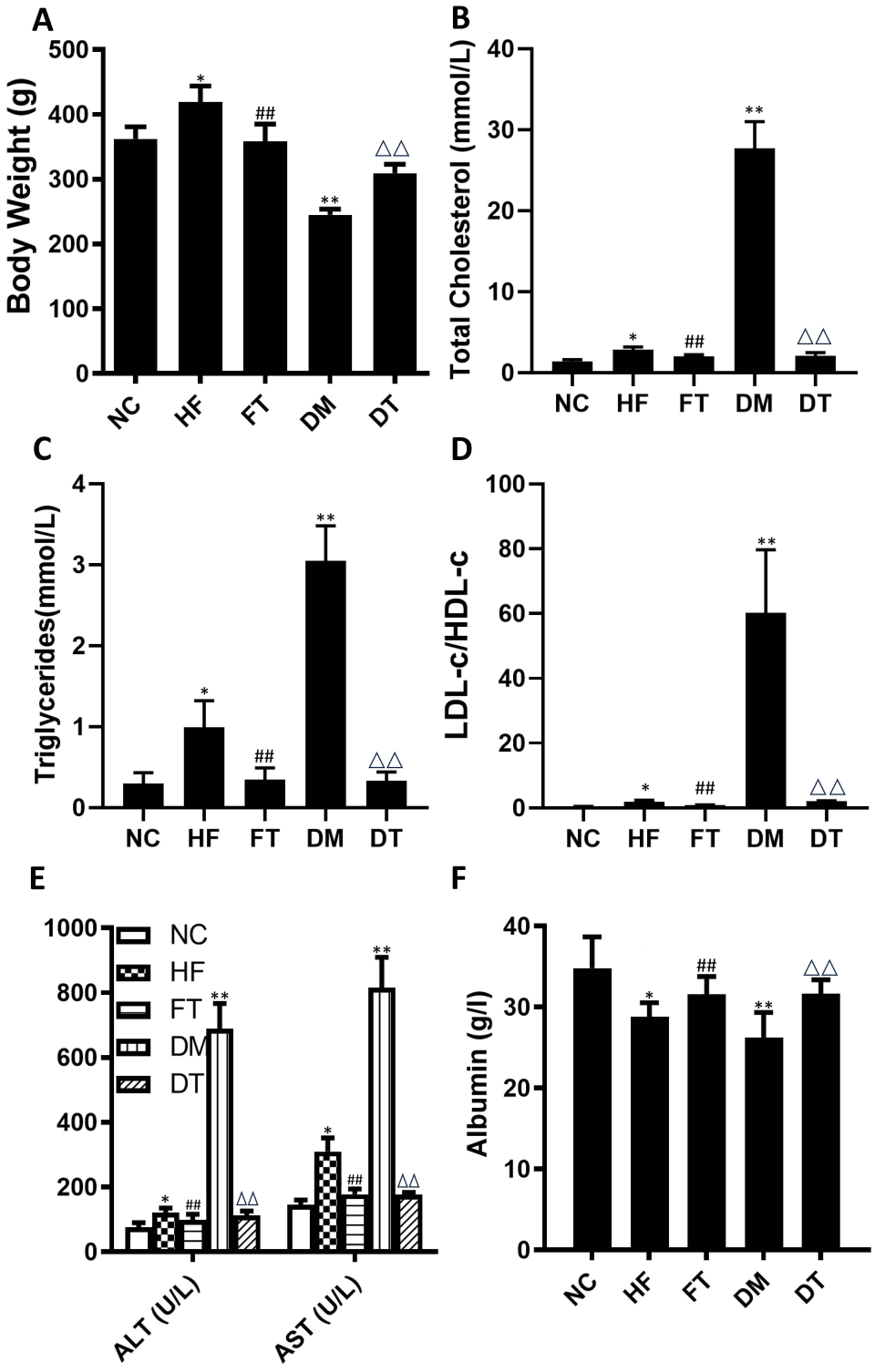


**Figure 2** This figure shows changes in body weight and biochemical analysis of SD rats in the NC, HF, FT, DM and DT. (**A**) Body weights of the rats. (**B**) Total Cholesterol Level. (**C**) Triglycerides Level. (**D**) LDL-c/HDL-c Level. (**E**) ALT and AST Level. (**F**) Albumin level.. Mean ± SD; *P<0.05 vs NC, **P<0.05 vs NC; ##P<0.05 vs HF; △△P<0.05 vs DM.
**Abbreviations**: TC, total cholesterol; TG, triglycerides; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; LDL-c, low-density lipoprotein-cholesterol; HDL-c, high-density lipoprotein-cholesterol; NC, Normal control; HF, High fat; FT, High fat treatment; DM, Diabetes mellitus; DT, Diabetes treatment.

Compared with those in the NC group (Figure 3A1, B1 and C1) the histological analysis of the liver structure revealed that rats in the HF group exhibited typical signs of obesity, including the presence of adipose droplets with varying sizes in the cytoplasm (as indicated by red Oil O staining in Figure 3B2, arrow), infiltration of inflammatory cells (lymphocytes,

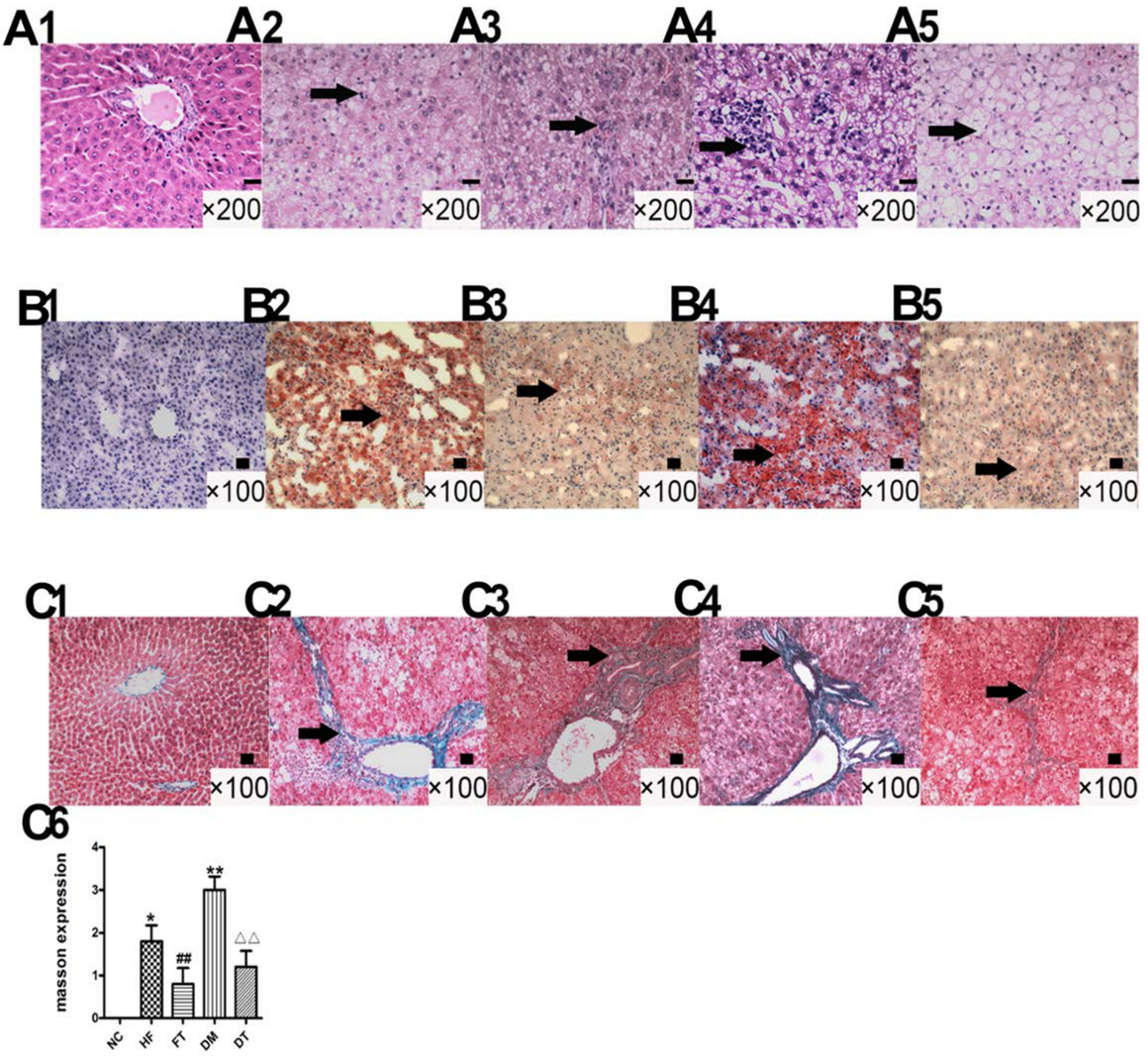


**Figure 3** Pathological changes in the liver of diabetic rats before and after L6H4 treatment upon HE (**A1**–**A5** Scale bar: 50 μm), Red Oil O (**B1**–**B5** Scale bar: 100 μm) and Masson (**C1**–**C5** Scale bar: 100 μm) staining and grading (**C6**). HE staining showed inflammation in the liver of the HF and DM group (Black arrow in **A2** and **A4**), which was reduced in rats treated with L6H4 (**A3** and **A5**). Red Oil O staining showed the diffuse distribution of adipose droplets (Black arrow in **B2** and **B4**), which was reduced in rats treated with L6H4 (**B3** and **B5**). Masson staining indicated hyperplasia of the collagen fibers (Black arrow in **C2** and **C4**), which was diminished in rats treated with L6H4 (**C3** and **C5**). Mean ± SD; *P<0.05 vs NC, **P<0.05 vs NC; $^{\#\#}$P<0.05 vs HF; $^{\Delta\Delta}$P<0.05 vs DM.
**Abbreviations**: NC, Normal control; HF, High fat; FT, High fat treatment; DM, Diabetes mellitus; DT, Diabetes treatment.

monocytes, and plasma cells, Figure 3A2, arrow), and mild collagen fiber hyperplasia and fibrosis (as graded by Masson staining in Figure 3C2, arrow). The severity of liver fibrosis is shown in Figure 3C6. The livers of rats in the DM group showed further damage, characterized by multifocal inflammatory cell infiltration, more pronounced fatty degeneration, and disruption of the normal hepatic cord (Figure 3A4, arrow), increased lipid droplets (Figure 3B4, arrow), and widespread collagen fiber deposition (even in the portal area) with thicker septa and increased fibrosis (Figure 3A4 and C4, arrow).

Ultrastructural examination revealed the formation of lipid particles within hepatic cells, with some fusing into large fat droplets in the HF and DM groups (Figure 4B and D, arrow), in contrast to the NC group (Figure 4A). Certain hepatocytes displayed pyknotic nuclei (indicating apoptosis), swollen rough endoplasmic reticulum, and mitochondria (Figure 4B and D).

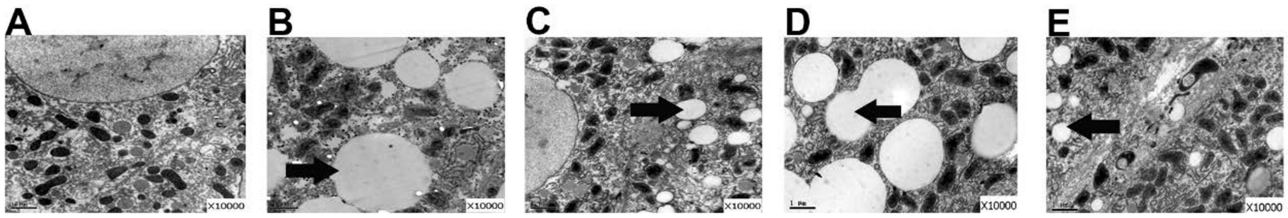


**Figure 4** Ultrastructural changes in the liver of diabetic rats before and after L6H4 treatment under scanning microscope (**A**–**E**) among various groups. Lipid particles fused into huge fat drops in the HF and DM groups (Black arrow in **B** and **D**), which was decreased in rats treated with L6H4 (**C** and **E**).
**Abbreviations**: HF, High fat; DM, Diabetes mellitus.

After eight weeks of L6H4 treatment, a significant improvement in liver structure was observed in the FT and DT groups, characterized by better tissue architecture under light and electron microscopy, reduced inflammatory cell infiltration (Figure 3A3 and A5, arrow), decreased presence of adipose droplets in the cytoplasm (Figure 3B3 and 3B5, arrow), and decreased collagen fibers (Figure 3C3 and C5, arrow). The ultrastructure of hepatocytes also showed a dramatic change (Figure 4C and E, arrow). Liver sections from diabetic rats treated with L6H4 displayed thinner septa and better-preserved hepatic parenchyma (Figure 4E, arrow).

## Analysis of TGF-β1, TIMP-2 and MMP-2 Before and After L6H4 Treatment

Immunohistochemistry was performed to evaluate the distribution and expression of TGF-β1, TIMP-2, and MMP-2 in the liver. The tissue sections stained with the respective antibodies (Figure 5) and the quantification of staining intensity (Figure 6) revealed that the expression of TGF-β1 and TIMP-2 was significantly increased in the liver tissue of rats in the

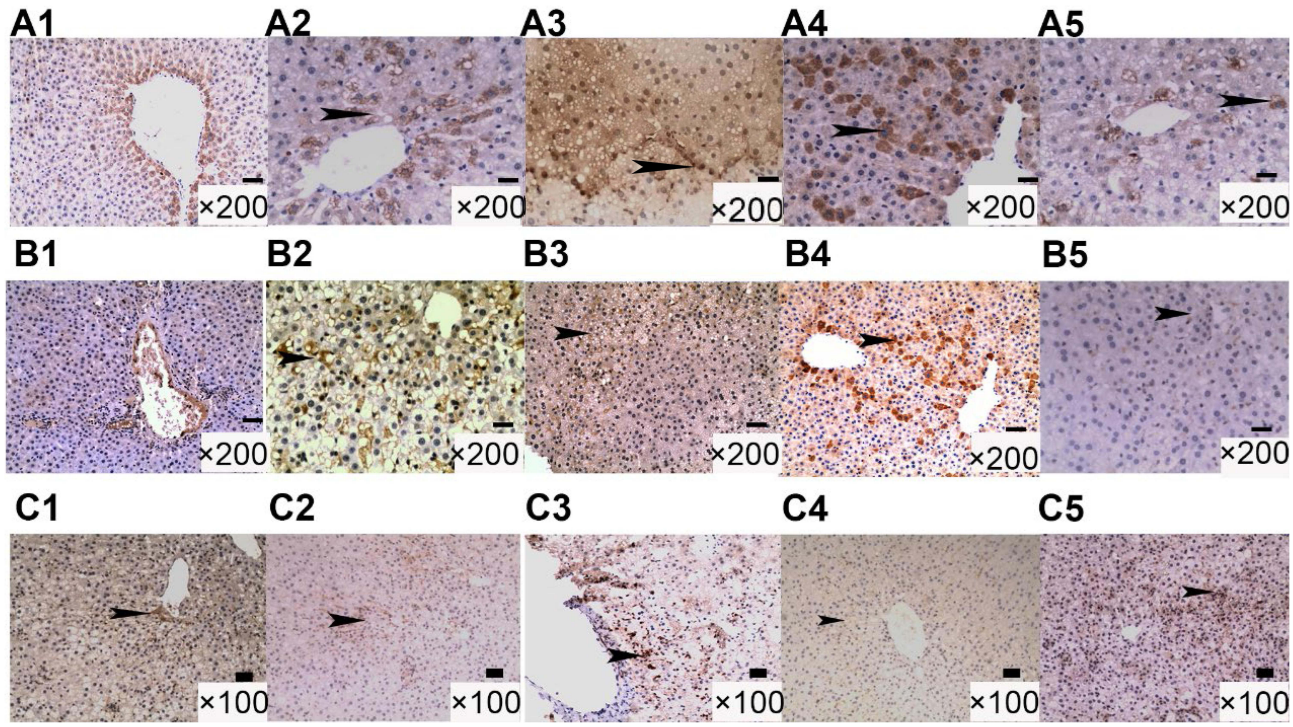


**Figure 5** Immunohistochemical stainings of TIMP-2 (**A1**–**A5** Scale bar: 50 μm), TGF-β1 (**B1**–**B5** Scale bar: 50 μm), and MMP-2 (**C1**–**C5** Scale bar: 100 μm) in the livers of diabetic rats before and after L6H4 treatment. Expression of TGF-β1 and TIMP-2 was significantly increased in the liver tissue of the rats in the HF and DM group (Black arrowhead in **A2** and **A4, B2** and **B4**), which was decreased in rats treated with L6H4 (Black arrowhead in **A3** and **A5, B3** and **B5**). MMP-2 expression decreased in the level of the HF and DM group (**C2** and **C4**), enhanced after the L6H4 invention (**C3** and **C5**).
**Abbreviations**: TIMP-2, tissue inhibitors of metalloproteinase-2; TGF-β, transforming growth factor-β; MMP-2, matrix metalloproteinase-2; HF, High fat; DM, Diabetes mellitus.

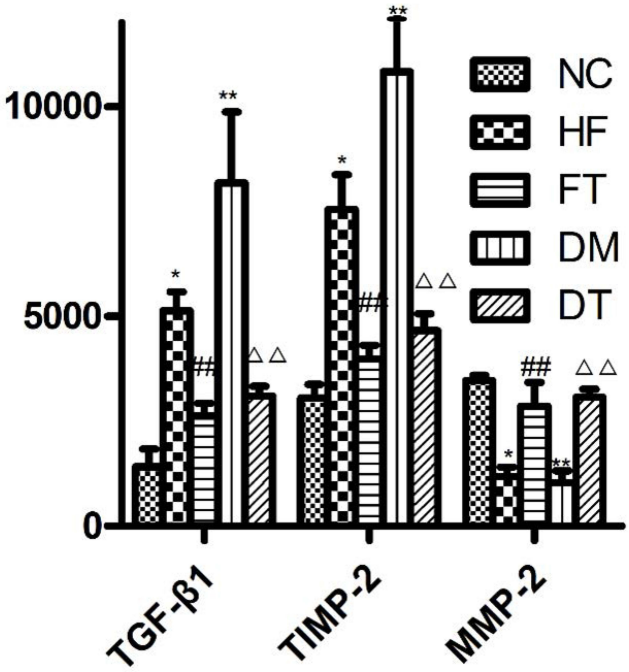


**Figure 6** The expression levels of TIMP-2, TGF-β1, and MMP-2 in the livers of diabetic rats before and after L6H4 treatment. Mean ± SD; *P>0.05 vs NC, **P<0.05 vs NC; ##P<0.05 vs HF; ΔΔP<0.05 vs DM.

**Abbreviations**: TIMP-2, tissue inhibitors of metalloproteinase-2; TGF-β, transforming growth factor-β; MMP-2, matrix metalloproteinase-2; NC, Normal control; HF, High fat; FT, High fat treatment; DM, Diabetes mellitus; DT, Diabetes treatment.

HF and DM groups (Figure 5A2, A4, B2, and B4, arrowhead). In contrast, MMP-2 expression was significantly decreased in these groups (Figure 5C2 and C4, arrowhead) compared to the NC group (Figure 5A1, B1, and C1).

After administration of L6H4 to the rats in the FT and DT groups, the expressions of TGF-β1 and TIMP-2 were downregulated (Figure 5A3, A5, B3, and B5), and MMP-2 expression was restored almost back to the same level as the NC group (Figure 5C3 and C5).

Correlation analysis revealed a significant and positive correlation between the expression of TGF-β1 and TIMP-2 in liver tissues (r=0.88, P<0.01).

## Attenuation of Insulin Resistance

The levels of fasting plasma glucose (FPG), free blood insulin (FIN), and homeostasis model assessment-estimated insulin resistance (HOMA-IR) were measured at the end of oral L6H4 treatment. In rats of the HF and DM groups (Figure 7), the levels of FPG, FIN, and HOMA-IR were significantly higher compared to the normal control group (NC) ($P < 0.05$), indicating the presence of mild and severe insulin resistance in the HF and DM rats, respectively.

However, treatment with L6H4 significantly decreased the levels of FPG, FIN, and HOMA-IR ($P < 0.05$), indicating the potential effectiveness of L6H4 in improving insulin sensitivity.

# Discussion

The versatile compound curcumin, found in turmeric (Curcuma longa), is known for its various health benefits. However, it has a bioavailability issue. In this study, we evaluated the performance of L6H4, an analogue of curcumin with a potency greater than curcumin, in treating type 2 diabetic rats.[16] L6H4 showed an IC50 of 7.32 and 8.22, respectively, in inhibiting TNF-α and IL-6 production from macrophages upon LPS stimulation.[16] The effects of L6H4 were compared to those of curcumin[19] and its other reported analogues, but the exact mechanism(s) of how L6H4 exerts its effects remain to be elucidated.[20–27]

To create an obese rat model, we fed rats a high-fat diet for 4 weeks, resulting in significant weight gain, abnormal liver function (notably elevated AST levels), and histological changes (including varied sizes of lipid droplets in the liver

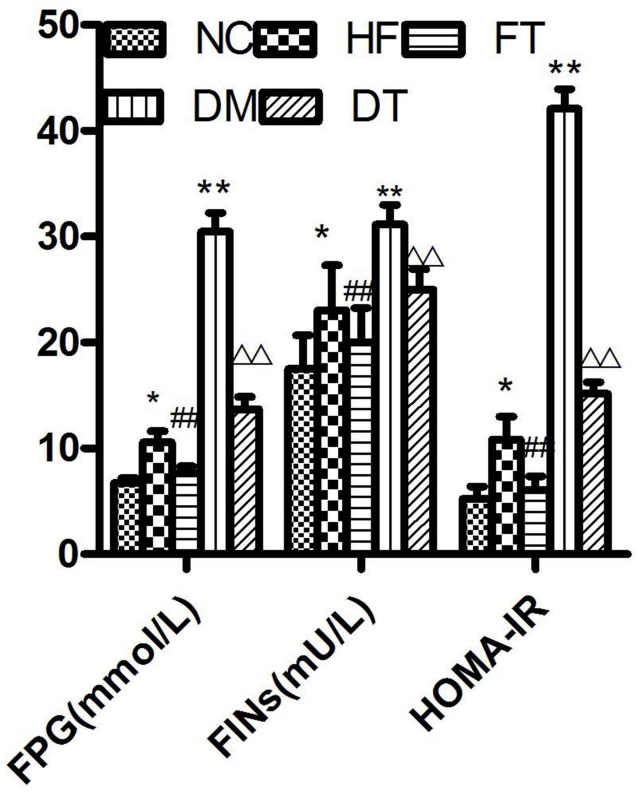


**Figure 7** Changes of FPG, FINs, and HOMA-IR of the diabetic rats before and after L6H4 treatment. NC: Normal control; HF: High fat; FT: High-fat treatment; DM: Diabetes mellitus; DT: Diabetes treatment. Mean ± SD; *P<0.05 vs NC, **P<0.05 vs NC; ##P<0.05 vs HF; △△P<0.05 vs DM.

**Abbreviations**: FPG, Fasting plasma glucose; FINs, Fasting insulin; HOMA-IR, blood indexes of insulin resistance homeostasis model assessment-estimated insulin resistance; NC, Normal control; HF, High fat; FT, High fat treatment; DM, Diabetes mellitus; DT, Diabetes treatment.

cytoplasm). Administration of L6H4 by gavage for 8 weeks effectively restored body weight, AST level, blood glucose, free insulin, and insulin resistance to normal, showing a similar effect to curcumin[19] and its analogues.[20–27] However, further research is needed to understand the exact mechanisms by which L6H4 achieves this effect.[19]

In another set of experiments, we induced a type 2 diabetes (T2D) model in obese rats with a single dose of streptozotocin injection, resulting in more remarkable markers (as discussed in comparison to L6H4 treatment) than those observed in the obese rats. However, L6H4 treatment for 8 weeks effectively brought the underweight diabetic rats back to a nearly normal weight. Furthermore, L6H4 improved liver function, as evidenced by the AST and ALT values (Figure 3E), and reduced total cholesterol (TC) and LDL-C levels (Figure 3C and D). L6H4 also significantly reduced FPG, FIN, and HOMA-IR in diabetic rats, although it did not completely restore them to the control level (Figure 3B). These findings contrast with an earlier study on db/db mice, which showed that curcumin had no effect on blood glucose levels.[28] Additionally, L6H4 treatment reduced the number of lipid droplets and inflammatory cells, as well as the amount of collagen fibers, thereby reducing fibrosis in the liver and restoring both the general liver microscopic structure and its mitochondrial ultrastructure.

Hepatic fibrosis is one of the complications of T2D. In the current model, we observed signs of liver fibrosis with elevated expression of TGF-β1 and TIMP-2, along with decreased expression of MMP-2 in the livers of diabetic rats. L6H4 treatment decreased the expression of TGF-β1 and TIMP-2 but increased that of MMP-2 in the livers of diabetic rats. The increase of TGF-β1 expression in liver fibrosis has been observed in several studies on rats[29–32] and mice,[33,34] even though the experimental design varies in terms of rat species, inducing agent (streptozotocin vs CCl4), dose (single or multiple), and duration of the experiment. Our findings are consistent with previous studies that analyzed TGF-β1 expression at both the mRNA level using RT-PCR[29,35] or qPCR,[30] and the protein level using Western blot[29,30] or immunohistochemical (IHC) staining.[30,35] Similar findings were found in mice work, where hepatic TGF-β1 and MMP-2

levels were elevated in fibrosis models induced by the methionine choline-deficient (MCD) diet or thioacetamide (TAA) treatment,[33] as well as in C57BL/6J mice fed with a high-fat diet for 17 weeks.[34] The decreased expression of MMP-2 and increased expression of TIMP-2 at the gene and protein levels in the hearts of diabetic rats were consistent with our findings in the livers of the diabetic rats.[36]

Previous studies have investigated the effects of various drug candidates on T2D and its complications, including liver fibrosis.[37,38] In our study, L6H4 treatment of T2D rats for almost 2 months significantly increased their body weight and MMP-2 expression in the liver. It also significantly decreased collagen deposition in the liver and various parameters, such as ALT, AST, FPG, HOMA-IR, FINS, TC, LDL, TGF-β1, and TIMP-2. Although some agents (RhoA/Rho kinase inhibitor, curcumin, active vitamin D3, oleoylethanolamide, angiotensin II type 1 receptor blocker, RAS inhibitor, SalA, etc.) have been shown to ameliorate liver fibrosis by inhibiting TGF-β, our study is the first to describe the changes in MMP-2 and TIMP-2 levels in the liver before and after curcumin (analogue) treatment of T2D.[33,34,36–39]

There is growing interest in using natural products like curcumin to treat NAFLD due to their efficacy, availability, low cost, and minimal side effects as compared to pharmaceutical drugs, as evidenced by recent preclinical and clinical studies. Given curcumin's potential, our study provides evidence that its synthetic analog L6H4 effectively treats complications of T2D like liver fibrosis by modulating key markers like MMP-2 and TIMP-2. Further research on curcumin and its analogues as natural therapeutic options for NAFLD and related conditions is warranted.[40]

While not tested in the current study, L6H4 was found to inhibit the release of TNF-α and IL-6, as well as the transcription of a group of cytokines (TNF-α, IL-1β, IL-6, IL-12, and COX-2), and the activation of ERK and p38 in LPS-stimulated macrophages.[17] Given the pleiotropic functions of curcumin and its analogues, future studies using -omics techniques could provide a panoramic view of their molecular mechanisms, which would be of great interest and significance.

## Conclusion

In Conclusion, the findings suggest that L6H4 has therapeutic potential in attenuating liver fibrosis and alleviating insulin resistance in streptozotocin-induced diabetic rats. The hepatoprotective effect of L6H4 may be attributed to its anti-inflammatory properties and its ability to target molecules involved in fibrosis. Further research is warranted to explore the potential of L6H4 as a treatment option for nonalcoholic fatty liver disease and type 2 diabetes.

## Abbreviations

TIMP-2, tissue inhibitors of metalloproteinase-2; TGF-β, transforming growth factor-β; MMP-2, matrix metalloproteinase-2; NAFLD, nonalcoholic fatty liver disease; CMC-Na, carboxyl methyl cellulose; STZ, Streptozotocin; FPG, Fasting plasma glucose; FINs, Fasting insulin; TC, total cholesterol; TG, triglycerides; LDL-c, low-density lipoprotein-cholesterol; HDL-c, high-density lipoprotein-cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; HOMA-IR, blood indexes of insulin resistance homeostasis model assessment-estimated insulin resistance; OD, Optical Density; IHC, immunohistochemical.

## Data Sharing Statement

The datasets used and analyzed during the current study are included in this published article.

## Ethics Approval

This study was conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health and with the approval of the Ethical Committee on Animal Research at Wenzhou Medical University (Wenzhou, Zhejiang, China).

## Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, analysis, and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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## Disclosure

Jun Ma and Deep K Vaishnani are co-first authors for this study. The authors declare that they have no conflicts of interest for this work.

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