WEP Warwick Evans Publishing

Transactions on Materials, Biotechnology and Life Sciences
ISSN: 2960-1932, eISSN: 2960-2319 | Volume 7
MISBP 2024

# Mechanism of Liraglutide Regulating the Expression of PI3K/AKT/NF-KB Pathway-related Proteins on Nonalcoholic Fatty Liver Disease

Yao Sang*

School of Life Sciences, Jilin University, Changchun City 130012, China

*Corresponding author: Yao Sang, e-mail: 13309173169@163.com

**Abstract.** Background and Objective: GLP-1RAs can effectively prevent hepatic steatosis. It was to investigate the mechanism by which the GLP-1RA Liraglutide ameliorates non-alcoholic fatty liver disease (NAFLD) and its impact on PI3K/AKT/NF-κB pathway. Materials and Methods: eight-week-old, clean-grade male C57BL/6J mice were assigned to Normal group (NG, fed standard chow), NAFLD group (high-calorie, high-cholesterol), and Liraglutide group (LG, high-calorie, high-cholesterol for 12 weeks + Liraglutide treatment at 1 mg/kg). Serum samples were collected from the mice to assess changes in glucose and lipid metabolism and liver function (LF) indicators. Histopathological changes in liver tissues (LTs) were visualized. Real-time quantitative PCR measured expression levels (ELs) of inflammatory genes in LTs. Western blotting was conducted to detect EL of proteins related to PI3K/AKT/NF-κB pathway in LTs. Results: NAFLD group exhibited markedly elevated levels of blood glucose (BG), blood lipids, and LF indicators relative to NG. Histopathological examination revealed increased scores for steatosis, lobular inflammation, and hepatocellular ballooning. The ELs of inflammatory genes IL-1β, TNF-α, and NF-κB p65 in LTs, were greatly elevated ($P<0.05$). In contrast, relative to NAFLD group, LG showed notably reduced levels of BG, blood lipids, and LF indicators, along with decreased histopathological scores. Furthermore, the ELs of inflammatory genes and protein ELs of p-PI3K, p-AKT, and p-NF-κBp65 in LTs were drastically lowered ($P<0.05$). Conclusion: Liraglutide ameliorates glucose and lipid metabolism disorders and suppresses inflammatory responses in liver by inhibiting PI3K/AKT/NF-κB signaling, thereby improving NAFLD in mice.

**Keywords:** NAFLD; Liraglutide; glucose and lipid metabolism; inflammation; PI3K/AKT/NF-κB signaling.

## 1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a metabolic stress-related liver injury, with its incidence rising dramatically each year [1]. Additionally, NAFLD is implicated in the progression of type 2 diabetes and atherosclerosis [2,3]. Currently, there are no specific therapeutic drugs for NAFLD, making the identification of therapeutic targets and drugs to improve NAFLD a critical focus of research.

NAFLD is closely associated with factors such as insulin resistance and genetic susceptibility [4]. The use of antidiabetic drugs can improve liver biochemical parameters to some extent. GLP-1RAs can mitigate lipid-induced endoplasmic reticulum stress by regulating the expression of Sirtuin 1, effectively preventing hepatic steatosis [5]. Liraglutide, a GLP-1 analog, has been shown to be effective in treating type 2 diabetes-related NAFLD [6]. Liraglutide has been clinically applied as a subcutaneously injected medication for treating T2DM, obesity, NAFLD, and for reducing risk of cardiovascular events in patients with T2DM. Recent research on Liraglutide has highlighted its multifaceted therapeutic effects and its potential applications in various metabolic and cardiovascular conditions. PI3K/AKT pathway is involved in maintaining normal cellular physiological functions, and inhibiting it can suppress hepatic stellate cell activation and promote apoptosis [7]. Nuclear factor (NF)-κB is a key downstream transcription factor in the PI3K/AKT signaling, involved in processes such as inflammation, immunity, and cell proliferation. Notably, NF-κB activation is prominent in

NAFLD [8]. Recent research has described Liraglutide as a versatile medication with broad therapeutic potential in the management of T2DM, obesity, NAFLD, cardiovascular disease, and potentially neurodegenerative disorders. Its multifaceted effects on metabolism, inflammation, and cardiovascular health make it an attractive option for the treatment of these complex and interconnected conditions.

This study induced a NAFLD mouse model to analyze the effects of Liraglutide on metabolism disorders, liver function (LF), and inflammatory responses in NAFLD mouse model. Additionally, the study investigated changes in the PI3K/AKT/NF-κB pathway. The research aimed to provide experimental data to understand the potential mechanisms by which Liraglutide treats NAFLD.

## 2. Materials and methodologies

### 2.1 Experimental animals

Forty-five male C57BL/6J mice (8 weeks, 20-25 g) from Beijing Vital River Laboratory Animal Technology Co., Ltd., were housed at (22.5±1.5)°C, with a relative humidity of (50±10)%, and a 12-hour light/dark cycle. Experiments began after one-week. All procedures involving animal housing, experimentation, and sample collection were implemented in compliance with the relevant regulations governing laboratory animal management and welfare.

### 2.2 Reagents and instruments

The utilized reagents included high-fat, high-cholesterol diet from Research Diets, Inc. (Canada); Liraglutide from Novo Nordisk Biopharmaceuticals (Denmark); lipid profile assay kits supplied by Tianjin Yueteng Biotechnology Co., Ltd. (China); insulin assay kits by Wuhan EIAab Science Co., Ltd. (China); LF assay kits by Beijing Annoron Biotech Co., Ltd. (China); sodium pentobarbital and enhanced chemiluminescence (ECL) kits by Sigma-Aldrich (USA); 4% paraformaldehyde by Beijing Biorigin Biotechnology Co., Ltd. (China); xylene by Sinopharm Chemical Reagent Co., Ltd. (China); hematoxylin-eosin (HE) staining kits and RIPA buffer by Solarbio Science&Technology Co., Ltd. (China); cDNA reverse transcription kits and real-time quantitative PCR kits by Beijing Biolab Technology Co., Ltd. (China); BCA kits by Thermo Fisher Scientific (China); anti-PI3K, anti-p-PI3K, anti-AKT, anti-p-AKT, and anti-NF-κB p65 polyclonal Abs by Cell Signaling Technology (USA); anti-p-NF-κB p65, anti-β-actin polyclonal Abs, and horseradish peroxidase (HRP)-conjugated IgG Abs by Abcam (UK).

The instruments used in this study included a Multiskan FC automated microplate reader (Thermo Fisher Scientific, USA); a CX33 microscope (Olympus, Japan); a DYY-6C electrophoresis apparatus (Beijing Liuyi Biotechnology Co., Ltd., China); a CFX96 Touch real-time PCR system, a T100 thermal cycler, and a Gel Doc EZ gel imaging system (BIO-RAD, USA).

### 2.3 Modeling and grouping

Forty-five C57BL/6 mice were rolled into Normal group (NG), NAFLD group, and Liraglutide group (LG) (each n=15). NG was fed a standard diet with a total caloric content of 14.5 kJ/g, consisting of 66.9% carbohydrates, 24.8% protein, and 8.3% fat. The NAFLD group and LG were fed a high-calorie, high-cholesterol diet, comprising 49% standard chow, 20% lard, 20% sucrose, 10% egg yolk powder, and 1% cholesterol. After 12 weeks on the high-calorie, high-cholesterol diet, the LG received intraperitoneal injections of Liraglutide at 1 mg/kg, twice daily, for 4 weeks.

### 2.4 Glucose tolerance test (GTT)

At conclusion of treatment, five mice from each group were chosen for the GTT. Mice underwent a 12-hour fasting period before the GTT. Subsequently, they received an oral glucose load of 2 g/kg. Blood samples (5 μL) were harvested from tail vein at 0, 0.5, 1.0, 1.5, and 2.0 hours after glucose adoption to measure blood glucose (BG) levels.

### 2.5 Serological indicator testing

At conclusion of treatment, the left 10 mice from each group were used for subsequent experiments. Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), low-DL-C (LDL-C), and free fatty acids (FFA), as well as LF indicators alanine aminotransferase (ALT) and aspartic transaminase (AST), and BG indicators fasting BG (FBG) and fasting insulin (FINS) were determined according to the instructions provided with the assay kits. Additionally, the HOMA-IR was calculated as FINS×FBG/22.5.

### 2.6 HE staining of liver tissue (LT)

Mice were anesthetized with 50 mg/kg 0.3% sodium pentobarbital at the end of treatment. Fresh LTs were harvested after laparotomy, and blood was flushed out. The LTs were then blotted dry with filter paper and weighed. The LTs were assigned into three portions: two portions were stored in liquid nitrogen for future use, and the remaining portion was fixed in 4% paraformaldehyde solution. Then, tissues were dehydrated in ethanol (graded series), cleared in xylene, and embedded in paraffin to prepare tissue slices. Following deparaffinization in xylene, rehydration in ethanol (graded series), and rinsing, the tissue slices were stained with hematoxylin for 5-10 minutes, rinsed, differentiated in hydrochloric acid ethanol for 15-30 seconds, counterstained with eosin for 1 minute after immersion in concentrated ammonia water for 5-10 seconds. Subsequently, the tissue slices were dehydrated in ethanol, cleared in xylene, and mounted with neutral resin. Pathological changes were visualized, and NAFLD activity score (NAS) was assessed based on established criteria.

### 2.7 PCR detection

One portion of the LT was retrieved from liquid nitrogen and homogenized in Trizol reagent. Total RNA was extracted. cDNA was synthesized. The cDNA served as template for the detection of the target genes IL-1β, TNF-α, and NF-κB p65 expression levels (ELs) utilizing assay kit. Quantitative primers were designed and synthesized by Shanghai Sangon Biotech Co., Ltd. as follows: IL-1β (F: 5'-GCAACTGTTCCTGAACTCAACT-3', R: 5'-ATCTTTTGGGGTCCGTCAACT-3'), TNF-α (F: 5'-CTGAACTTCGGGGTGATCGG-3', R: 5'-GGCTTGTCACTCGAATTTTGAGA-3'), NF-κB p65 (F: 5'-AGGCTTCTGGGCCTTATGTG-3', R: 5'-TGCTTCTCTCGCCAGGAATAC-3'), GAPDH (F: 5'-AGGTCGGTGTGAACGGATTTG-3', R: 5'-TGTAGACCATGTAGTTGAGGTCA-3'). ELs of target genes were calculated by 2-ΔΔCT with GAPDH as internal control.

### 2.8 Western blotting

One portion of LT was retrieved from liquid nitrogen and homogenized in RIPA. BCA assay determined total protein concentration. Proteins were loaded for electrophoresis, and the target protein bands were then transferred onto PVDF membrane using wet transfer methodology. The membrane was blocked for 1 hour. Subsequently, primary Abs against PI3K, p-PI3K, AKT, p-AKT, NF-κB p65, p-NF-κB p65, and β-actin were applied at a 1:1000 dilution and incubated overnight at 4°C. After membrane rinsing, HRP-conjugated IgG secondary Abs, diluted at 1:5000, were incubated at 25°C for 1.5 hours. Following membrane washing, chemiluminescent signals were developed employing the ECL methodology. The relative ELs of the target proteins were analyzed using ImageJ with β-actin as internal control.

### 2.9 Statistical processing

Data were denoted as mean ± standard deviation using SPSS 23.0. One-way analysis of variance was adopted for intergroup comparisons, while LSD-t test was for post hoc multiple comparisons. $P<0.05$ was statistically considerable.

## 3. Results

### 3.1 Impact of Liraglutide on lipid metabolism in NAFLD mice

The differences in serum lipid metabolism-related indicators among the three groups of mice were assessed, as illustrated in Figure 1. In comparison to NG, TC, TG, LDL-C, and FFA were greatly elevated in mice of NAFLD group, while HDL-C was reduced (P<0.05). Conversely, relative to NAFLD group, TC, TG, LDL-C, and FFA were drastically decreased in mice of LG, while HDL-C was greatly increased (P<0.05).

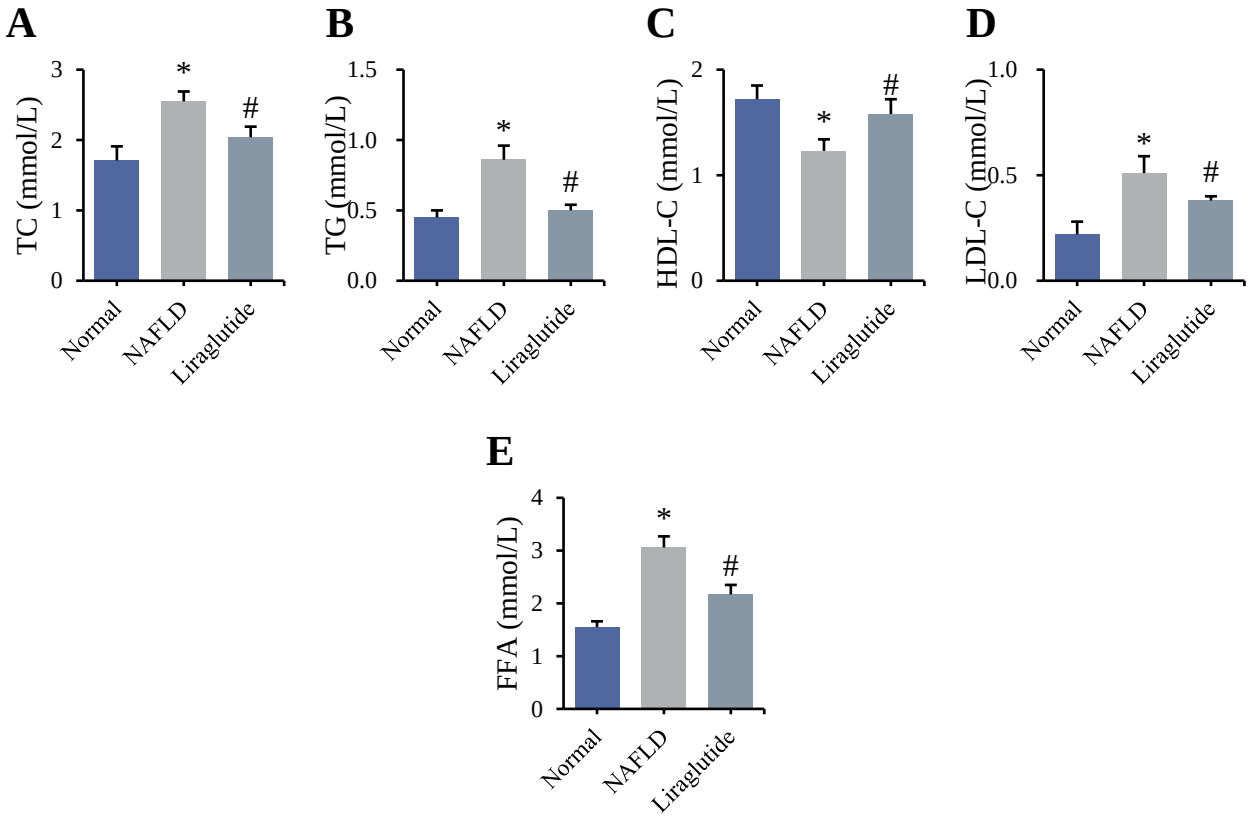


Figure. 1 Changes in serum lipid metabolism indicators among the groups of mice.

Note: A represents TC, B represents TG, C represents HDL-C, D represents LDL-C, E represents FFA; * meant P<0.05 vs. NG and # meant P<0.05 vs. NAFLD group (also in figures below).

### 3.2 Impact of Liraglutide on glucose metabolism in NAFLD mice

Differences in glucose metabolism-related parameters in the serum of the three mouse groups were assessed, as depicted in Figure 2. The GTT experiment revealed that the BGs of mice in the Normal, NAFLD, and LGs peaked at 0.5 hours, with NAFLD group consistently displaying the highest BG levels at all time points. In comparison to NG, the levels of FBG, FINS, and HOMA-IR in mice of NAFLD group were markedly elevated (P<0.05). Conversely, relative to NAFLD group, the levels of FBG, FINS, and HOMA-IR in mice of LG were substantially reduced (P<0.05).

### 3.3 Influence of Liraglutide on LF and pathological morphology in NAFLD mice

Initially, the distinctions in LF-related parameters in the serum of the three mouse groups were analyzed, as depicted in Figure 3A~C. In comparison to NG, the LT weight, ALT, and AST levels in mice of NAFLD group exhibited a drastic increase (P<0.05). Conversely, relative to NAFLD group, the LT weight, ALT, and AST levels in mice of LG showed a great decrease (P<0.05). Subsequently, histopathological alterations of the three mouse groups were examined using HE staining and scored, as illustrated in Figure 3D~F. Relative to NG, the scores of steatosis, lobular inflammation, and

hepatocellular ballooning in LTs of mice in NAFLD group were considerably elevated (P<0.05); relative to NAFLD group, the scores of steatosis, lobular inflammation, and hepatocellular ballooning in LTs of mice in LG displayed a prominent reduction (P<0.05).

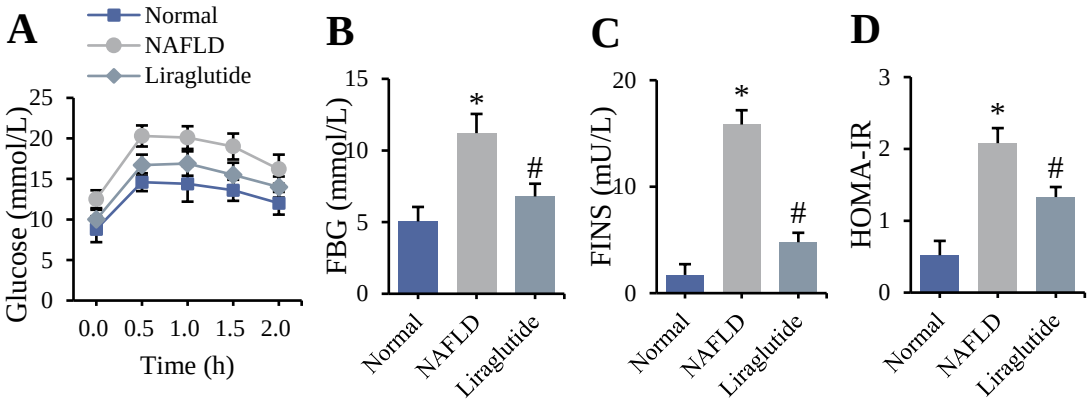


Figure. 2 Changes in serum glucose metabolism indicators among the groups of mice.

Note: A represents the GTT experiment detecting BG change curve, B represents FBG, C represents FINS, D represents HOMA-IR.

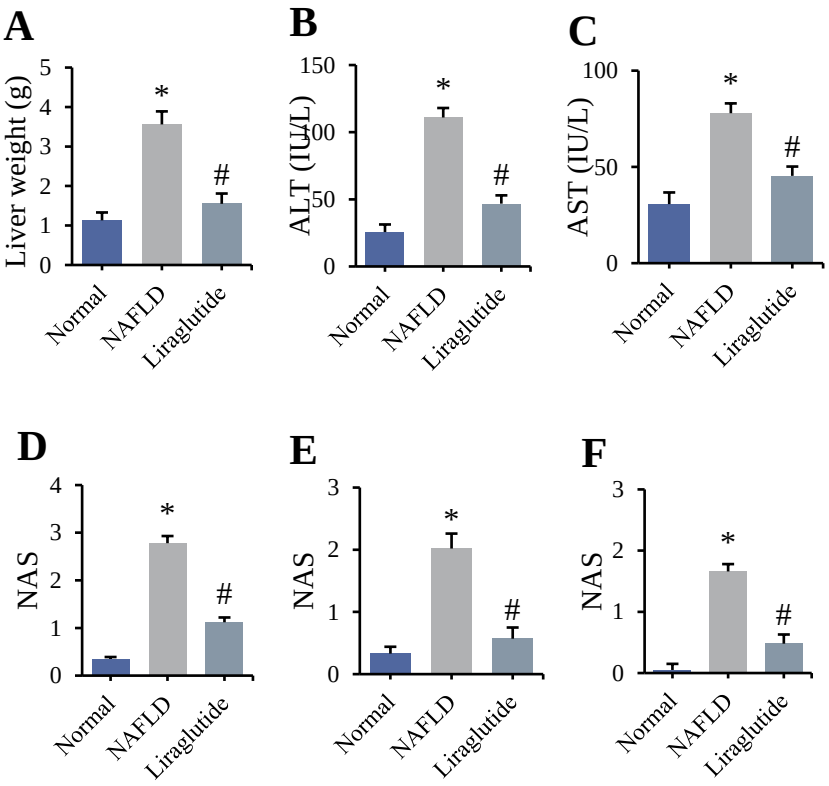


Figure. 3 Changes in LF and histopathology among the groups of mice.

Note: A represents LT weight, B represents ALT, C represents AST, D represents steatosis score, E represents lobular inflammation score, F represents hepatocellular ballooning score.

### 3.4 Impact of Liraglutide on the EL of inflammatory genes

Variations in the ELs of IL-1β, TNF-α, and NF-κB p65 in LTs of the three mouse groups were assessed, as depicted in Figure 4. The relative ELs of IL-1β, TNF-α, and NF-κB p65 in the LTs of mice in NAFLD group exhibited a drastic increase versus NG (P<0.05). Relative ELs of IL-1β, TNF-α, and NF-κB p65 in LTs of mice in LG demonstrated a remarkable decrease versus NAFLD group (P<0.05).

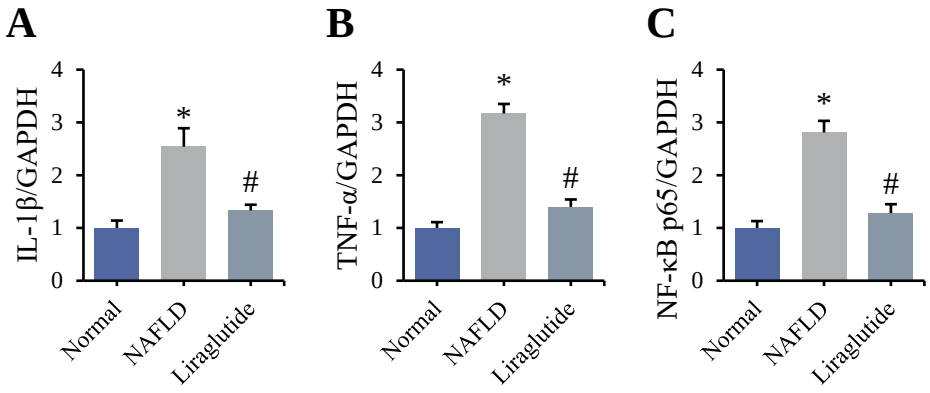


Figure. 4 Changes in the ELs of inflammation-related genes in the LTs of mice among the groups.

Note: A represents IL-1β, B represents TNF-α, C represents NF-κB p65.

### 3.5 Influence of Liraglutide on the status of PI3K/AKT/NF-κB pathway in LT of NAFLD mice

In Figure 5, phosphorylation levels of PI3K, AKT, and NF-κB p65 in LTs of mice in NAFLD group showed a great increase relative to NG ($P<0.05$). In contrast, those levels in LG exhibited a considerable decrease versus NAFLD group ($P<0.05$).

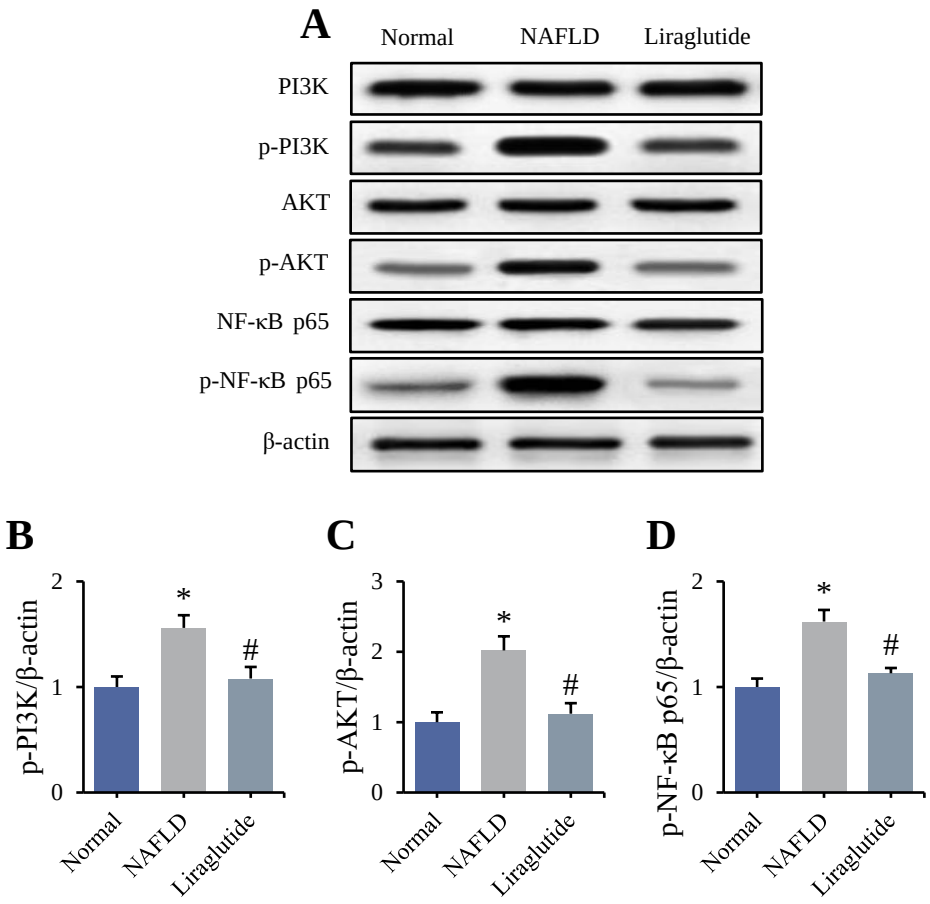


Figure. 5 Changes in the ELs of PI3K/AKT/NF-κB pathway-related proteins in LTs of mice among groups.

Note: A represents the western blotting analysis, B represents phosphorylation level of PI3K, C represents the phosphorylation level of AKT, D represents the phosphorylation level of NF-κB p65.

## 4. Discussion

NAFLD is a metabolic stress-related disease associated with genetic susceptibility, encompassing a spectrum of liver damage conditions [9,10]. Incidence of NAFLD has been gradually increasing, attracting widespread attention from the public. Dietary modifications are crucial for the management of NAFLD. Patients are advised to follow a balanced diet with reduced calorie intake, focusing on fruits, vegetables, whole grains, and lean proteins. Weight loss through calorie restriction and increased physical activity is a cornerstone of NAFLD treatment. Losing 5-10% of total body weight enhances liver health and reduces liver fat accumulation. Regular physical activity, such as aerobic exercise and resistance training, is beneficial for patients with NAFLD. Exercise helps to improve insulin sensitivity, promote weight loss, and reduce liver fat accumulation. Patients with NAFLD should abstain from alcohol consumption, as even moderate alcohol intake can exacerbate liver damage. The clinical therapy of NAFLD involves a comprehensive approach that includes lifestyle modifications, pharmacological interventions, management of comorbidities, and, in some cases, liver transplantation. Tailored treatment plans should be developed based on individual patient characteristics, disease severity, and comorbid conditions. Early intervention and sustained lifestyle changes are crucial for effectively managing NAFLD and preventing disease progression.

The “two-hit” theory, as a classic pathogenic mechanism, has been widely accepted. This theory suggests that accumulation of lipid substances in cytoplasm of liver cells (1st hit) triggers cytotoxic events (2nd hit), leading to liver inflammation, necrosis, or fibrosis [11]. It has been confirmed that the initial process of excessive lipid accumulation in liver parenchyma is associated with insulin resistance [12]. Insulin resistance causes disturbances in triglyceride synthesis and transport functions within liver cells. This study found that the levels of FBG, FINS, and HOMA-IR were greatly increased in serum of NAFLD mice. Watanabe et al. (2020) confirmed that baseline HOMA-IR is a positive predictive factor for decreased hepatic steatosis index in patients with NAFLD [13]. Xue et al. (2022) recruited 1,727 healthy and nutritionally screened adult populations and found differences in HOMA-IR indices among patients with NAFLD, metabolically associated fatty liver disease, and liver fibrosis. HOMA-IR was found to be more suitable for monitoring metabolic risks and disease progression in patients with NAFLD [14]. HOMA-IR plays a crucial role in the assessment, diagnosis, and management of NAFLD. Elevated HOMA-IR values are indicative of insulin resistance, which is a key driver of NAFLD pathogenesis. Monitoring HOMA-IR levels is essential for risk stratification, disease monitoring, and treatment optimization in patients with NAFLD. Therefore, it is believed that changes in HOMA-IR may be involved in the formation of NAFLD. Monitoring changes in HOMA-IR over time can help predict risk of disease progression in patients with NAFLD.

Liraglutide, a GLP-1 analog, plays a crucial role in reducing BG and lipids, controlling appetite, and enhancing insulin sensitivity. Liraglutide can reduce BG and stimulate fat consumption [15]. Moreover, Liraglutide can significantly enhance hepatic steatosis in patients and delay the progression of liver fibrosis [16,17]. This study found that after Liraglutide treatment, TC, TG, LDL-C, FFA, FBG, FINS, and HOMA-IR were markedly reduced in NAFLD mice. Cuatrecasas et al. (2024) confirmed that Liraglutide used in the treatment of obese patients can significantly reduce body weight and visceral, omental, and perirenal fat, and also lower TC and LDL-C levels [18]. An et al. (2023) also found that Liraglutide could alleviate the dyslipidemia in a type 2 diabetes mouse model and improve the activation of astrocytes in the hippocampal region while suppressing neuroinflammatory responses [19]. This indicates that Liraglutide can improve lipid metabolism abnormalities and insulin resistance by regulating FBG and FINS levels. Furthermore, this study confirmed that after Liraglutide treatment, ALT, AST, and LT NAS scores were drastically reduced in NAFLD mice. This is consistent with Guo et al. (2023), who observed that Liraglutide improved LF in mice with type 2 diabetes-related NAFLD [20]. This suggests that Liraglutide can improve NAFLD LF by regulating ALT and AST activity. Lowering HOMA-IR through lifestyle modifications (e.g., weight loss, dietary changes, increased physical activity) and pharmacological interventions is a key therapeutic strategy in the management of NAFLD.

Hepatic stellate cell activation and transformation act in chronic liver diseases, and PI3K/AKT signaling activation is involved in regulating activation process of hepatic stellate cells [21]. AKT, a serine/threonine kinase, is an imperative downstream target kinase in the signaling transduction process. NF-κB p65 belongs to the inflammatory signaling pathway; however, once activated, it can regulate the synthesis and transcription of a series of inflammation-related mediators such as IL-6, TNF-α, thereby inducing a cascade of inflammatory responses [22,23]. This study found that mRNA ELs of IL-1β, TNF-α, NF-κB p65, and protein ELs of p-PI3K, p-AKT, and p-NF-κB p65 were markedly increased in the LTs of NAFLD mice. This indicates notable inflammatory response in NAFLD LTs, and the PI3K/AKT/NF-κB pathway is activated, which is similar to the results found by Li et al. (2021) in the livers of NAFLD rats [24]. Activation of NF-κB can promote the synthesis and secretion of ROSs and inflammatory factors. Stimulation of hepatic stellate cell activation accelerates the progression of NAFLD [25-27]. This study found that after Liraglutide treatment, the mRNA ELs of IL-1β, TNF-α, NF-κB p65, and protein ELs of p-PI3K, p-AKT, and p-NF-κB p65 were notably decreased in LTs of NAFLD mice. Liraglutide, as a long-acting agonist, can improve IL-1β-induced generation of ROSs and inhibit triglyceride accumulation [28]. This indicates that Liraglutide can alleviate inflammatory reactions through the PI3K/AKT/NF-κB pathway [29], thereby improving NAFLD.

## 5. Conclusion

Liraglutide markedly improves the abnormal metabolism in NAFLD mice, while also alleviating hepatic inflammatory reactions and reducing the extent of liver damage. This effect is achieved through regulation of PI3K/AKT/NF-κB pathway. However, this work has certain limitations, and future research should focus on preparing animal models to explore whether Liraglutide can inhibit the progression of liver fibrosis. Our work provides valuable insights for the selection of therapeutic targets and drugs for NAFLD treatment.

## References


[1] Duell PB, Welty FK, Miller M, Chait A, Hammond G, Ahmad Z, Cohen DE, Horton JD, Pressman GS, Toth PP; American Heart Association Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Hypertension; Council on the Kidney in Cardiovascular Disease; Council on Lifestyle and Cardiometabolic Health; and Council on Peripheral Vascular Disease. Nonalcoholic Fatty Liver Disease and Cardiovascular Risk: A Scientific Statement From the American Heart Association. Arterioscler Thromb Vasc Biol. 2022 Jun;42(6):e168-e185. doi: 10.1161/ATV.0000000000000153. Epub 2022 Apr 14. PMID: 35418240.

[2] Loomba R, Friedman SL, Shulman GI. Mechanisms and disease consequences of nonalcoholic fatty liver disease. Cell. 2021 May 13;184(10):2537-2564. doi: 10.1016/j.cell.2021.04.015. PMID: 33989548.

[3] Liu C, Liu T, Zhang Q, Jia P, Song M, Zhang Q, Ruan G, Ge Y, Lin S, Wang Z, Xie H, Shi J, Han R, Chen Y, Zheng X, Shen L, Deng L, Wu S, Shi H. New-Onset Age of Nonalcoholic Fatty Liver Disease and Cancer Risk. JAMA Netw Open. 2023 Sep 5;6(9):e2335511. doi: 10.1001/jamanetworkopen.2023.35511. PMID: 37747732; PMCID: PMC10520743.

[4] Muzica CM, Sfarti C, Trifan A, Zenovia S, Cuciureanu T, Nastasa R, Huiban L, Cojocariu C, Singeap AM, Girleanu I, Chiriac S, Stanciu C. Nonalcoholic Fatty Liver Disease and Type 2 Diabetes Mellitus: A Bidirectional Relationship. Can J Gastroenterol Hepatol. 2020 Dec 28;2020:6638306. doi: 10.1155/2020/6638306. PMID: 33425804; PMCID: PMC7781697.

[5] Stenlid R, Cerenius SY, Manell H, Küçükemre Aydin B, Mörwald K, Gomahr J, Höghammar Mitkas M, Eriksson I, Ciba I, Geiersberger S, Thivel D, Weghuber D, Bergsten P, Forslund A. Screening for Inflammatory Markers Identifies IL-18Rα as a Potential Link between Exenatide and Its Anti-Inflammatory Effect: New Results from the Combat-JUDO Randomized Controlled Trial. Ann Nutr Metab. 2023;79(6):522-527. doi: 10.1159/000534725. Epub 2023 Oct 26. PMID: 37883939.

[6] Yan J, Yao B, Kuang H, Yang X, Huang Q, Hong T, Li Y, Dou J, Yang W, Qin G, Yuan H, Xiao X, Luo S, Shan Z, Deng H, Tan Y, Xu F, Xu W, Zeng L, Kang Z, Weng J. Liraglutide, Sitagliptin, and Insulin Glargine Added to Metformin: The Effect on Body Weight and Intrahepatic Lipid in Patients With Type 2 Diabetes Mellitus and Nonalcoholic Fatty Liver Disease. Hepatology. 2019 Jun;69(6):2414-2426. doi: 10.1002/hep.30320. Epub 2019 Feb 22. PMID: 30341767; PMCID: PMC6594101.

[7] Lin X, Li Y, Zhang X, Wei Y, Wen S, Lu Z, Huang Q, Wei J. Tormentic acid inhibits hepatic stellate cells activation via blocking PI3K/Akt/mTOR and NF-κB signalling pathways. Cell Biochem Funct. 2021 Jan;39(1):77-87. doi: 10.1002/cbf.3564. Epub 2020 Jun 21. Retraction in: Cell Biochem Funct. 2021 Oct;39(7):921. doi: 10.1002/cbf.3666. PMID: 32564421.

[8] Huang X, Yao Y, Hou X, Wei L, Rao Y, Su Y, Zheng G, Ruan XZ, Li D, Chen Y. Macrophage SCAP Contributes to Metaflammation and Lean NAFLD by Activating STING-NF-κB Signaling Pathway. Cell Mol Gastroenterol Hepatol. 2022;14(1):1-26. doi: 10.1016/j.jcmgh.2022.03.006. Epub 2022 Mar 31. PMID: 35367665; PMCID: PMC9117819.

[9] Paternostro R, Trauner M. Current treatment of non-alcoholic fatty liver disease. J Intern Med. 2022 Aug;292(2):190-204. doi: 10.1111/joim.13531. Epub 2022 Jul 7. PMID: 35796150; PMCID: PMC9546342.

[10] Heeren J, Scheja L. Metabolic-associated fatty liver disease and lipoprotein metabolism. Mol Metab. 2021 Aug;50:101238. doi: 10.1016/j.molmet.2021.101238. Epub 2021 Apr 20. PMID: 33892169; PMCID: PMC8324684.

[11] Rong L, Zou J, Ran W, Qi X, Chen Y, Cui H, Guo J. Advancements in the treatment of non-alcoholic fatty liver disease (NAFLD). Front Endocrinol (Lausanne). 2023 Jan 16;13:1087260. doi: 10.3389/fendo.2022.1087260. PMID: 36726464; PMCID: PMC9884828.

[12] Tanase DM, Gosav EM, Costea CF, Ciocoiu M, Lacatusu CM, Maranduca MA, Ouatu A, Floria M. The Intricate Relationship between Type 2 Diabetes Mellitus (T2DM), Insulin Resistance (IR), and Nonalcoholic Fatty Liver Disease (NAFLD). J Diabetes Res. 2020 Jul 31;2020:3920196. doi: 10.1155/2020/3920196. PMID: 32832560; PMCID: PMC7424491.

[13] Watanabe M, Risi R, Camajani E, Contini S, Persichetti A, Tuccinardi D, Ernesti I, Mariani S, Lubrano C, Genco A, Spera G, Gnessi L, Basciani S. Baseline HOMA IR and Circulating FGF21 Levels Predict NAFLD Improvement in Patients Undergoing a Low Carbohydrate Dietary Intervention for Weight Loss: A Prospective Observational Pilot Study. Nutrients. 2020 Jul 18;12(7):2141. doi: 10.3390/nu12072141. PMID: 32708435; PMCID: PMC7400878.

[14] Xue Y, Xu J, Li M, Gao Y. Potential screening indicators for early diagnosis of NAFLD/MAFLD and liver fibrosis: Triglyceride glucose index-related parameters. Front Endocrinol (Lausanne). 2022 Sep 2;13:951689. doi: 10.3389/fendo.2022.951689. PMID: 36120429; PMCID: PMC9478620.

[15] Kelly AS, Auerbach P, Barrientos-Perez M, Gies I, Hale PM, Marcus C, Mastrandrea LD, Prabhu N, Arslanian S; NN8022-4180 Trial Investigators. A Randomized, Controlled Trial of Liraglutide for Adolescents with Obesity. N Engl J Med. 2020 May 28;382(22):2117-2128. doi: 10.1056/NEJMoa1916038. Epub 2020 Mar 31. PMID: 32233338.

[16] Tan Y, Zhen Q, Ding X, Shen T, Liu F, Wang Y, Zhang Q, Lin R, Chen L, Peng Y, Fan N. Association between use of liraglutide and liver fibrosis in patients with type 2 diabetes. Front Endocrinol (Lausanne). 2022 Aug 10;13:935180. doi: 10.3389/fendo.2022.935180. PMID: 36034438; PMCID: PMC9399467.

[17] Somm E, Montandon SA, Loizides-Mangold U, Gaïa N, Lazarevic V, De Vito C, Perroud E, Bochaton-Piallat ML, Dibner C, Schrenzel J, Jornayvaz FR. The GLP-1R agonist liraglutide limits hepatic lipotoxicity and inflammatory response in mice fed a methionine-choline deficient diet. Transl Res. 2021 Jan;227:75-88. doi: 10.1016/j.trsl.2020.07.008. Epub 2020 Jul 22. PMID: 32711187.

[18] Cuatrecasas G, Calbo M, Rossell O, Dachs L, Aguilar-Soler G, Coves MJ, Patrascioiu I, Benito CE, March S, Balfegó M, Cuatrecasas G, Di Gregorio S, Marina I, Garcia-Lorda P, Munoz-Marron E, De Cabo F. Effect of Liraglutide in Different Abdominal Fat Layers Measured by Ultrasound: The Importance of Perirenal Fat Reduction. Obes Facts. 2024 Apr 20:1-8. doi: 10.1159/000538996. Epub ahead of print. PMID: 38643760.

[19] An JR, Liu JT, Gao XM, Wang QF, Sun GY, Su JN, Zhang C, Yu JX, Yang YF, Shi Y. Effects of liraglutide on astrocyte polarization and neuroinflammation in db/db mice: focus on iron overload and oxidative stress. Front Cell Neurosci. 2023 Jun 1;17:1136070. doi: 10.3389/fncel.2023.1136070. PMID: 37323581; PMCID: PMC10267480.

[20] Guo T, Yan W, Cui X, Liu N, Wei X, Sun Y, Fan K, Liu J, Zhu Y, Wang Z, Zhang Y, Chen L. Liraglutide attenuates type 2 diabetes mellitus-associated non-alcoholic fatty liver disease by activating AMPK/ACC signaling and inhibiting ferroptosis. Mol Med. 2023 Sep 28;29(1):132. doi: 10.1186/s10020-023-00721-7. PMID: 37770820; PMCID: PMC10540362.

[21] Chen Z, Lin Z, Yu J, Zhong H, Zhuo X, Jia C, Wan Y. Mitofusin-2 Restrains Hepatic Stellate Cells' Proliferation via PI3K/Akt Signaling Pathway and Inhibits Liver Fibrosis in Rats. J Healthc Eng. 2022 Jan 17;2022:6731335. doi: 10.1155/2022/6731335. PMID: 35083025; PMCID: PMC8786480.

[22] Zheng Y, Li Y, Ran X, Wang D, Zheng X, Zhang M, Yu B, Sun Y, Wu J. Mettl14 mediates the inflammatory response of macrophages in atherosclerosis through the NF-κB/IL-6 signaling pathway. Cell Mol Life Sci. 2022 May 22;79(6):311. doi: 10.1007/s00018-022-04331-0. PMID: 35598196; PMCID: PMC9124663.

[23] Phusuntornsakul P, Jitpukdeebodintra S, Pavasant P, Leethanakul C. Vibration activates the actin/NF-κB axis and upregulates IL-6 and IL-8 expression in human periodontal ligament cells. Cell Biol Int. 2020 Feb;44(2):661-670. doi: 10.1002/cbin.11267. Epub 2019 Dec 4. PMID: 31769560.

[24] Li J, Wang T, Liu P, Yang F, Wang X, Zheng W, Sun W. Hesperetin ameliorates hepatic oxidative stress and inflammation via the PI3K/AKT-Nrf2-ARE pathway in oleic acid-induced HepG2 cells and a rat model of high-fat diet-induced NAFLD. Food Funct. 2021 May 11;12(9):3898-3918. doi: 10.1039/d0fo02736g. PMID: 33977953.

[25] Sommerhalder C, Cummins CB, Wang X, Ramdas D, Lopez ON, Gu Y, Zhou J, Radhakrishnan RS. HJC0416 Attenuates Fibrogenesis in Activated Hepatic Stellate Cells via STAT3 and NF-κB Pathways. J Surg Res. 2021 May;261:334-342. doi: 10.1016/j.jss.2020.12.045. Epub 2021 Jan 21. PMID: 33486415; PMCID: PMC8634552.

[26] Lee SM, Koh DH, Jun DW, Roh YJ, Kang HT, Oh JH, Kim HS. Auranofin attenuates hepatic steatosis and fibrosis in nonalcoholic fatty liver disease via NRF2 and NF- κB signaling pathways. Clin Mol Hepatol. 2022 Oct;28(4):827-840. doi: 10.3350/cmh.2022.0068. Epub 2022 Jun 22. PMID: 35730208; PMCID: PMC9597229.

[27] Zhang L, Tian J, Diao S, Zhang G, Xiao M, Chang D. GLP-1 receptor agonist liraglutide protects cardiomyocytes from IL-1β-induced metabolic disturbance and mitochondrial dysfunction. Chem Biol Interact. 2020 Dec 1;332:109252. doi: 10.1016/j.cbi.2020.109252. Epub 2020 Sep 6. PMID: 32898504.

[28] Zou Z, Wang Z. Liraglutide attenuates intestinal ischemia/reperfusion injury via NF-κB and PI3K/Akt pathways in mice. Life Sci. 2022 Nov 15;309:121045. doi: 10.1016/j.lfs.2022.121045. Epub 2022 Oct 4. PMID: 36206837.