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Posted Date: 3 June 2026

doi: 10.20944/preprints202508.0696.v2

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Protective Effects of 17β-Estradiol on Metabolic Dysfunction and Hepatic Steatosis in T2DM Mice with NAFLD Through Modulation of the PGC-1α/ERRα Axis

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Abstract

Introduction: This study investigated the protective effects and potential mechanisms of 17β-estradiol (E2) in a mouse model of type 2 diabetes mellitus (T2DM) complicated by non-alcoholic fatty liver disease (NAFLD). **Methods:** A T2DM+NAFLD model was established in male C57BL/6J mice by high-fat diet feeding followed by streptozotocin administration. Animals were randomly assigned to control, model, E2, E2 + small interfering negative control (siNC), and E2 + PGC-1α knockdown (siPGC-1α) groups. E2 was administered intraperitoneally for 8 weeks. Fasting blood glucose (FBG), fasting insulin (FINS), homeostasis model assessment of insulin resistance (HOMA-IR), serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured. Body weight and liver index were recorded. Hepatic lipid accumulation was evaluated by Oil Red O staining. The expression of PGC-1α and ERRα was determined by Western blotting, and the transcriptional regulation of ERRα by PGC-1α was assessed using chromatin immunoprecipitation quantitative PCR (ChIP-qPCR). **Results:** E2 significantly reduced FBG, FINS, HOMA-IR, serum TC, TG, LDL-C, ALT, and AST levels compared with the model group ($P < 0.05$). E2 treatment also decreased body weight, liver index, and hepatic lipid deposition. Western blot analysis demonstrated that E2 markedly increased hepatic PGC-1α and ERRα expression, whereas PGC-1α knockdown largely abolished these beneficial effects. ChIP-qPCR further confirmed enhanced binding of PGC-1α to the ERRα promoter region following E2 treatment. **Discussion:** E2 improved glucose and lipid metabolism, alleviated hepatic steatosis, and enhanced PGC-1α/ERRα signaling in T2DM+NAFLD mice. The attenuation of these effects after PGC-1α knockdown suggests that PGC-1α plays a critical role in mediating the metabolic benefits of E2. **Conclusion:** E2 ameliorates metabolic dysfunction and hepatic steatosis in T2DM+NAFLD mice through activation of the PGC-1α/ERRα signaling axis, providing experimental evidence for the therapeutic potential of estrogen-related interventions in metabolic liver disease.

Keywords: Type 2 diabetes mellitus; non-alcoholic fatty liver disease; 17β-estradiol; PGC-1α; ERRα

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic condition marked by persistent hyperglycemia, representing roughly 90–95% of all diabetes diagnoses. Over the past decades, its global prevalence has shown a steady upward trend [1]. The disease is primarily driven by insulin resistance, which disrupts glucose homeostasis and contributes to a spectrum of metabolic abnormalities. Among its complications, non-alcoholic fatty liver disease (NAFLD) is particularly

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common and is recognized as a major precursor to advanced hepatic disorders, including cirrhosis and hepatocellular carcinoma [2]. Epidemiological data indicate that NAFLD affects up to 70% of individuals with T2DM [3]. The onset and progression of NAFLD involve a multifactorial pathogenesis, encompassing excessive hepatic lipid storage, insulin resistance, oxidative injury, and chronic inflammation [4]. When T2DM coexists with NAFLD, the metabolic burden is amplified, accelerating diabetes progression, exacerbating hepatic injury, and impairing overall prognosis and quality of life [5]. Thus, identifying strategies to mitigate liver damage in T2DM-related NAFLD is of considerable clinical importance for optimizing metabolic control and improving therapeutic outcomes.

17β-estradiol (E2), a principal form of estrogen, exerts diverse physiological and pathological regulatory roles in the human body. Beyond its well-established functions in reproductive health, E2 has been reported to confer cardiovascular and neuroprotective benefits [6,7]. Notably, E2 acts on multiple metabolic tissues, including the liver, skeletal muscle, and adipose tissue, to enhance insulin sensitivity, improve glucose utilization, suppress lipid accumulation, and attenuate hepatic inflammation [8]. While previous research has clarified its general influence on glucose and lipid metabolism, the precise role and mechanistic pathways of E2 in the context of T2DM complicated by NAFLD remain incompletely defined.

Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a central transcriptional coactivator that modulates mitochondrial biogenesis, oxidative metabolism, and cellular antioxidant defense. By partnering with multiple transcription factors, it maintains energy balance in metabolically active tissues, including the liver and muscle [9]. Estrogen-related receptor α (ERRα), a nuclear receptor family member, regulates genes pivotal to intracellular energy metabolism.

PGC-1α directly interacts with ERRα to enhance its transcriptional activity, driving mitochondrial oxidative capacity and cellular energy production—functions with promising implications for metabolic disease therapy [10]. Previous evidence suggests that suppression of PGC-1α and ERRα contributes to metabolic dysfunction, whereas restoration of their activity improves fatty acid oxidation, mitochondrial function, and hepatic metabolic homeostasis [11]. Building on these findings, the present study investigates whether E2 supplementation in a T2DM+NAFLD mouse model can improve glucose and lipid metabolic homeostasis, reduce hepatic steatosis, and modulate the PGC-1α/ERRα signaling axis, thereby providing mechanistic insights and potential therapeutic targets for metabolic liver disease.

2. Materials and Methods

2.1. Experimental Animals

A total of 40 specific pathogen-free (SPF) grade male C57BL/6J mice, aged 7–8 weeks and weighing 18–22 g, were housed at the institutional animal facility under controlled environmental conditions: constant temperature (22 ± 1 °C), relative humidity (50 ± 10%), and a 12-hour light/dark cycle. Mice were provided ad libitum access to food and water and acclimatized for one week prior to experimentation.

2.1.1. Study Design

This randomized, controlled experimental animal study investigated the protective effects and mechanisms of 17β-estradiol (E2) in a mouse model of type 2 diabetes mellitus (T2DM) with comorbid non-alcoholic fatty liver disease (NAFLD). Male

C57BL/6J mice (8 weeks old, 20–22 g) were obtained from the Laboratory Animal

Center of Xuzhou Medical University (Xuzhou, Jiangsu, China) and acclimatized for

7 days under specific pathogen-free (SPF) conditions (22 ± 2 °C, 50 ± 10% relative humidity, 12 h light/dark cycle) with ad libitum access to food and water.

A total of 30 mice were randomly assigned to five groups (n = 6 per group): control, model, E2, E2+siNC, and E2+siPGC-1α. The T2DM+NAFLD model was induced by feeding a high-fat diet (HFD)

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for 8 weeks, followed by intraperitoneal injections of streptozotocin (STZ, 60 mg/kg, dissolved in citrate buffer, pH 4.5) once daily for 3 consecutive days; control mice received a standard diet and citrate buffer. Mice in the E2, E2+siNC, and E2+siPGC-1α groups were administered E2 (20 μg/kg/day, i.p., dissolved in corn oil) for 8 weeks. In weeks 1 and 5, the E2+siNC and E2+siPGC-1α groups received tail-vein injections of either an empty lentiviral vector (siNC) or a

PGC-1α-targeting vector (siPGC-1α) at 2 × 10^7 TU in 200 μL sterile PBS.

Study period: 10 Jun 2023 – 15 Jun 2025.

2.1.2. Ethics Statement, Health Monitoring and Humane Endpoints, and Sample Size

All procedures complied with the ARRIVE guidelines and the US National Research Council *Guide for the Care and Use of Laboratory Animals*. The protocol was reviewed and approved by the Experimental Animal Ethics Committee of Xuzhou Medical University, Jiangsu, China (Approval No. XZMC2023-021, approved on 10 June 2023). Animals were monitored at least once daily for general health (body weight, posture, grooming, mobility, and food/water intake). Predefined humane endpoints included >20% body-weight loss from baseline, severe/persistent lethargy or recumbency, inability to access food or water, self-mutilation/ulceration, or severe dehydration. Animals meeting endpoint criteria were humanely euthanized by cervical dislocation (performed by trained personnel; following deep anesthesia where required by institutional policy). Randomization was applied at group allocation, and histological/biochemical assessments were performed by investigators blinded to group assignments.

Sample size (n = 6 per group) was determined with reference to prior studies using comparable T2DM+NAFLD models and expected effect sizes, providing approximately ≥80% power to detect differences in primary metabolic and hepatic steatosis-related outcomes at $\alpha = 0.05$.

2.2. Reagents and Materials

Standard and high-fat diets were purchased from Suzhou Xietong Technology Co., Ltd. Streptozotocin (STZ) and 17β-estradiol (E2) were obtained from Sigma-Aldrich

(USA). Lentiviral vectors for PGC-1α knockdown and empty controls were purchased from Shanghai GeneChem Co., Ltd. Mouse insulin (FINS) ELISA kits were from Nanjing Jiancheng Bioengineering Institute. Kits for serum TC, TG, LDL-C, ALT, and AST were obtained from Roche (USA). Oil Red O staining kits were obtained from Wuhan Boster Biological Technology Co., Ltd. Protein extraction reagents were supplied by Shanghai Biyuntian Biotechnology Co., Ltd. BCA protein quantification kits, ECL reagents, and bovine serum albumin (BSA) were also from Biyuntian. Human HepG2 hepatocyte cell line was obtained from Wuhan Nuojing Gene Technology. Chromatin immunoprecipitation (ChIP) kits were purchased from eBioscience (USA). Primary antibodies against PGC-1α, ERRα, and GAPDH were from Abcam (UK). HRP-conjugated secondary antibodies were obtained from Cell Signaling Technology (USA).

2.3. Methods

2.3.1. Animal Model, Grouping, and Treatment

Male C57BL/6J mice (8 weeks old) were housed under standard laboratory conditions (22 ± 2 °C, 12 h light/dark cycle) with ad libitum access to food and water. Following the protocol described in [12], the T2DM+NAFLD model was induced by feeding a high-fat diet (HFD; 60% fat, 20% protein, 20% carbohydrate) for 8 weeks, followed by intraperitoneal streptozotocin (STZ, 60 mg/kg, dissolved in citrate buffer, pH 4.5) once daily for 3 consecutive days; control mice received a standard diet and an equivalent volume of citrate buffer.

After the final STZ injection, mice were fasted for 12 h and fasting blood glucose (FBG) was measured. Mice with FBG between 15–25 mmol/L were considered successfully modeled.

A total of 30 mice were then randomly assigned to five groups (n = 6 per group): control, model, E2, E2 + siNC, and E2 + siPGC-1α. The E2, E2 + siNC, and E2 + siPGC-1α groups received 17β-

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estradiol (E2, 20 μg/kg/day, intraperitoneally, dissolved in corn oil) for 8 weeks. In weeks 1 and 5, the E2 + siNC and E2 + siPGC-1α groups received tail-vein injections of either an empty lentiviral vector (siNC) or a PGC-1α-targeting lentiviral vector (siPGC-1α) at 2×10^7 transducing units in 200 μL sterile PBS.

2.3.2. Blood Glucose and Insulin Measurement

At the end of treatment, mice were fasted for 12 h. Tail vein blood was collected to measure FBG using a glucometer. Serum fasting insulin (FINS) was determined by

ELISA, and insulin resistance was calculated as HOMA-IR = (FBG × FINS) / 22.5.

2.3.3. Serum Lipid Profile and Liver Function Tests

Blood collected from the abdominal aorta was centrifuged at 3000 rpm for 10 min to obtain serum. Total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were quantified using commercial assay kits according to the manufacturers' instructions.

2.3.4. Body Weight and Liver Index

Body weight was recorded prior to sacrifice. Livers were excised, blotted dry, and weighed immediately. Liver index (%) was calculated as:

$$Liver\ Index = \frac{\text{Liver Weight}}{\text{Body Weight}} \times 100\%$$

2.3.5. Oil Red O Staining for Hepatic Lipid Deposition

Fresh liver tissues were embedded in OCT compound and cryosectioned. Frozen sections were stained with Oil Red O according to the manufacturer's instructions. Lipid accumulation was visualized as red-stained droplets under a light microscope. Quantitative analysis of lipid-positive areas was performed using ImageJ software and expressed as the percentage of Oil Red O-positive area relative to the total tissue area.

2.3.6. Western Blotting

Liver proteins were extracted and quantified using a BCA protein assay kit. Equal amounts of protein (40 μg) were separated by SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked with 5% skim milk for 1.5 h at room temperature and incubated overnight at 4 °C with primary antibodies against PGC-1α (1:1000), ERRα (1:1000), and GAPDH (1:1000).

Following washing, membranes were incubated with HRP-conjugated secondary antibodies (1:5000) for 30 min at room temperature. Protein bands were visualized using enhanced chemiluminescence (ECL) reagents and quantified with ImageJ software.

2.3.7. Chromatin Immunoprecipitation (ChIP)-qPCR

HepG2 cells were cross-linked with 1% formaldehyde and sonicated to obtain chromatin fragments. Immunoprecipitation was performed with either anti-PGC-1α antibody or normal IgG (negative control). DNA fragments were purified and subjected to qPCR to quantify enrichment of the ERRα promoter region.

2.4. Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 8.0. Multiple group comparisons were conducted using one-way ANOVA followed by the LSD-t test. Differences were considered statistically significant at $P < 0.05$.

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3. Results

3.1. FBG, FINS, and HOMA-IR Levels Across Groups

Following the intervention, fasting blood glucose (FBG), fasting insulin (FINS), and the homeostasis model assessment of insulin resistance (HOMA-IR) were determined for each group. Relative to the control group, the model group exhibited marked elevations in all three parameters (**P** < 0.05). Administration of 17β-estradiol (E2) led to significant reductions in FBG, FINS, and HOMA-IR when compared with the model group (**P** < 0.05). However, animals in the E2 + siPGC-1α group displayed higher values for these indices than those in the E2 + siNC group (**P** < 0.05), suggesting that silencing PGC-1α partially reversed the metabolic benefits induced by E2. Detailed data are presented in **Table 1**.

Table 1. Comparison of FBG, FINS, and HOMA-IR levels among groups ($\bar{x} \pm s$).

Group	FBG (mmol/L)	FINS (mU/L)	HOMA-IR
Control	5.73 ± 0.61	7.75 ± 0.76	1.97 ± 0.16
Model	27.69 ± 2.83*	17.08 ± 1.69*	21.02 ± 2.07*
E2	12.55 ± 1.19#	10.88 ± 1.41#	6.07 ± 0.59#
E2+siNC	13.06 ± 1.27a	10.64 ± 1.03a	6.18 ± 0.73a
E2+siPGC-1α	29.47 ± 2.90*b	18.19 ± 1.82*b	23.82 ± 2.43*b

*P < 0.05 vs. Control group; # P < 0.05 vs. Model group; a P < 0.05 vs. Model group; b P < 0.05 vs. E2+siNC group.

3.2. Serum Lipid Profiles and Hepatic Enzyme Activities

Following treatment, serum levels of TC, TG, LDL-C, ALT, and AST were quantified. The model group displayed markedly elevated concentrations of all five parameters compared with the control group ($P < 0.05$). Administration of E2 resulted in significant decreases in these lipid and liver injury markers relative to the model group ($P < 0.05$). However, animals in the E2+siPGC-1α group exhibited notably higher values for these indicators than those in the E2+siNC group ($P < 0.05$), suggesting that suppression of PGC-1α partially attenuated the beneficial effects of E2 on lipid metabolism and liver function. (see Table 2).

Table 2. Comparison of Serum TC, TG, LDL-C, ALT, and AST Levels among Groups ($\bar{x} \pm s$).

Group	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	ALT (U/L)	AST (U/L)
Control	3.56 ± 0.34	1.22 ± 0.13	0.37 ± 0.04	79.50 ± 7.88	122.84 ± 13.17
Model	8.15 ± 0.82*	3.57 ± 0.36*	1.12 ± 0.13*	184.36 ± 20.87*	267.91 ± 25.41*
E2	6.04 ± 0.57#	1.92 ± 0.18#	0.71 ± 0.09#	97.33 ± 8.72#	160.98 ± 19.47#
E2+siNC	6.12 ± 0.63a	1.99 ± 0.17a	0.73 ± 0.10a	99.48 ± 6.01a	162.20 ± 17.39a

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E2+siPGC-1α	8.71 ± 0.89*b	3.61 ± 0.38*b	180.52 ± 17.94*b	1.16 ± 0.11*b	265.79 ± 25.87*b

* P < 0.05 vs. Control group; #P < 0.05 vs. Model group; a P < 0.05 vs. Model group; b P < 0.05 vs. E2+siNC group.

3.3. Body Weight and Hepatic Index

Measurements of body weight and liver index revealed that the model group had significantly greater values than the control group ($P < 0.05$). Treatment with E2 led to notable reductions in both parameters compared with the model group ($P < 0.05$). In contrast, mice in the E2+siPGC-1α group exhibited significantly elevated body weight and liver index relative to those in the E2+siNC group ($P < 0.05$), implying that silencing PGC-1α weakened the modulatory effects of E2 (see Table 3).

Table 3. Comparison of Body Weight and Liver Index among Groups ($\bar{x} \pm s$).

Group	Body weight (g)	Liver/body weight (%)
Control	22.47 ± 2.34a	4.08 ± 0.39a
Model	27.08 ± 2.81*	6.30 ± 0.56*
E2	22.54 ± 2.17#	4.32 ± 0.41#
E2+siNC	23.11 ± 2.06a	4.37 ± 0.44a
E2+siPGC-1α	28.23 ± 2.93*b	6.71 ± 0.74*b

*P < 0.05 vs. Control group; #P < 0.05 vs. Model group; a P < 0.05 vs. Model group; b P < 0.05 vs. E2+siNC group.

3.4. Hepatic Lipid Accumulation Assessed by Oil Red O Staining

Oil Red O staining was performed to evaluate hepatic lipid accumulation in each group. As shown in Figure 1, extensive lipid deposition was observed in the model group, characterized by large areas of red-stained lipid droplets within hepatocytes. Quantitative analysis demonstrated a significantly higher Oil Red O-positive area in the model group compared with the control group (P < 0.05).

E2 treatment markedly reduced hepatic lipid accumulation, as evidenced by a significant decrease in the proportion of Oil Red O-positive areas compared with the model group (P < 0.05). Similar results were observed in the E2+siNC group. In contrast, PGC-1α knockdown significantly increased hepatic lipid deposition relative to the E2+siNC group (P < 0.05), indicating that suppression of PGC-1α attenuated the lipid-lowering effects of E2 (Figure 1).

These findings suggest that E2 alleviates hepatic steatosis in T2DM+NAFLD mice and that activation of the PGC-1α pathway contributes to this protective effect.

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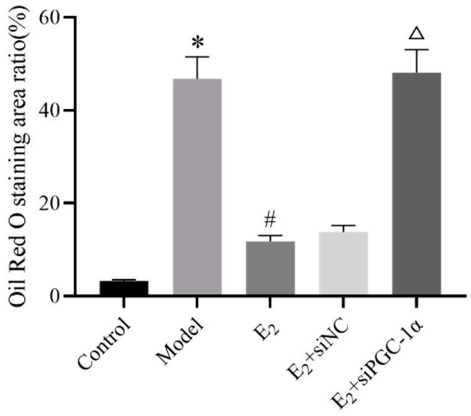

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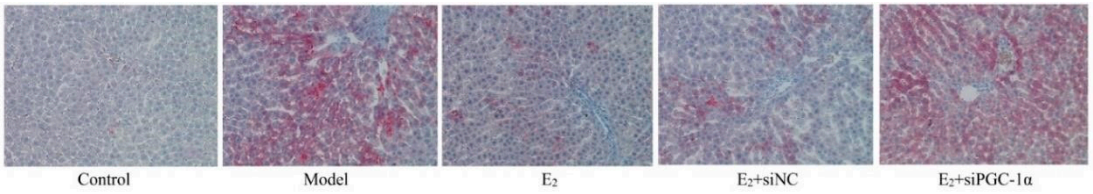

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Figure 1. Hepatic lipid accumulation assessed by Oil Red O staining. Quantification of Oil Red O–positive area (% of field area) in Control, Model, E2, E2+siNC, and E2+siPGC-1α groups. Representative Oil Red O micrographs (×100). Red staining indicates neutral lipid deposition; extensive lipid droplets are seen in the Model group, reduced after E2 treatment and in E2+siNC, and increased again in E2+siPGC-1α. Data are mean ± SD (n = 6 per group). One-way ANOVA with LSD post hoc test. * $P < 0.05$ vs. Control; # $P < 0.05$ vs. Model; △ $P < 0.05$ vs. E2+siNC.

3.5. Protein Expression of the PGC-1α/ERRα Signaling Pathway in Liver Tissue

Western blot results demonstrated that hepatic PGC-1α and ERRα protein levels were markedly reduced in the model group compared with the control group ($P < 0.05$). Administration of E2 significantly increased the expression of both proteins relative to the model group ($P < 0.05$). In contrast, the E2+siPGC-1α group showed a pronounced reduction in PGC-1α and ERRα expression when compared with the E2+siNC group ($P < 0.05$), suggesting that PGC-1α plays an important role in mediating the regulatory effects of E2 on ERRα expression (Figure 2).

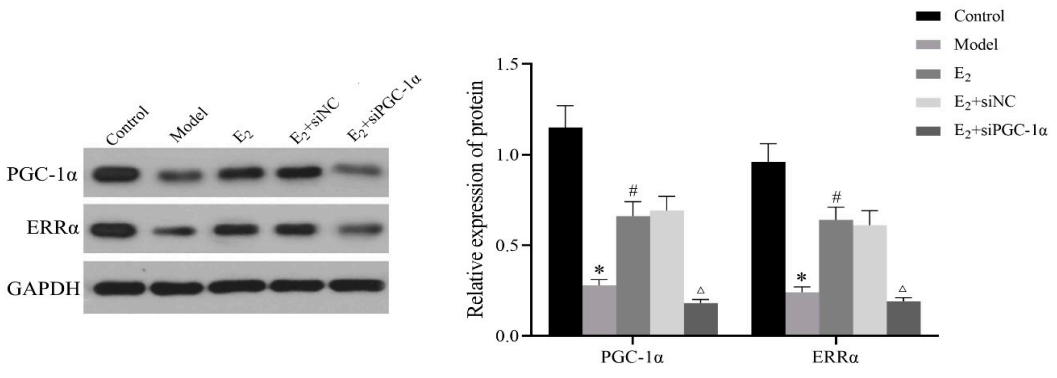

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Figure 2. Hepatic PGC-1α and ERRα protein expression across groups (Western blot). (A) Representative Western blot bands for PGC-1α and ERRα with GAPDH as the loading control from the Control, Model, E2, E2+siNC, and E2+siPGC-1α groups. **(B)** Densitometric quantification of PGC-1α and ERRα, normalized to GAPDH and expressed relative to the Control group (set to 1.0). Bars show mean ± SD (n = 6 per group). Statistics: one-way ANOVA followed by LSD post hoc test. * $P < 0.05$ vs. Control; # $P < 0.05$ vs. Model;△ $P < 0.05$ vs. E2+siNC. E2 treatment increased hepatic PGC-1α and ERRα compared with the Model group, whereas PGC-1α knockdown (E2+siPGC-1α) attenuated these increases.

3.6. ChIP-qPCR Analysis of PGC-1α Binding to the ERRα Promoter

Chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) was performed to investigate the interaction between PGC-1α and the ERRα promoter. Compared with the IgG negative control, immunoprecipitation using a PGC-1α-specific antibody resulted in significantly greater enrichment of the ERRα promoter region ($P < 0.05$), indicating direct association of PGC-1α with the ERRα promoter.

These findings suggest that PGC-1α may regulate ERRα transcription through promoter binding, providing mechanistic support for the involvement of the PGC-1α/ERRα signaling pathway in the metabolic effects of E2(Figure 3).

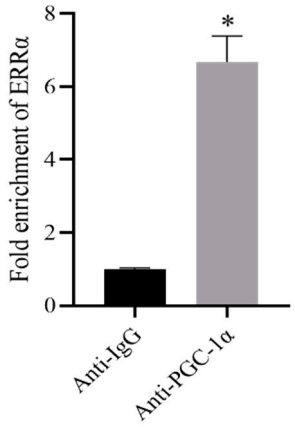

Figure 3. Enrichment of the ERRα promoter by PGC-1α determined using chromatin immunoprecipitation quantitative PCR (ChIP-qPCR).

4. Discussion

The relationship between type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) is both complex and bidirectional, with a substantial proportion of patients presenting with both conditions. Insulin resistance is a central pathological link, triggering disturbances in glucose and lipid metabolism that drive disease progression. Standard management—comprising dietary modification, increased physical activity, and pharmacotherapies such as insulin, metformin, and glucagon-like peptide-1 (GLP-1) receptor agonists—can improve glycemic control and insulin responsiveness. However, these measures often show limited efficacy in reversing hepatic steatosis and metabolic dysfunction. This highlights the necessity of uncovering precise molecular mechanisms and identifying novel therapeutic targets for T2DM complicated by NAFLD.

17β-Estradiol (E2), the predominant estrogen in premenopausal females, has emerged as a modulator of multiple metabolic disorders, including obesity, diabetes, and NAFLD. It exerts protective actions by enhancing fatty acid oxidation, stimulating browning of white adipose tissue, and increasing energy expenditure. E2 also improves insulin signaling, promotes glucose utilization, preserves endothelial function, and mitigates oxidative stress and lipid peroxidation. In this study, E2 administration significantly improved glycemic and lipid profiles, reducing FBG, FINS, HOMA-IR, TC, TG, LDL-C, ALT, and AST levels while lowering body weight and liver index. Oil Red O

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staining further demonstrated a significant reduction in hepatic lipid accumulation following E2 treatment, suggesting improvement of hepatic steatosis.

At the molecular level, T2DM+NAFLD mice exhibited marked downregulation of PGC-1α and ERRα in liver tissue, indicating dysfunction of this signaling axis. E2 supplementation restored the expression of both proteins, consistent with previous reports demonstrating that activation of PGC-1α improves mitochondrial quality control and protects against liver injury [20], while stimulation of the AMPK–PGC-1α/Nrf2 pathway alleviates hepatic oxidative stress and metabolic dysfunction [21]. In addition, ERRα has been recognized as a key regulator of energy metabolism and fatty acid oxidation in metabolic diseases [22]. Our findings, together with the direct association of PGC-1α with the ERRα promoter demonstrated by ChIP-qPCR, support the involvement of the PGC-1α/ERRα signaling pathway in mediating the metabolic benefits of E2.

Importantly, PGC-1α knockdown largely abolished the protective effects of E2, resulting in reduced expression of PGC-1α and ERRα, increased hepatic lipid accumulation, and worsening metabolic abnormalities. These findings indicate that the PGC-1α/ERRα axis is a critical mediator of E2 action and may represent a promising therapeutic target for T2DM-associated NAFLD.

Conclusion

This study demonstrates that 17β-estradiol (E2) improves glucose and lipid metabolism, alleviates hepatic steatosis, and improves liver function in a mouse model of T2DM with NAFLD. These protective effects are mediated, at least in part, through activation of the PGC-1α/ERRα signaling axis, which contributes to the maintenance of metabolic homeostasis. Silencing PGC-1α largely abolishes these benefits, confirming its important role in the mechanism of action of E2. These findings provide new mechanistic insights and identify the PGC-1α/ERRα axis as a potential therapeutic target for T2DM-associated NAFLD, warranting further investigation in future preclinical and clinical studies.

Study Limitations

This study has certain limitations that should be acknowledged. First, the findings are based on a single mouse model of T2DM with NAFLD induced by a high-fat diet and streptozotocin; therefore, the results may not fully represent the complexity and heterogeneity of the disease in humans. Second, only male mice were used, which may overlook potential sex-specific differences in the metabolic and hepatic responses to 17β-estradiol. Third, while the study confirmed the involvement of the PGC-1α/ERRα axis in mediating the protective effects of E2, other signaling pathways and downstream targets were not extensively explored. In addition, mechanistic evidence was primarily derived from Western blot and ChIP-qPCR analyses, and further studies are needed to provide a more comprehensive characterization of the cellular interactions involved in PGC-1α/ERRα signaling. The relatively small sample size, although statistically powered, may also limit the generalizability of the results. Finally, as this work was conducted in an experimental setting, further validation in clinical populations is warranted to confirm the translational relevance of these findings.

Author Contributions: Conceptualization: Q L. Methodology: Q L. Investigation: W X, Q N, Q Y. Data Curation: Q N. Formal Analysis: Q N, W X, Q L. Writing – Original Draft: Q N. Writing – Review & Editing: Q L, W X. Visualization: Q Y. Supervision: Q L. Funding Acquisition: Q L.

Funding: This work was supported by the Science and Technology Project Fund of Xuzhou City (Grant No. SHE20250017). The funding agency had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Ethics Approval and Consent to Participate: All procedures involving animals were approved by the Experimental Animal Ethics Committee of Xuzhou Medical University, Jiangsu, China (Approval No. XZMC2023-021) and conducted in accordance with the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals (US National Research Council).

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Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to institutional data retention policies and availability of archived records.

Acknowledgments: The authors thank the Department of Pharmaceutical Analysis, School of Pharmacy, Xuzhou Medical University, for providing laboratory facilities and technical assistance. We also appreciate the constructive discussions with colleagues in the field of pharmacology and metabolic disease research.

Conflicts of Interest: The authors declare no conflicts of interest.

List of Abbreviations

Abbreviation	Full term
AMPK	AMP-activated protein kinase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BSA	Bovine serum albumin
ChIP-qPCR	Chromatin immunoprecipitation quantitative polymerase chain reaction
E2	17β-estradiol
ECL	Enhanced chemiluminescence
ELISA	Enzyme-linked immunosorbent assay
ERRα	Estrogen-related receptor alpha
FBG	Fasting blood glucose
FINS	Fasting insulin
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GLP-1	Glucagon-like peptide-1
HOMA-IR	Homeostasis model assessment of insulin resistance
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
LDL-C	Low-density lipoprotein cholesterol
LSD	Least significant difference (test)
NAFLD	Non-alcoholic fatty liver disease
PBS	Phosphate-buffered saline
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator-1 alpha
PVDF	Polyvinylidene difluoride
SD	Standard deviation
siNC	Small interfering negative control
siPGC-1α	Small interfering RNA targeting PGC-1α
STZ	Streptozotocin
TC	Total cholesterol
TG	Triglycerides

 doi:10.20944/preprints202508.0696.v2

T2DM	Type 2 diabetes mellitus

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