Zheng L et al (2024) PROTECTIVE EFFECTS OF CHENODEOXYCHOLIC ACID ON RENAL FIBROSIS IN HIGH-FAT DIET-INDUCED CHRONIC KIDNEY INJURY: IMPLICATIONS FOR PHYSICAL RESILIENCE AND RECOVERY. Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte vol. 24 (98.1) pp. 352-370
DOI: https://doi.org/10.15366/rimcafd2024.98.1.024

ORIGINAL

PROTECTIVE EFFECTS OF CHENODEOXYCHOLIC ACID ON RENAL FIBROSIS IN HIGH-FAT DIET-INDUCED CHRONIC KIDNEY INJURY: IMPLICATIONS FOR PHYSICAL RESILIENCE AND RECOVERY

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Recibido 13 de abril de 2024 **Received** April 13, 2024
Aceptado 27 de diciembre de 2024 **Accepted** December 27, 2024

ABSTRACT

Objective: To investigate the protective effects of chenodeoxycholic acid (CDCA), a potent physiological agonist of the farnesoid X receptor (FXR), on renal fibrosis in 5/6 nephrectomized (I-PNx) mice fed a high-fat diet and to explore its implications for physical resilience and recovery in chronic kidney disease (CKD). **Methods:** I-PNx mice were randomly assigned to high-fat or low-fat diet groups. After four weeks of dietary intervention, mice were treated with CDCA or placebo for an additional four weeks. Renal function was assessed by monitoring biochemical markers, pathological changes, and fibrosis-related factor expression. Primary mesangial cells (P-MCs) were isolated from C57/BL6 mice and exposed to exogenous TGF-β to induce fibrosis. These cells were treated with CDCA and Fxrα2 adenovirus (adv-FXRα2) to activate FXR. Biochemical, molecular, and histological analyses were performed to evaluate the effects of CDCA on renal fibrosis. **Results:** Mice in the high-fat diet group exhibited significantly increased body weight, deteriorated renal function, and elevated albuminuria compared to those on a low-fat diet. CDCA treatment significantly reduced levels of urea, creatinine, and urinary protein, with the most pronounced effects observed in high-fat diet-fed mice. Mechanistically, CDCA downregulated the expression of fibrotic markers and Smad3 in kidney tissues, while upregulating Smad7 expression.

Similar results were observed in TGF-β-induced P-MCs following CDCA and adv-FXRα2 treatment, confirming the role of FXR activation in mitigating fibrosis. **Conclusion:** CDCA protects against renal fibrosis by modulating the TGF-β/Smad signaling pathway, reducing Smad3 expression and increasing Smad7 expression, thereby delaying the progression of chronic kidney disease. These findings highlight the potential of FXR agonists like CDCA as therapeutic options for obesity-associated CKD. By improving renal function and reducing fibrosis, CDCA may enhance physical resilience and recovery, supporting its integration into multidisciplinary approaches for managing CKD, particularly in populations where physical performance and rehabilitation are critical.

KEYWORDS: FXR, CDC, 5/6 Nephrectomy, High-Fat Feeding, Tgfβ; Chronic Kidney Disease

1. INTRODUCTION

Chronic kidney disease (CKD) is a global health concern, affecting millions of people and contributing significantly to morbidity, mortality, and healthcare costs. Renal fibrosis, characterized by excessive deposition of extracellular matrix proteins in the kidney, is a hallmark of CKD progression. This pathological condition leads to structural and functional deterioration of the kidneys, ultimately resulting in end-stage renal disease (ESRD). Patients with CKD, particularly those associated with obesity or metabolic disorders, often face additional challenges (Boor et al., 2010; Gotoh et al., 2013; Raj & Kumar, 2010), including reduced physical function, fatigue, and impaired quality of life, further highlighting the need for effective therapeutic interventions. The farnesoid X receptor (FXR) (Gonzalez et al., 2005; Stemmer et al., 2012), a nuclear receptor primarily expressed in the liver, kidneys, and intestines, plays a crucial role in regulating lipid metabolism, bile acid homeostasis, and inflammatory responses. Activation of FXR has been shown to exert protective effects against renal fibrosis by modulating key molecular pathways involved in fibrosis and inflammation. Chenodeoxycholic acid (CDCA), a physiological agonist of FXR, has emerged as a promising candidate for targeting renal fibrosis. Its ability to activate FXR and regulate downstream signaling pathways, such as the transforming growth factor-beta (TGF-β)/Smad pathway, makes it an attractive therapeutic option for CKD management (Hall et al., 2002; Iseki et al., 2004). The TGF-β/Smad pathway is a central mediator of renal fibrosis, driving the expression of profibrotic genes and promoting extracellular matrix accumulation. Smad3, a key signaling molecule in this pathway, facilitates fibrotic processes, while Smad7 acts as a natural inhibitor, counterbalancing the effects of Smad3 (Kambham et al., 2001; Praga et al., 2000). Dysregulation of this pathway contributes to the progression of renal fibrosis, making it a critical target for therapeutic intervention. Previous studies have indicated that CDCA can modulate the TGF-β/Smad signaling cascade, but its efficacy in the context of obesity-related CKD remains underexplored. Obesity, a major risk

factor for CKD, exacerbates renal fibrosis through mechanisms such as increased oxidative stress, inflammation, and altered lipid metabolism. High-fat diets, often used in experimental models, mimic the metabolic disturbances observed in obesity, providing a relevant framework for studying potential therapies. Addressing renal fibrosis in obesity-associated CKD is particularly important in the context of sports and physical rehabilitation, as CKD-related physical impairments can significantly impact mobility, endurance, and overall quality of life (Praga & Morales, 2006; Y. Wang et al., 2008). This study aims to investigate the protective effects of CDCA on renal fibrosis in a 5/6 nephrectomized (l-PNx) mouse model fed a high-fat diet, focusing on its ability to modulate the TGF-β/Smad signaling pathway. By evaluating biochemical markers, pathological changes, and molecular mechanisms, the research seeks to provide a comprehensive understanding of CDCA's therapeutic potential. Furthermore, the study explores the broader implications of these findings for enhancing physical resilience and recovery in CKD patients, aligning with the goals of sports and rehabilitation medicine to optimize functional outcomes and quality of life. The underlying mechanisms of obesity-induced renal damage are unclear. Renal fibrosis is a common feature of CKD progression; it is characterized by glomerulosclerosis and tubulointerstitial fibrosis. The pathological factors mediating progressive renal fibrosis include various growth factors (GF), cytokines, metabolic toxins, and the byproducts of oxidative stress (OS). Of these factors, transforming growth factor β1 (TGF-β1) seems to have a critical role in renal fibrosis, which is achieved through either the Smad-dependent or Smad-independent pathways(Meng et al., 2016). Farnesol X receptor (FXR) is a member of the nuclear receptor superfamily, mainly expressed in the liver, small intestine and kidney (Ding et al., 2015). FXR is involved in the regulation of bile acid metabolism (Y.-D. Wang et al., 2008), glucose metabolism (Zhang et al., 2006), kidney water and salt metabolism (Xu et al., 2018; Zhang et al., 2014). Physiological levels of bile acids, such as bovine deoxycholic acid (CDCA), have been found to be endogenous ligands of FXR, so FXR is considered to be a nuclear receptor for bile acids. Primary bile acid, cholic acid (CA) and andeoxycholic acid (CDCA) are strong agonists of FXR. 6-ecdca is currently in Phase II clinical trials for the treatment of primary biliary cirrhosis and non-alcoholic fatty liver disease. We investigated the effects of FXR on obese individuals with chronic kidney failure. To this end, ligation based 5/6 nephrenectomy (l-PNx) mice were generated and fed either a high fat or low-fat diet to establish a model of CKD. We have found in previous studies that FXR levels in blood and urine gradually decrease in CKD patients with renal function progression. We then monitored biochemical markers, pathological and fibrotic factor levels to determine the effect of CDCA in the body. Primary mesangial cells (P-MCs) were isolated from C57/BL6 mice and exogenous TGF-β was exposed to cells that mimicked fibrosis. TGF-β-induced cells were then treated with CDCA and Fxrα2 adenovirus (adv-FXRα2) to activate FXR. CDCA inhibits TGF-β-induced fibrosis by down-regulating Smad3

expression and up-regulating Smad7 expression, thereby delaying the progression of chronic kidney disease. FXR agonist CDCA may be a suitable treatment option for patients with obesity-associated CKD.

2. Materials and Methods

2.1 5/6 Nephrectomy Mouse Model

A total of 32 8-week-old male C57BL/6 mice weighing 22-25 g were used in all experiments. All the animals used in this study were housed on a daily 12-h light/black cycle under controlled temperature (22–24°C) and humidity (50–65%) in the animal facility of Peking University Health Science Center. All experiments were reviewed and approved by the Animal Care and Use Review Committee of Peking University Health Science Center. To construct the ligation-based 5/6 nephrectomy (l-PNx) model, the right kidney was exposed and removed under anesthesia (1% pentobarbital sodium) in aseptic conditions. After 1 week, the left kidney was exposed and both the upper and lower poles were ligated with a 3–0 non-absorbable suture, until the coil diameter was 1/2 of that of the kidney.

2.2 Experimental Mouse Groups

Male 1-pnx mice (n = 32) were randomly divided into high fat (Madison, 45% high fat) and low-fat diet (Madison, 10% low fat) groups. After 4 weeks of dietary intervention, each group was further randomized into two sub-groups and administered either placebo control (water) or CDCA (0.5% CDCA dissolved in drinking water) for 4 weeks. The final four groups were as follows: Control low fat diet, Con-LF (n=6); CDCA low fat diet, CDCA-LF (n=10); control high fat diet, Con-HF (n=6); and CDCA high fat diet, CDCA-HF (n=10).

2.3 Urine Collection, Osmolarity Analysis and MRI Analysis

Mice were housed in individual metabolic cages (Tecniplast) for 24 h prior to measurement, with free access to water and food. Urine was collected over 24-h periods. Body weight, urine excretion and water consumption were measured for each animal. By using metabolic cage (Metabolic cages (3600M021; Tecniplast) were used for the collection of 24-h urine. Urine samples were centrifuged at 3,000 g at 4° C for 5 min, and the supernatants were saved for osmolality analysis using a freezing-point depression osmometer (Micro- Osmometer 3300; Advanced Instruments). Then, the mice were fixed to avoid the influence of their activities on the measurements, and MRI examinations were carried out to collect body-fat weights.

2.4 Biochemical Analyses

Mice in each group were exposed to specific treatments within the same

12-hour light cycle. The body weight, urine output, osmotic pressure and body fat of each mouse were monitored every 4 weeks. After 8 weeks, blood samples were collected from each animal, and urine and serum samples were analyzed in the third people's Hospital of Peking University to detect triglyceride (TG), total cholesterol (TC), urea, creatinine (CR), uric acid (UA) and urine protein levels.

2.5 In Vitro Wild-Type Primary Mesangial Cells (P-MC) Culture

Wild type primary mesangial cells (p-mcs) were isolated from C57BL / 6 mice and cultured as previously described. Briefly, the renal cortex was isolated from 4-6-week-old male mice and chopped up, and then immersed in the enzyme solution (collagenase A: 1mg / ml) under continuous stirring at 37 ° C for 40 minutes. Then, 70 μ M U.M sieve to collect the supernatant, and then 40 μ M U.M sieve to filter the supernatant again. P-Mc cells were then cultured in Dulbecco's modified eagle's medium high glucose (dmem-hg) supplemented with 4500mg / L glucose. After 2-3 days of culture in an incubator at 37 ° C and 5% CO2, the medium was changed when the cells slid around the glomeruli. After about one week, the passaged cells formed into long spindle shape; Endothelial cells and epithelial cells gradually died after ~ 2 weeks, and oval mesangial cells crawled out. After about 4 weeks of culture, the cells of passages 5-6 were purified, and then the cells of passages 8-12 were collected for further analysis.

2.6 Cell Experiments

Chenodeoxycholic acid (CDCA) and TGF β were purchased from Sigma. Mouse anti-farnesoid X receptor (FXR) was provided by Perseus Proteomics. Primary antibodies against p-Smad3 and Smad3 used in this study were obtained from Santa Cruz Biotechnology. Antibodies against Smad7 and collagen Ⅰ were derived from ABclonal. Antibodies against SHP, CTGF, α-SMA and SDHA were bought from Abcam. The adenoviruses expressing FXR α1 (Ad-FXR α1), FXR α2 (Ad-FXR α2) and their control adenoviruses (Ad-VP16) were provided by Dr. Peter Edwards from the University of California (Los Angeles). Initial ‘dose-response’ experiments for the FXR agonist CDCA (Sigma Aldrich, USA) were performed to determine the optimal CDCA concentration to maximally stimulate FXR protein expression with the minimal cytotoxicity. Exposure of P-MC cells to 100 nM CDCA resulted in no cytotoxicity. After reaching 60-70% confluence, the P-MC cells were exposed to the following experimental conditions for 24 h prior to RNA extraction and for 48 h before protein extraction: 1) DMSO (vehicle control); 2) 5 ng/ml TGF β; and 3) 5 ng/ml TGF β + 100 nM CDCA. The adenovirus α2 titers of FXR (Ad-VP16, University of California, Los Angeles) were accurately measured according to the KARBER statistical method. After reaching 60–70% confluence, the P-MC cells were also exposed to the following experimental conditions for 24 h prior

to RNA extraction and for 48 h before protein extraction: 1) GFP (vehicle control); 2) Ad-FXR α 2; 3) 5 ng/ml TGF β 1+ GFP; and 4) 5 ng/ml TGF β 1+ Ad-FXR α 2.

2.7 mRNA Isolation, RT-PCR, and Real-Time PCR

Total mRNA was extracted from cells or kidney tissues using a commercial RNA isolation kit (Biotek, Beijing, China) and then reverse transcribed to cDNA using a RevertAidTM First Strand cDNA Synthesis Kit (Fermentas) according to the manufacturer's instructions. The resultant cDNA was used as the template in a PCR reaction with SYBR Green 1 (Bio-rad). The following primers were used in this study:

TGFβ1_forward: 5′-AGC CCG AAG CGG ACT ACT AT-3′; TGFβ1_reverse: 5′- CTG TGT GAG ATG TCT TTG GTT TTC -3′;

FXRα_forward: 5′- CCG AGA GAA GAA CCG AGT T-3′; FXRα_reverse: 5′- TAG ATG CCA GGA GAA TAC CAG-3′;

FXRβ_forward: 5′-ATG CAG TTT CAG GGC TTA GAA-3′; FXRβ_reverse: 5′-CGG GAC ATT GTT GTA TGG G-3′;

β-actin_forward: 5′-TGT TAC CAA CTG GGA CGA CA-3′; β-actin_reverse: 5′-GGG GTG TTG AAG GTC TCA AA-3′;

18S_forward: 5′-GAA ACG GCT ACC ACA TCC AAG G-3′; and 18S_reverse: 5′-GCC CTC CAA TGG ATC CTC GTT A-3′.

The PCR cycling conditions were as follows: 94 °C for 5 min, followed by 35 cycles of 94°C for 30 s, 59°C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. β-Actin and 18S were used as internal controls.

2.8 Protein Extraction and Western Blotting

Renal or cellular proteins were solubilized in lysis buffer (20 mM Tris-Cl, pH 7.4, 1 mM EDTA, 0.1% SDS, and 1% Triton X-100) supplemented with phosphatase inhibitor (P1260, PPLYGEN) and 0.2 mg/mL PMSF. Nuclear extracts were isolated using an NE-PER Kit, in accordance with the manufacturer's instructions (78833, Pierce Biotechnology, Inc.). Protein concentrations were determined by bicinchoninic acid assay (P001, Vigorous Biotechnology). Then, 40-80 μg proteins mixed with loading buffer were separated by 10% (wt/vol) SDS/PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5% (wt/vol) skim milk and incubated with specific primary antibodies overnight at 4 °C. The primary antibodies used in this study included mouse anti-FXR (1:1000); rabbit anti-TGFβ1 (1:1000); mouse anti–β-actin (1:1000); rabbit anti-p-Smad3, Smad3, Smad7 and collagen1, and CTGF (1:1000). After washing, the membranes were further incubated for 1 h at room temperature with HRP-conjugated secondary antibodies (Santa Cruz Biotechnology). Then, the membranes were washed again, and antibody binding was detected using a chemiluminescence

substrate (sc-2048, Santa Cruz Biotechnology), followed by exposure to X-ray film (Kodak XBT-1).

2.9 Immunohistochemistry (IHC), Masson Staining and Immunofluorescence (IF)

For IHC studies, kidneys were fixed with 4% (wt/vol) paraformaldehyde (PFA) in PBS overnight, followed by dehydration, paraffin embedding, and slicing into 4-μm sections. Then, the sections were incubated with specific primary antibodies overnight at 4 ° C, and then with an HRP-conjugated secondary antibody (Zhongshan Golden Bridge) for 30 min at 37 ° C, followed by hematoxylin counterstaining. For Masson's trichrome staining, renal tissues were fixed with 4% (wt/vol) PFA and embedded in paraffin, and fibrosis severity was measured using a Masson's trichrome staining kit (MST-8004, purchased from Fuzhou Maixin). For immunofluorescence, cultured P-MC cells were fixed with 4% (wt/vol) PFA for 15 min at room temperature on a rocking platform. After three washes with PBS, the sections were permeabilized with 0.1% Triton X-100 in PBS for 10 min and then blocked with 0.5% BSA in PBS for 10 min. The sections were then incubated with primary antibodies overnight at 4 ° C and then with the appropriate DyLight 488 (green) or Dy Light 594(red)-conjugated secondary antibodies (Jackson Immuno Research Laboratories) for 1 h at room temperature. After washing, the nuclei were counterstained with DAPI and the cells were visualized under a confocal microscope. All images were captured under a Virtual Slide Microscope (VS120-S6-W, Olympus, Japan) at 400 x magnification.

2.10 Statistical Analysis

All data were analyzed using the Prism software package version 6.0 (GraphPad Software) and are expressed as the means ± SEM. The individual differences were analyzed by two-sided Student’s t-test. A P-value < 0.05 was considered to be statistically significant. At least 3 biological replicates were performed for each assay.

3. Results

3.1 CDCA Retards Renal Function and Proteinuria Deterioration in the Context of a High Fat Diet

Nephrectomy affects the expression of kidney genes in high-fat diet-induced obese mice, but how is unknown. We thus investigated the effects of treating 1-PNx mice with the FXR agonist CDCA, and characterized whether these effects depended on the diet at the phenotypic level. We found that CDCA prevented body weight and body fat gains in mice fed a high-fat diet but did not prevent weight gain in mice fed a low-fat diet. (Fig. 1A, B). The urine volume and urine osmotic pressure were also increased in both Con-LF and Con-HF

group (Fig. 1C, D). Finally, the body weight, body fat, and urine osmotic pressure in Con-HF group were higher than in the Con-LF group, while the urine volume was lower (Fig. 1A, B), body fat, renal function, and proteinuria in I-PNx mice, especially for CDCA-HF group. In terms of biochemical differences between the four groups, we found that the Con-HF group had higher Urea, Cr and urine protein levels than those in Con-LF group (Table 1).

The Urea, Cr and urine protein levels in CDCA-HF group were significantly reduced compared to those in control group, while the Urea and urine protein levels in the CDCA-LF group were significantly reduced compared to the Con-LF group, with no significant difference in Cr levels (Table 1). These data indicate that a high-fat diet increased the body weight and body fat nutrition index in I-PNx mice, which further deteriorates renal function and aggravates increased proteinuria. CDCA ameliorated the gains in body weight and body fat, further loss of renal function and proteinuria in I-PNx mice, in the context of a high-fat diet group.

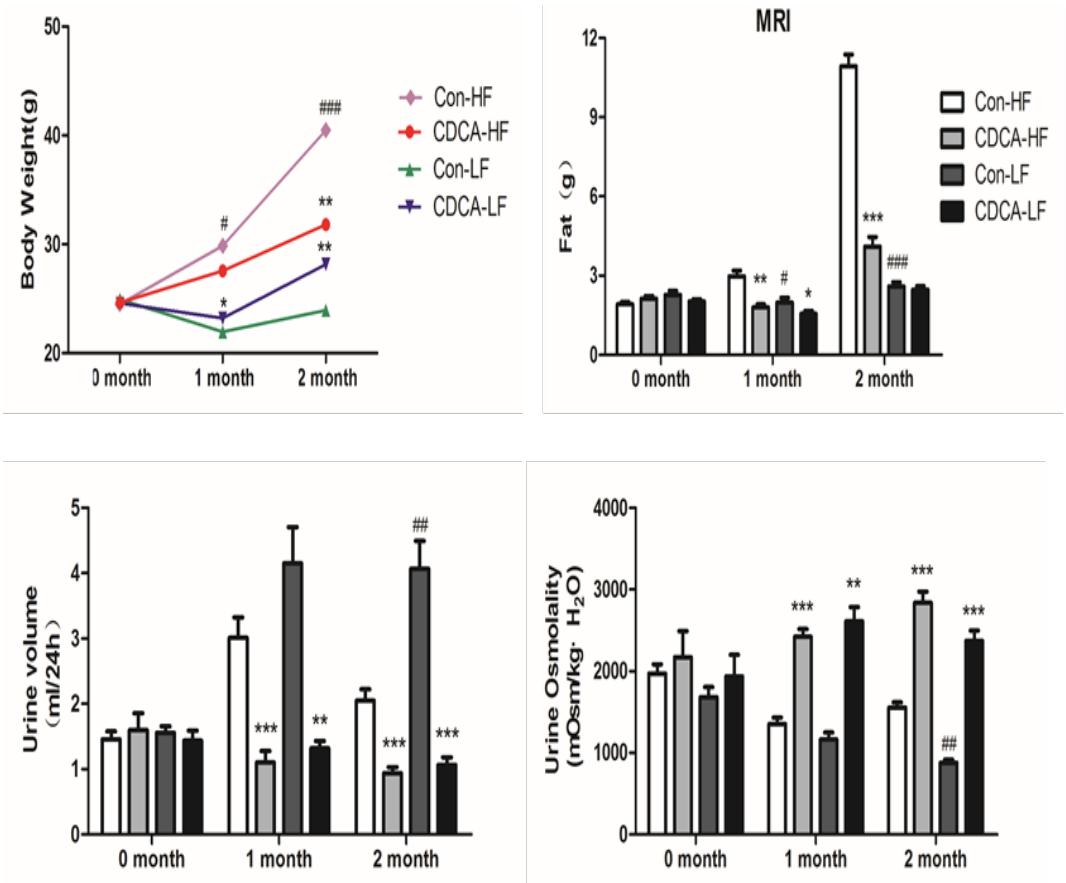


Figure 1: The effects of the FXR agonist CDCA on body weight, body fat and biochemistry in 5/6 nephrectomized mice fed a high-fat or low-fat diet. (A,B) The body weights (A) and body fat weights of the four groups, determined by MRI (B). (C,D) 24 h urine volume (C) and urine osmolality (D).The data represent the means±SEM : *p <0.05, **p <0.01, ***p <0.001 vs. Con; #p <0.05, ## p <0.01, ### p <0.001 vs. HF. Abbreviations: HF,a high-fat ;LF, low-fat diet.

Table 1: Changes in measurements for different groups

	CON-HF	CDCA-HF	CON-LF	CDCA-LF
BODY WEIGHT (G)	37.7±5.8a	31.5±1.2b	26.8±3.0	29.1±1.5
KIDNEY WEIGHT (MG)	205.0±12.9a	244.3±28.2b	182.9±13.8	157.5±9.6b
KW / BW (%)	0.6±0.07	0.8±0.09b	0.7±0.06	0.5±0.06b
PLASMA TG (MG / DL)	1.0±0.2	1.0±0.2	1.1±0.1	1.1±0.4
PLASMA TC (MG / DL)	3.3±0.8	2.9±0.5	2.9±0.4	2.4±0.5
UREA (MMOL/L)	25.2±2.4a	19.0±1.9b	11.8±1.3	8.6±0.7b
CR (MMOL/L)	43.4±3.1 a	37.7±4.5b	34.8±5.0	34.5±2.6
UA (MMOL/L)	276.6±88.2	180.0±76.2	282.8±106.7	297.8±98.2
PROTEINURIA (MG)	3065.3±356.4a	1397.4±179.3b	1601.5±156.5	801.5±120.7 b

TC, total cholesterol; TG, triglyceride. The data represent the means ± SD：aP <0.05 vs. Con-LF, bP < 0.05 vs. Con.

3.2 CDCA Ameliorates the Effects of a High Fat Diet on Renal Fibrosis in 5/6 Nephrectomized Mice

To confirm the severity level of renal fibrosis in Con-HF, CDCA-HF, Con-LF and CDCA-LF groups, we performed IHC of α-SMA and Masson staining (Fig.2A). First, we found that the severity of renal fibrosis in high-fat diet group was greater than that in low-fat diet group. Consistent with our previous findings, we found that the FXR agonist CDCA reduced a-SMA levels in kidneys of mice fed a high fat diet (CDCA-HF) after 4 weeks of treatment compared to mice fed a high-fat without CDCA treatment (Con-HF). Semi-quantitative analysis of the Masson trichrome and α-SMA staining (blue) confirmed these differences in fibrosis between the groups (Fig.2 B, C). These data support that a high-fat diet aggravates renal fibrosis in I-PNx mice, and that CDCA can ameliorate the effects of the high-fat diet in terms of restricting renal fibrosis.

3.3 CDCA Represses Fibrosis in 5/6 Nephretomized Mice by Down-Regulating Smad3 and Up-Regulating Smad7 Expression

Our previous analyses showed that CDCA mitigated renal fibrosis start at 8 weeks after surgery. To determine the severity of renal fibrosis in Con-HF, CDCA-HF, Con-LF and CDCA-LF groups, we monitored the protein and mRNA levels of the classic fibrotic factors, FXR, Smad3 and Smad7 by western blotting and PCR (Fig.3 A, B). We found that renal fibrosis was more severe in the high-fat diet group than in low-fat diet group in the absence of CDCA treatment (Fig.3 A, B). Exposure to the FXR agonist CDCA for 4 weeks, however, caused a down-regulation of the renal fibrosis factors and Smads, and an up-regulation of FXR and Smad7 in CDCA-HF and CDCA-LF groups (Fig.3 A, B). We confirmed these findings at the mRNA level (Fig.3C). These data suggest that a high-fat diet aggravates the severity of renal fibrosis in I-PNx mice. The FXR agonist CDCA can mitigate renal fibrosis in these I-PNx mice, as evidenced by

the down-regulation of Smad3 and the up-regulation of Smad7.Smad7 is one of the most important negative regulators of TGF-β signaling pathway in cells, which is closely related to renal fibrosis.

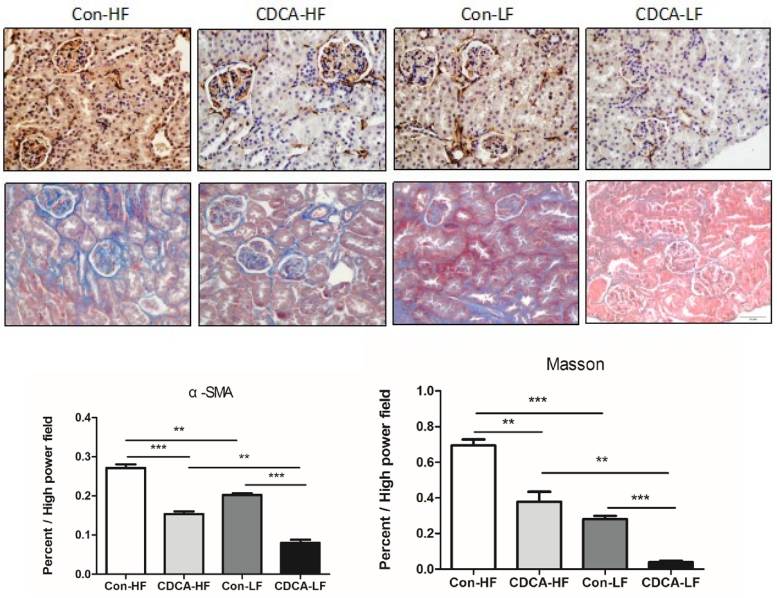
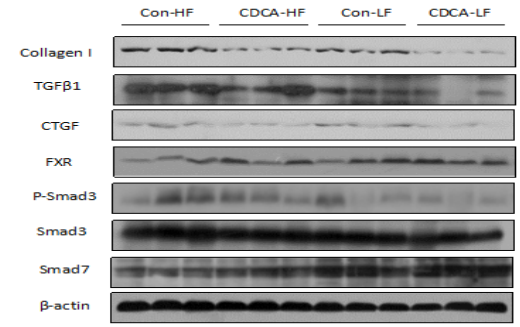
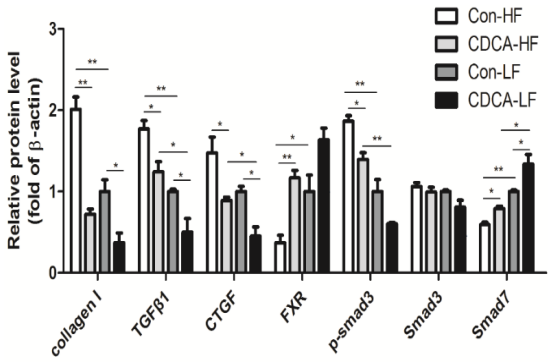


Figure 2: Overt renal fibrosis in I-PNx mice with or without CDCA treatment. (A) IHC analysis of α-SMA and Masson staining in the kidneys of Con-HF, CDCA-HF, Con-LF and CDCA-LF groups (400 x magnification). (B) The percent/high power field of α-SMA IHC in each group. (C) The percent/high power field of Masson staining IHC in each group. The data represent the means ± SD: *p < 0.05, **p <0 .01, ***p <0 .001.



(A)



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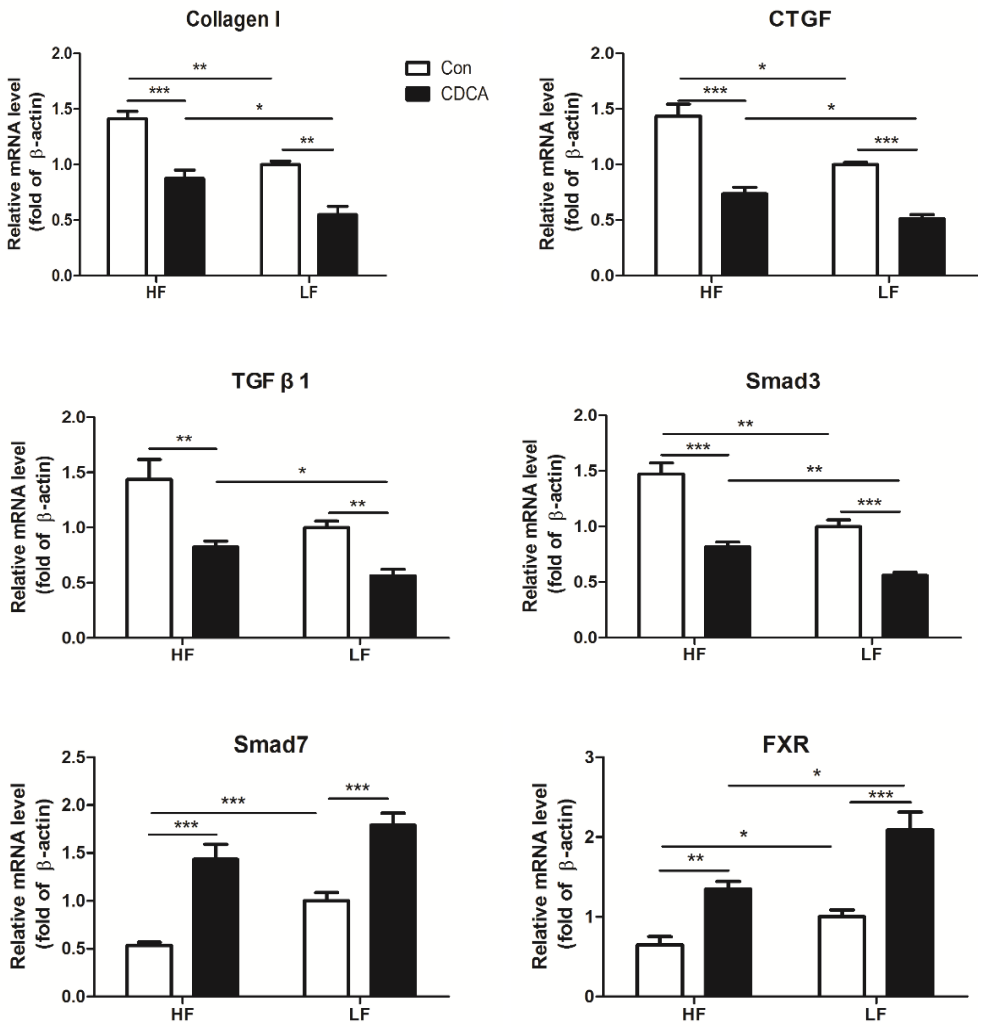


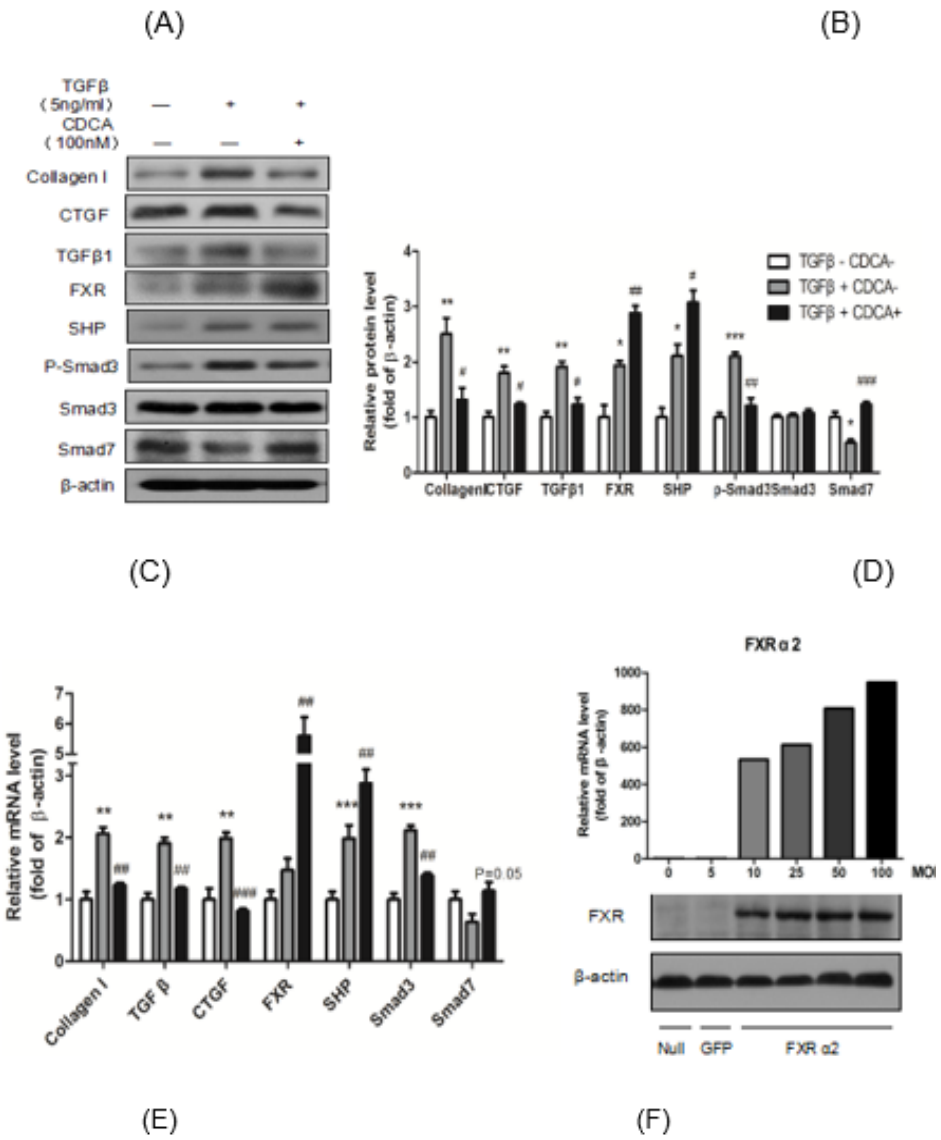
(C)

Figure 3: FXR represses fibrosis in l-PNx mice by down-regulating Smad3 and up-regulating Smad7 expression. (A, B) The protein levels of fibrotic factors, FXR, Smad3 and Smad7 in the renal tissues of Con-HF, CDCA-HF, Con-LF and CDCA-LF groups. (C) The mRNA levels of fibrotic factors, FXR, Smad3 and Smad7 in the renal tissues of four groups. The data represent the means ± SD: *p < 0.05, **p <0 .01, ***p <0 .001.

3.4 FXR Activation Inhibits Tgfβ1-Induced Cell Fibrosis

TGFβ1 plays a key role in renal fibrosis. To investigate the protective mechanism of FXR induction on renal fibrosis, we further cultured mouse P-MCs and treated them with CDCA with or with our TGFβ1. We found that FXR activation down-regulated TGFβ1-induced protein and mRNA expression of fibro genic factors （Collagen I、CTGF、TGF β 1） in P-MC cells while up-regulating Smad7 expression compared to vehicle treatment (Fig. 4A-D). We repeated the study by treating the cells with an FXR adenovirus α 2, to confirm that the effects of CDCA were indeed via FXR up-regulation. Consistent with CDCA treatment, adv-FXR α 2 down-regulated the TGF β 1-induced mRNA and protein expression of related fibro genic factors （Collagen I、CTGF、TGF

β1）in P-MC cells, and up-regulated Smad7 expression (Fig. 4E-G). These data suggest that FXR activation inhibits TGFβ1-induced cell fibrosis by reducing Smad3 expression and increasing Smad7 expression.



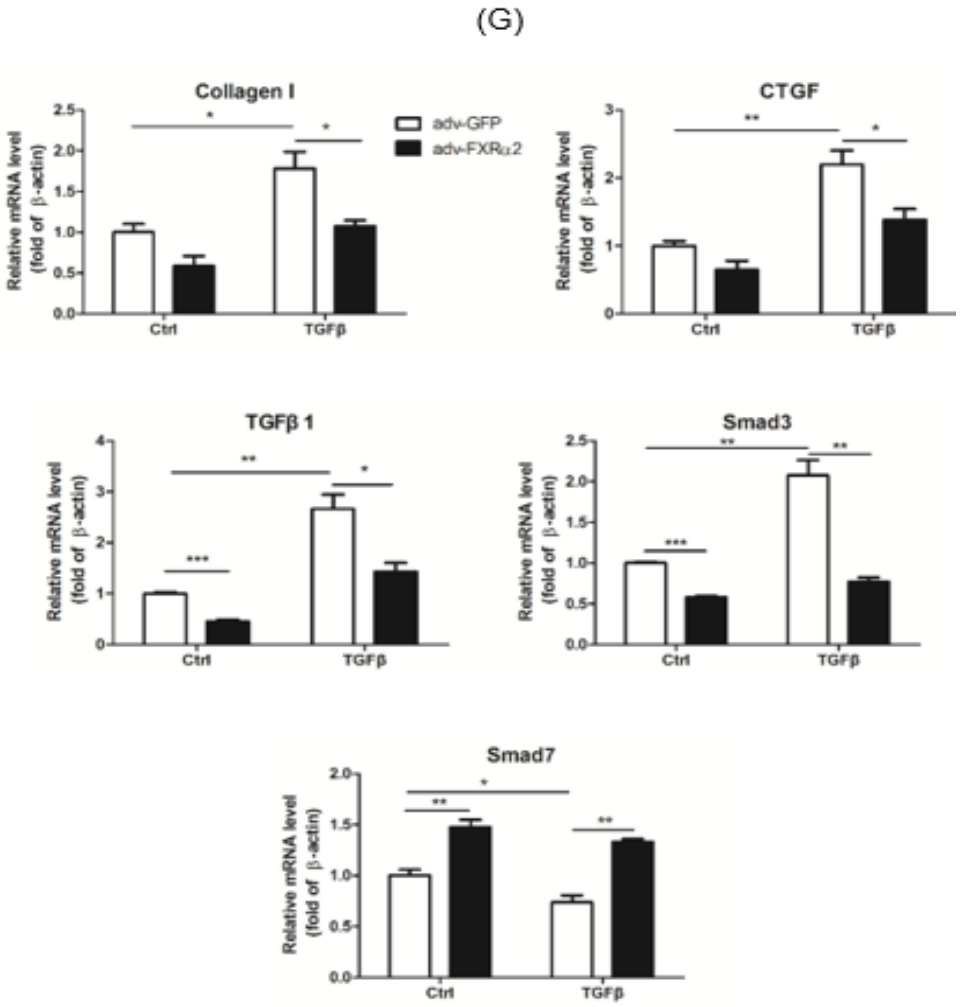


Figure 4: FXR represses fibrosis in P-MCs by down-regulating Smad3 and up-regulating Smad7 expression. (A, B) The protein levels of fibrosis factors, FXR, SHP, Smad3 and Smad7 in P-MCs after 24 h of CDCA (100 nM) treatment or 24 h of DMSO treatment following TGFβ1 (5 ng/ml) pre-treatment. (C) The mRNA levels of each group. *p <0.05, **p <0.01, ***p <0.001vs. TGFβ-CDCA-; # p <0.05, ## p <0.01, ### p <0.001vs. TGFβ+CDCA-. (D) Western blot and QPCR results of fibrosis factors expression after FXR adenovirus α2. (E, F) The protein levels of fibrosis factors, Smad3 and Smad7 in P-MCs after 24 h of adv-FXRα2 (10 MOI) or GFP treatment after 24 h of TGFβ1 (5 ng/ml) pretreatment. *p <0.05, **p <0.01, ***p <0.001 vs. TGFβ-GFP+; #p <0.05, ## p <0.01, ### p <0.001 vs. TGFβ+GFP+. (G) mRNA levels of each group. *p < 0.05, **p <0 .01, ***p <0 .001. The data represent the means ± SD in all cases.

4. Discussion

We demonstrated that a high-fat diet led to a significant increase in body weight and body fat nutrition index in I-PNx mice, which further worsened renal function and increased nephrectomy induced albuminuria. The excretion levels of urea, Cr and urinary protein were reduced after CDCA treatment, indicating that kidney damage was alleviated. We also detected reduced protein and

mRNA expression levels of fibrosis markers including type I collagen, CTGF, and TGFβ1, suggesting that CDCA improved renal fibrosis in PNx mice. The reduction of fibrosis level was pathologically supported by three-color masson pine staining and α-SMA immunohistochemistry. In vitro, we treated TGF-β-induced P-MCs with CDCA or FXR adenovirus (adv-FXRα2). FXR activation stimulates downregulation of TGFβ, which in turn leads to downregulation of Smad3 and upregulation of Smad7, ultimately restoring the balance between Smad3 and Smad7 and alleviating fibrosis. In summary, we suggest that FXR has a protective effect on renal fibrosis in 5/6 nephrectomized mice on a high fat diet. The primary bile acid, andeoxycholic acid, is the strongest endogenous ligand of FXR. In recent years, semi-synthetic ligands such as obercholic acid (OCA), INT-767 and synthetic ligands such as GW4604 play an increasingly obvious role in improving renal lipid metabolism, anti-inflammatory and fibrosis regulation, and laboratory and clinical studies have been extensively carried out(Xu et al., 2018). OCA is a 6-site ethylation product of goandeoxycholic acid, which has been used in clinical studies as a drug for the treatment of type 2 diabetes mellitus and non-alcoholic fatty liver disease (Wong, 2018) However, the clinical trials of INT-767 showed that the blood sugar of diabetic patients rose instead of falling, and the reasons for its occurrence remain to be further studied(Hodge & Nunez, 2016; Jiang et al., 2007). The systemic effect of FXR in diabetes nephropathy is still controversial. The FXR agonist GW4064 can improve the proteinuria, pro fibrosis and pro inflammation changes of db/db mice after 3 months of treatment, and improve the renal lipid metabolism. These changes are also related to the reduction of lipid hydroperoxide in the kidney. Similar beneficial effects have been shown in other organs, including the pancreas β Cell hypertrophy, liver steatosis and medial aortic hypertrophy recovery, more differentiated phenotypic changes in adipose tissue, myocardial cell disorder and improvement of left ventricular mass index (Han et al., 2021).The bioavailability of GW4064 is not high, and the stilbene structure in the molecular structure has potential toxicity and off-target effect (Xin et al., 2014). In the past few decades, understanding and intervening the progression of obesity-induced kidney damage in CKD has been the focus of research(Gai et al., 2014). Here, we generated an l-PNx model to simulate traditional 5/6 nephrectomy (Tan et al., 2019) to investigate the mechanism by which FXR induction affects the progression of CKD. FXR is involved in regulating lipid metabolism (Wang et al., 2009). High-fat diet may cause lipid metabolism disorder, which may induce or contribute to diabetic nephropathy. Jiang et al. found that FXR inhibits fatty acid and triglyceride synthesis in high-fat diet-induced type 2 diabetes mice by negatively regulating SREBP-1c. This effect reduces lipid accumulation in the kidneys. They also noted downregulation of TGF beta and CTGF, which reduced fibrosis. SREBP-1 is a transcription factor that regulates expression of genes involved in fatty acid synthesis. The classical result of SREBP-1 activation is increased fatty acid and triglyceride synthesis, leading to lipotoxicity and ultimately fibrosis, although lipotoxicity-independent

mechanisms are also known to play a role(Dorotea et al., 2020; Shimano & Sato, 2017). The exact mechanism of regulating lipid metabolism, especially in kidney diseases, is still unclear. The guidelines for the treatment of CKD lipid lowering are controversial. Some promising drugs, such as FXR/TGR5 double agonist INT-767, can improve renal lipid metabolism disorder and delay CKD progress (Zuzda et al., 2022).We suggest that the improvement of renal fibrosis in PNx mice by CDCA may be due to the negative regulation of SREBP-1c by FXR, but the specific mechanism needs to be further clarified in future studies. Rnal fibrosis is the common converging end point of kidney diseases. Indeed, the antifibrotic effects of decreasing lipotoxicity, oxidative stress, ER stress, and inflammation have all been well documented. However, emerging research indicates that FXR also has a direct role in decreasing renal fibrosis. FXR inhibited Src phosphorylation, thereby preventing Src-mediated activation of the profibrotic Yap both by directly decreasing Yap phosphorylation and indirectly decreasing Yap nuclear localization (Libby et al., 2021). FXR is involved in regulating water metabolism. We consistently found that urine volume was higher in untreated mice fed either high fat or low-fat diets (Con-HF and Con-LF) than in mice fed both diets (CDCA-hf and CDCA-lf). However, urinary osmolality was significantly reduced, so these findings may be attributed to FXR expression. In fact, FXR gene deletion resulted in impaired urinary concentration in mice, showing a polyuria phenotype. Activation of FXR restored urine concentration in these mice. FXR expressed in the collecting duct can bind to the FXRE response element on the promoter of aquaporin 2 (AQP2) gene of the target gene to induce increased AQP2 gene expression and regulate water reabsorption in vivo. Compared with CDCA-HF group, mice in Con-HF group showed no significant increase in body weight, TG and TC, which may be due to edema caused by severe kidney damage. Obesity is an independent risk factor for CKD (Garofalo et al., 2017); therefore, these patients should control their body weight. The mechanism of obesity aggravating CKD is still unclear. Some studies believe that obesity is related to the increase of GFR and renal blood flow (Chagnac et al., 2000; Henegar et al., 2001; Wahba & Mak, 2007). The role of the renin angiotensin system (RAS) may persist in dominant CKD. Consistent with this hypothesis, the post hoc analysis of the renal efficacy of ramipril (rein) study in CKD patients showed that the use of Ras inhibitors could achieve greater renal protection compared with non-obese individuals (Mallamaci et al., 2011). This study demonstrates that chenodeoxycholic acid (CDCA), a potent agonist of the farnesoid X receptor (FXR), effectively mitigates renal fibrosis in a 5/6 nephrectomized (l-PNx) mouse model fed a high-fat diet. CDCA exerts its protective effects by modulating the TGF-β/Smad signaling pathway, specifically through the downregulation of Smad3 expression and upregulation of Smad7 expression, thereby reducing fibrotic activity and preserving renal function. The findings highlight CDCA's potential as a therapeutic agent for managing chronic kidney disease (CKD), particularly in cases associated with obesity and metabolic

dysfunction. CDCA's ability to reduce fibrosis and improve renal function has significant implications for physical resilience and recovery in CKD patients. By mitigating disease progression and improving metabolic health, CDCA offers a pathway to enhance patients' physical capacity, enabling greater participation in rehabilitation programs and physical activity. This is especially relevant for individuals facing CKD-related fatigue, mobility limitations, and reduced quality of life. The study also underscores the importance of addressing obesity-related CKD within a multidisciplinary framework that integrates pharmacological interventions like CDCA with lifestyle modifications, including dietary management and structured physical activity programs. Such a comprehensive approach can optimize renal health, reduce disease burden, and improve overall patient outcomes. Future research should focus on translating these preclinical findings into clinical settings by evaluating CDCA's safety, efficacy, and long-term impact in human populations. Additionally, studies exploring the integration of CDCA with rehabilitation strategies and its role in promoting physical performance and recovery could further enhance its application in sports and rehabilitation medicine. In conclusion, CDCA represents a promising therapeutic option for CKD management, with the potential to protect against renal fibrosis, improve metabolic health, and support physical resilience. Its integration into treatment protocols could significantly enhance the quality of life and functional independence of CKD patients, aligning with the broader goals of improving health outcomes through multidisciplinary care and rehabilitation.

Author Contributions

Y.Q, Y.Y, and L.Z. designed research; Y.Q., Y.Y, X.W., N.K. and J.C. performed research; Y.Q and L.Z. contributed new reagents/analytic tools; Y.Q. and L.Z. analyzed data; and Y.Q, Y.Y. and L.Z wrote the paper.

Studies Involving Animal Subjects

Generated Statement: The studies involving animal participants were approved by the Animal Care and Use Review Committee of Peking University Health Science Center(P20200315-004).

Funding

The present study was supported by Nantong Municipal Health Commission (QN2022040 to Y.Q.).

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