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Original Article

# Ursolic Acid Modulates Estrogen Conversion to Relieve Inflammation in Metabolic Dysfunction-associated Steatotic Liver Disease via HSD17B14

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## Abstract

***Background and Aims:*** The incidence of metabolic dysfunction-associated steatotic liver disease (MASLD) has been escalating annually, positioning it as the leading cause of chronic liver disease worldwide. Ursolic acid has demonstrated promising therapeutic efficacy in managing MASLD, thereby justifying the need for an in-depth exploration of its pharmacological mechanisms. This study aimed to investigate elucidate the therapeutic mechanisms by which ursolic acid modulates estrogen conversion in the treatment of MASLD. ***Methods:*** Building upon prior studies that have highlighted the potent anti-inflammatory effects of ursolic acid and its specific targeting of 17β-hydroxysteroid dehydrogenase 14 (HSD17B14), this investigation employed a western diet to induce MASLD in murine models with varying severities over different time intervals. ***Results:*** The protein expression of HSD17B14 initially increased, followed by a subsequent decrease. This trend was accompanied by corresponding changes in 17β-estradiol (E2) and estrone (E1) levels. Intervention with ursolic acid resulted in a reduction in HSD17B14 and E1 levels during the phase of high HSD17B14 expression, while simultaneously elevating E2 levels. In steatotic hepatocytes, E1 promoted cellular inflammation, whereas E2 exhibited anti-inflammatory effects. However, the alleviated effects of E2 were antagonized by HSD17B14. As expected, ursolic acid modulated HSD17B14, thereby mitigating the inflammatory response in steatotic hepatocytes. ***Conclusions:*** HSD17B14, a crucial enzyme regulating the balance between E1 and E2, catalyzes the conversion of estrogen E2 into E1, thereby exacerbating tissue inflammation induced by metabolic stress. Ursolic acid, by modulating HSD17B14-mediated estrogen conversion, appears to ameliorate immune-related inflammation in MASLD.

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## Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) represents a complex form of hepatic injury induced by multifactorial metabolic stress.[1] In the context of the current global health landscape, the incidence of MASLD has been steadily escalating, positioning it as the most prevalent chronic liver disease worldwide.[2] Notably, the affected demographic is progressively skewing younger, implying that an increasing number of individuals may endure the long-term ramifications of MASLD and its associated complications, further exacerbating the burden on global public health systems.[3,4] Recent cohort studies have highlighted that even mild steatosis is significantly associated with an increased risk of all-cause mortality, with the risk closely tied to the severity of the disease.[5] In response to these alarming trends, a recent consensus statement formulated by a consortium of global multidisciplinary experts has classified MASLD as a chronic metabolic public health disease, highlighting its significance in global health and the urgent need for effective interventions to address this growing issue.[6] In light of the formidable challenges posed by this widespread health issue, current guidelines recommend lifestyle modifications as the cornerstone of intervention, supplemented by pharmacological treatments targeting metabolic syndrome, along with educational initiatives aimed at mitigating additional insults and improving hepatic health.[7,8] While strategies for managing MASLD are rapidly evolving, substantial technical gaps persist, and the development of effective pharmacological agents remains a priority for both academic and pharmaceu-

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tical circles.[9]

Ursolic acid, a naturally occurring pentacyclic triterpenoid widely distributed in various plants, has attracted considerable attention due to its remarkable bioactivities, including anti-inflammatory, antioxidant, and antitumor properties.[10,11] In the context of therapeutic intervention in MASLD, ursolic acid has been shown to enhance hepatic lipid metabolism by promoting fatty acid oxidation and inhibiting lipogenesis.[12,13] Meanwhile, its potent anti-inflammatory and antioxidant properties, as well as its ability to attenuate the activation of hepatic stellate cells and the accumulation of extracellular matrix, have been demonstrated to impede the progression of hepatic fibrosis.[14–16] Our previous research has extensively investigated the anti-inflammatory mechanisms of ursolic acid in MASLD, identifying multiple pharmacological targets through HuProt™ 20K human proteome microarray screening, thereby highlighting its multi-target and synergistic effects.[17,18] These findings were further validated by surface plasmon resonance assays and GST-pull down experiments, confirming the binding affinity of ursolic acid to specific protein targets. Further studies have substantiated that ursolic acid can bind to decorin, regulating the IGF-IR and HIF-1 signaling pathways, thereby offering dual protection against both metabolic dysfunction and hepatic hypoxic injury during MASLD progression.[17] Additionally, ursolic acid has been found to target secreted phosphoprotein 1, resulting in notable suppression of the protein's activity, which plays a critical role in metabolic inflammation. This presents a promising therapeutic avenue for ameliorating the immunoinflammatory trajectory in MASLD.[18]

Moreover, our research has revealed that ursolic acid exhibits a strong affinity for 17β-hydroxysteroid dehydrogenase 14 (HSD17B14).[18] It should be noticed that HSD17B14 is known to possess NAD-dependent 17β-hydroxysteroid dehydrogenase activity, capable of converting the highly bioactive 17β-estradiol (E2) into the less active estrone (E1). This conversion may attenuate the biological activity of estrogen, potentially affecting the intensity and duration of estrogen signaling.[19] In light of recent research indicating the critical role of estrogen metabolism in the progression of MASLD, this study focuses on the HSD17B14-mediated estrogen conversion, revealing it as an additional pharmacodynamic mechanism by which ursolic acid mitigates immune inflammation in MASLD.[20,21]

## Methods

### *Animal model*

Six-week-old male C57BL/6J mice were obtained from Jiangsu GemPharmatech Co., Ltd. All animals were handled humanely, and the experiments were performed under a project license (2023023) approved by the Institutional Animal Ethics Committee of Shanghai Hospital of Traditional Chinese Medicine, in compliance with the ARRIVE guidelines.

All mice were housed under standard environmental conditions with ad libitum access to food and water. Following a one-week acclimatization period, the mice were randomized into different experimental groups. A western diet (WD, D09100310, Research Diets, USA) was administered to induce the MASLD mouse model. The treatment group received ursolic acid (U820363, Macklin, China) via oral gavage at a dose of 100 mg/kg/d as intervention therapy, starting at the beginning of the experiment and continuing throughout the entire experimental period, while the WD and normal chow diet (NCD) groups were administered normal saline. Body weights were recorded weekly, and blood samples were drawn from the heart under anesthesia. Livers were excised, either immediately snap-frozen in liquid nitrogen or fixed in 4% paraformaldehyde (BL539A, Biosharp, China) for further detection.

### *Cell culture*

AML12 cells were obtained from Pricella Life Science & Technology Co., Ltd. The cells were cultured in specialized medium (CM-0602, Pricella, China) containing insulin, transferrin, sodium selenite, and dexamethasone. To establish a steatotic cell model, palmitic acid (P5585, Sigma, USA) and oleic acid (O1008, Sigma, USA) were utilized in this study. Meanwhile, 17β-estradiol (E8875, Sigma, USA), estrone (E9750, Sigma, USA), HSD17B14 (ICA175Hu01, LMAI, China), and ursolic acid were employed for intervention.

### *Quantitative real-time polymerase chain reaction (PCR)*

Total RNA was extracted using the TRIzol reagent (15596026, Invitrogen, USA), following the manufacturer's protocols. RNA concentrations were quantified using the Thermo Scientific NanoDrop 2000c spectrophotometer. For quantitative real-time PCR, PrimeScript RT reagent (RR036A, Takara, Japan) and TB Green Premix Ex Taq II (RR420A, Takara, Japan) were used. The reactions were performed on a Bio-Rad CFX96 Real-Time PCR System. The relative mRNA levels of the target genes were calculated using the 2-ΔΔCt method, with beta-actin serving as the internal control. All primer sequences used in this study are provided in Supplementary Table 1.

### *Western blotting*

Western blotting was performed as described previously.[18] Liver tissues were homogenized in RIPA lysis buffer (P0013B, Beyotime, China) supplemented with protease and phosphatase inhibitors (P1045, Beyotime, China) to extract total protein. Protein concentrations were determined using the BCA protein assay kit (P0010, Beyotime, China), and equal amounts of protein were loaded onto 10% SDS-polyacrylamide gels for electrophoresis. Following separation, the proteins were transferred onto polyvinylidene difluoride membranes (ISEQ00010, Millipore, USA). Membranes were blocked in 5% milk (232100, BD Biosciences, USA) dissolved in Tris-buffered saline containing 0.1% Tween-20 (TBST, ST825, Beyotime, China) for 1 h at room temperature. Subsequently, the membranes were incubated overnight at 4°C with primary antibodies specific to HSD17B14 (1:1,000, ab198013, Abcam, USA) and β-actin (1:5,000, abs171598, Absin, China). After primary antibody incubation, the membranes were washed thrice with TBST and then incubated with horseradish peroxidase-conjugated secondary antibodies (GB23303, 1:10,000, Servicebio, China) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence detection system (1705060, Bio-Rad, USA), and quantification was performed using ImageJ 1.8.0 software.

### *Histological staining*

Histological staining was performed as described previously.[18] Liver sections from paraffin blocks were deparaffinized, rehydrated, and stained with hematoxylin and eosin (G1005, Servicebio, China) following standard procedures. Frozen samples were embedded in optimal cutting temperature (OCT, 4583, Sakura, USA), and sections were stained with Oil Red O (C0157S, Beyotime, China) to visualize lipid droplets. Immunohistochemistry was carried out using a

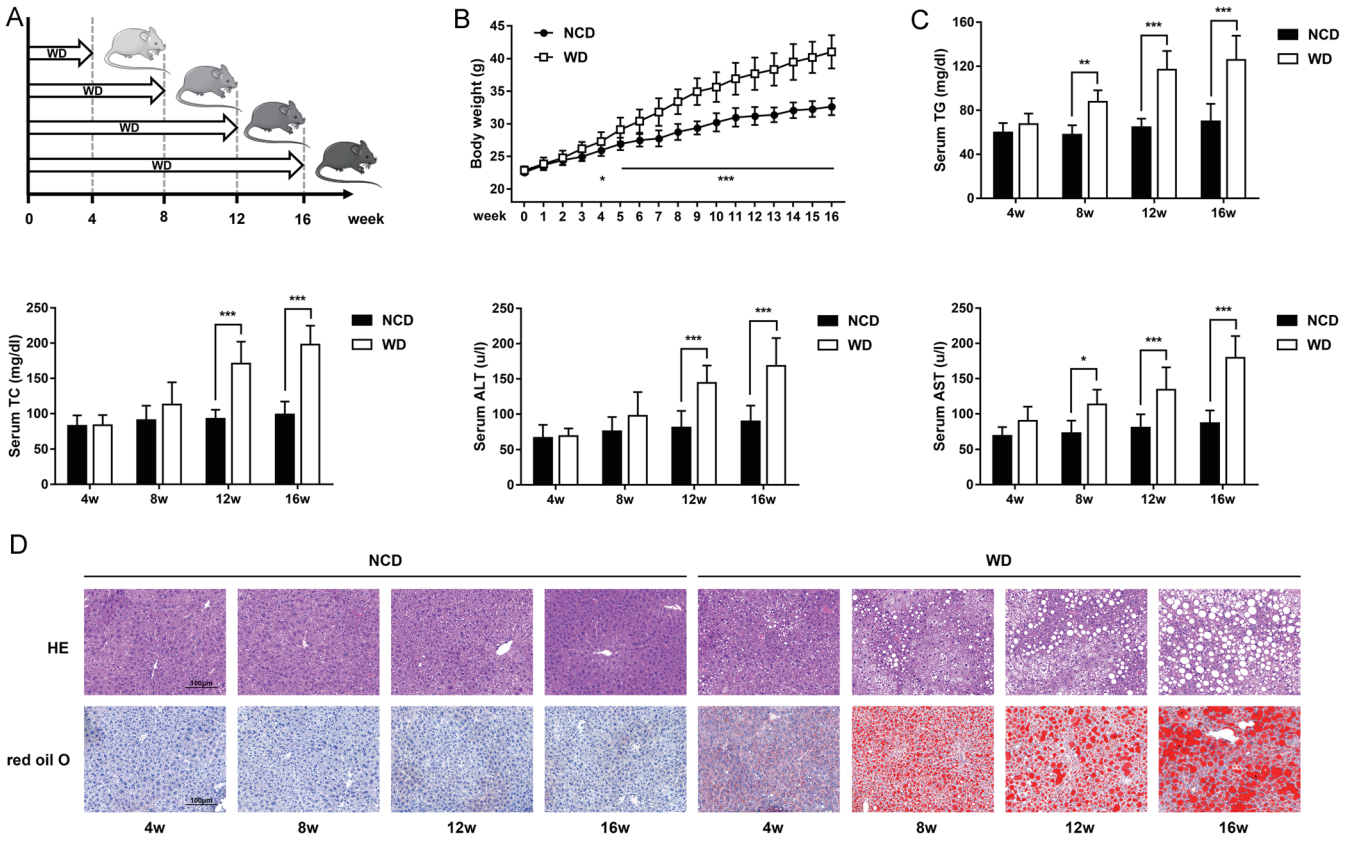


**Fig. 1. Characteristics of MASLD mouse induced by WD.** (A) Schematic representation of the experimental modeling process; (B) Weekly body weight measurements (n = 8); (C) Serum biochemical analyses of TC, TG, ALT, and AST were conducted by an automatic biochemical analyzer (n = 6); (D) Histological assessment of liver tissues using HE and oil red O staining to evaluate lipid droplet accumulation and inflammatory cell infiltration. Data are presented as means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. NCD, normal chow diet; WD, western diet; TG, triglycerides; TC, total cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MASLD, metabolic dysfunction-associated steatotic liver disease.

commercially available kit (G1215, Servicebio, China). After deparaffinization and hydration, antigen retrieval was performed using a sodium citrate solution. The sections were then incubated with a primary antibody against HSD17B14 (1:100). The color reaction was developed using biotinylated immunoglobulin G, horseradish peroxidase-streptavidin, and diaminobenzidine. Images were captured using an Olympus BX-50 microscope.

### *Statistical analysis*

All quantitative data generated in this study are presented as mean ± standard deviation. Data visualization and statistical analyses were performed using GraphPad Prism 7.0 software. One-way and two-way analysis of variance was used to analyze differences among groups, with a $p$-value < 0.05 considered statistically significant.

## Results

### *HSD17B14 mediates estrogen conversion in MASLD mice*

A recent study identified HSD17B14 as a pivotal enzyme responsible for regulating the balance between E1 and E2, where E2, with its anti-inflammatory properties, is converted into the more pro-inflammatory E1. This conversion suggests that HSD17B14 may promote inflammation induced by metabolic stress, thereby contributing to the progression of MASLD.[20] However, an analysis of transcriptomic microarray data pointed out a significant reduction in HSD17B14 expression, a finding that contradicts the previously hypothesized expectations.[18] A further literature review indicates that, while there exists a theoretical basis for the involvement of HSD17B14 and estrogens in MASLD progression, the existing research is limited and inconclusive.[21] In light of these discrepancies, the present experimental study seeks to elucidate the expression patterns of E1, E2, and HSD17B14 during MASLD progression. To this end, MASLD mouse models of varying severity were induced by administering a WD over different time intervals (four, eight, twelve, and sixteen weeks) (Fig. 1A).

Body weight measurements during modeling revealed that mice in the WD group exhibited significantly faster weight gain compared to those in the control group fed an NCD, with significant differences emerging by the fourth week (Fig. 1B). Serum biochemical tests revealed no significant differences in the levels of serum triglycerides (TG), total cholesterol (TC), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) between the two groups at the fourth week of induction. However, by the eighth week, the WD group displayed significantly elevated serum TG and AST levels compared to the NCD group, while TC and ALT levels showed slight increases, albeit without statistical significance. From the twelfth week onwards, serum levels of TG, TC, ALT, and AST were all significantly elevated in the WD

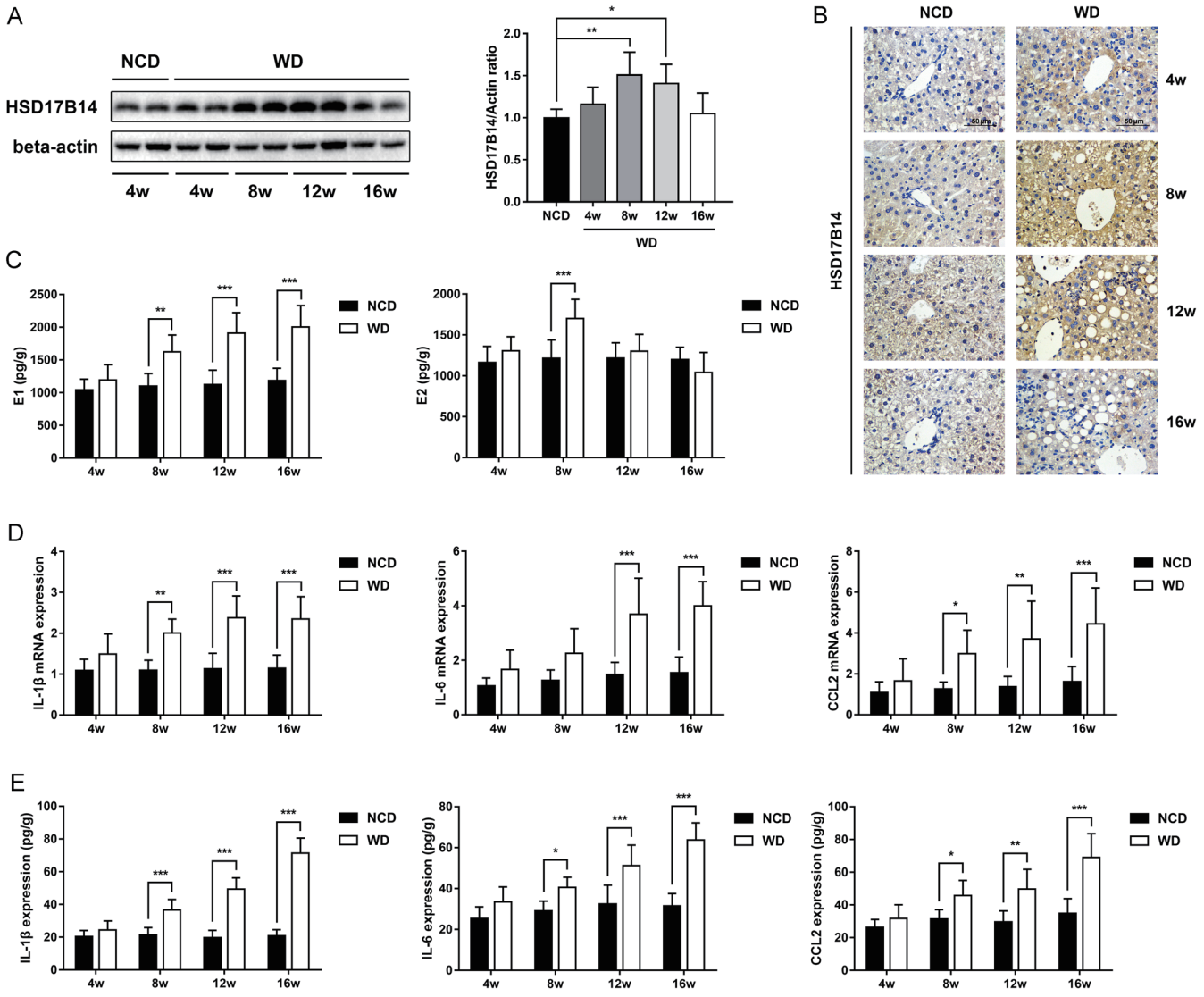


**Fig. 2. HSD17B14 effects on estrogen conversion in MASLD mice.** (A) Western blot analysis of HSD17B14 protein expression in liver tissues (n = 6); (B) Immunohistochemical staining of HSD17B14; (C) Hepatic concentrations of E1 and E2 were measured using ELISA (n = 6); (D) Relative mRNA expression levels of inflammatory cytokines IL-1β, IL-6, and CCL2 in liver tissues were quantified by qRT-PCR. (E) Protein levels of inflammatory cytokines IL-1β, IL-6, and CCL2 were measured using ELISA. Data are presented as means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. NCD, normal chow diet; WD, western diet; HSD17B14, 17β-hydroxysteroid dehydrogenase 14; E1, estrone; E2, 17β-estradiol; MASLD, metabolic dysfunction-associated steatotic liver disease.

group (Fig. 1C). Histopathological staining further unveiled that liver tissues from the NCD group maintained a well-preserved hepatic lobular architecture, with regularly arranged hepatocytes and minimal intracellular lipid droplets. In contrast, liver tissues from the WD group represented obvious lipid droplet accumulation, fat vacuole formation, ballooning degeneration, and inflammatory cell infiltration, which began to appear at the fourth week and became progressively more evident as the modeling period advanced (Fig. 1D).

Notably, western blot analysis demonstrated a biphasic trend in HSD17B14 protein expression in the liver tissues of MASLD mice fed with WD. Specifically, a slight but statistically insignificant increase was observed at the fourth week, followed by a significant elevation by the eighth week. This elevated expression remained stable at the twelfth week, only to markedly decline by the sixteenth week (Fig. 2A). Consistently, immunohistochemical staining further showcased increased HSD17B14 expression in the liver tissues of the WD group at both the eighth and twelfth weeks, with a significant reduction at the sixteenth week (Fig. 2B).

Hepatic E1 and E2 levels were determined by ELISA assays. At the fourth week of modeling, no significant differences were found between the two groups. However, both E1 and E2 levels were significantly elevated in the WD group by the eighth week. From the twelfth week onwards, while E1 levels continued to rise, E2 levels markedly decreased, which is likely due to the increased expression of HSD17B14, facilitating the conversion of E2 to E1. This suggests that HSD17B14 indeed plays a crucial role in mediating estrogen conversion in the liver during MASLD progression (Fig. 2C). Building upon our previous findings, which demonstrated the notable anti-inflammatory effects of ursolic acid in the treatment of MASLD, and considering literature suggesting that HSD17B14-mediated estrogen conversion may accelerate

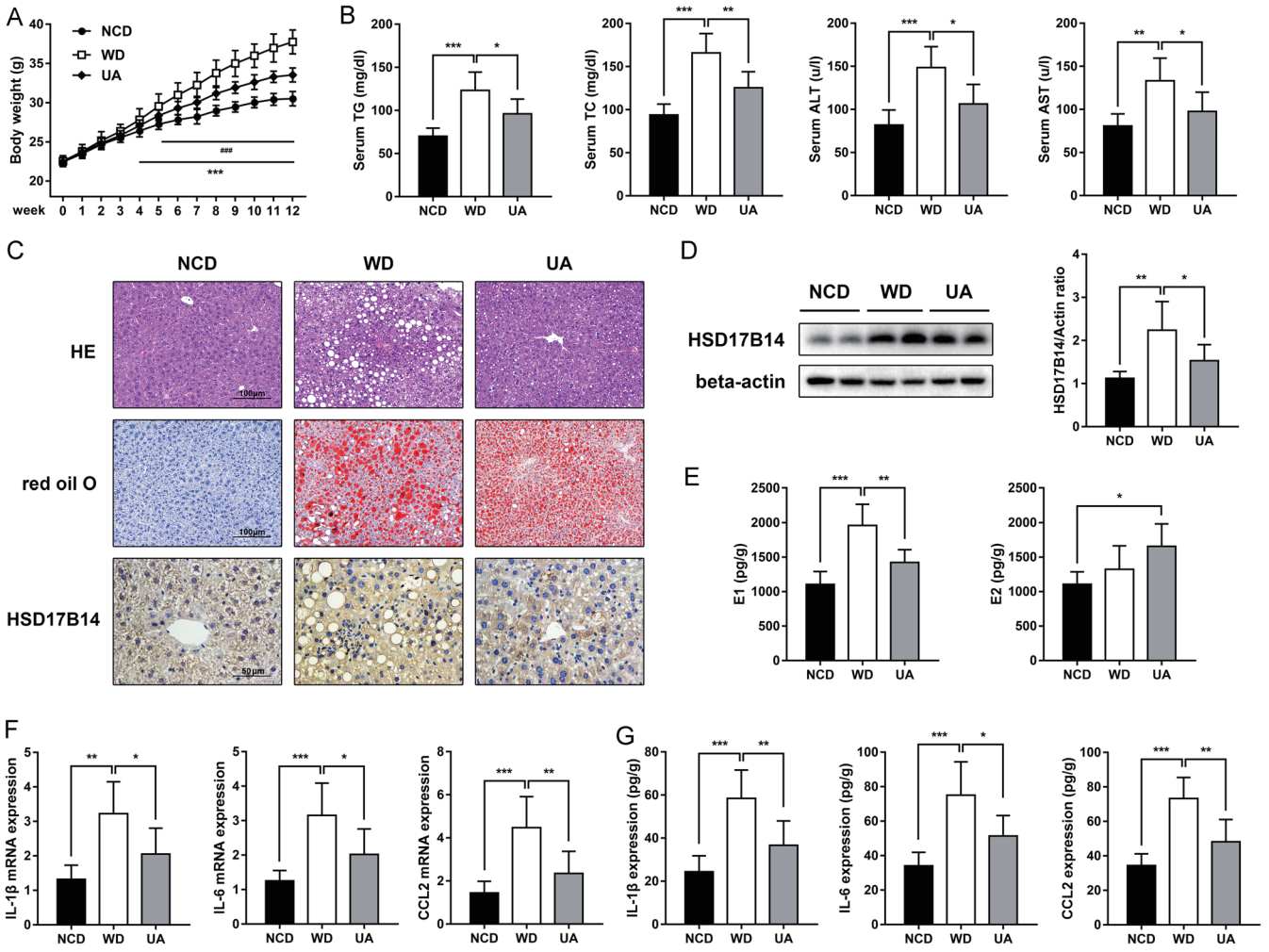


**Fig. 3. Therapeutic effects of ursolic acid on HSD17B14-mediated estrogen conversion in MASLD mice.** (A) Weekly body weight measurements (n = 8); (B) Serum biochemical analyses of TC, TG, ALT, and AST were measured at the end of the experiment (n = 6); (C) Histological staining of HE and oil red O and immunohistochemical staining of HSD17B14; (D) Western blot analysis of HSD17B14 protein expression in liver tissues (n = 6); (E) Hepatic concentrations of E1 and E2 measured by ELISA (n = 6); (F) Relative mRNA expression levels of inflammatory cytokines IL-1β, IL-6, and CCL2 in liver tissues were quantified by qRT-PCR; (G) Protein levels of inflammatory cytokines IL-1β, IL-6, and CCL2 were measured using ELISA. Data are presented as means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. NCD, normal chow diet; WD, western diet; TG, triglycerides; TC, total cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; E1, estrone; E2, 17β-estradiol; HSD17B14, 17β-hydroxysteroid dehydrogenase 14; UA, ursolic acid; MASLD, metabolic dysfunction-associated steatotic liver disease.

metabolic stress-induced tissue inflammation, we proceeded to examine the expression levels of key inflammatory cytokines. In alignment with previous findings, the pro-inflammatory cytokines IL-1β, IL-6, and CCL2 were distinctly upregulated in the WD group (Fig. 2D, E).

### *Ursolic acid modulates HSD17B14-mediated estrogen conversion in MASLD mice*

Based on the aforementioned experimental results, a twelve-week modeling and intervention period was employed, considering both the effectiveness of the MASLD model and the temporal expression peaks of HSD17B14 and E2, to investigate the pharmacological mechanisms underlying the therapeutic effects of ursolic acid in the treatment of MASLD. Drawing from prior studies, a dose of 100 mg/kg of ursolic acid was selected for administration. Throughout the modeling period, body weight was monitored, and the results revealed that ursolic acid effectively attenuated weight gain in MASLD mice, with statistically significant differences emerging by the fifth week (Fig. 3A). At the conclusion of the twelve-week experiment, serum biochemical analyses demonstrated that the intervention with ursolic acid also led to reductions in serum lipid levels and improvements in liver function (Fig. 3B). These findings were further corroborated by histopathological staining, which revealed that ursolic acid treatment alleviated hepatic lipid droplet accumulation, fat vacuole formation, ballooning degeneration, and inflammatory cell infiltration (Fig. 3C).

Furthermore, western blot analysis represented a significant reduction in the protein expression of HSD17B14 in the liver tissue of MASLD mice following ursolic acid treatment, with these results being validated by immunohistochemical staining (Fig. 3C, D). Notably, the reduced HSD17B14 expression in the ursolic acid-treated group was accompanied by an increase in E1 levels and a decrease in E2 levels, indicating that ursolic acid may modulate HSD17B14 to restore the balance between E2 and E1 (Fig. 3E). Concurrently, the detection of pro-inflammatory cytokines IL-1β, IL-6, and

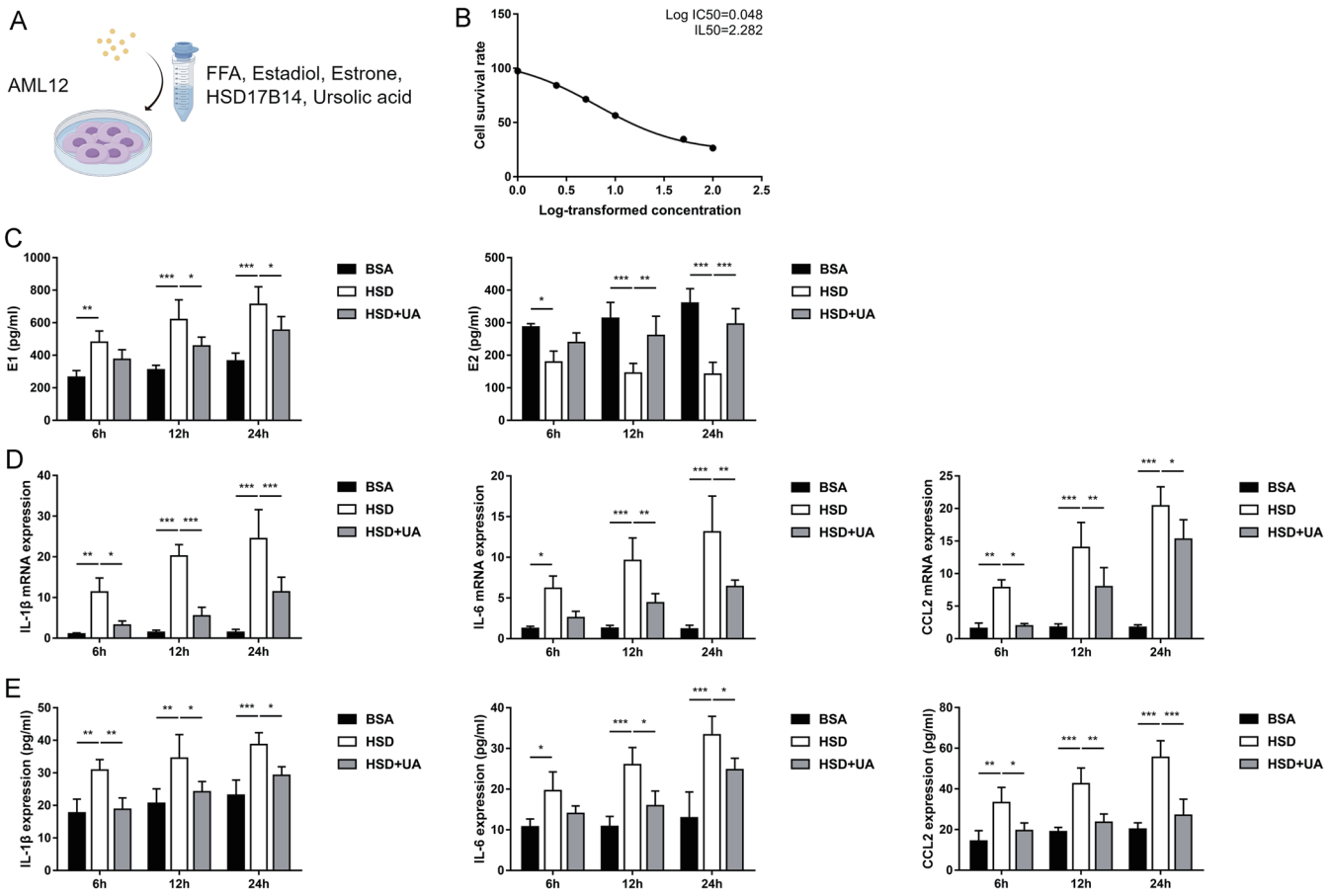


**Fig. 4. Therapeutic effects of ursolic acid on HSD17B14-mediated estrogen conversion in AML12 cells.** (A) Schematic representation of the experimental induction protocol; the schematic diagram was created by Figdraw; (B) CCK8 assay was used to ascertain the optimal dosage of recombinant HSD17B14 protein; (C) Hepatocytes were incubated with HSD17B14 and ursolic acid, with BSA as a control; protein levels of E1 and E2 in the culture supernatant were measured by ELISA (n = 3, technical replicates); (D) Relative mRNA expression levels of inflammatory cytokines IL-1β, IL-6, and CCL2 in hepatocytes were performed by qRT-PCR (n = 3, technical replicates); (E) Protein levels of inflammatory cytokines IL-1β, IL-6, and CCL2 in culture supernatant were measured using ELISA (n = 3, technical replicates). Data are presented as means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. E1, estrone; E2, 17β-estradiol; HSD, 17β-hydroxysteroid dehydrogenase 14; UA, ursolic acid; BSA, bovine serum albumin.

CCL2 in liver tissues affirmed the anti-inflammatory efficacy of ursolic acid intervention (Fig. 3F, G).

### *Ursolic acid exerts anti-inflammatory effects by modulating HSD17B14-mediated estrogen conversion in AML12 cells*

To further elucidate the role of HSD17B14-mediated estrogen conversion in hepatocellular inflammatory responses, AML12 cells were stimulated with a combination of palmitic acid (250 μM) and oleic acid (500 μM) to mimic free fatty acid (FFA) exposure.[22] Interventions with E1 (10 nM), E2 (10 nM), recombinant HSD17B14 protein (2 ng/mL), and ursolic acid (5 μM) were applied to assess the effects of various treatments and durations on cellular inflammatory responses (Fig. 4A, B).[20] The results demonstrated that HSD17B14 intervention led to an increase in E1 levels, accompanied by a concomitant reduction in E2 levels, with the conversion process being effectively suppressed in the presence of ursolic acid (Fig. 4C). Furthermore, HSD17B14 intervention significantly elevated the expression of pro-inflammatory cytokines IL-1β, IL-6, and CCL2, whereas ursolic acid attenuated this response, likely by modulating HSD17B14-dependent E2 to E1 conversion to mitigate inflammation (Fig. 4D, E).

In addition, FFA stimulation significantly upregulated the gene and protein expression of pro-inflammatory cytokines IL-1β, IL-6, and CCL2. Although E2 intervention exhibited a partial protective effect, this effect was antagonized by HSD17B14, further confirming that HSD17B14 facilitates the estrogen conversion of E2 to E1, thereby diminishing the anti-inflammatory effect of E2 (Fig. 5A, B). Given that the effects of HSD17B14 intervention were most pronounced at 24 h, particularly manifesting as a complete antagonism of the therapeutic effects of E2, a 24-h intervention period was implemented in subsequent experiments. Finally, upon simultaneous administration of FFA, E2, and HSD17B14, ursolic acid effectively reduced the gene and protein expression levels of IL-1β, IL-6, and CCL2 in AML12 cells, indicating its potential to ameliorate the inflammatory response in steatotic hepatocytes (Fig. 5C, D). Notably, this effect was particularly significant in the context of HSD17B14 regulation, further suggesting that HSD17B14 serves as a critical target in the therapeutic mechanism.

## Discussion

The pathophysiological mechanisms underlying MASLD are

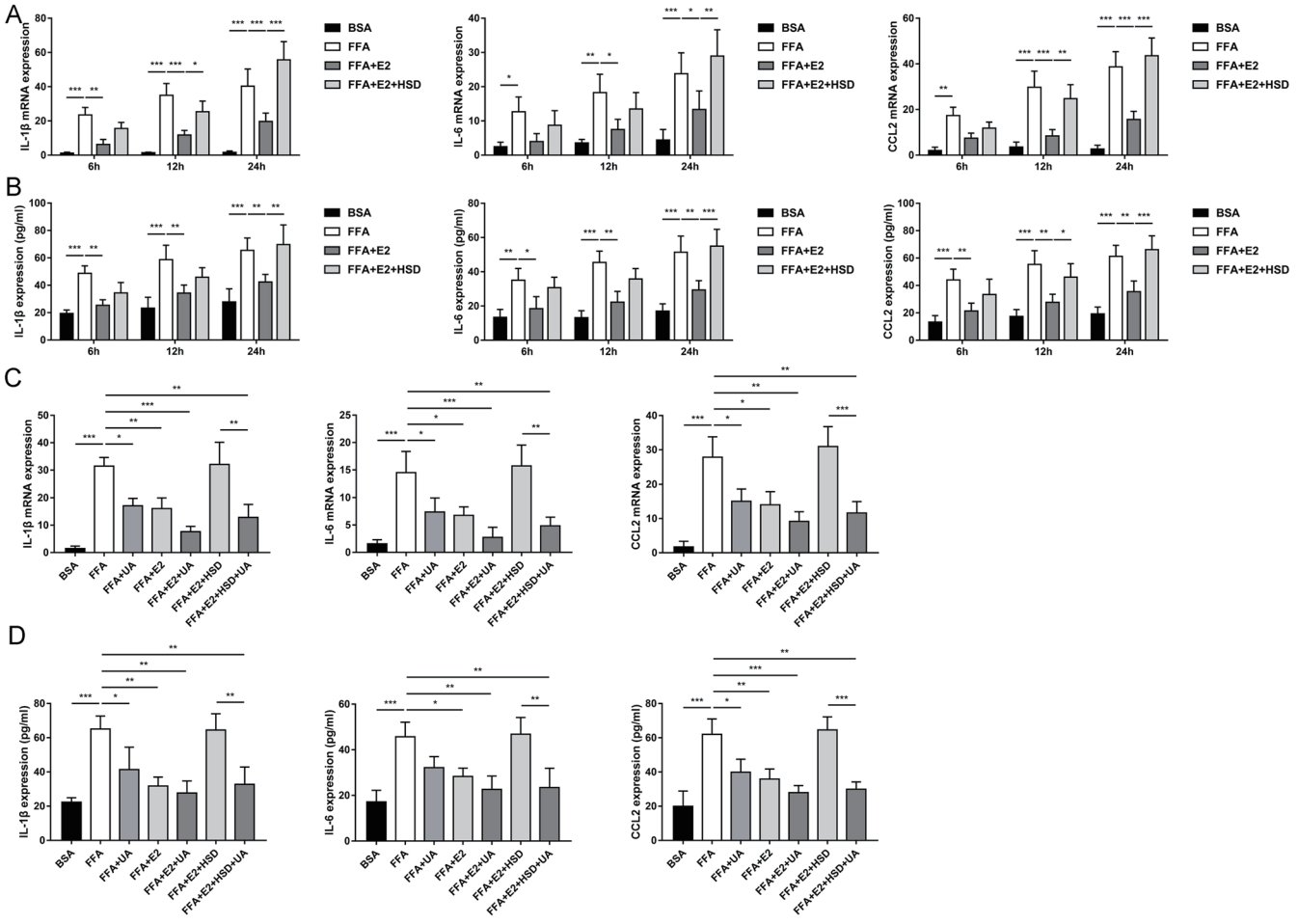


**Fig. 5. Ursolic acid effects on HSD17B14-mediated inflammation in AML12 cells.** (A) Hepatocytes were incubated with FFA, E2, and HSD17B14, with BSA as a control; relative mRNA expression levels of inflammatory cytokines IL-1β, IL-6, and CCL2 in AML12 cells were performed by qRT-PCR (n = 3, technical replicates); (B) Protein levels of IL-1β, IL-6, and CCL2 in culture supernatant were measured using ELISA (n = 3, technical replicates); (C) Hepatocytes were incubated with FFA, E2, HSD17B14, and ursolic acid for 24 h, with BSA as a control; relative mRNA expression levels of inflammatory cytokines IL-1β, IL-6, and CCL2 in AML12 cells were performed by qRT-PCR (n = 3, technical replicates); (D) Protein levels of IL-1β, IL-6, and CCL2 in culture supernatant were measured using ELISA (n = 3, technical replicates). Data are presented as means ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. E1, estrone; E2, 17β-estradiol; HSD, 17β-hydroxysteroid dehydrogenase 14; UA, ursolic acid; BSA, bovine serum albumin; FFA, free fatty acid.

intricate and remain incompletely elucidated. Extensive research has highlighted the pivotal role of estrogens in lipid metabolism and hepatic inflammatory responses.[23,24] Specifically, E2, a potent estrogen, activates estrogen receptors to regulate numerous genes involved in lipid metabolism, promoting lipolysis while inhibiting lipogenesis, thus conferring a protective effect on lipid metabolism.[25] The reduction in E2 levels has been associated with dysregulated lipid metabolism, contributing to hepatic lipid accumulation and disease progression.[26] In contrast, E1, exhibiting lower biological activity compared to E2, tends to increase during MASLD progression. This shift may reflect impaired hepatic metabolic function and disrupted steroid hormone metabolism, resulting in a concurrent decrease in E2 and elevation in E1 levels.[27] Recent findings by Joyce *et al*. have pointed out that E1 and E2 can bidirectionally monitor metabolic inflammation, with E1 promoting pro-inflammatory responses, whereas E2 exerts anti-inflammatory effects.[20] Hence, the balance between E2 and E1 may serve as a critical determinant in modulating the tissue microenvironment during MASLD progression.

HSD17B14, a member of the 17β-hydroxysteroid dehydrogenase family, is widely expressed in tissues such as the liver, kidneys, brain, and mammary glands.[28] This enzyme primarily participates in redox reactions involving steroid hormones, lipid metabolism intermediates, fatty acids, and other bioactive molecules.[19] In this experimental study, particular focus was placed on the catalytic role of HSD17B14 in mediating the conversion of E2 to E1. Given the liver's central function in energy metabolism and its critical involvement in steroid hormone, lipid, and carbohydrate metabolism, the hepatic expression and metabolic activity of HSD17B14 are of paramount importance and may influence the overall metabolic state.[29] However, current research on HSD17B14 in this context remains limited, and its expression patterns and mechanistic involvement in MASLD progression have yet to be fully characterized.

Our experimental findings indicate that HSD17B14 expression increases during the early stages of MASLD, facilitating the conversion of E2 to E1, thereby exacerbating metabolic stress-induced lipid dysregulation and inflammatory responses. However, as the disease progresses to more

advanced stages characterized by pronounced inflammation and potential fibrosis, HSD17B14 expression declines, possibly as a consequence of the progressive decrease in hepatocellular function. Additionally, given that HSD17B14 is implicated in the metabolism of not only estrogens but also other steroid hormones and lipids, its dynamic expression changes may also reflect hepatic compensatory mechanisms regulating hormonal and lipid metabolic imbalances.[30,31] Accordingly, considering the fluctuating expression patterns of HSD17B14 and its critical role in MASLD progression, we propose that alterations in HSD17B14 expression could serve as a potential biomarker. Furthermore, elucidating the specific mechanisms may position HSD17B14 as a novel therapeutic target.

Ursolic acid, a naturally occurring bioactive compound, has attracted considerable interest in biomedical research due to its broad-spectrum pharmacological properties.[32] It has been demonstrated to exert anti-inflammatory, antioxidant, and antifibrotic properties, contributing to the prevention and treatment of MASLD.[15,33] Notably, liver fibrosis is a strong independent predictor of mortality in MASLD, and ursolic acid may hold considerable therapeutic potential in preventing or even reversing fibrosis progression. Its mechanisms of action are believed to involve suppression of inflammation, attenuation of collagen deposition, modulation of the TGF-β/Smad signaling pathway, and potential interactions with estrogen signaling. Our previous studies have shown that ursolic acid can modulate decorin to provide dual protection against metabolic dysfunction and hepatic hypoxia in MASLD, as well as target secreted phosphoprotein 1 to regulate Th17 cell differentiation.[17,18] However, its role in hormone metabolism regulation remains largely unexplored. In this study, we identified that ursolic acid can modulate HSD17B14-mediated conversion of E2 to E1, unveiling a previously unrecognized mechanism through which ursolic acid exerts its multifaceted protective effects.

The limitations of this study are primarily related to the lack of an in-depth exploration of the precise downstream molecular mechanisms by which ursolic acid targets HSD17B14 to regulate estrogen conversion. This constraint arises largely from the exploratory nature of this study; however, gene knockdown and overexpression models have been incorporated into our follow-up research to address this gap. Another notable limitation is that all experiments were conducted exclusively on male animals. This decision was made based on the fact that estrogen levels in female mice fluctuate with the estrous cycle, potentially introducing variability into experimental parameters and leading to greater individual differences. The use of male mice helps mitigate variability caused by hormonal fluctuations, thereby yielding clearer and more reproducible results. Nevertheless, this approach may restrict the generalizability of the findings to female subjects. Therefore, future studies should aim to validate these results in female models to provide a more comprehensive understanding of sex-specific differences in MASLD progression and response to ursolic acid. Moreover, considering the critical role of hormone administration timing in clinical practice, as well as the circadian rhythm in hormone metabolism, further investigations are warranted to evaluate the efficacy of ursolic acid administration at different times and dosing frequencies. Such studies would provide deeper insights into its potential pharmacological mechanisms in hormone metabolism regulation and contribute to optimizing therapeutic strategies for MASLD intervention.

## Conclusions

Taken together, our findings, in conjunction with previous research, illustrate that ursolic acid exerts its regulatory effects by targeting HSD17B14 to modulate its protein activity. Collectively, these results support the conclusion that HSD17B14-mediated estrogen conversion plays a pivotal role in MASLD progression, serving as a central mechanism underlying the anti-inflammatory efficacy of ursolic acid in ameliorating the disease.

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## Conflict of interest

The authors have no conflict of interest related to this publication.

## Author contributions

Study concept and design (YZ, YL), performance of experiments (SG, HZ), analysis and interpretation of data (ZX, CC, JW), manuscript writing (SG, DF, YZ), and critical funding (HZ, ZX, YZ, YL). All authors have read and agreed to the published version of the manuscript.

## Ethical statement

All animals were handled humanely, and the experiments were performed under a project license (2023023) approved by the Institutional Animal Ethics Committee of Shanghai Hospital of Traditional Chinese Medicine, in compliance with the ARRIVE guidelines. All animals received human care.

## Data sharing statement

All the relevant data are provided within the paper, and data from the current study are available from the corresponding author.

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