



Research Article

***Cornus officinalis* vinegar alters the gut microbiota, regulating lipid droplet changes in nonalcoholic fatty liver disease model mice**

Li Cao^{1,2}, Ying Wu^{1,2,3}, Kai-Yu Liu¹, Ning-Xian Qi¹, Jie Zhang^{1,2}, Shan-Shan Tie^{1,3}, Xuan Li^{1,3}, Ping-Ping Tian^{1,2}, Shao-Bin Gu^{1,2,3} 

¹ College of Food and Bioengineering, Henan University of Science and Technology, Luoyang 471023, China

² Henan Engineering Research Center of Food Microbiology, Luoyang 471023, China

³ National Demonstration Center for Experimental Food Processing and Safety Education, Luoyang 471023, China

Received: 8 April 2024 / Revised: 22 April 2024 / Accepted: 24 April 2024

Abstract: This study investigated the effect of *Cornus officinalis* vinegar on gut microbiota and hepatic lipid droplet in nonalcoholic fatty liver disease (NAFLD) model mice. The C57BL/6J male mice were divided into three treatment groups: Control group (CON group) fed with a purified diet; high fat diet (HFD) group and *C. officinalis* vinegar treatment (CVT) group fed with high fat diet. After 10 weeks, the CVT group was orally administered with *C. officinalis* vinegar once a day for 6 weeks. We found that *C. officinalis* vinegar improved gut microbiota, significantly reduced body weight and liver injury of HFD-fed mice. Observation of histopathology and ultrastructure showed that *C. officinalis* vinegar improved histological changes, reduced size and number of lipid droplets. Additionally, *C. officinalis* vinegar significantly inhibited the expression of perilipin 2 (PLIN-2) and increased the expression of adipose triglyceride lipase (ATGL). In conclusion, *C. officinalis* vinegar could exert beneficial effects on NAFLD through modulation of gut microbiota and lipid droplets.

Keywords: nonalcoholic fatty liver disease; lipid droplets; gut microbiota; *C. officinalis* vinegar

Citation: Cao L., Wu Y., Liu K. Y., et al. *Cornus officinalis* vinegar alters the gut microbiota, regulating lipid droplet changes in nonalcoholic fatty liver disease model mice. *Food & Medicine Homology*, 2024, 1: 9420002. <https://doi.org/10.26599/FMH.2024.9420002>

1 Introduction

Nonalcoholic fatty liver disease (NAFLD) is a metabolic liver disorder disease^[1], and has become a major public health problem worldwide^[2]. Risk factors are associated with NAFLD include increased age, male sex, ethnicities, unhealthy dietary patterns, lack of certain nutrients, genetic factors and metabolic diseases such as obesity, diabetes and insulin resistance^[3]. Recently, the most studies have indicated that gut microbiota could modify the pathology of NAFLD^[4,5] and influence NAFLD progression^[6,7], as well as coordinate host metabolism^[8]. Dysbiosis is defined as any change in the composition of the microbiota and is commonly reflected by a reduction of the species number. Some studies have revealed the association between gut microbiota dysbiosis and obesity, diabetes, NAFLD and metabolic disease^[9-12]. Therefore, it is critical to maintain the balance and homeostasis of gut microbiota in the treatment of metabolic diseases, such as NAFLD and diabetes.

A key feature of NAFLD is excessive accumulation of lipids in liver^[13]. In cells, lipids are stored in organelles called lipid droplets (LDs). LDs are surrounded by a phospholipid monolayer and filled with triacylglycerols and sterol esters in their core^[14,15]. LDs are present in almost all cell types and play an important role in balancing intracellular lipid homeostasis, even the whole body^[16].

In hepatocytes, fusion, growth and shrinking of LDs play a regulatory role in lipid and energy homeostasis^[17,18]. Abnormal lipid accumulation in LDs in the liver caused hepatic steatosis, a pathological feature of NAFLD^[18], implicating that modulate the formation and breakdown of LDs will help understand the progression of NAFLD and guide intervention strategies for the treatment of NAFLD. In fact, the pathological mechanisms of NAFLD are still incompletely understood. Despite the increase in the prevalence of NAFLD and the morbidity associated with NAFLD, there are still not satisfactory treatment drugs for NAFLD nowadays.

Cornus officinalis (Cornus, *C. officinalis*) a deciduous tree distributed in Eastern Asia. The fruits of *C. officinalis* are often used as a medicinal plant to treat liver and kidney disease^[19]. The fruit of *C. officinalis* and its extracts can prevent hepatic injuries^[20]. In recent years, the yield of *C. officinalis* fruit is on the increase in China, but the fruits cannot be preserved for a long time. Based on health function of *C. officinalis*, it have been developed into various dietary supplements such as *C. officinalis* wine, oral solution and other functional beverage. *C. officinalis* vinegar had been made in our team, and we found that *C. officinalis* vinegar contained some important bioactive substances like morroniside, loganin, and numerous organic acids and preliminary study have also showed that *C. officinalis* vinegar attenuates the development

of hepatic steatosis in mouse model of nonalcoholic fatty liver^[21]. However, it is unclear whether *C. officinalis* vinegar regulates hepatic lipid metabolism by influencing gut microbiota and changes of hepatic LDs. Therefore, the effects of *C. officinalis* vinegar on the changes of gut microbiota and hepatic LDs were investigated in a high fat diet (HFD)-induced mouse model of NAFLD. The findings of this study might contribute to elucidate the mechanism of action of *C. officinalis* vinegar and provide well-established evidence in hepatoprotective effect of *C. officinalis* vinegar.

2 Materials and methods

2.1 Preparation of *C. officinalis* vinegar

C. officinalis vinegar was obtained from our laboratory, and the acidity was 5.12%. Prior to experiments, the *C. officinalis* vinegar samples were stored in the laboratory at a constant temperature of around 25 °C.

2.2 Animal experiments

All animal experiments were conducted according to the Guide for the Care and Use of Laboratory Animals: Eighth Edition, ISBN-10: 0-309-15396-4, and they were approved by the Institutional Animal Care and Use Committee of Henan University of Science and Technology (SYXK(Yu)2021-0020). Male C57BL/6J (6 weeks old) mice were selected from the Zhengzhou University Laboratory Animal Center (Zhengzhou, China). They were acclimated to the environment for 1 week. Mice were then randomly divided into three groups ($n=12/\text{group}$) as follows: Control group (CON group) fed with standard chow diet (LAD3001M, purchased from Trophic Animal Feed High-Tech Co., Ltd. Nantong, China); HFD group and *C. officinalis* vinegar treatment group (CVT group) fed with high fat diet (60% kcal from fat, TP23400, purchased from Trophic Animal Feed High-Tech Co., Ltd.). Mice were given ad libitum access to food and water. After 10 weeks, mice in CVT group were gavaged the *C. officinalis* vinegar (2 mL/kg body weight) once a day, accordingly, mice in CON group and HFD group were gavaged with the same volume of saline, for 6 weeks. The body weight was measured weekly. At the end of the experiment, the mice were anaesthetized with pentobarbital sodium after 12 h overnight fasting, blood from hearts was collected in clean Ethylene Diamine Tetraacetic Acid tubes, serum samples were obtained from the collected blood samples through 10 min of centrifugation ($3,500 \times g$, 4 °C). And then, liver tissues were removed, weighed, processed for subsequent analyses. The time schedule of procedure in this study is shown in Fig. 1.

2.3 Analysis of gut microbiota

At the end of the experiment, Mouse feces were collected and

the microbial DNA was extracted using QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration and purity of DNA were determined with a NanoDrop One Microvolume UV-Vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and the bacterial genomic DNA integrity of was detected by agarose gel electrophoresis. the bacterial 16S rRNA V3–V4 region was amplified using the following barcoded primers: 338F 5' -ACTCCTACGGGAGGCAGCAG-3' and 806R 5' -GGACTACHVGGGTWTCTAAT-3'. Then, the PCR amplification products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA). The DNA library was constructed, the purified amplification products were sequenced using the Illumina MiSeq system and Data analysis was performed on the network cloud platform at Majorbio BioPharm Technology Co., Ltd. (Shanghai, China). The information of operational taxonomic units (OTUs) in each group was analyzed and classified according to 97% similarity. Then chao index and shannon index were used to evaluate alpha diversity of gut microbiota at phylum level.

2.4 Evaluation of AST, ALT and ALP Activities in serum and triglyceride concentration in liver

Activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in serum were detected using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). The liver tissue was homogenized, and the total lipids were extracted as previously described^[22]. Then, the triglyceride (TG) concentration in livers was measured by commercial kits (Nanjing Jiancheng Bioengineering Institute).

2.5 Histopathology observation and analysis

Liver were removed and were fixed in 10% neutral formalin solution. After 48 h, fixed liver tissues were dehydrated and then embedded in paraffin. Serial sections were cut at 5 µm, deparaffinized in xylene, rehydrated, and stained with hematoxylin and eosin. Sections were observed and scored by an experienced pathologist without prior knowledge of the treatment groups using light microscopy^[23], and the Leica Application Suite 3.0 software was employed for morphometric analysis.

2.6 Ultrastructural observation and analysis in livers

For ultrastructural observation, liver samples fixed in 2.5% glutaraldehyde were postfixed in 1% osmium tetroxide (in pH 7.4 of phosphate buffer for 1.5 h at 4 °C). After dehydration in a graded ethanol series, samples were embedded in epoxy resin (Epon 812, Hede BioInc, Beijing, China). Ultrathin sections were cut and were stained with uranyl acetate and lead citrate (Hede), and images were captured and examined in a *Transmission*

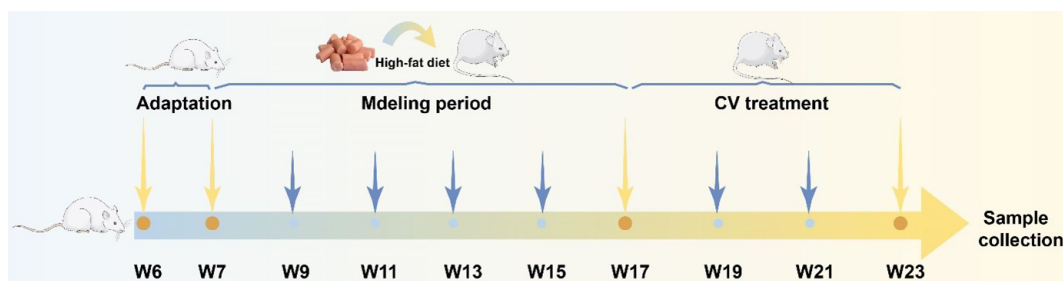


Figure 1 The schedule of procedure of the present study.

electron microscope (TEM) (JEM-1400, JEOL Ltd, Tokyo, Japan), the ImageJ software was used for analysis of number and diameters of LDs.

2.7 Real-time PCR

Total RNA was extracted from the liver tissue using TRIzol isolation reagent (TaKaRa BioInc, Otsu City, Japan) according to the manufacturer's protocol. The mRNA was then reverse transcribed into cDNA using an oligo-dT primer and PrimeScript RT enzyme (TaKaRa). The cDNA was used as a template for real-time PCR analysis. Real-time PCR was performed for determine of LD-associated genes using SYBR Premix ExTaq II (TaKaRa) in a Bio-Rad iQ5 detection system (Bio-Rad Laboratories, Hercules, CA, USA). Each real-time PCR was performed in triplicate and normalized with the housekeeping gene β -actin. Primer sequences are shown in Table 1. Relative gene expression data were determined by the $2^{-\Delta\Delta C_t}$ method^[24].

2.8 Statistical analysis

All data are expressed as the mean $\pm$ standard deviation (SD). The results were statistically analyzed by one-way ANOVA, followed by Tukey's multiple comparison test. The criterion for significance was a $P < 0.05$. Analysis was performed using SPSS 16.0 software.

3 Results and discussion

3.1 Effect of *C. officinalis* vinegar on the microbiota composition in HFD-fed mice

The action of gut microecology recently attracted more people's attention. Dysbiosis of gut microbiota is associated with obesity, NAFLD and other metabolic diseases^[25]. Recent evidences have revealed the abnormality of the gut microbiota in patients with NAFLD and HFD-induced animal model^[26,27]. Modulation of gut microbiota have been proposed for treatment of NAFLD^[28]. Environmental factors including the type of diet, drugs presence can alter the composition of the gut microbiota^[29]. However,

whether *C. officinalis* vinegar caused changes of gut microbiota remains to be determined. In the studies, the effects of *C. officinalis* vinegar on the composition of fecal microbiota were assessed through a 16S rRNA (V4 region) sequence analysis. As shown in Fig. 2a, the composition of fecal microbiota of mice in different groups differed significantly. At the genus level, amounts of *Faecalibaculum*, *Bifidobacterium*, *Lactobacillus*, *Ruminococcus*, *Turicibacter* were significantly decreased in the HFD group compared with control group. Oppositely, amounts of *Bacteroides* and norank-*Lachnospiraceae* were significantly increased in the HFD group compared with control group. However, *C. officinalis* vinegar treatment group showed a significant rising in *Faecalibaculum*, *Bifidobacterium*, *Lactobacillus*, while a declining trend in *Bacteroides* norank-*Lachnospiraceae*. *Lactobacillus*' abundance was negatively correlated with liver fat and body weight^[30], some vinegar including pineapple vinegar and nipa vinegar can reduce body fat and bodyweight^[31]. Taken together, the positive effects of *C. officinalis* vinegar on NAFLD may be an independent action, or may be mediated by regulating gut microbiota. Notably, HFD lead almost to disappearing of *Ruminococcus* and *Turicibacter* genus, but *C. officinalis* vinegar could not recover the alteration. To date, it's not clear whether the occurrence of NAFLD was associated with decreased abundance of *Ruminococcus* and *Turicibacter*. As shown in Fig. 2b, the diversity (Chao and Shannon indices) of intestinal microbiota was lower in the HFD group than in the CON group, and the CVT group showed a significant increase in diversity compared with the HFD group, these results are similar to previous studies^[32,33]. Therefore, it's certain that the diversity and abundance of gut microflora were associated with progression of NAFLD.

3.2 Effect of *C. officinalis* vinegar on body weight, liver weight and hepatic TG

At the beginning of the experiment (W7), the body weight of mice in the different group is no differences ($P > 0.05$). After 10 weeks (W17), body weight of HFD feeding mice is higher than that of the standard chow diet feeding mice (Figs. 3a and 3b). After treatment with *C. officinalis* vinegar for 6 weeks (W23), mice

Table 1 Primer sequences for quantitative real-time PCR

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
PLIN-2	TTGCAGTTGCCAATACCTATGC	CCAGTCACAGTAGTCGTCACA
ATGL	TGCTACCCGTCTGCTCTTTC	GACCTGATGACCACCTTTTC
β -actin	AGAGGGAAATCGTGCGTGAC	CAATAGTGATGACCTGGCCGT

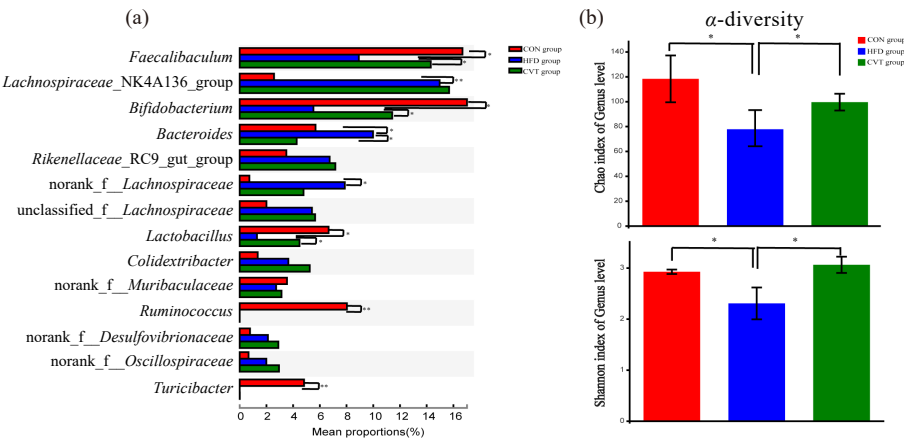


Figure 2 Changes in gut microbiota of mice. (a) Changes in gut microbiota of mice from each group at the genus level, (b) The alpha diversity of the gut microbiota. Data are expressed as mean $\pm$ SD. * means the difference is significant at the 0.05 level, ** means the difference is significant at the 0.01 level.

in the HFD group had higher body weight and liver weight than mice in the CON group. However, the body weight and liver weight of mice in the CVT groups was lower than that of mice in HFD group (Fig. 3c). As shown in Fig. 3d, hepatic TG significantly increased in the HFD group compared with the CON group. After *C. officinalis* vinegar treatment, hepatic TG exhibited a significant decrease compared with those in the HFD group. Previous studies have shown that obesity is strictly related to the development of NAFLD^[34], and body weight loss can be beneficial for NAFLD^[35]. These results implied that *C. officinalis* vinegar was effective in management of HFD-induced NAFLD by losing weight.

3.3 Effect of *C. officinalis* vinegar on liver injury

As shown in Fig. 4, compared with the CON group, the serum levels of AST, ALT and ALP in HFD group were significantly increased. However, the serum levels of AST, ALT and ALP were significantly decreased in the CVT group, suggesting that

C. officinalis vinegar treatment may have beneficial effects on the prevention and progression of NAFLD.

3.4 *C. officinalis* vinegar alleviated histopathological changes

The histopathological analysis revealed greater steatosis area ($P < 0.001$), and accumulation of greater levels lipid droplets and cell swelling in the livers of HFD group mice compared to the CON group (Fig. 5a). However, following treatment with the *C. officinalis* vinegar, the increase of lipid droplets was moderate and the number and the size of hepatocyte with fat vacuoles were decreased, and steatosis area were reduced from 51% of HFD group to 28% of the CVT group, respectively (Fig. 5b).

Together with results of the microbiota composition, body weight, hepatic fat content and liver injury, we thought that *C. officinalis* vinegar could inhibit the development of NAFLD by

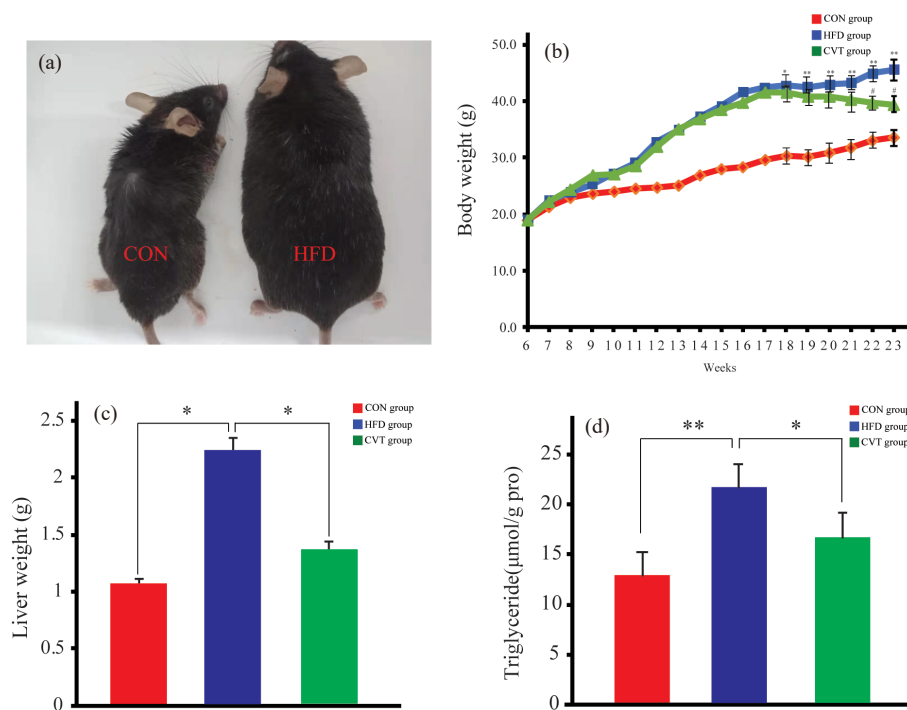


Figure 3 Effects of the COVs on body weight, liver weight and TG. (a) Physical appearance of mice fed standard chow diet and high-fat diet for 10 weeks, (b) Body weight changes of mice from 6 weeks old to 23 weeks old, (c) Liver weight in the different groups, (d) liver TG in the different groups. Data are expressed as mean $\pm$ SD. * means the difference is significant at the 0.05 level compared with CON group, ** means the difference is significant at the 0.01 level compared with CON group, # means the difference is significant at the 0.05 level compared with HFD group in figure 3B; * means the difference is significant at the 0.05 level, ** means the difference is significant at the 0.01 level in other figures.

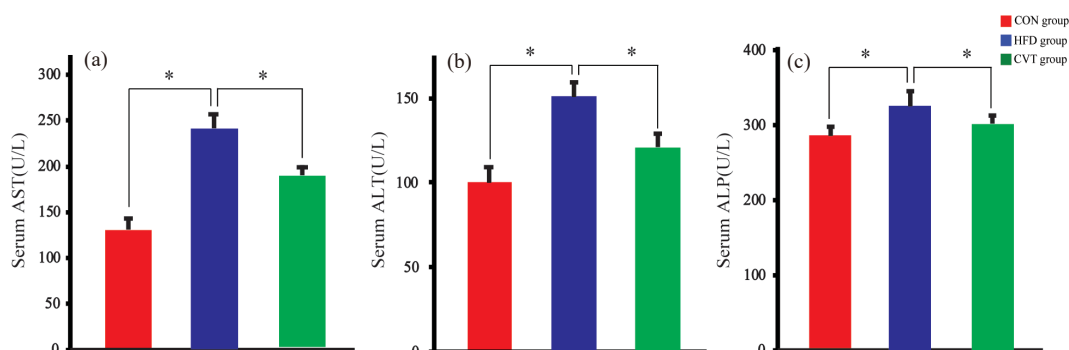


Figure 4 Effects of the COVs on liver injury. Serum levels of AST (a), ALT (b) and ALP (c). Data are expressed as mean $\pm$ SD. * means the difference is significant at the 0.05 level.

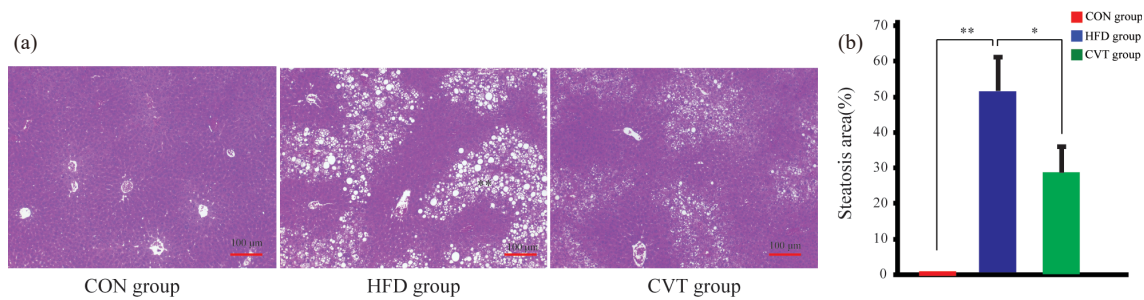


Figure 5 Effect of the COV on histopathological changes. (a) Hematoxylin and eosin (H&E) staining of liver samples of mice from the different groups, (b) Steatosis area of liver from the different groups. ** means the difference is significant at the 0.01 level.

altering the dominant species within the gut and our results also support the viewpoint that manipulation of gut microbiota can be an effective in the treatment of NAFLD^[36,37] and may be therapeutic target for NAFLD^[28,38].

3.5 *C. officinalis* vinegar altered lipid droplet in hepatocytes

Lipid droplets are lipid-rich organelles within the cells, and are associated with hepatic lipid metabolism. Excess lipids accumulate within hepatocytes can be a pathological condition of occurrence of NAFLD and progression to nonalcoholic steatohepatitis (NASH)^[39]. The quantity, sizes and dynamics of the LDs reflect different biological progresses, and are correlate with the development of metabolic diseases^[40]. *C. officinalis* vinegar may protect against the progression of NAFLD by inhibiting formation of LDs or facilitating the breakdown of LDs in the liver. In this study, we observed LDs in liver tissue samples using TEM. As illustrated in Fig. 6, under the ultrastructural observation,

hepatocytes in HFD group exhibited larger LDs ($P < 0.001$) and more LDs ($P < 0.01$) compared to the CON group. When treated with the *C. officinalis* vinegar, lipid droplet size and number of lipid droplets per cell were significantly reduced, suggesting that the *C. officinalis* vinegar can inhibit the accumulation of LDs in liver and hepatocytes, and facilitate lipid droplet degradation. However, further researches are needed to reveal the mechanisms of lipid droplet degradation in hepatocytes by the regulatory of the *C. officinalis* vinegar.

3.6 Effect of *C. officinalis* vinegar on the expression of LD-associated genes in liver

LD-related proteins including the perilipins (PLINs) and fat-specific storage protein 27 (FSP27), localize to the LD surface, and govern the formation and breakdown of LDs^[41,42]. Perilipin (PLIN)-2 is one member of the PLIN protein family which is the main structural proteins of LDs and regulates LD assembly and formation^[43]. PLIN-2-deficient reduced liver steatosis, meanwhile

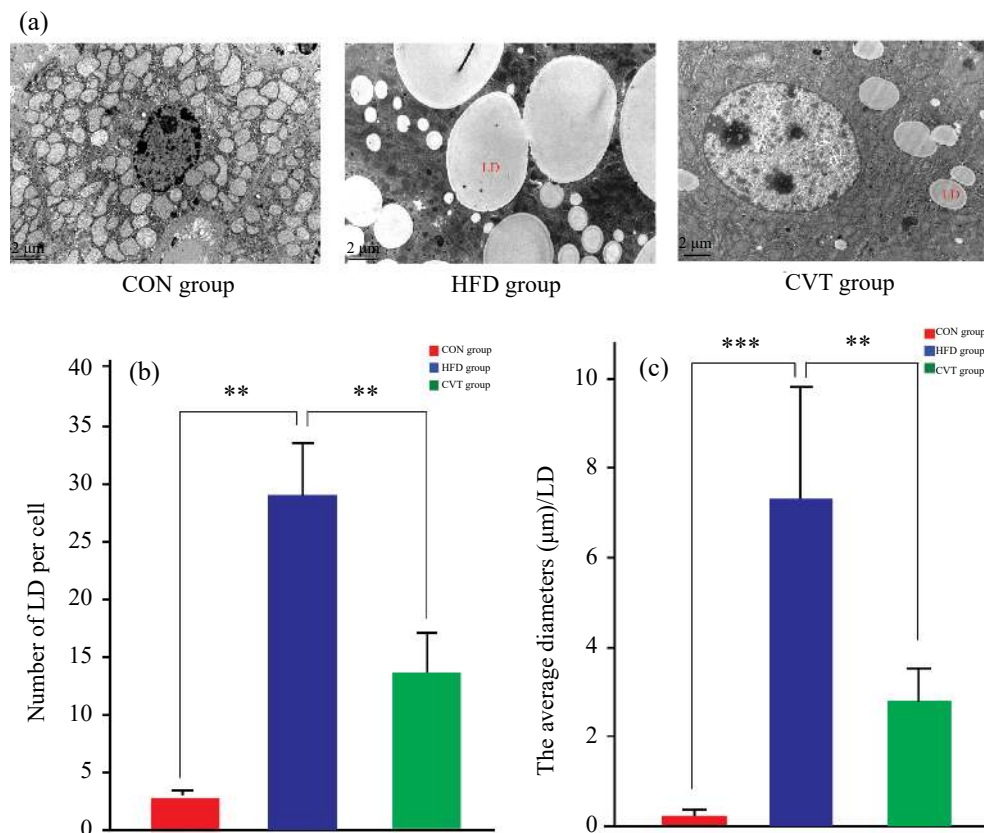


Figure 6 Effect of the COV on LD changes. (a) Transmission electron microscope of LDs from the different groups, (b) Number of LD per cell, (c) The average diameters of LD from the different groups. ** means the difference is significant at the 0.01 level. *** means $P < 0.001$ versus CON group.

increased lipolysis in mice^[44]. A previous study demonstrated that PLIN-2 deficiency decreased the lipid content and the size and number of LD in macrophages^[45]. These findings indicate that PLIN-2 is a key regulator in promoting larger LDs, steatosis, and the pathogenesis of NAFLD. Moreover, ATGL is a key enzyme in degradation of TG stored in LDs. ATGL first hydrolyzes TG into diacylglycerol (DG) and DG is sequentially acted by hormone-sensitive lipase (HSL) and monoacyl-glycerol lipase (MGL)^[18,46]. In the whole lipolytic process, the free fatty acids (FFAs) were released for mitochondrial energy production or re-esterizing into TG. Therefore, function of ATGL is important for maintaining balance of LD storage and metabolism^[47].

In this study, the *C. officinalis* vinegar on the expression of LD-associated genes including PLIN-2 and ATGL was evaluated. As shown in Fig. 7, HFD increased expression levels of PLIN-2 gene and decreased expression levels of ATGL gene in the liver tissue, compared to the CON group ($P < 0.05$). When treated with the *C. officinalis* vinegar, the expression of PLIN-2 was inhibited and the expression of ATGL was increased, compared to the HFD group ($P < 0.05$). these results indicated that *C. officinalis* vinegar could facilitate lipolysis pathway of LDs, led to larger LDs turned into small LDs. It may be more amenable for lipophagic internalization^[19].

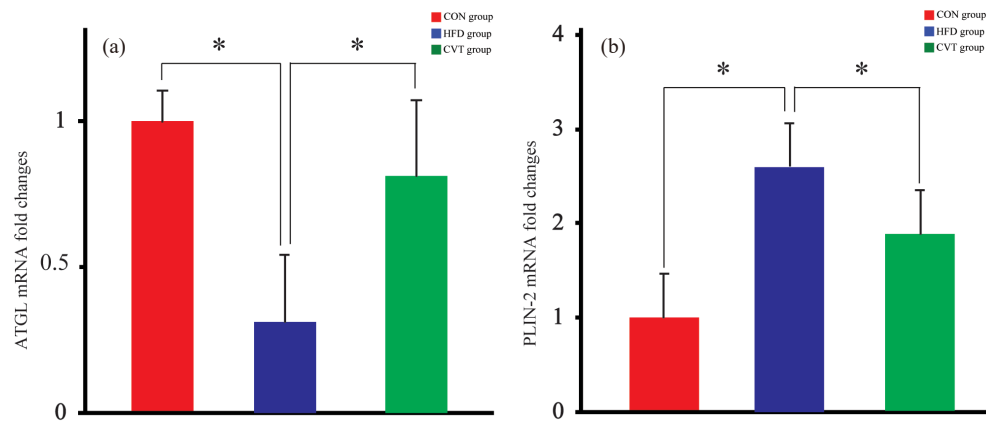


Figure 7 Effect of the COV on the expression of LD-associated genes. (a) ATGL gene expression, (b) PLIN-2 gene expression.

The disease spectrum of NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis, hepatic fibrosis, and final cirrhosis and liver failure. Although *C. officinalis* vinegar reversed hepatic steatosis in mice with NAFLD mice fed a HFD under the present study, it was unclear whether *C. officinalis* vinegar was effective for hepatic fibrosis or cirrhosis. Therefore, further studies are required. Subsequently, a human study is also required to confirm the data obtained in this mouse model of NAFLD.

4 Conclusion

In conclusion, this study demonstrated that *C. officinalis* vinegar exerted beneficial effects on hepatic steatosis in NAFLD model mice by reducing liver TG levels, liver weight and body weight, improving the serum levels of AST, ALT and ALP. *C. officinalis* vinegar also reduced hepatic LD sizes by modulating gut microbiota and decreasing the expression of PLIN-2 and ATGL, which provided new evidence to support that *C. officinalis* vinegar could be considered to be functional food.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

We are grateful for the financial support from Major Science and Technology Special Projects in Henan Province (No. 231100310200) and Key Science Research Projects of Higher Education of Henan Province (No. 20A550007).

Abbreviations

NAFLD, nonalcoholic fatty liver disease; CON, control; HFD,

high fat diet; CVT, *C. officinalis* vinegar treatment; PLIN-2, perilipin 2; ATGL, adipose triglyceride lipase; LDs, lipid droplets; OTUs, operational taxonomic units; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; TG, triglyceride; TEM, Transmission electron microscope; SD, standard deviation; NASH, nonalcoholic steatohepatitis; ESP27, fat-specific storage protein 27; DG, diacylglycerol; HSL, hormone-sensitive lipase; MGL, monoacyl-glycerol lipase; FFAs, free fatty acids.

References

- [1] Dhami-Shah, H., Vaidya, R., Udipi, S., et al. Picoside II attenuates fatty acid accumulation in HepG2 cells via modulation of fatty acid uptake and synthesis. *Clinical and Molecular Hepatology*, **2018**, 24: 77–87. <https://doi.org/10.3350/cmh.2017.0039>
- [2] Ni, M. Z., Zhang, B. B., Zhao, J. N., et al. Biological mechanisms and related natural modulators of liver X receptor in nonalcoholic fatty liver disease. *Biomedicine & Pharmacotherapy*, **2019**, 113: 108778. <https://doi.org/10.1016/j.biopha.2019.108778>
- [3] Seto, W. K., Yuen, M. F. Nonalcoholic fatty liver disease in Asia: Emerging perspectives. *Journal of Gastroenterology*, **2017**, 52: 164–174. <https://doi.org/10.1007/s00535-016-1264-3>
- [4] Caussy, C., Hsu, C., Lo, M. T., Liu, et al. Link between gut-microbiome derived metabolite and shared gene-effects with hepatic steatosis and fibrosis in NAFLD. *Hepatology*, **2018**, 68: 918–932. <https://doi.org/10.1002/hep.29892>
- [5] Miyazaki, T., Honda, A., Ikegami, T., et al. Human-specific dual regulations of FXR-activation for reduction of fatty liver using *in vitro* cell culture model. *Journal of Clinical Biochemistry and Nutrition*, **2019**, 64: 112–123. <https://doi.org/10.3164/jcbs.18-80>
- [6] Ballestri, S., Zona, S., Targher, G., et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *Journal of Gastroenterology and Hepatology*, **2016**, 31: 936–944. <https://doi.org/10.1111/jgh.13264>

- [7] Cani, P. D., Van Hul, M., Lefort, C., et al. Microbial regulation of organismal energy homeostasis. *Nature Metabolism*, **2019**, 1: 34–46. <https://doi.org/10.1038/s42255-018-0017-4>
- [8] Rinninella, E., Raoul, P., Cintoni, M., et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*, **2019**, 7: 14. <https://doi.org/10.3390/microorganisms7010014>
- [9] Turnbaugh, P. J., Backhed, F., Fulton, L., et al. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host & Microbe*, **2008**, 3: 213–223. <https://doi.org/10.1016/j.chom.2008.02.015>
- [10] Qin, J. J., Li, Y. R., Cai, Z. M., et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, **2012**, 490: 55–60. <https://doi.org/10.1038/nature11450>
- [11] Mouzaki, M., Comelli, E. M., Arendt, B. M., et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology*, **2013**, 58: 120–127. <https://doi.org/10.1002/hep.26319>
- [12] Knight, R., Callewaert, C., Marotz, C., et al. The microbiome and human biology. *Annual Review of Genomics and Human Genetics*, **2017**, 18: 65–86. <https://doi.org/10.1146/annurev-genom-083115-022438>
- [13] Cazzo, E., Pareja, J. C., Chaim, E. A. Nonalcoholic fatty liver disease and bariatric surgery: A comprehensive review. *Sao Paulo Medical Journal*, **2017**, 135: 277–295. <https://doi.org/10.1590/1516-3180.2016.0306311216>
- [14] Martins, A. S., Martins, I. C., Santos, N. C. Methods for lipid droplet biophysical characterization in *Flaviviridae* infections. *Frontiers in Microbiology*, **2018**, 9: 1951. <https://doi.org/10.3389/fmicb.2018.01951>
- [15] Jackson, C.L. Lipid droplet biogenesis. *Current Opinion in Cell Biology*, **2019**, 59: 88–96. <https://doi.org/10.1016/j.celb.2019.03.018>
- [16] Mokhtari, Z., Gibson, D. L., Hekmatdoost, A. Nonalcoholic fatty liver disease, the gut microbiome, and diet. *Advances in Nutrition*, **2017**, 8: 240–252. <https://doi.org/10.3945/an.116.013151>
- [17] Missaglia, S., Coleman, R. A., Mordente, A., et al. Neutral lipid storage diseases as cellular model to study lipid droplet function. *Cells*, **2019**, 8: 187. <https://doi.org/10.3390/cells8020187>
- [18] Schott, M. B., Weller, S. G., Schulze, R. J., et al. Lipid droplet size directs lipolysis and lipophagy catabolism in hepatocytes. *J Cell Biol*, **2019**, 218: 3320–3335. <https://doi.org/10.1083/jcb.201803153>
- [19] Ji, L. L., Wang, X., Li, J. J., et al. New iridoid derivatives from the fruits of *Cornus officinalis* and their neuroprotective activities. *Molecules*, **2019**, 24: 625. <https://doi.org/10.3390/molecules24030625>
- [20] Lee, N. H., Seo, C. S., Lee, H. Y., et al. Hepatoprotective and antioxidative activities of *Cornus officinalis* against acetaminophen-induced hepatotoxicity in mice. *Evid Based Complement Alternat Med*, **2012**, 2012: 804924. <https://doi.org/10.1155/2012/804924>
- [21] Cao, L., Wu, Y., Li, W. W., et al. *Cornus officinalis* vinegar reduces body weight and attenuates hepatic steatosis in mouse model of nonalcoholic fatty liver disease. *Journal of Food Science*, **2022**, 87: 3248–3259. <https://doi.org/10.1111/1750-3841.16178>
- [22] Head, B., Bionaz, M., Cherian, G. Flaxseed and carbohydrase enzyme supplementation alters hepatic n-3 polyunsaturated fatty acid molecular species and expression of genes associated with lipid metabolism in broiler chickens. *Veterinary Sciences*, **2019**, 6: 25. <https://doi.org/10.3390/vetsci6010025>
- [23] Sakamoto, M., Tsujikawa, H., Effendi, K., et al. Pathological findings of nonalcoholic steatohepatitis and nonalcoholic fatty liver disease. *Pathology International*, **2017**, 67: 1–7. <https://doi.org/10.1111/pin.12485>
- [24] Livak, K. J., Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. *Methods*, **2001**, 25: 402–408. <https://doi.org/10.1006/meth.2001.1262>
- [25] Wieland, A., Frank, D. N., Harnke, B., et al. Systematic review: Microbial dysbiosis and nonalcoholic fatty liver disease. *Alimentary Pharmacology & Therapeutics*, **2015**, 42: 1051–1063. <https://doi.org/10.1111/apt.13376>
- [26] Saltzman, E. T., Palacios, T., Thomsen, M., et al. Intestinal microbiome shifts, dysbiosis, inflammation, and non-alcoholic fatty liver disease. *Frontiers in Microbiology*, **2018**, 9: 61. <https://doi.org/10.3389/fmicb.2018.00061>
- [27] Mukai, R., Handa, O., Naito, Y., et al. High-fat diet causes constipation in mice via decreasing colonic mucus. *Digestive Diseases and Sciences*, **2020**, 65: 2246–2253. <https://doi.org/10.1007/s10620-019-05954-3>
- [28] Suk, K. T., Kim, D. J. Gut microbiota: Novel therapeutic target for nonalcoholic fatty liver disease. *Expert Review of Gastroenterology & Hepatology*, **2019**, 13: 193–204. <https://doi.org/10.1080/17474124.2019.1569513>
- [29] Donaldson, G. P., Lee, S. M., Mazmanian, S. K. Gut biogeography of the bacterial microbiota. *Nature Reviews Microbiology*, **2016**, 14: 20–32. <https://doi.org/10.1038/nrmicro3552>
- [30] Yuan, G. F., Tan, M. J., Chen, X. E. Punicic acid ameliorates obesity and liver steatosis by regulating gut microbiota composition in mice. *Food & Function*, **2021**, 12: 7897–7908. <https://doi.org/10.1039/d1fo01152a>
- [31] Mohamad, N. E., Yeap, S. K., Ky, H., et al. Pineapple vinegar regulates obesity-related genes and alters the gut microbiota in high-fat diet (HFD) C57BL/6 obese mice. *Evidence-Based Complementary and Alternative Medicine*, **2020**, 2020: 1257962. <https://doi.org/10.1155/2020/1257962>
- [32] Turnbaugh, P. J., Hamady, M., Yatsunenko, T., et al. A core gut microbiome in obese and lean twins. *Nature*, **2009**, 457: 480–484. <https://doi.org/10.1038/nature07540>
- [33] Dalby, M. J., Ross, A. W., Walker, A. W., et al. Dietary uncoupling of gut microbiota and energy harvesting from obesity and glucose tolerance in mice. *Cell Reports*, **2017**, 21: 1521–1533. <https://doi.org/10.1016/j.celrep.2017.10.056>
- [34] Papandreou, D., Karavetian, M., Karabouta, Z., et al. Obese children with metabolic syndrome have 3 times higher risk to have nonalcoholic fatty liver disease compared with those without metabolic syndrome. *Int J Endocrinol*, **2017**, 2017: 2671692. <https://doi.org/10.1155/2017/2671692>
- [35] Younossi, Z. M., Loomba, R., Rinella, M. E., et al. Current and future therapeutic regimens for nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology*, **2018**, 68: 361–371. <https://doi.org/10.1002/hep.29724>
- [36] Cassard, A. M., Ciocan, D. Microbiota, a key player in alcoholic liver disease. *Clinical and Molecular Hepatology*, **2018**, 24: 100–107. <https://doi.org/10.3350/cmh.2017.0067>
- [37] Bakhshimoghaddam, F., Alizadeh, M. Modulation of the gut microbiota represents a new management for non-alcoholic fatty liver disease. *Hepatobiliary Surgery and Nutrition*, **2020**, 9: 223–226. <https://doi.org/10.21037/hbsn.2019.10.01>
- [38] Li, Z. P., Yang, S. Q., Lin, H. Z., et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology*, **2003**, 37: 343–350. <https://doi.org/10.1053/jhep.2003.50048>
- [39] Seebacher, F., Zeigerer, A., Kory, N., et al. Hepatic lipid droplet homeostasis and fatty liver disease. *Seminars in Cell & Developmental Biology*, **2020**, 108: 72–81. <https://doi.org/10.1016/j.semcdb.2020.04.011>
- [40] Muoio, D. M. Revisiting the connection between intramyocellular lipids and insulin resistance: A long and winding road. *Diabetologia*, **2012**, 55: 2551–2554. <https://doi.org/10.1007/s00125-012-2597-y>
- [41] Zhang, L. Y., Ding, L., Shi, H. H., et al. Eicosapentaenoic acid-enriched phospholipids suppressed lipid accumulation by specific inhibition of lipid droplet-associated protein FSP27 in mice. *Journal of the Science of Food and Agriculture*, **2020**, 100: 2244–2251. <https://doi.org/10.1002/jsfa.10250>
- [42] Čopić, A., Antoine-Bally, S., Giménez-Andrés, M., et al. A giant amphipathic helix from a perilipin that is adapted for coating lipid droplets. *Nature Communications*, **2018**, 9: 1332. <https://doi.org/10.1038/s41467-018-03717-8>

- [43] Huang, X. C., Sun, J., Bian, C. C., et al. *Perilipin 1–3* in grass carp *Ctenopharyngodon idella*: molecular characterization, gene structure, tissue distribution, and mRNA expression in DHA-induced lipid droplet formation in adipocytes. *Fish Physiology and Biochemistry*, **2020**, 46: 2311–2322. <https://doi.org/10.1007/s10695-020-00857-x>
- [44] Xu, S. M., Zou, F., Diao, Z. Q., et al. Perilipin 2 and lipid droplets provide reciprocal stabilization. *Biophysics Reports*, **2019**, 5: 145–160. <https://doi.org/10.1007/s41048-019-0091-5>
- [45] Larigauderie, G., Cuaz-Pérolin, C., Younes, A. B., et al. Adipophilin increases triglyceride storage in human macrophages by stimulation of biosynthesis and inhibition of β -oxidation. *The FEBS Journal*, **2006**, 273: 3498–3510. <https://doi.org/10.1111/j.1742-4658.2006.05357.x>
- [46] Huang, Y. F., Chen, H., Yang, P., et al. Hepatic lipid droplet breakdown through lipolysis during hibernation in Chinese Soft-Shelled Turtle (*Pelodiscus sinensis*). *Aging*, **2019**, 11: 1990–2002. <https://doi.org/10.18632/aging.101887>
- [47] Cerk, I. K., Wechselberger, L., Oberer, M. Adipose triglyceride lipase regulation: An overview. *Current Protein & Peptide Science*, **2017**, 19: 221–233. <https://doi.org/10.2174/1389203718666170918160110>