



2020

Food preservative sorbic acid deregulates hepatic fatty acid metabolism

Follow this and additional works at: <https://www.jfda-online.com/journal>

 Part of the [Food Science Commons](#), [Medicinal Chemistry and Pharmaceutics Commons](#), [Pharmacology Commons](#), and the [Toxicology Commons](#)



This work is licensed under a [Creative Commons Attribution-Noncommercial-No Derivative Works 4.0 License](#).

Recommended Citation

Chen, Chia-Hui; Ho, Sin-Ni; Hu, Po-An; Kou, Yu Ru; and Lee, Tzong-Shyuan (2020) "Food preservative sorbic acid deregulates hepatic fatty acid metabolism," *Journal of Food and Drug Analysis*: Vol. 28 : Iss. 2 , Article 2.

Available at: <https://doi.org/10.38212/2224-6614.1055>

This Original Article is brought to you for free and open access by Journal of Food and Drug Analysis. It has been accepted for inclusion in Journal of Food and Drug Analysis by an authorized editor of Journal of Food and Drug Analysis.

Food preservative sorbic acid deregulates hepatic fatty acid metabolism

Chia-Hui Chen ^{a,1}, Sin-Ni Ho ^{b,1}, Po-An Hu ^{a,1}, Yu Ru Kou ^b, Tzong-Shyuan Lee ^{a,*}

^a Graduate Institute and Department of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan

^b Department of Physiology, School of Medicine, National Yang-Ming University, Taipei, Taiwan

Abstract

Sorbic acid (SA) is one of the most commonly used food preservatives worldwide. Despite SA having no hepatotoxicity at legal dosages, its effect on hepatic lipid metabolism is still unclear. We investigated the effect of SA on hepatic lipid metabolism and its mechanism of action in C57BL/6 mice. Daily treatment with SA (1 g/kg in diet) for 4 weeks did not alter the body weight, organ weight, and blood lipids in mice. However, hepatic lipid accumulation, particularly that of triglycerides, fatty acids, and glycerol, but not cholesteryl ester and free cholesterol, was increased with SA treatment. Mechanistically, SA decreased the expression of proteins related to *de novo* fatty acid lipogenesis, fatty acid internalization, and very low-density lipoprotein (VLDL) secretion-related pathways, including sterol regulatory element-binding proteins, acetyl-coA carboxylase, fatty acid synthase, liver fatty acid-binding protein, CD36, and apolipoprotein E. In contrast, SA increased the expression of diacylglycerol O-acyltransferase 2, the key enzyme for triacylglycerol synthesis. Moreover, SA downregulated the protein expression of autophagy-related and β -oxidation-related pathways, the two major metabolic pathways for lipid metabolism, including LC-3, beclin-1, autophagy related protein 5 (ATG-5) and ATG-7, acyl-CoA synthetase long chain family member 1, carnitine palmitoyltransferase 1 α , peroxisome proliferator-activated receptor α (PPAR α), PPAR γ , and PPAR γ coactivator-1. Collectively, SA deregulates *de novo* lipogenesis and fatty acid internalization, VLDL secretion, autophagy, and β -oxidation in the liver, leading to impaired lipid clearance and ultimately, resulting in lipid accumulation in the liver.

Keywords: Autophagy, β -oxidation, Fatty acid metabolism, Liver, Sorbic acid

1. Introduction

Sorbic acid (SA), also known as 2,4-hexadienoic acid, is a natural organic compound. It is derived from the berries of the mountain rowan tree. Since the 1930s, SA has been used as a preservative for food and wine due to its ability to prevent spoilage caused by yeast, fungi and molds, as well as some bacteria. To cater to huge demand, SA is produced by a chemical synthesis method. Currently, preservatives are being widely used with the purpose to extend the expiration date of foods [1]. In Taiwan, SA can be used legally in 34 specific food items and

regulations limit the concentration to 0.5–2 g/kg of food [2]. For instance, SA cannot exceed 2 g/kg in meat products, in soy sauce, miso, jam, and ketchup to a maximum concentration of 1 g/kg and in drinks to a maximum concentration of 0.5 [2]. Ling et al. reported that the use of SA at the current dosage in the Taiwanese diet does not pose a risk for public health and safety [2]. However, an elegant study by Luo et al. demonstrated that diets supplemented with SA (2 g/kg) for 35 days increased plasma levels of albumin, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in pigs [3].

The liver is the major organ for controlling lipid homeostasis in the human body including that of

Received 7 September 2019; accepted 27 November 2019.
Available online 27 June 2020

* Corresponding author at: Graduate Institute and Department of Physiology, College of Medicine, National Taiwan University, Taipei 10051, Taiwan. Fax: +886-2-2396-4350.

E-mail address: ntutslee@ntu.edu.tw (T.-S. Lee).

¹ These authors equally contributed to this work.

<https://doi.org/10.38212/2224-6614.1055>

1021-9498/© 2020 Food and Drug Administration. This is an open access article under the CC BY NC ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

cholesterol and triglycerides, with fluxes in *de novo* lipid synthesis in the liver, and from dietary, circulating, and peripheral local lipid pools [4,5]. It is well-established that *de novo* lipogenesis in the liver and lipids from dietary fats are the main sources for maintaining physiological function [4,5]. Under physiological conditions, about 70–80% of total daily cholesterol and fatty acids are produced in the liver [6,7]. Hepatic lipid homeostasis is dynamically regulated by integrating several aspects of metabolic pathways including *de novo* lipogenesis, fatty acid uptake, triglyceride synthesis, β -oxidation of fatty acids, secretion of very-low-density lipoprotein (VLDL), and lipoprotein internalization (chylomicron remnants, VLDL, intermediate-density lipoprotein, LDL, and HDL) from circulation [8–12]. It has been well-documented that the balance between *de novo* lipogenesis and lipid metabolism is a crucial factor for maintaining whole-body lipid homeostasis [8,12]. For example, increased hepatic lipogenesis and decreased β -oxidation of fatty acids may result in excessive lipid accumulation in the liver and hyperlipidemia, which are important risk factors in the development of fatty liver and related metabolic diseases [13,14]. During the past decades, adequate evidence suggests that the deregulation of hepatic lipid metabolism by genetic factors, clinical drugs, and environmental pollutants leads to hyperlipidemia, fatty liver, and metabolic disorders [15–18]. However, less is known about the effect of SA on hepatic lipid metabolism. To this end, investigations delineating the effect and molecular mechanisms of SA in hepatic lipid metabolism and related diseases are warranted.

Given the impact of SA in increasing the level of plasma lipid, we aimed to characterize the effect and molecular mechanisms of SA regarding hepatic lipid metabolism. We first investigated the effect of SA on plasma lipid level and hepatic lipid accumulation in wild-type (WT) mice and then examined whether SA affected the molecular mechanisms in hepatic lipid metabolism. Our findings demonstrated that SA might reduce the production and uptake of fatty acids in the liver and decrease the lipid clearance and metabolism, resulting in hepatic lipid accumulation.

2. Materials and methods

2.1. Reagents

SA and oil red O dyes were obtained from Sigma–Aldrich (St. Louis, USA). Mouse antibody for carnitine palmitoyltransferase 1 α (CPT1 α), rabbit antibodies for acyl-coA synthetase α (ACC α), acyl-

Abbreviations

SA	sorbic acid
ATG5	autophagy related protein 5
ATG7	autophagy related protein 7
VLDL	very low-density lipoprotein
PPAR α	peroxisome proliferator-activated receptor α
PPAR γ	peroxisome proliferator-activated receptor γ
HDL	high-density lipoprotein
LDL	low-density lipoprotein
CPT1 α	carnitine palmitoyltransferase 1 α
ACC α	acyl-CoA synthetase α
ACOX1	acyl-CoA oxidase
ApoB	apolipoprotein B
PGC1 α	peroxisome proliferator-activated receptor γ coactivator 1– α
SREBP1	sterol regulatory element-binding protein 1
SREBP2	sterol regulatory element-binding protein 2
ACSL1	acyl-CoA synthetase
DGAT1	diacylglycerol acyltransferase 1
DGAT2	diacylglycerol acyltransferase 2
FAS	fatty acid synthase
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase
L-FABP	liver fatty acid-binding protein
DGAT2	diacylglycerol acyltransferase 2
MTP	microsomal triglyceride transfer protein
WAT	white adipose tissue
BAT	brown adipose tissue
HDL-c	HDL-cholesterol

CoA oxidase (ACOX1), apolipoprotein B (ApoB), and peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assay kits were procured from Abcam (Cambridge, UK). Mouse antibodies for sterol regulatory element-binding protein 1 (SREBP1) and sterol regulatory element-binding protein 2 (SREBP2) were procured from BD Bioscience (San Jose, CA, USA). Mouse antibodies for β -actin, peroxisome proliferator-activated receptor γ (PPAR γ), rabbit antibodies for acyl-CoA synthetase (ACSL1), beclin-1, diacylglycerol acyltransferase 1 (DGAT1), fatty acid synthase (FAS), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), liver fatty acid-binding protein (L-FABP), and peroxisome proliferator-activated receptor α (PPAR α), goat antibodies for diacylglycerol acyltransferase 2 (DGAT2), and microsomal triglyceride transfer protein (MTP) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit antibodies for autophagy related protein 5 (ATG5), autophagy related protein 7 (ATG7), CD36 and low-density lipoprotein receptor (LDLR) were from Novus Biologicals (Littleton, Co, USA). Rabbit antibody for LC3A/B was purchased from Cell Signaling Technology (Danvers, MA, USA). Rabbit antibody for apolipoprotein E (ApoE) was

from Epitomics (Burlingame, CA, USA). Cholesterol and triglyceride assay kits were obtained from Randox (Crumlin, Co Antrim, UK). Total cholesterol, free cholesterol, cholesteryl ester, triglyceride fatty acid and glycerol fluorometric assay kits were purchased from BioVision (Milpitas, CA, USA).

2.2. Mice

This study conformed to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, eighth edition, 2011), and all animal experiments were approved by the Animal Care and Utilization Committee of National Yang-Ming University. Eight-week old C57BL/6 mice purchased from National Laboratory Animal Center (Taipei, Taiwan) were randomly divided into two groups and treated with either vehicle or SA. Mice were housed in barrier facilities, maintained in a 12-h/12-h light–dark cycle. Temperature (22 °C) and humidity (40–60%) of the vivarium were tightly controlled. Mice were group-housed 5 per cage and fed a regular chow diet containing 4.5% fat by weight (0.02% cholesterol) (Newco Distributors, Redwood, CA, USA). Two-month-old male C57BL/6 mice received daily treatment with SA (1 g/kg in diet) or vehicle for four weeks. At the end of the experiment, mice were euthanized with CO₂. The liver, white adipose tissue (WAT), and brown adipose tissue (BAT) were isolated and weighed. These were then subjected to histological analysis or stored at –80 °C. The isolated liver, WAT, and BAT were homogenized, and lysates were subjected to Western blot analysis.

2.3. Histological examination

The liver, white adipose tissue (WAT), and brown adipose tissue (BAT) blocks were cut into 8- μ m sections and subjected to histological examination. Cryosections were subjected to oil red O staining and deparaffinized sections were stained with hematoxylin and eosin (H&E), and then viewed under a Motic TYPE 102 M microscope (Motic Images, China).

2.4. Western blotting analysis

The liver samples were lysed using immunoprecipitation lysis buffer (50 mmol/L Tris, pH 7.5, 5 mmol/L EDTA, 300 mmol/L NaCl, 1% Triton X-100, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μ g/mL leupeptin, and 10 μ g/mL aprotinin). Aliquots of lysates were separated by SDS-PAGE and transblotted onto an Immobilon-P membrane, blocked

with 5% skim milk for 1 h at room temperature, and then incubated with primary antibodies overnight and then with corresponding secondary antibody for 1 h. The protein bands were detected by using an enhanced chemiluminescence kit (PerkinElmer, Boston, MA, USA) and quantified using ImageQuant 5.2 (Healthcare Bio-Sciences, PA, USA).

2.5. Serum lipid profile analysis

Blood was collected by cardiac puncture. After clotting and centrifugation, serum was isolated and levels of triglycerides, total cholesterol, HDL-cholesterol (HDL-c), and non-HDL-c in serum were measured using Spotchem EZ SP 4430 (ARKRAY, Inc., Kyoto, Japan).

2.6. Determination of hepatic lipids and glycerol

The levels of total cholesterol, free cholesterol, cholesteryl ester, triglycerides, fatty acids, and glycerol were measured using fluorescence assay kits (BioVision, Milpitas, CA, USA).

2.7. Statistical analysis

Data are presented as mean \pm SEM. Data from mice were evaluated by parametric tests. The unpaired *t*-test was used to compare two independent groups. SPSS software v20.0 (SPSS Inc, Chicago, IL, USA) was used for analysis. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. SA increases lipid accumulation in the liver without changing body weight, organ weight and blood lipid profile of mice

The liver is the major organ for lipid metabolism in humans and animals [6,7]. We examined the effect of SA on hepatic lipid metabolism and its potential mechanism(s) of action. Results of H&E and oil red O staining showed that daily treatment of mice with SA for 4 weeks increased lipid accumulation (Fig. 1A). In addition, treatment with SA increased hepatic triglyceride levels, free fatty acid, and glycerol but not total cholesterol, free cholesterol, and cholesteryl ester (Fig. 1B–G). These results suggest that increased lipid accumulation in the liver due to SA treatment might be attributed to the elevation of triglycerides, free fatty acid, and glycerol.

Intriguingly, we demonstrated that daily treatment of mice with SA for four weeks did not alter

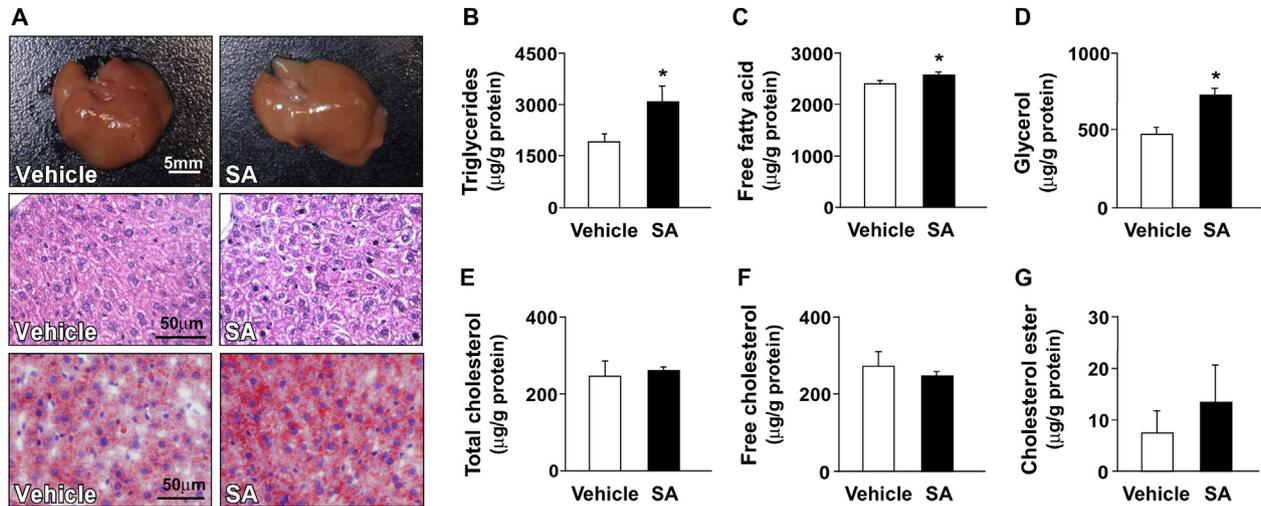


Fig. 1. SA increases lipid accumulation in the liver. Eight-week old C57BL/6 mice were treated with SA (1 g/kg diet/day) or vehicle (oil) for four weeks. (A) The liver appearance, representative H&E and oil red O staining of liver tissue. (B–G) Lipid profile in liver was assessed by fluorometric assay kits. (B–D) Hepatic levels of (B) triglycerides, (C) fatty acids, and (D) glycerol, (E) total cholesterol, (F) free cholesterol and (G) cholesteryl ester. Data are represented as mean \pm SEM from five mice. * $P < 0.05$ vs. vehicle.

their appearance (Supplementary Fig. S1A), body weight, the liver weight, and the ratio of the liver weight to body weight as compared to vehicle treatment (Supplementary Fig. S1B, C and F). SA treatment showed an increasing trend in WAT weight, BAT weight, and their ratios with respect to body weight; however, there was no statistical significance (Supplementary Fig. S1D, E, G, and H). Results of H&E staining also showed that SA treatment did not affect the histology of WAT and BAT (Supplementary Fig. S1I and J). In addition, SA did not affect the circulating levels of triglycerides, total cholesterol, HDL-c, and non-HDL-c in C57BL/6 mice (Supplementary Fig. S2A–D).

3.2. Effects of SA on *de novo* hepatic lipogenesis and fatty acid metabolism-related protein expression

The lipid homeostasis in the liver is tightly controlled by several metabolic pathways including *de novo* lipogenesis, fatty acid uptake, triglyceride synthesis, secretion of VLDL, and lipoprotein internalization [8–12]. We next examined the mechanisms underlying the detrimental effect of SA on lipid accumulation in the liver by evaluating the protein expression of hepatic lipid metabolic pathways. As compared to vehicle treatment, daily SA treatment decreased the protein expression of SREBP-1 and its downstream proteins ACC α and FAS, the key regulators for fatty acid synthesis [8,9], without affecting the expression of SREBP-2 and its downstream proteins LDLR and HMGCR, the key

regulators for cholesterol synthesis (Fig. 2A–C). Furthermore, treatment with SA increased the protein expression of DGAT2, but not DGAT1 (Fig. 3A–C), the key regulators of triglyceride synthesis in the liver [19]. On the other hand, treatment with SA decreased the protein expression of L-FABP and CD36 (Fig. 3A–C), two crucial hepatic regulators in the internalization of fatty acids from circulation [20,21]. Moreover, SA treatment decreased the protein expression of apoE, but not apoB and MTP (Fig. 3A–C), key players in the process of VLDL assembly and secretion [22,23]. These results suggest that increased triglyceride synthesis by upregulation of DGAT-2 and impaired VLDL secretion by downregulation of apoE might result in the disturbance of hepatic lipid metabolism and thereby lead to lipid accumulation in the liver.

3.3. SA deregulates catabolic metabolism of hepatic lipid droplets in mice

Autophagy, lipolysis, and β -oxidation of fatty acids are key metabolic processes in regulating intracellular lipid droplets [24,25]. Thus, we next evaluated the effects of SA on the expression of proteins related to catabolic metabolism of hepatic lipid droplets in the liver. We demonstrated that SA markedly decreased the expression of proteins involved in autophagy flux, including beclin-1, SQSTM1, LC3, ATG5, and ATG7 without altering the protein expression of ATGL and HSL, the two major regulators in the process of lipolysis (Fig. 4A–C). Moreover, treatment with SA

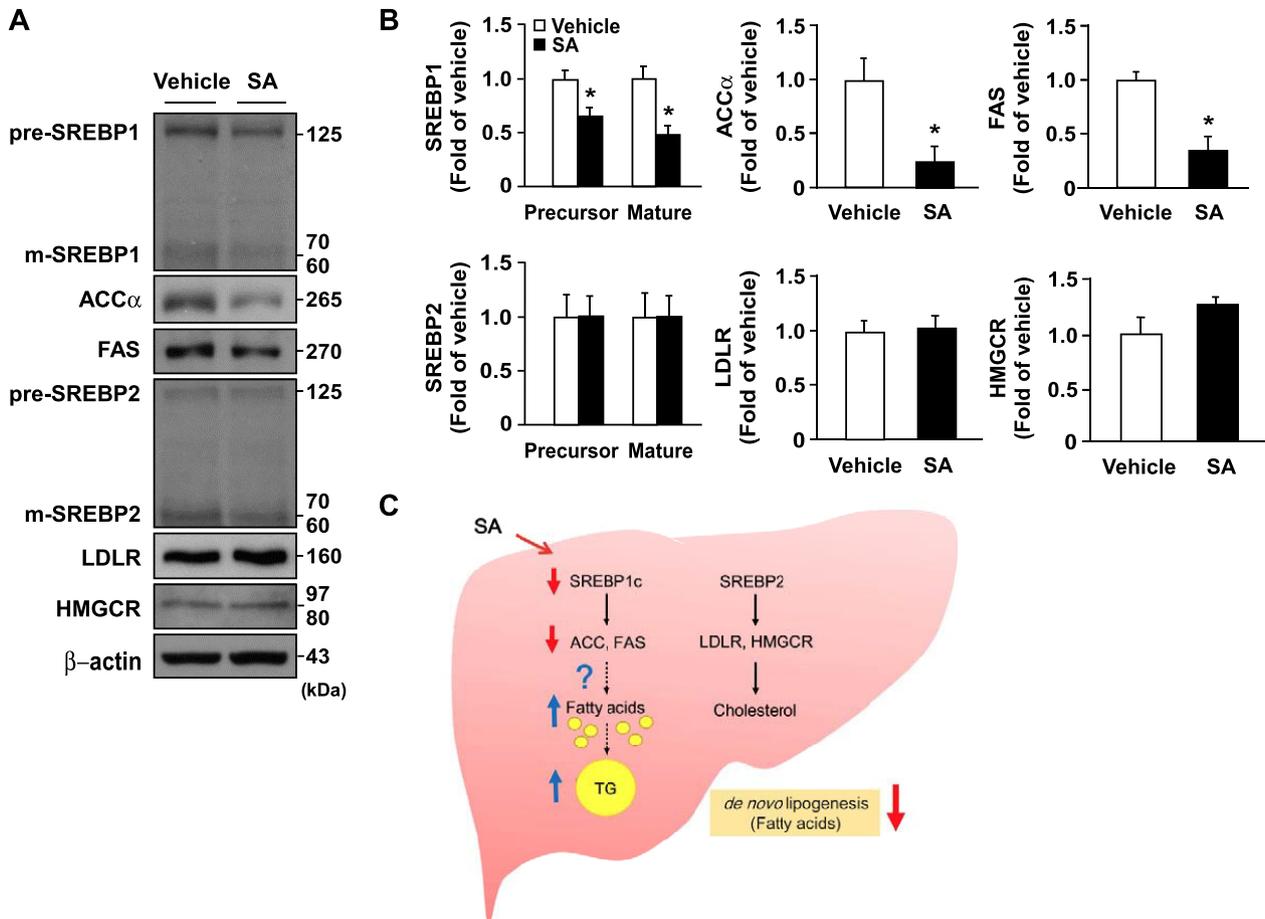


Fig. 2. Effect of SA on *de novo* fatty acid and cholesterol synthesis-related protein expression in the liver. Eight-week old C57BL/6 mice were treated with SA (1 g/kg diet/day) or vehicle (oil) for four weeks. (A and B) Western blot analysis of protein levels of SREBP1, ACC α , FAS, SREBP2, LDLR, HMGCR, and β -actin, in the liver. (C) Summary for the effect of SA on hepatic *de novo* lipogenesis. Data are represented as mean \pm SEM from five mice. * $P < 0.05$ vs. vehicle.

significantly downregulated the protein expression of ACSL1, CPT1 α , PPAR α , PPAR γ , and PGC1 α , all of which are crucial regulators involved in β -oxidation of fatty acids and energy release (Fig. 5A–C). These findings suggest that deregulation of catabolic metabolism of lipid droplets by decreasing autophagy and β -oxidation is the key factor for SA-induced lipid accumulation in the liver.

4. Discussion

Based on this study, we provided new insights into the effect of SA on hepatic lipid metabolism and the underlying molecular mechanisms. We used a mouse model to investigate the effect of SA on hepatic lipid metabolism and found that chronic treatment with SA (1 g/kg diet/day) for four weeks increased the glycerol and the lipid accumulation, particularly that of triglycerides and free fatty acids in the liver. While the severity of hepatic lipid

accumulation by SA did not reach the degree of fatty liver; the plasma levels of ALT and AST were not different with that in control group. However, our knowledge about the molecular mechanism behind the detrimental effect of SA on hepatic lipid metabolism is limited. We then explored the effect of SA on *de novo* lipogenesis, fatty acid internalization, autophagy, and β -oxidation in this C57BL/6 mouse model. We found that SA decreased the protein expression related to *de novo* lipogenesis and fatty acid internalization and VLDL secretion in the liver. Moreover, SA downregulated the expression of proteins involved in autophagy and β -oxidation pathways, which in turn limited lipid clearance. These key events might work in concert to promote lipid accumulation in the liver (Fig. 6). Intriguingly, we demonstrated that treatment with SA did not affect the body weight, organ weight, and blood lipid profile of wild type C57BL/6 mice, which, however, was inconsistent with a previous finding

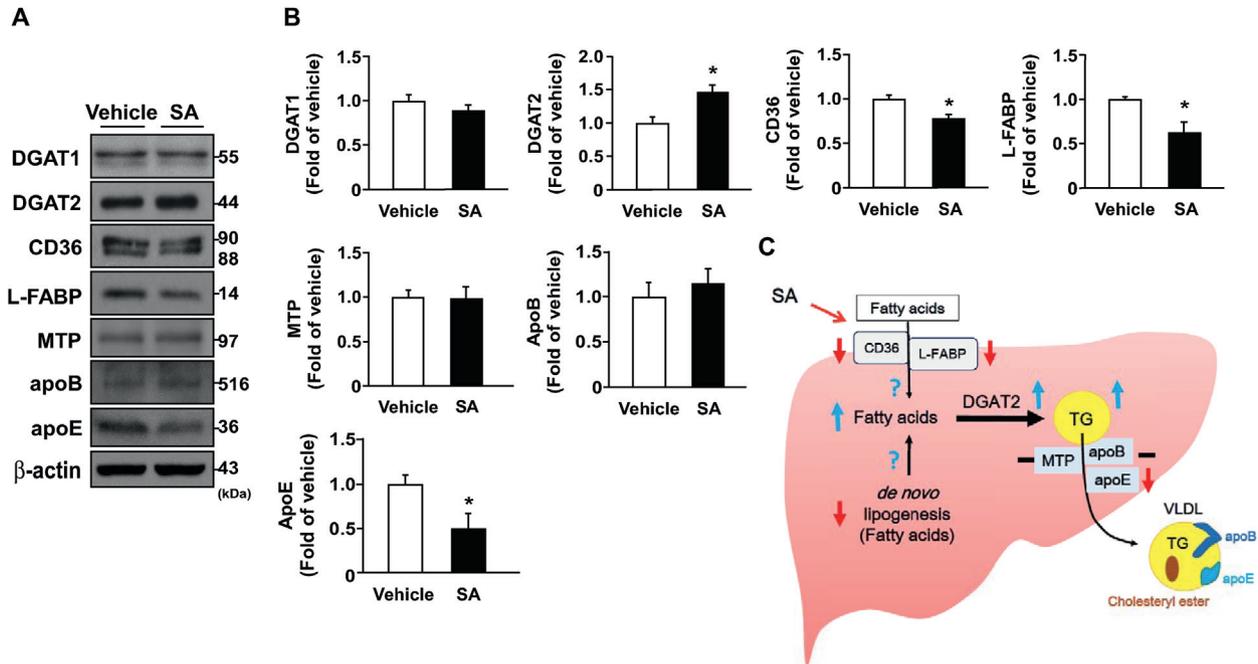


Fig. 3. Effect of SA on fatty acid metabolism-related protein expression in the liver. Eight-week old C57BL/6 mice were treated with SA (1 g/kg diet/day) or vehicle (oil) for four weeks. (A and B) Western blot analysis of protein levels of DGAT1, DGAT2, CD36, L-FABP, MTP, apoB, apoE, and β -actin, in the liver. (C) Summary of the effect of SA on fatty acid metabolism in the liver. Data are represented as mean \pm SEM from five mice. * $P < 0.05$ vs. vehicle.

reporting that treatment with SA (2 or 4 g/kg diet/day) for 35 days increased the body weight and circulating levels of total cholesterol or triglycerides in piglets [3]. Although the exact mechanism is unclear, the reason for the discrepancy between our study and the study by Luo et al. could be the difference in the dosage of SA and the species of experimental animals. On the other hand, Itagaki et al. reported that the degree of fatty liver and steatohepatitis induced by methionine- and choline-deficient diet was returned to normal condition when switched back to the chow diet for 16 weeks [26]. Therefore, we believe that the effect of SA on hepatic lipid deregulation is reversible once the mice are switched back on the regular chow diet.

Regarding with the concentration of SA used in this study, the legal dosages (0.5–2.0 g/kg) of SA used in food have been established [2]. Therefore, we simply adopted the average dosage (1 g/kg) and tried to address the effect of SA at the legal concentration on lipid metabolism. We demonstrated that there was no difference in food intake daily among the vehicle group and SA group (vehicle group: SA group = 4.03 ± 0.32 g: 3.98 ± 0.28 g/mouse [20 g]). Therefore, the consumption amount of SA daily is about 4 mg/mouse. After calculating, the concentration of SA used in our study is about

200 mg/kg body weight for mice. By Meeh-Rubner equation, this concentration of 200 mg/kg for mice could be comparable to the concentration of 4–8 mg/kg for human consumers. The consumption amount in our study is lower than the average intake level of SA at 37 mg/kg for human consumers, suggesting the detrimental effect of SA at legal concentration on lipid metabolism could be an important issue for human health.

SA is a well-known food preservative for preventing microbial growth, thereby extending the shelf life of food. It is widely used in soy sauce, jam, frozen meat products, pickled vegetables, canned foods, and beverages [1,2]. Excessive use of SA may cause hepatotoxicity [27]; therefore, it is necessary to control and use it at legally permissible dosages in preparation and for preservation of food. Indeed, the legally permissible concentration of SA to be used in various food items has been established during the past decades [2]. However, whether the legal concentration of SA used in food disturbs hepatic lipid metabolism and the potential molecular mechanism is largely unknown. The impact of food preservatives on human health and metabolic diseases is still incompletely understood. There have been epidemiological studies reporting that exposure to SA and benzoic acid might be hazardous to

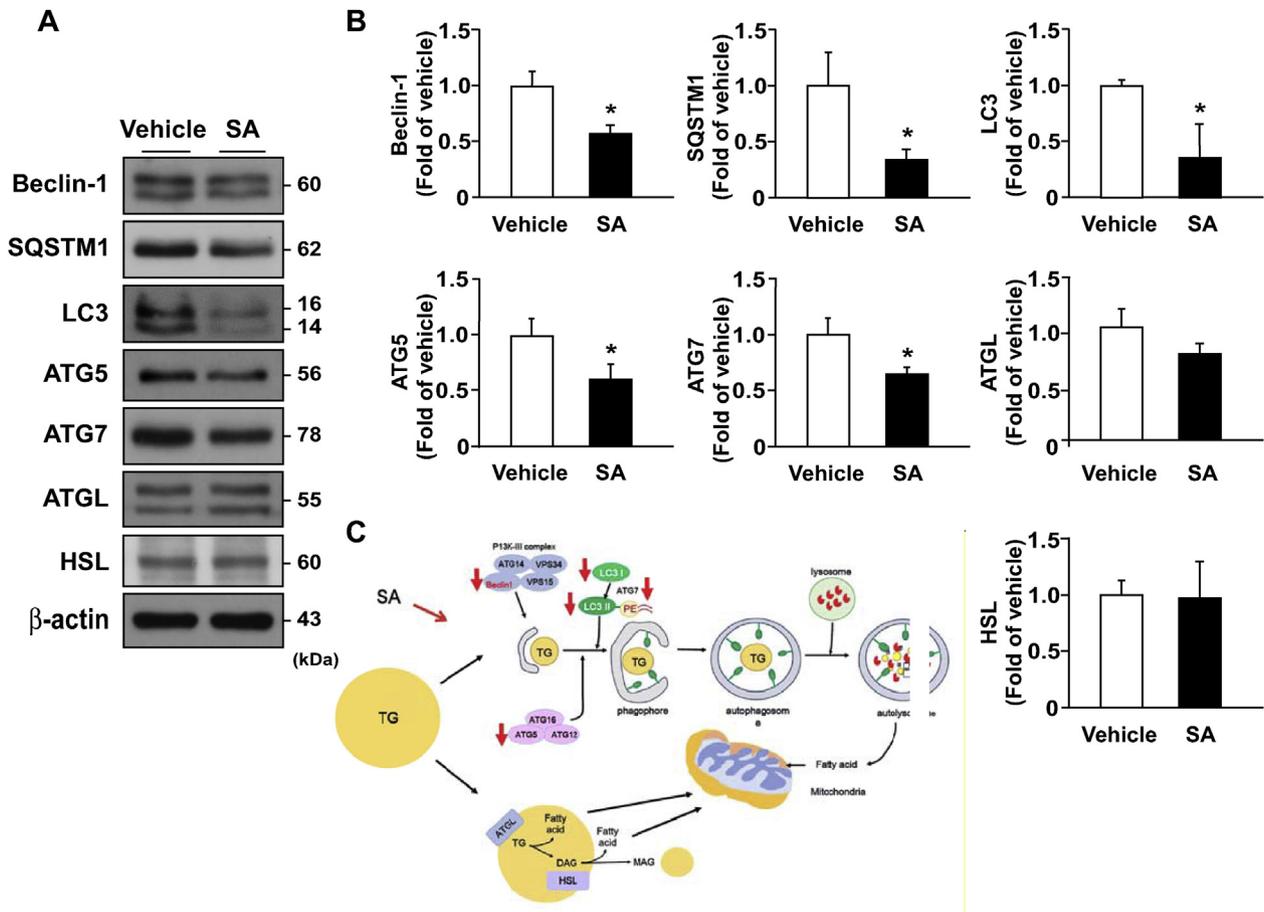


Fig. 4. SA decreases the protein expression of indispensable regulators involved in autophagy and lipolysis. Eight-week old C57BL/6 mice were treated with SA (1 g/kg diet/day) or vehicle (oil) for four weeks. (A and B) Western blot analysis of protein levels of beclin-1, SQSTM1, LC3, ATG5, ATG7, ATGL, HSL and β -actin, in the liver. (C) Summary of the effect of SA on autophagy and lipolysis in the liver. Data are represented as mean \pm SEM from five mice. * $P < 0.05$ vs. vehicle.

human health [2,28]. However, the relationship between the use of food preservatives and human diseases is still unclear. Here, we provided new evidence to support the idea that SA, at legal dosage, increased lipid accumulation in the liver of mice by decreasing autophagy and β -oxidation, two crucial events in fatty acid metabolism. In this study, our findings show that treatment with SA significantly increased the lipid content in the liver, particularly triglycerides, free fatty acids, and glycerol, but not total cholesterol, free cholesterol, and cholesteryl ester, suggesting the unfavorable effect of SA on hepatic lipid metabolism. However, the circulating levels of total cholesterol and triglycerides were not changed. It has been well-documented that dietary fat and *de novo* lipogenesis in the liver are the main sources of circulating total cholesterol and triglycerides, which are carried in circulation as VLDL [29–31]. In this study, no difference was observed in food intake between

vehicle-treated mice and SA-treated mice (data not shown). Therefore, upon SA treatment, elevated levels of triglycerides in the liver might be attributed to increased lipid synthesis, or decreased lipid clearance, or both.

Under physiological conditions, the lipid content in the liver is tightly regulated by the integration of several aspects of metabolic pathways, including *de novo* lipogenesis, lipoprotein internalization, VLDL synthesis, β -oxidation of fatty acids, and catabolic metabolism of lipid droplets including lipolysis and autophagy [8,10,11,32]. Therefore, we investigated how SA affected hepatic lipid metabolism. Our findings demonstrated that SA-induced accumulation of triglycerides, fatty acid, and glycerol in the liver might not be due to increased *de novo* lipogenesis or fatty acid uptake as evidenced by the observation that SA decreased the protein expression of SREBP-1, FAS, and ACC, all crucial regulators in the *de novo* synthesis of hepatic triglycerides

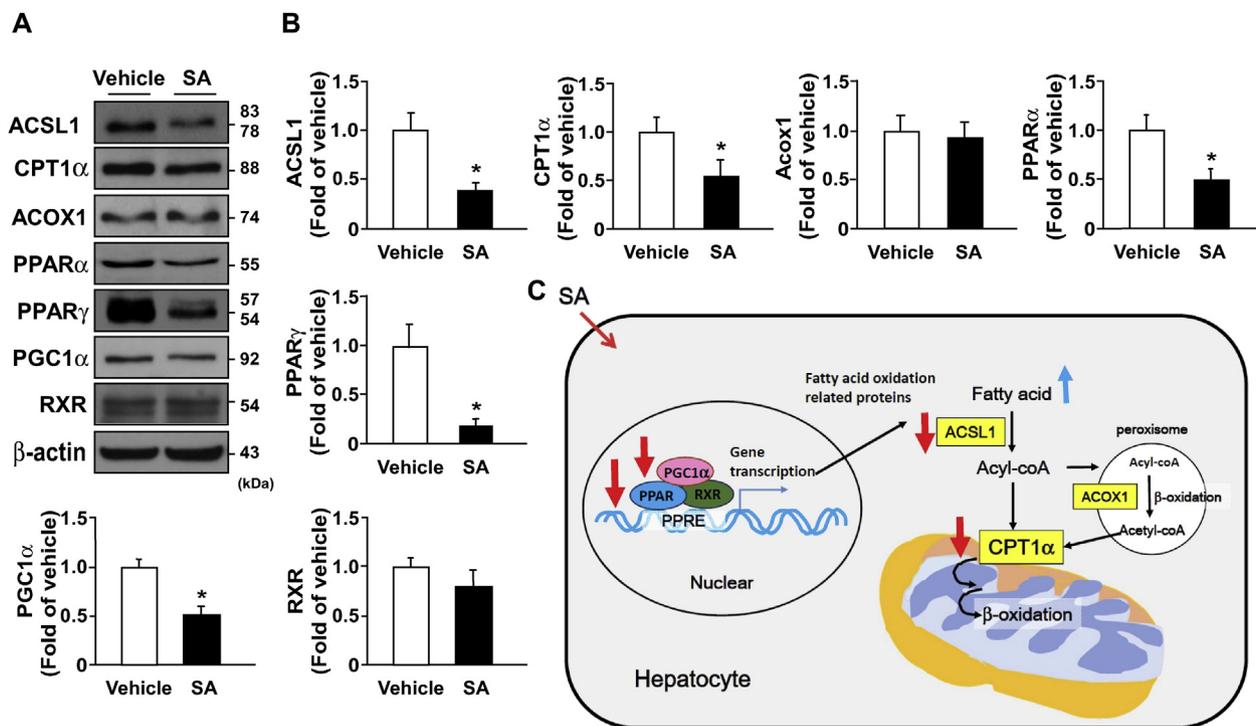


Fig. 5. SA downregulates the protein expression of key regulators involved in β -oxidation pathway. Eight-week old C57BL/6 mice were treated with SA (1 g/kg diet/day) or vehicle (oil) for four weeks. (A and B) Western blot analysis of protein levels of ACSL1, CPT1 α , Acox1, PPAR α , PPAR γ , PGC1 α , and β -actin in the liver. (C) Summary of the effect of SA on β -oxidation of fatty acids in the liver. Data are represented as mean \pm SEM from five mice. * $P < 0.05$ vs. vehicle.

[33–35]. In addition, SA decreased the uptake of fatty acids from circulation, as evidenced by the downregulation of L-FABP and CD36, which are key transporters of fatty acids into hepatocytes [36,37]. Moreover, the expression level of the key enzyme DGAT2 for triglyceride synthesis was increased; in contrast, the key regulator apoE for VLDL secretion

was decreased by SA treatment. Thus, these deregulations in lipid metabolism with respect to triglyceride synthesis and VLDL secretion might contribute to the elevated levels of triglycerides, free fatty acids, and glycerol in the liver. Moreover, the interaction of the liver-mediated lipid metabolism with peripheral local lipid pools such as WAT and BAT plays a principal role in regulating whole-body lipid homeostasis [30,31], we think that the SA-induced deregulation of lipid metabolism may be not a liver-specific effect. Additionally, SA is a di-unsaturated, six carbon-atom fatty acid, can be rapidly broken down quickly and utilized similarly as fatty acids, and ultimately be metabolized to carbon dioxide [1]. Thus, the effects observed on protein expression of different lipid metabolism pathways were unlikely to be direct effects of SA. Despite our unique findings, the detailed mechanisms by which SA modulates lipid metabolism within the liver merit further study.

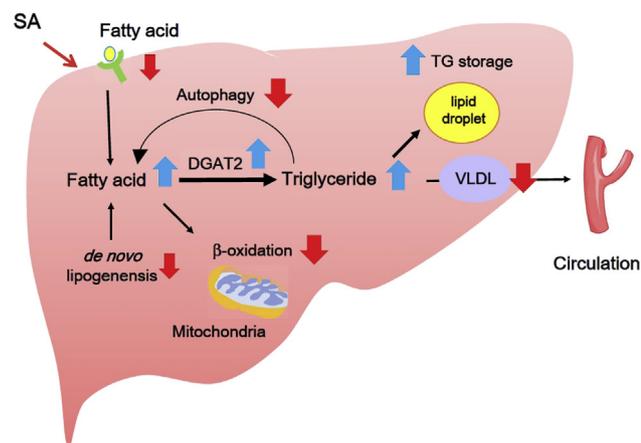


Fig. 6. Proposed model of mechanisms by which SA accelerates lipid accumulation in the liver. SA deregulates de novo lipogenesis and fatty acid internalization and VLDL secretion and interferes with autophagy and β -oxidation in the liver, leading to impaired lipid clearance and ultimately resulting in lipid accumulation in the liver.

Notably, we demonstrated that SA downregulated the expression of proteins related to autophagy including beclin-1, SQSTM1, ATG5, and ATG7. Autophagy is a conserved self-eating process that is important for cellular homeostasis under various stress conditions. It allows cells to self-degrade intracellular components including lipid droplets

and damaged organelles in the lysosomes for balancing energy sources upon nutrient shortage or various cytotoxic insults [38,39]. Our findings are consistent with studies by Du et al. and Xie et al. who found that inhibition of autophagy disturbs lipid homeostasis and worsens the progression of fatty liver [40,41]. More importantly, SA also decreased the protein expression of ACSL1, CPT1 α , PPAR α , PPAR γ , and PGC-1 α , all implicated in the β -oxidation pathway for energy metabolism in mitochondria [42–46], which are in line with previous findings that suggest that inhibition of fatty acid oxidation damages energy metabolism and accelerates the development of non-alcoholic fatty liver [47,48]. These findings suggest that SA impaired the functions of autophagy and β -oxidation pathways in catabolic metabolism of triglyceride-rich lipid droplets, leading to lipid accumulation in the liver. This observation agrees with previous reports that impairment of autophagy or β -oxidation disturbs hepatic lipid metabolism and accelerates the progression of fatty liver [48–51]. In view of the function of these proteins, SA might disturb lipid homeostasis by impairing the catabolic metabolism of lipid droplets and energy metabolism. Nevertheless, the molecular mechanism behind autophagy machinery contributing to SA-mediated deregulation of lipid homeostasis remains incompletely understood. Overall, congruous data have been reported suggesting the alteration of key molecules regulating lipid metabolism, which again demonstrates the detrimental effects of SA on hepatic lipid metabolism. The disturbed hepatic lipid metabolism appears to be the key event in SA-induced lipid accumulation.

Response or adaption to environmental stress such as the change in pH or food preservatives is important for the survival and proliferation of living organisms [52]. For example, upon weak acid exposure, the membrane permeability and anion extrusion in *Saccharomyces cerevisiae* were decreased and the gene-related to metal metabolism and vacuolar H⁺-ATPase were upregulated to adapt the environmental changes in pH value [53]. In mammalian cells, autophagy is reported to be protective for gastric cancer cells against the acidic environment [54]. However, there is no study investigating the acid stress response in hepatocytes. Although SA is a weak organic acid, we thought that SA may not induce acid stress response in hepatocytes. Because plasma or tissue fluid has a strong capacity of buffering to maintain the pH value within normal range under physiological or pathological condition, to prove our hypothesis, we additionally performed in vitro study to examine

whether SA affects the pH value in the culture system of murine hepatocyte cell line AML12 cells. Our results demonstrated that treatment with SA (1 g/L) did not change the pH value of culture medium at time 0 (vehicle group: SA group = 7.49: 7.49) and 18 h after SA treatment (vehicle group: SA group = 7.34: 7.34). This finding suggests that SA may not induce acid stress response in hepatocytes.

Metabolic diseases including fatty liver disease are complex, progressive disease processes with multiple etiologies [55–57]. Although SA did not have a significant impact on appearance and weight; it indeed disturbed the regulation of hepatic lipid metabolism leading to lipid accumulation in the liver. This observation suggests that SA intake might be a risk factor in the development of metabolic diseases under certain circumstances, in developed countries. In this study, we found that SA decreases lipoprotein secretion, autophagy and β -oxidation. All these events induced by SA could speed up the progression of fatty liver. In addition, our results from a cellular model showed that SA exacerbated oleic acid-induced lipid accumulation in Huh7 hepatoma cells, a fatty liver cell model (data not shown). Nevertheless, our study has several limitations since, for experimental analysis, we did not use hyperlipidemic or fatty liver animal models to mimic the pathogenesis of metabolic diseases. Using specific hyperlipidemic mouse models will be helpful to clarify the effects of SA on lipid homeostasis and disorders under these pathological conditions. In addition, the sample size of this study is rather small ($n = 5$), the experimental design may not have enough power to detect differences between the 2 groups. Indeed, upon SA treatment for 4 weeks, there is an increasing trend in the WAT weight, BAT weight, and their ratios with respect to body weight, as well as the plasma level of HDL-c; however, the p value didn't reach the level of statistically significant. Increasing the number of samples may improve such a defect in this study. Moreover, we do not have clinical data to support our observations from animal studies. To this end, further investigations describing the implications of food preservatives in hepatic lipid metabolism and related metabolic diseases are warranted.

5. Conclusion

Collectively, our study provides new evidence that SA has detrimental effects on hepatic lipid metabolism by decreasing *de novo* lipogenesis, fatty acid internalization, VLDL secretion, autophagy, and β -oxidation, thereby promoting lipid accumulation in the liver. Moreover, we discovered a link between a

food preservative and hepatic lipid metabolism, which broadens the biomedical implications of SA in the development of metabolic diseases.

Conflict of interest

The authors disclose no potential conflict of interest.

Acknowledgements

This study was supported by grants from the Ministry of Science and Technology of Taiwan (105-2320-B-010-036, 105-2811-B-010-022, 106-2320-B-002-056, 106-2320-B-002-057-MY3, 107-2320-B-002-062, 107-2811-B-002-585 and 107-2320-B-010-027-MY3).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.38212/2224-6614.1055>.

References

- [1] Hu S, Yu J, Wang Z, Li L, Du Y, Wang L, et al. Effects of sorbic acid-chitosan microcapsules as antimicrobial agent on the properties of ethylene vinyl alcohol copolymer film for food packaging. *J Food Sci* 2017;82:1451–60.
- [2] Ling MP, Lien KW, Wu CH, Ni SP, Huang HY, Hsieh DP. Dietary exposure estimates for the food preservatives benzoic acid and sorbic acid in the total diet in Taiwan. *J Agric Food Chem* 2015;63:2074–82.
- [3] Luo ZF, Fang XL, Shu G, Wang SB, Zhu XT, Gao P, et al. Sorbic acid improves growth performance and regulates insulin-like growth factor system gene expression in swine. *J Anim Sci* 2011;99:2356–64.
- [4] Sanders FW, Griffin JL. De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. *Biol Rev Camb Phil Soc* 2016;91:452–68.
- [5] Hellerstein MK, Schwarz JM, Neese RA. Regulation of hepatic de novo lipogenesis in humans. *Annu Rev Nutr* 1996;16:523–57.
- [6] Ipsen DH, Lykkesfeldt J, Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. *Cell Mol Life Sci* 2018;75:3313–27.
- [7] Hodson L. Hepatic fatty acid synthesis and partitioning: the effect of metabolic and nutritional state. *Proc Nutr Soc* 2019;78:126–34.
- [8] Ferré P, Foufelle F. Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. *Diabetes Obes Metabol* 2010;12:83–92.
- [9] Yap F, Craddock L, Yang J. Mechanism of AMPK suppression of LXR-dependent Srebp-1c transcription. *Int J Biol Sci* 2011;7:645–50.
- [10] Park HM, Russo KA, Karateev G, Park M, Dubikovskaya E, Kriegsfeld LJ, et al. A system for in vivo imaging of hepatic free fatty acid uptake. *Gastroenterology* 2017;152:78–81.
- [11] Li D, Cheng M, Niu Y, Chi X, Liu X, Fan J, et al. Identification of a novel human long non-coding RNA that regulates hepatic lipid metabolism by inhibiting SREBP-1c. *Int J Biol Sci* 2017;13:349–57.
- [12] Alves-Bezerra M, Cohen DE. Triglyceride metabolism in the liver. *Comp Physiol* 2017;8:1–8.
- [13] Softic S, Cohen DE, Kahn CR. Role of dietary fructose and hepatic de novo lipogenesis in fatty liver disease. *Dig Dis Sci* 2016;61:1282–93.
- [14] Mansouri A, Gattoliat CH, Asselah T. Mitochondrial dysfunction and signaling in chronic liver diseases. *Gastroenterology* 2018;155:629–47.
- [15] Tian Y, Wong VW, Chan HL, Cheng AS. Epigenetic regulation of hepatocellular carcinoma in non-alcoholic fatty liver disease. *Semin Canc Biol* 2013;23:471–82.
- [16] Chen CH, Shyue SK, Hsu CP, Lee TS. Atypical antipsychotic drug olanzapine deregulates hepatic lipid metabolism and aortic inflammation and aggravates atherosclerosis. *Cell Physiol Biochem* 2018;50:1216–29.
- [17] Feng D, Zou J, Zhang S, Li X, Li P, Lu M. Bisphenol A promotes cholesterol absorption in Caco-2 cells by up-regulation of NPC1L1 expression. *Lipids Health Dis* 2017;16:2.
- [18] Yang Q, Xiao T, Guo J, Su Z. Complex relationship between obesity and the fat mass and obesity locus. *Int J Biol Sci* 2017;13:615–29.
- [19] Irshad Z, Chmel N, Adya R, Zammit VA. Hepatic VLDL secretion: DGAT1 determines particle size but not particle number, which can be supported entirely by DGAT2. *J Lipid Res* 2019;60:111–20.
- [20] Bass NM. Fatty acid-binding protein expression in the liver: its regulation and relationship to the zonation of fatty acid metabolism. *Mol Cell Biochem* 1990;98:167–76. <https://doi.org/10.1007/BF00231381>.
- [21] Wilson CG, Tran JL, Erion DM, Vera NB, Febbraio M, Weiss EJ. Hepatocyte-specific disruption of CD36 attenuates fatty liver and improves insulin sensitivity in HFD-fed mice. *Endocrinology* 2016;157:570–85.
- [22] Wang S, Chen Z, Lam V, Han J, Hassler J, Finck BN, et al. IRE1 α -XBPs induces PDI expression to increase MTP activity for hepatic VLDL assembly and lipid homeostasis. *Cell Metabol* 2012;16:473–86.
- [23] Dergunov AD, Visvikis-Siest S, Siest G. Statins as effectors of key activities involved in apoE-dependent VLDL metabolism: review and hypothesis. *Vasc Pharmacol* 2008;48:70–5.
- [24] Zhang Q, Li Y, Liang T, Lu X, Zhang C, Liu X, et al. ER stress and autophagy dysfunction contribute to fatty liver in diabetic mice. *Int J Biol Sci* 2015;11:559–68.
- [25] Quiroga AD, Lehner R. Pharmacological intervention of liver triacylglycerol lipolysis: the good, the bad and the ugly. *Biochem Pharmacol* 2018;155:233–41.
- [26] Itagaki H, Shimizu K, Morikawa S, Ogawa K, Ezaki T. Morphological and functional characterization of non-alcoholic fatty liver disease induced by a methionine-choline-deficient diet in C57BL/6 mice. *Int J Clin Exp Pathol* 2013;6:2683–96.
- [27] Hendy RJ, Hardy J, Gaunt IF, Kiss IS, Butterworth KR. Long-term toxicity studies of sorbic acid in mice. *Food Chem Toxicol* 1976;14:381–6.
- [28] Chaleshtori FS, Arian A, Chaleshtori RS. Assessment of sodium benzoate and potassium sorbate preservatives in some products in Kashan, Iran with estimation of human health risk. *Food Chem Toxicol* 2018;120:634–8.
- [29] Gordon T, Fisher M, Ernst N, Rifkind BM. Relation of diet to LDL cholesterol, VLDL cholesterol, and plasma total cholesterol and triglycerides in white adults. The lipid research clinics prevalence study. *Arteriosclerosis* 1982;2:502–12.
- [30] Vedala A, Wang W, Neese RA, Christiansen MP, Hellerstein MK. Delayed secretory pathway contributions to VLDL-triglycerides from plasma NEFA, diet, and de novo lipogenesis in humans. *J Lipid Res* 2006;47:2562–74.
- [31] Fu J, Ma S, Li X, An S, Li T, Guo K, et al. Long-term stress with hyperglucocorticoidemia-induced hepatic steatosis with VLDL overproduction is dependent on both 5-HT2 receptor and 5-HT synthesis in liver. *Int J Biol Sci* 2016;12:219–34.
- [32] Paglialunga S, Dehn CA. Clinical assessment of hepatic de novo lipogenesis in non-alcoholic fatty liver disease. *Lipids Health Dis* 2016;15:159.
- [33] Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 1997;89:331–40.

- [34] Griffin MJ, Sul HS. Insulin regulation of fatty acid synthase gene transcription: roles of USF and SREBP-1c. *IUBMB Life* 2004;56:595–600.
- [35] Mounier C, Bouraoui L, Rassart E. Lipogenesis in cancer progression (review). *Int J Oncol* 2014;45:485–92.
- [36] Newberry EP, Xie Y, Kennedy S, Han X, Buhman KK, Luo J, et al. Decreased hepatic triglyceride accumulation and altered fatty acid uptake in mice with deletion of the liver fatty acid-binding protein gene. *J Biol Chem* 2003;278:51664–72.
- [37] Miqulena-Colina ME, Lima-Cabello E, Sánchez-Campos S, García-Mediavilla MV, Fernández-Bermejo M, Lozano-Rodríguez T, et al. Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in non-alcoholic steatohepatitis and chronic hepatitis C. *Gut* 2011;60:1394–402.
- [38] Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, et al. Autophagy regulates lipid metabolism. *Nature* 2009;458:1131–5.
- [39] Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science* 2004;306:990–5.
- [40] Du X, Liu G, Loor JJ, Fang Z, Bucktrout R, Yang Y, et al. Impaired hepatic autophagic activity in dairy cows with severe fatty liver is associated with inflammation and reduced liver function. *J Dairy Sci* 2018;101:11175–85.
- [41] Xie ZY, Xiao ZH, Wang FF. Inhibition of autophagy reverses alcohol-induced hepatic stellate cells activation through activation of Nrf2-Keap1-ARE signaling pathway. *Biochimie* 2018;147:55–62.
- [42] Li LO, Ellis JM, Paich HA, Wang S, Gong N, Altshuler G, et al. Liver-specific loss of long chain acyl-CoA synthetase-1 decreases triacylglycerol synthesis and beta-oxidation and alters phospholipid fatty acid composition. *J Biol Chem* 2009;284:27816–26.
- [43] Orellana-Gavaldà JM, Herrero L, Malandrino MI, Pañeda A, Sol Rodríguez-Peña M, Petry H, et al. Molecular therapy for obesity and diabetes based on a long-term increase in hepatic fatty-acid oxidation. *Hepatology* 2011;53:821–32.
- [44] Nakamuta M, Kohjima M, Morizono S, Kotoh K, Yoshimoto T, Miyagi I, et al. Evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med* 2005;16:631–5.
- [45] Wenz T. PGC-1alpha activation as a therapeutic approach in mitochondrial disease. *IUBMB Life* 2009;61:1051–62.
- [46] Xu M, Wu H, Li M, Wen Y, Yu C, Xia L, et al. DJ-1 deficiency protects hepatic steatosis by enhancing fatty acid oxidation in mice. *Int J Biol Sci* 2018;14:1892–900.
- [47] Koo SH. Nonalcoholic fatty liver disease: molecular mechanisms for the hepatic steatosis. *Clin Mol Hepatol* 2013;19:210–5.
- [48] Shen T, Chen X, Li Y, Tang X, Jiang X, Yu C, et al. Interleukin-17A exacerbates high-fat diet-induced hepatic steatosis by inhibiting fatty acid β -oxidation. *Biochim Biophys Acta Mol Basis Dis* 2017;1863:1510–8.
- [49] Zhang Q, Li Y, Liang T, Lu X, Zhang C, Liu X, et al. ER stress and autophagy dysfunction contribute to fatty liver in diabetic mice. *Int J Biol Sci* 2015;11:559–68.
- [50] Qiang X, Xu L, Zhang M, Zhang P, Wang Y, Wang Y, Zhao Z, Chen H, Liu X, Zhang Y. Demethyleneberberine attenuates non-alcoholic fatty liver disease with activation of AMPK and inhibition of oxidative stress. *Biochem Biophys Res Commun* 2016;472:603–9.
- [51] Yan H, Gao Y, Zhang Y. Inhibition of JNK suppresses autophagy and attenuates insulin resistance in a rat model of nonalcoholic fatty liver disease. *Mol Med Rep* 2017;15:180–6.
- [52] Martani F, Fossati T, Posterl R, Signori L, Porro D, Branduardi P. Different response to acetic acid stress in *Saccharomyces cerevisiae* wild-type and l-ascorbic acid-producing strains. *Yeast* 2013;30:365–78.
- [53] Kawahata M, Masaki K, Fujii T, Iefuji H. Yeast genes involved in response to lactic acid and acetic acid: acidic conditions caused by the organic acids in *Saccharomyces cerevisiae* cultures induce expression of intracellular metal metabolism genes regulated by Aft1p. *FEMS Yeast Res* 2006;6:924–36.
- [54] Sheng J, Sun LB, Zhao SF, Qi WW, Lv J, Zhang ZG, et al. Acidic stress induces protective autophagy in SGC7901 cells. *J Int Med Res* 2018;46:3285–95.
- [55] Bugianesi E, McCullough AJ, Marchesini G. Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology* 2005;42:987–1000.
- [56] Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 2010;51:679–89.
- [57] Begriche K, Massart J, Robin MA, Bonnet F, Fromenty B. Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. *Hepatology* 2013;58:1497–507.