

Lycopene counteracts palmitate-induced “two-hit” in mouse primary hepatocytes via triggering NAMPT/Sirt1 pathway

Yaoliang Zhu¹, Yafang Chen², Lei Zhu², Xian Zheng², Xiaoying Zhou^{1,*}

¹School of Pharmacy, School of Medicine, Changzhou University, Changzhou 213164, Jiangsu, China

²Department of Pharmacy, Affiliated Kunshan Hospital of Jiangsu University, Kunshan 215300, China

*Corresponding author: xiaoyingzhou@cczu.edu.cn

Abstract: Previous research demonstrated that tomato powder presented impressed anti-nonalcoholic fatty liver disease (NAFLD) efficacy through restoring NAMPT/Sirt1 activity. Lycopene, the principal monomer component of tomato powder, also effectively attenuated NAFLD pathological features. However, whether lycopene exerted its beneficial effects via tuning NAMPT/Sirt1 activity is still unknown. To solve this problem, mouse primary hepatocytes were isolated and challenged with palmitate to induce the *in vitro* model of NAFLD. The cytotoxicity of palmitate and lycopene was tested by MTT assay. Then the “first hit” (i.e., hepatosteatosis) and the “second hit” (i.e., inflammation) parameters were examined following lycopene treatment. Mechanisms involved NAMPT/Sirt1-regulated lipogenesis were determined by western blotting and qRT-PCR. Our results found that lycopene had a high safety profile (no cytotoxicity at 100 μ M), while palmitate exhibited potent cytotoxicity (significant cytotoxicity at 0.0625 mM). Finally, we choose 0.5 mM of palmitate and 20, 40 μ M of lycopene as the model and intervention conditions, respectively. Under the model condition, lycopene effectively rescued the decrease in cell viability, ameliorate the lipid accumulation, and mitigated the inflammatory response. Further exploration found that activation of NAMPT/Sirt1 and blockage of downstream lipogenesis mediated its favorable effects. In summary, lycopene is sufficient to undo palmitate-induced “two-hit” in primary hepatocytes and is a prospective candidate for NAFLD therapy.

Keywords: Lycopene, Nonalcoholic fatty liver disease, Two-hit, NAMPT/Sirt1

1. Introduction

With the global prevalence of obesity, the incidence of nonalcoholic fatty liver disease (NAFLD) is on a rapid rise. The global epidemic of NAFLD is 25%, among which 27% occurred in Asia [1]. NAFLD is characterized by an ectopic accumulation of triglyceride in the liver (5%), initially being benign steatosis, while if unattended, it may further progress into hepatic fibrosis, cirrhosis, and even hepatic carcinoma (HCC) [2-4]. Till date, no efficacious medications specific for NAFLD have been approved, and lifestyle modification (i.e., diet control and physical exercise) remains the ideal and proverbial approach, while less than 50% of patients can tolerate the interventions and obtain the desired outcomes [5]. Accordingly, pursuit of safe and effective agents against NAFLD is highly urgent.

The pathogenesis of NAFLD is intricate and still undeciphered. One of the most recognized pathogenesis is the “two-hit” hypothesis, which comprises insulin resistance-evoked hepatic steatosis (the “first hit”) and subsequently occurred mitochondrial dysfunction, oxidative stress, and inflammatory response (the “second hit”) [6,7]. In this context, strategies defense against the process of the “two-hit” show great treatment promise for NAFLD.

Lycopene, an acyclic and lipophilic carotenoid, is widely spread in red-colored fruits and vegetables such as tomato, watermelon, etc., presenting potent anti-inflammatory as well as anti-oxidant traits (high defense potential against the “second hit”), and showing ameliorative effects on obesity-related complications and the aberration of liver function [8,9]. Research also found that lycopene can effectively alleviate high-fat diet-induced insulin resistance and hepatic steatosis in mice, exerting high defense potential against the “first hit” [10,11]. Nevertheless, the accurate mechanism mediating lycopene’s anti-NAFLD action has not yet been fully elucidated. Of particular note, tomato powder in which lycopene is the major monomer component displaying considerable anti-NAFLD efficacy via restoring nicotinamide phosphoribosyl-transferase (NAMPT) and Sirtuin1 (Sirt1) activity [12]. Based on the above-mentioned

findings, we surmised that lycopene might counteract the “two-hit” that happened in NAFLD by triggering NAMPT/Sirt1. Herein, we introduced palmitate-induced lipid accumulation model in primary hepatocytes to validate lycopene’s counteracting effects on the “two-hit” and explore the possible mechanism focusing on NAMPT/Sirt1 pathway.

2. Materials and methods

2.1. Reagents and antibodies

HBSS buffer (Ca²⁺ and Mg²⁺ free), MTT, and PBS were obtained from KeyGEN BioTECH (Nanjing, China). Insulin-Transferrin-Selenium (ITS), L-Glutamine, HEPES, DMEM, and Willian’s Medium E were purchased from Gibco (Carlsbad, CA, USA). Fetal bovine serum (FBS) was procured from Hyclone (Boston, MA, USA). EGTA, and penicillin/streptomycin (100×) were acquired from Solarbio (Beijing, China). Collagenase, Type IV, dexamethasone, Oil Red O, and sodium palmitate (PA) were offered by Sigma-Aldrich (St Louis, MO, USA). Lycopene (HPLC ≥98%, BR, MW: 536.87, CAS: 502-65-8) was bought from MedChemExpress (Monmouth Junction, NJ, USA). TG kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Primary antibodies against Sirt1, NAMPT, SREBP-1c, ACC1, and FAS were acquired from Cell Signaling Technology (Beverly, MA, USA). Antibody specific against β-action was obtained from Proteintech (Wuhan, China).

2.2. Isolation and cultivation of mouse primary hepatocytes (MPHs)

MPHs were isolated from healthy ICR mice referring to Salem *et al.* established method with minor modification [13]. More specifically, mice were anesthetized with intraperitoneal injection of mixed anesthetic agents (80 mg/kg Ketamine plus 5 mg/kg Xylazine). Then, the mice were sterilized with 75% alcohol, transferred to an ultra-clean table, and fixed on an absorbent pad using sterile medical tape. Carefully make a “U” type incision in the abdomen, remove abdominal viscera to the left side, and completely expose the portal vein (PV) and inferior vena cava (IVC). An indwelling needle (24G) was applied to insert into PV at 1-1.5 cm of porta hepatis, prudently open the thoracic diaphragm, and clamp the thoracic IVC and hepatic artery using an artery clip. Buffer 1 (Ca²⁺ and Mg²⁺ free HBSS, 0.5 mM EGTA) and buffer 2 (Ca²⁺ and Mg²⁺ free HBSS, 0.04% Collagenase IV, 5 nM dexamethasone) were successively perfused into the liver at a flow rate of 6 mL/min for 7 min and 5 min, respectively. Following that, the liver was carefully resected, and placed into a 100-mm-diameter dish containing 20 mL of culture medium (Willian’s Medium E, ITS, 5 nM dexamethasone, 1 penicillin/streptomycin, 5% FBS). Strip the liver capsule and quickly shake it to produce the cell suspensions. To purify isolated MPHs, the cell suspensions were ordinally filtered through a 70-μm cell strainer, washed with DMEM twice (centrifugation: 50 g, 2 min, 4 °C), and centrifuged at 800 g for 2 min with 45% gradient percoll solution. Finally, MPHs were resuspended with culture medium, stained with Trypan blue, counted, and seeded into 6-well or 96-well plates at an appropriate density. After culturing for 4 h, the medium was replaced by fresh maintenance medium (Willian’s Medium E, ITS, 5 nM dexamethasone, 1×penicillin/streptomycin), and then MPHs can use for experiments.

2.3. MTT assay

The MTT assay was performed with reference to the previously reported method [14]. Briefly, MPHs were seeded into 96-well plates at a density of 1.2×10^4 cells/well, and cultivated overnight. Then, MPHs were co-incubated with lycopene (Lyc), palmitate (PA), or Lyc plus PA for 24 h. Following that, each well was added 10 μL of MTT solution (5 mg/mL), and then transferred into an incubator continuously incubated for 4 h at 37°C. Pipette the medium and add 150 μL of DMSO into each well, followed by shaking for 15 min to sufficiently dissolve formazan crystals. At last, absorbance was recorded at 490 nm with a multi-functional microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). Cell viability was measured with the following formula: cell viability (%) = $(OD_{\text{treatment}} - OD_{\text{blank}}) / (OD_{\text{control}} - OD_{\text{blank}}) \times 100\%$

2.4. Oil Red O staining

MPHs were seeded into 6-well plates at a density of 4×10^5 cells/well. Following interventions, MPHs were successively fixed in 4% paraformaldehyde for 15 min and stained with Oil Red O solution for 20 min, both at room temperature. Subsequently, MPHs were washed with isopropanol for 10s, followed by

counterstaining with hematoxylin for 3 min at room temperature. After being thoroughly cleaned, the images of stained MPHs were photographed using an optical microscope (Carl Zeiss, Jena, Germany).

2.5. ELISA and biochemical test

IL-6, IL-1 β and TNF- α levels in the supernatant of MPHs were determined by specific ELISA kits based on the manufacturer's protocol. TG content in MPHs was detected by a commercial biochemical kit following the manufacturer's instructions. Additionally, the protein concentration of MPHs was examined with a BCA Assay kit, and was used to normalize the TG level.

2.6. Western blotting

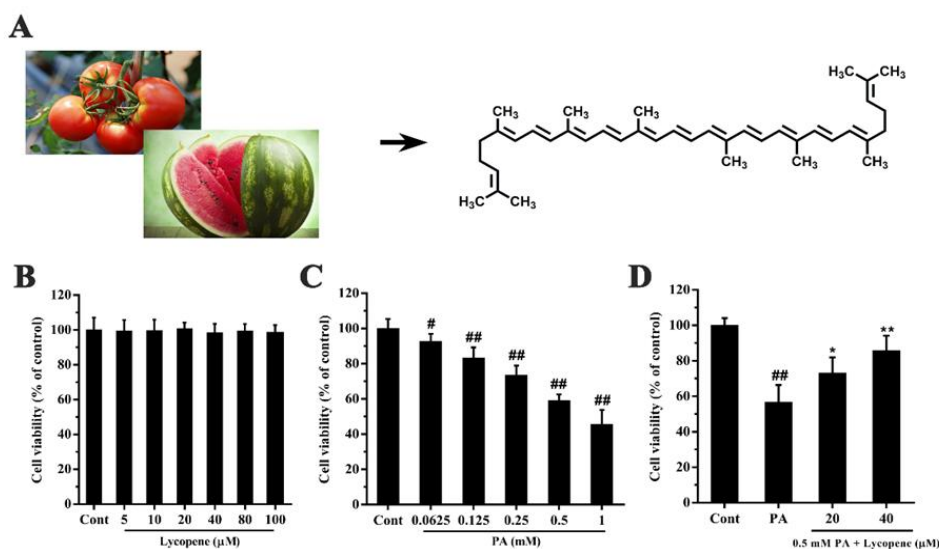
Western blotting was performed referring strictly to the online protocol of Cell Signaling Technology. Briefly, MPHs were lysed in chilled RIPA (containing protease inhibitor cocktail). Protein supernatant was acquired by centrifuging cell lysates at 12000 rpm for 15 min, followed by quantifying the protein concentration, adding the SDS-PAGE loading buffer, and boiling for 5 min to produce the protein samples. Equal amounts of protein were loaded into each well of 10% SDS-PAGE gel. Following electrophoresis, proteins were transferred onto the NC membranes, followed by blocking for 1 h at room temperature. Then the membranes were incubated with the primary antibodies with appropriate dilution overnight at 4°C. The next day, an IRDye® 800CW-conjugated second antibody was utilized to label the membranes for 1 h at room temperature. After being thoroughly washed, the membranes were put into the Azure Biosystems C600, and scanned to obtain the visible strips. Finally, the bands were quantitatively analyzed with the ImageJ software (National Institutes of Health).

2.7. Statistical analysis

All data were analyzed by GraphPad 8.0 (GraphPad Software, Inc.) and presented as the mean \pm SD. One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test was used to determine the statistical significance between the individuals. Values of $p < 0.05$ were considered statistically significant.

3. Results and analysis

3.1. Lycopene reverses PA-induced the decrease in MPHs viability



(A) Chemical structure of lycopene. (B) Cell viability of MPHs was measured with MTT when incubated with (B) lycopene, (C) PA, and (D) lycopene plus PA. Data were presented as mean \pm SD. * $P < 0.05$, ** $P < 0.01$ vs. Control (Cont); # $P < 0.05$, ## $P < 0.01$ vs. PA.

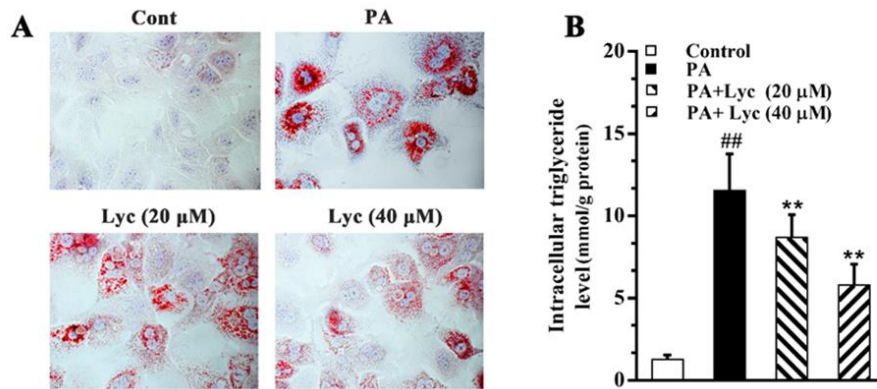
Figure 1: Lycopene alleviated PA-induced cytotoxicity.

First, MTT assay was performed to determine the safety concentration range of lycopene and optimal

model condition. As shown in Fig. 1B and C, no cytotoxicity was observed when incubated with lycopene at the doses of 5~100 μM for 24 h, while PA showed high cytotoxicity to MPHs and significant cytotoxicity was noted at the doses of ≥ 0.0625 mM. Based on the above results, PA at the dose of 0.125 mM and lycopene at the doses of 20, 40 μM were respectively selected as the model and intervention conditions. More importantly, Fig. 1D shows that under the model condition, lycopene dose-dependently rescued PA-induced the decrease in cell viability (20 μM , increased by $13.49 \pm 5.24\%$; 40 μM , increased by $25.37 \pm 4.65\%$), indicating the ameliorative effects of lycopene on hepatocellular damage happened in NAFLD.

3.2. Lycopene attenuated PA-induced MPHs steatosis

To investigate the effect of lycopene on the “first hit” in NAFLD (i.e., hepatosteatosis), lipid accumulation was determined by Oil O red staining and TG kit. As illustrated in Fig. 2A, lipid droplets were apparently increased in MPHs following PA challenge, while markedly alleviated in a dose-dependent manner after treatment with lycopene. Consistently, the increment of TG level in MPHs was also significantly reduced after lycopene treatment (20 μM , reduced by 2.87 ± 0.86 mmol/g.protein; 40 μM , reduced by 5.36 ± 1.43 mmol/g.protein). Collectively, lycopene effectively counteracts PA-evoked the “first hit” in MPHs.



(A) Oil Red O staining of MPHs. (B) TG levels in MPHs. Data were presented as mean \pm SD. ** $P < 0.01$ vs. Control; # $P < 0.01$ vs. PA.

Figure 2: Lycopene mitigated PA-induced lipid accumulation in MPHs.

3.3. Lycopene mitigated PA-induced inflammation in MPHs

Aggrandized inflammation is one of the major characteristics of the “two-hit” in NAFLD. Fig. 3 shows that compared with the control group, incubation with PA substantially augmented the levels of IL-6, IL-1 β and TNF- α (IL-6, increased by 105.01 ± 10.23 ng/L; IL-1 β , increased by 116.92 ± 12.63 ng/L; TNF- α , increased by 133.11 ± 16.32 ng/L). However, lycopene intervention could remarkably suppress these increased inflammatory cytokines in a dose-dependent manner (20 μM : IL-6, reduced by 14.56 ± 4.36 ng/L, IL-1 β , reduced by 17.69 ± 3.94 ng/L, TNF- α , reduced by 28.44 ± 6.28 ng/L; 40 μM : IL-6, reduced by 42.09 ± 5.87 ng/L, IL-1 β , reduced by 45.95 ± 8.29 ng/L, TNF- α , reduced by 61.31 ± 13.82 ng/L). Taken together, lycopene presents considerable anti-inflammation activity, that is, defense against the “second hit” in NAFLD.

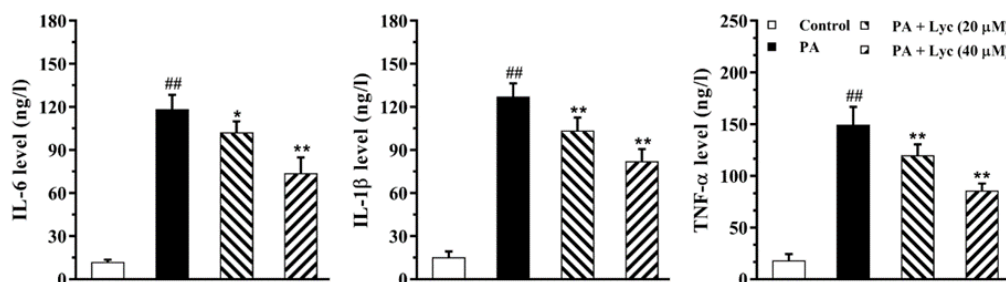
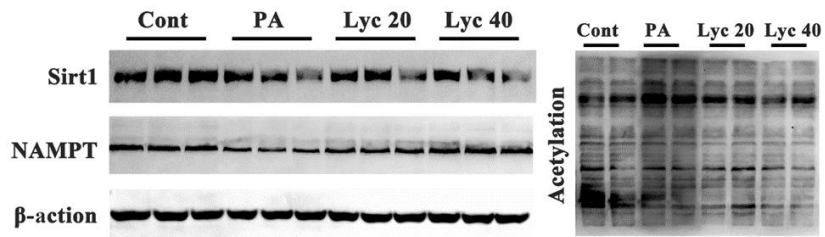


Figure 3: Lycopene alleviated PA-induced inflammation in MPHs. Levels of IL-6, IL-1 β , and TNF- α in cell supernatant. Data were presented as mean \pm SD. * $P < 0.05$, ** $P < 0.01$ vs. Control; # $P < 0.01$ vs. PA.

3.4. Regulating NAMPT/Sirt1-regulated lipogenesis might mediate lycopene’s favorable effects

To explore the involved mechanism underlying lycopene’s action, NAMPT/Sirt1-regulated lipogenesis pathway was investigated. As shown in Fig. 4 and Table 1, although lycopene did not affect PA-induced the decrease in Sirt1 expression, it could dose-dependently aggrandize the expression of NAMPT which had been reported showing the ability to indirectly activate Sirt1 through increasing the NAD⁺ pool [14]. Consistently, compared with the PA group, the acetylation levels in MPHs were significantly suppressed following lycopene treatment, indicating the activation of Sirt1. Sirt1 is a key modulator in regulating the homeostasis of lipid metabolism, including lipogenesis [15,16]. Accordingly, lipogenesis-related proteins (SREBP-1c, ACC1, and FAS) were examined. As demonstrated in Fig. 5 and Table 2, in contrast to the control group, PA challenge markedly increased the expression of SREBP-1c, ACC1, and FAS, which were significantly inhibited by lycopene treatment. Taken together, the potential action mechanism of lycopene might be through regulating NAMPT/Sirt1-regulated lipogenesis.



(A) Expression levels of Sirt1 and NAMPT in MPHs. (B) Acetylation levels in MPHs.

Figure 4: Effects of lycopene on NAMPT/Sirt1 pathway.

Table 1: Semiquantitative analysis of Sirt1, NAMPT and acetylation

Group	Sirt1	NAMPT	Acetylation
Cont	100±9.32	100±5.68	100±7.63
PA	67.04±7.65	46.42±4.99 ^{##}	312.23±20.11 ^{##}
Lyc20	70.02±10.32	67.89±7.32 ^{**}	188.45±18.72 ^{**}
Lyc40	66.99±8.92	95.14±6.65 ^{**}	110.78±15.65 ^{**}

^{**}P <0.01 vs. Control; ^{##}P <0.01 vs. PA.

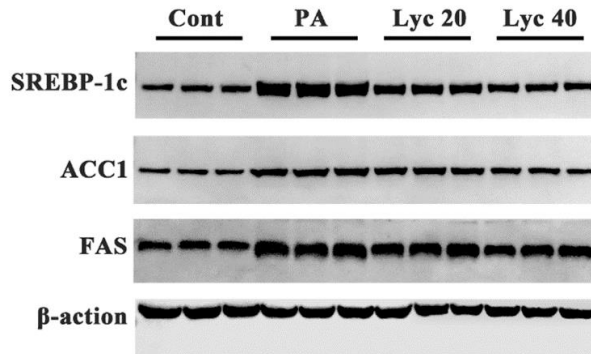


Figure 5: Effects of lycopene on the expression of lipogenesis-related proteins including SREBP-1c, ACC1 and FAS.

Table 2: Semiquantitative analysis of SREBP-1c, ACC1 and FAS

Group	SREBP-1c	ACC1	FAS
Cont	100±5.44	100±4.28	100±6.98
PA	467.34±18.45 ^{##}	265.36±21.14 ^{##}	378.48±10.99 ^{##}
Lyc20	257.46±19.89 ^{**}	200.11±14.67 ^{**}	259.45±15.78 ^{**}
Lyc40	145.67±15.27 ^{**}	156.23±17.34 ^{**}	189.49±13.77 ^{**}

^{**}P <0.01 vs. Control; ^{##}P <0.01 vs. PA.

4. Discussion

NAFLD is becoming a global health problem, afflicting nearly 200 million people. At the same time,

treatment NAFLD with natural products has been a research hotspot. Moreover, Li *et al.* found that tomato power ameliorated NAFLD via triggering NAMPT/Sirt1 pathway, and lycopene is the main component in tomato power [12]. Therefore, we assumed that lycopene harbored the anti-NAFLD efficacy might be through NAMPT/Sirt1 pathway. In the present study, our data showed that lycopene could effectively counteract PA-induced the “first hit” (i.e., steatosis) and the “second hit” (i.e., inflammation) in MPHs, and further exploration found that these beneficial effects were closely associated with NAMPT/Sirt1-regulated lipogenesis.

Sirt1, an NAD⁺-dependent deacetylase, plays an imperative role in tuning the homeostasis of lipid metabolism [17]. Extensive research indicated that activating Sirt1 presented appreciable ameliorative effects on high-fat diet-induced NAFLD [15,18]. Sirt1 activators or increase in Sirt1 expression (direct), or increment in NAD⁺ pool (indirect) all can boost the deacetylation activity of Sirt1, and show inhibitory action on lipogenesis [19,20]. Emerging evidence has been indicating that activation of NAMPT, a rate-limiting enzyme for NAD⁺ synthesis, can indirectly trigger Sirt1 through restoring NAD⁺ pool, showing comparable intervention effectiveness with Sirt1 activators in NAFLD treatment [16,21]. According, NAMPT is another prospective target in the management of NAFLD, and has gradually attracted the attention of scholars. In our study, we found that lycopene dose-dependently upregulated NAMPT expression and repressed the acetylation level in MPHs, which indicated the activation of Sirt1, and the reason of which might be due to increment of NAMPT catalytic product (i.e., NAD⁺). SREBP-1c is a key transcription factor downstream of Sirt1, responsible for modulating de novo lipogenesis-related proteins, such as ACC1 and FAS [22]. Our data showed that the expression of SREBP-1c, ACC1, and FAS was significantly inhibited following incubation with lycopene, which contributed to lycopene’s anti-steatosis effects. Collectively, lycopene can effectively impede the “first hit” in NAFLD through NAMPT/Sirt1-regulated lipogenesis. As

Inflammatory response is a critical promotor that impelled the progression from NAFLD to NASH, and a principal participant in the “second hit” [23]. Zheng *et al.* found that PA could induce a substantial increase in inflammatory cytokines in MPHs, participating in the “second hit” process [24]. In this study, our results showed that PA markedly augmented the levels of IL-6, IL-1 β , and TNF- α , consistent with the previously reported [24]. However, treatment with lycopene was sufficient to reduce the increased levels of IL-6, IL-1 β , and TNF- α , thereby effective defense against the “second hit”. As Sirt1 can deacetylate NF- κ B subunit, and inhibit its transcriptional activity for inflammatory cytokines, the anti-inflammation effects of lycopene might also be attributed to its action on NAMPT/Sirt1 pathway [25].

5. Conclusion

On the whole, lycopene could substantially ameliorate PA-induced lipid accumulation and inflammation in MPHs (i.e., the “two-hit”). Increment in the expression of NAMPT, and indirectly activating Sirt1 (i.e., NAMPT/Sirt1 pathway) might contribute to lycopene’s beneficial effects. Based on the above findings, we propose that lycopene is a promising candidate in NAFLD treatment, and holds good application prospects when in combination with Sirt1 activators.

References

- [1] Younossi ZM, Koenig AB, Abdelatif D, *et al.* Global epidemiology of nonalcoholic fatty liver disease- Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. 2016; 64: 73-84.
- [2] Yatsuji S, Hashimoto E, Tobari M, *et al.* Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. *J Gastroenterol Hepatol*. 2009; 24: 248-54.
- [3] Dong R, Yang X, Wang C, *et al.* Yangonin protects against non-alcoholic fatty liver disease through farnesoid X receptor. *Phytomedicine*. 2019; 53: 134-42.
- [4] Guo WW, Wang X, Chen XQ, *et al.* Flavonones from *Penthorum chinense* Ameliorate Hepatic Steatosis by Activating the SIRT1/AMPK Pathway in HepG2 Cells. *Int J Mol Sci*. 2018; 19.
- [5] Han X, Cui ZY, Song J, *et al.* Acanthoic acid modulates lipogenesis in nonalcoholic fatty liver disease via FXR/LXRs-dependent manner. *Chem Biol Interact*. 2019; 311: 108794.
- [6] Yang J, Fernández-Galilea M, Martínez-Fernández L, *et al.* Oxidative Stress and Non-Alcoholic Fatty Liver Disease: Effects of Omega-3 Fatty Acid Supplementation. *Nutrients*. 2019; 11.
- [7] Nassir F, Ibdah JA. Sirtuins and nonalcoholic fatty liver disease. *World J Gastroenterol*. 2016; 22: 10084-92.
- [8] Kawata A, Murakami Y, Suzuki S, *et al.* Anti-inflammatory Activity of β -Carotene, Lycopene and Tri-

- n*-butylborane, a Scavenger of Reactive Oxygen Species. *In Vivo*. 2018; 32: 255-64.
- [9] Ni Y, Zhuge F, Nagashimada M, et al. Lycopene prevents the progression of lipotoxicity-induced nonalcoholic steatohepatitis by decreasing oxidative stress in mice. *Free Radic Biol Med*. 2020; 152: 571-82.
- [10] Wang J, Suo Y, Zhang J, et al. Lycopene supplementation attenuates western diet-induced body weight gain through increasing the expressions of thermogenic/mitochondrial functional genes and improving insulin resistance in the adipose tissue of obese mice. *J Nutr Biochem*. 2019; 69: 63-72.
- [11] Zeng Z, He W, Jia Z, et al. Lycopene Improves Insulin Sensitivity through Inhibition of STAT3/Srebp-1c-Mediated Lipid Accumulation and Inflammation in Mice fed a High-Fat Diet. *Exp Clin Endocrinol Diabetes*. 2017; 125: 610-7.
- [12] Li CC, Liu C, Fu M, et al. Tomato Powder Inhibits Hepatic Steatosis and Inflammation Potentially Through Restoring SIRT1 Activity and Adiponectin Function Independent of Carotenoid Cleavage Enzymes in Mice. *Mol Nutr Food Res*. 2018; 62: e1700738.
- [13] Salem ESB, Murakami K, Takahashi T, et al. Isolation of Primary Mouse Hepatocytes for Nascent Protein Synthesis Analysis by Non-radioactive L-azidohomoalanine Labeling Method. *J Vis Exp*. 2018.
- [14] Zheng X, Zhang X-G, Liu Y, et al. Arjunolic acid from *Cyclocarya paliurus* ameliorates nonalcoholic fatty liver disease in mice via activating Sirt1/AMPK, triggering autophagy and improving gut barrier function. *Journal of Functional Foods*. 2021; 86: 104686.
- [15] Colak Y, Yesil A, Mutlu HH, et al. A potential treatment of non-alcoholic fatty liver disease with SIRT1 activators. *J Gastrointestin Liver Dis*. 2014; 23: 311-9.
- [16] Salomone F, Barbagallo I, Godos J, et al. Silibinin Restores NAD⁺ Levels and Induces the SIRT1/AMPK Pathway in Non-Alcoholic Fatty Liver. *Nutrients*. 2017; 9.
- [17] Chyau CC, Wang HF, Zhang WJ, et al. Antrodan Alleviates High-Fat and High-Fructose Diet-Induced Fatty Liver Disease in C57BL/6 Mice Model via AMPK/Sirt1/SREBP-1c/PPAR γ Pathway. *Int J Mol Sci*. 2020; 21.
- [18] Liu X, Gao Y, Li M, et al. Sirt1 mediates the effect of the heme oxygenase inducer, cobalt protoporphyrin, on ameliorating liver metabolic damage caused by a high-fat diet. *J Hepatol*. 2015; 63: 713-21.
- [19] Liou CJ, Lee YK, Ting NC, et al. Protective Effects of Licochalcone A Ameliorates Obesity and Non-Alcoholic Fatty Liver Disease Via Promotion of the Sirt-1/AMPK Pathway in Mice Fed a High-Fat Diet. *Cells*. 2019; 8.
- [20] Tian Y, Ma J, Wang W, et al. Resveratrol supplement inhibited the NF- κ B inflammation pathway through activating AMPK α -SIRT1 pathway in mice with fatty liver. *Mol Cell Biochem*. 2016; 422: 75-84.
- [21] Hua X, Sun DY, Zhang WJ, et al. P7C3-A20 alleviates fatty liver by shaping gut microbiota and inducing FGF21/FGF1, via the AMP-activated protein kinase/CREB regulated transcription coactivator 2 pathway. *Br J Pharmacol*. 2021; 178: 2111-30.
- [22] Moon YA, Hammer RE, Horton JD. Deletion of ELOVL5 leads to fatty liver through activation of SREBP-1c in mice. *J Lipid Res*. 2009; 50: 412-23.
- [23] Li CX, Gao JG, Wan XY, et al. Allyl isothiocyanate ameliorates lipid accumulation and inflammation in nonalcoholic fatty liver disease via the Sirt1/AMPK and NF- κ B signaling pathways. *World J Gastroenterol*. 2019; 25: 5120-33.
- [24] Zheng X, Zhao MG, Jiang CH, et al. Triterpenic acids-enriched fraction from *Cyclocarya paliurus* attenuates insulin resistance and hepatic steatosis via PI3K/Akt/GSK3 β pathway. *Phytomedicine*. 2020; 66: 153130.
- [25] Yeung F, Hoberg JE, Ramsey CS, et al. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *Embo j*. 2004; 23: 2369-80.