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Sour cherry ameliorates hepatic lipid synthesis in high-fat diet-induced obese mice via activation of adenosine monophosphate-activated protein kinase signaling

Songhee Ahn, Minseo Kim, and Hyun-Sook Kim

Department of Food and Nutrition, Sookmyung Women's University, Seoul 04310, Korea

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Correspondence to
Hyun-Sook Kim
Department of Food and Nutrition, Sookmyung Women's University, 100 Cheongpa-ro 47-gil, Yongsan-gu, Seoul 04310, Korea.
Tel: +82-2-710-9469
Email: hskim@sookmyung.ac.kr

ORCID iDs
Songhee Ahn
https://orcid.org/0000-0002-0127-0251
Minseo Kim
https://orcid.org/0000-0002-3116-9779
Hyun-Sook Kim
https://orcid.org/0000-0002-1095-3660

Conflict of Interest
There are no financial or other issues that might lead to conflict of interest.

ABSTRACT

Purpose: Sour cherry (*Prunus cerasus L.*) contains abounding phytochemicals, such as polyphenols and anthocyanins, and has antioxidative effects. Adenosine monophosphate-activated protein kinase (AMPK) is a crucial regulator in enhancing the lipid metabolism. This study hypothesized that the intake of sour cherry affects AMPK signaling. Therefore, this study examined whether sour cherry regulates AMPK to balance the hepatic lipid metabolism and exert ameliorating effects.
Methods: Male C57BL/6J mice had obesity induced with a 45% fat diet. The mice were divided into four groups: control (CON), high-fat diet (HFD), low percentage sour cherry powder (LSC), and high percentage sour cherry powder (HSC). The mice in the sour cherry groups were fed 1% sour cherry or 5% sour cherry in their respective diets for 12 weeks.
Results: The body weight, visceral fat weight, and lipid droplet size significantly decreased in the treatment groups. The serum and hepatic triglyceride and total cholesterol levels improved significantly in the HSC group. The low-density lipoprotein cholesterol levels were also reduced significantly, whereas the high-density lipoprotein cholesterol levels were increased significantly in both treatment groups. The sterol regulator binding protein-1c and fatty acid synthase expression levels as fatty acid synthesis-related enzymes were significantly lower in the treatment groups than in the high-fat diet group. Furthermore, the adipose triglyceride lipase and hormone-sensitive lipase expression levels as lipolytic enzyme activity and AMPK/acetyl-CoA carboxylase/carnitine palmitoyltransferase-1 as fatty acid β-oxidation-related pathway were upregulated significantly in both sour cherry groups.
Conclusions: These results show that sour cherry intake improves hepatic lipid synthesis and chronic diseases by activating AMPK signaling. Therefore, this study suggests that phytochemical-rich sour cherry can be developed as a healthy functional food.

Keywords: *Prunus cerasus*; phytochemicals; lipid metabolism; chronic diseases

INTRODUCTION

The prevalence of dyslipidemia has been on the rise among adults aged 30 and above over the past decade. In the current in Korea, one out of every four adults have hypercholesterolemia, and two out of every five adults have dyslipidemia [1,2]. Healthy dietary habits are crucial

for improving chronic diseases. Recently, nutritional studies have focused on phytochemical foods for improving human health. The consumption of abundant phytochemicals and low-energy-dense foods, such as fruits and vegetables, decreases the risk of metabolic diseases. Many studies have concluded that phytochemical-rich foods can prevent the risk of developing chronic diseases [3,4]. These results suggest that phytochemical foods may be useful in improving long-term human health. This evidence suggests that additional strategies, such as dietary intervention with efficacious phytochemical foods, may be promising for alleviating obesity-related outcomes in the long run.

Sour cherry (*Prunus cerasus L.*) has high polyphenol contents like anthocyanins, kaempferol, and quercetin glucosides [5]. Also, sour cherry possesses beneficial effects, including antioxidant activity, decreasing body weight and abdominal fat, and lowering blood lipid and fasting blood sugar levels [6,7]. Anthocyanins in sour cherries are phytochemicals that belong to a class of polyphenols. Related studies found that anthocyanins decrease metabolic markers connected with body weight gain, insulin resistance, adipocyte size, lipid secretion, triglyceride (TG) levels, cholesterol levels, low-density lipoprotein cholesterol (LDL-C) levels, and very low-density lipoprotein cholesterol levels [8]. Cyanidin 3-O-glucoside (C3G), a natural anthocyanin, exhibits beneficial effects on lipid metabolism, type 2 diabetes, and inflammation. In particular, C3G upregulates ATP binding cassette transporter G1 and ATP binding cassette subfamily A member 1 expression in human aortic endothelial cells related to mediate oxysterols efflux to high-density lipoprotein cholesterol (HDL-C) [9]. As sour cherries contain approximately 1.6 times higher total anthocyanins than sweet cherries [10], they might affect antioxidative reactions more strongly and regulate the activities of lipid metabolic enzymes and the proper course of chemical reactions in the body. In addition, scientific background mechanisms indicate that sour cherries can potentially improve lipid profiles. However, previous reports were unable to elucidate how the mechanism in the body of sour cherry treatment improved lipid metabolism. This study confirmed the mechanism in the body of sour cherries.

The liver plays a pivotal role in lipid biosynthesis during both fed and fasting states. In a fed state, the liver undergoes increased lipid biosynthesis due to the abundance of substrates in portal vein blood and elevated insulin levels resulting from beta cell stimulation. This process simultaneously inhibits fatty acid oxidation and endogenous glucose production. Within the liver, sterol regulator binding protein-1c (SREBP-1c) serves as a crucial regulator of lipid synthesis. Over-expression of SREBP-1c in the liver significantly upregulates genes associated with cholesterol synthesis and fatty acid synthase (FAS), leading to the accumulation of both cholesterol and TG [11].

Activation of adenosine monophosphate-activated protein kinase (AMPK) inhibits the activities of hepatic lipid synthesis enzymes, such as SREBP-1c and FAS. Suppression of SREBP-1c and FAS leads to a decline in lipid accumulation and obesity-related metabolic diseases. AMPK phosphorylation contributes to fat decomposition by activating the adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) [12,13]. Phosphorylated AMPK (p-AMPK) also promotes the phosphorylation of acetyl-CoA carboxylase (p-ACC) and carnitine palmitoyltransferase-1 (CPT-1), allowing the transport of fatty acyl-CoA to the mitochondria to burn energy [14]. AMPK can regulate metabolism and reduce the risk of dyslipidemia and other chronic diseases by balancing lipid reserves in the body [15]. We noted that inhibiting lipid synthesis factors and activating fatty acid β-oxidation properly is associated with protecting against the imbalance of lipid metabolism and chronic diseases.

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Thus, we aimed to explore the effects of sour cherries on AMPK activity, which may be helpful for hepatic lipid metabolism. Therefore, we established a steady obese mouse model and investigated the effects of sour cherry on lipid accumulation for a 12-week duration. Furthermore, body weight, serum and liver lipid profiles, lipolysis activity, lipid droplet size, and expression of β-oxidation- and fatty acid synthesis-related proteins were analyzed to establish lipid mechanism of the sour cherry.

METHODS

Animals and experimental design

A total 56 male C57BL/6J mice (5-week-old, weight, 24.96 ± 1.48 g) were obtained from Saeronbio Inc., (Gyeonggi-do, Korea). Mice were housed at a temperature of 21 ± 1°C, relative humidity 55 ± 5%, in a 12-hour light/dark cycle, with drinking water and food ad libitum. All experiments were conducted with the approval of the Sookmyung Women's University in compliance with the regulations (SMWU-IACUC-2005-005). Mice were randomly assigned to the following treatment groups (n = 14 per group):

1) CON: Control normal diet group
2) HFD: The HFD group
3) LSC: HFD fed with the 1% sour cherry powder group
4) HSC: HFD fed with the 5% sour cherry powder group

We used the AIN-93G diet (Research Diet, New Brunswick, NJ, USA) for the CON group and the D12451 diet (Research Diet, New Brunswick, NJ, USA) for the HFD group. The sour cherry diets of LSC and HSC contained 1% and 5% sour cherry powder in the D12451 diet (w/w), respectively. The contents of fat, carbohydrate, dietary fiber, and protein in the dried sour cherry powder (Pureunbin, Busan, Korea) were 0.35 g/100 g, 94.81 g/100 g, 0.38 g/100 g, and 0.58 g/100 g, respectively. The total energy of the CON diet was approximately 4.0 kcal/g, while that of the HFD, LSC, and HSC diets was approximately 4.7 kcal/g. Mice were fed their respective diets for 12 weeks. Food intake and body weight of all groups were recorded daily and weekly, respectively.

Phenolic contents of sour cherry

The total phenol content of sour cherry powder was measured using a modified Folin–Ciocalteu method [16]. Approximately 200 μL of sour cherry sample was added to 1 mL of 2% Na_2CO_3 and incubated at room temperature (25–30°C) for 3 minutes. Next, 200 μL of 50% Folin–Ciocalteu solution was added and reacted for 30 minutes at room temperature 25°C. The measurement was repeated thrice, and the mean value was used as the total phenolic content. The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per 100 g of sour cherry.

The total anthocyanin content of the sour cherry was measured using the pH differential method [17]. First, 500 μL of sour cherry solution at a specific concentration (0.1:1, w/v) was diluted with 1 mL of 0.025 M KCl buffer (pH 1.0) and 1 mL of 0.4 M CH3COONa buffer (pH 4.5) and reacted at 25–30°C room temperature for 15 minutes. The measurement of anthocyanins at 515 nm and 700 nm was repeated thrice, and the mean value was used as the total anthocyanin content. The total anthocyanin content was expressed as mg of C3G equivalents (C3GE, dry basis) per 100 g of sour cherry.

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Serum and tissue sample preparation

After 12 weeks of feeding, the animals were fasted for 12 hours before euthanasia with CO_2 gas. Blood samples were separated using the cardiac puncture method [18], and serum was centrifuged at 3,000 rpm at 4 °C for 30 minutes (Combi-450R, Hanil Co. Ltd., Seoul, Korea). The liver, kidney, and visceral fat were removed from each mouse, washed with saline, and weighed. The serum and tissues were immediately frozen in liquid nitrogen and stored at –70°C until analysis.

Hematoxylin and eosin (H&E) staining

After treating visceral fat tissues of each group with 10% formalin solution and staining them with H&E, the fat tissues they were observed under a microscope (BX41, Olympus, Shinjuku, Japan), and the lipid droplet size was measured using Image J software (Ver. 1.45s, National Institutes of Health, Bethesda, MD, USA).

Serum and hepatic lipid analysis

Serum TG, total cholesterol (TC), and HDL-C levels were evaluated using commercial kits (Asan Pharmaceutical, Hwaseong, Korea). LDL-C levels were measured using the Friedewald [19] formula, as shown below:

Serum LDL-C (mg/dL) = Serum TC (mg/dL) – [Serum HDL-C (mg/dL) + Serum TG (mg/dL)] × 0.2 (Equation 1)

Hepatic TG and TC levels were evaluated using a modified Folch method [20]. Next, 2 mL of chloroform/methanol (2:1, v/v) was added to 0.1 g of the liver tissue and homogenized. The homogenized solution was then mixed using a roller mixer (Digisystem Laboratory Instruments Inc., New Taipei, Taiwan) for 20 minutes and centrifuged for 5 minutes at 1,000 rpm at 4°C. After carefully removing the lower layer of the solution separated by centrifugation into a new tube, the chloroform was removed using a rotary vacuum evaporator (Sunileyela Co., Ltd, Seongnam, Korea). Hepatic TG and TC levels were measured using commercial kits (Asan Pharmaceutical). After homogenization at 37°C and mixing 3 mL of enzyme solution with 0.02 mL of serum and 0.02 mL of standard solution, absorbance was measured using an enzyme-linked immunosorbent assay (ELISA) reader (Epoch Microplate Spectrophotometer; BioTek Instruments, Winooski, VT, USA) within 1 hour.

ELISA

To examine the effects of lipolytic activity, ATGL and HSL in the liver were analyzed using ELISA kits (MyBioSource, San Diego, CA, USA) on a competitive enzyme immunoassay. First, 0.3 g of frozen liver tissue was washed, homogenized with 500 μL phosphate-buffered saline, and centrifuged at 4°C for 15 minutes at 5,000 rpm. The supernatant was used for the ATGL and HSL assays and added to the HRP (horseradish peroxidase) conjugate solution and polyclonal antibody (ATGL and HSL antibodies, respectively). The absorbance was measured at 450 nm using a spectrophotometer (Epoch Microplate Spectrophotometer; BioTek Instruments).

Western blotting analysis

The liver tissue (10 mg) was homogenized to extract proteins using PRO-PREP™ (iNtRON Biotechnology, Seongnam, Korea). The Bradford method was used with bovine serum albumin (iNtRON Biotechnology) as a standard to determine protein concentration [21]. The protein samples (30 μg/mL) were loaded onto 8–10% sodium dodecyl sulfate-polyacrylamide gel and transferred onto the polyvinylidene difluoride membranes (Merck Millipore,

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Burlington, MA, USA), blocked with 5% blocking buffer, and incubated for 1 hour at 4°C. The samples were incubated with primary AMPK (1:500, Cell Signaling Technology, Inc., Danvers, MA, USA), p-AMPKα (Thr172) (1:1,000, Cell Signaling Technology, Inc.), ACC (Ser79) (1:500, Cell Signaling Technology, Inc.), p-ACC (1:1,000, Cell Signaling Technology, Inc.), carnitine palmitoyl transferase-1 (D3B3) (1:1,000, Cell Signaling Technology, Inc.), FAS (C20G5) (1:1,000, Cell Signaling Technology, Inc.), and sterol regulatory element-binding protein-1c (1:1,000, Abcam, Cambridge, UK) overnight. Glyceraldehyde-3-phosphate dehydrogenase (1:3,000, GeneTex, Inc., Irvine, CA, USA) was used for normalization. The secondary antibody was incubated at 4°C for 2 hours using anti-rabbit immunoglobulin G horseradish peroxidase-linked antibodies (Cell Signaling Technology, Inc.). An image analyzer (AmershamTM Imager 600; GE Healthcare, Chicago, IL, USA) was used to visualize the protein bands, and quantification was performed using the ImageJ software (version 1.45s; National Institutes of Health).

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics software (version 25.0; IBM Corp., Armonk, NY, USA). Results are expressed as the mean ± standard deviation. All data from each group were compared using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Total phenol and anthocyanin contents of sour cherry, food intake and food efficiency ratio

Total phenol and anthocyanin contents in sour cherry were determined to be 264.27 mg GAE/100 g and 89.97 mg CGE/100 g, respectively. The results of food intake are shown in **Table 1**. Sour cherry did not affect food intake, with no significant difference in food intake (g/day) between groups ($p = 0.686$). The FER (%) in the CON and HSC groups was significantly lower than that in the HFD and LSC groups ($p < 0.001$).

Effects of sour cherry powder on body and organ weights

The results of body and organ weights are shown in **Fig. 1**, **Table 2**. The initial weight (g) of all the groups was not different ($p = 0.431$). However, the final body weight of the LSC group decreased by 2.8%, while that of the HSC group significantly decreased by 11% compared to that of the HFD group ($p < 0.001$). Body weight changes (g) were greater in the HSC group than the HFD group ($p < 0.001$). Kidney weight (g) did not differ between the groups. The liver weight (g) of the HSC group was significantly higher than that of the HFD group ($p < 0.001$), while the visceral fat weight (g) of the HSC group was decreased compared to that of the HFD group ($p < 0.01$).

Table 1. Food intake and food efficiency ratio

Variables	CON	HFD	LSC	HSC
Food intake (g/day)	2.74 ± 0.23^{ns}	2.67 ± 0.37	2.71 ± 0.39	2.73 ± 0.33
FER (%)	3.22 ± 0.88^{b}	6.09 ± 1.76^{a}	5.53 ± 1.44^{a}	4.12 ± 1.20^{b}

Data are presented as the mean ± standard deviation (n = 14 each). All data from each group were compared using one-way analysis of variance (ANOVA). Different letters (a > b > c) with a column indicate significant differences ($p < 0.05$) as determined by Tukey's post hoc tests (ns: not significant).
CON, control normal diet group; HFD, high-fat diet group; LSC, high-fat diet + 1% sour cherry powder group; HSC, high-fat diet + 5% sour cherry powder group; FER, food efficiency ratio.
FER = Total Body Weight Gain (g)/Total Intake of Food (g) × 100.

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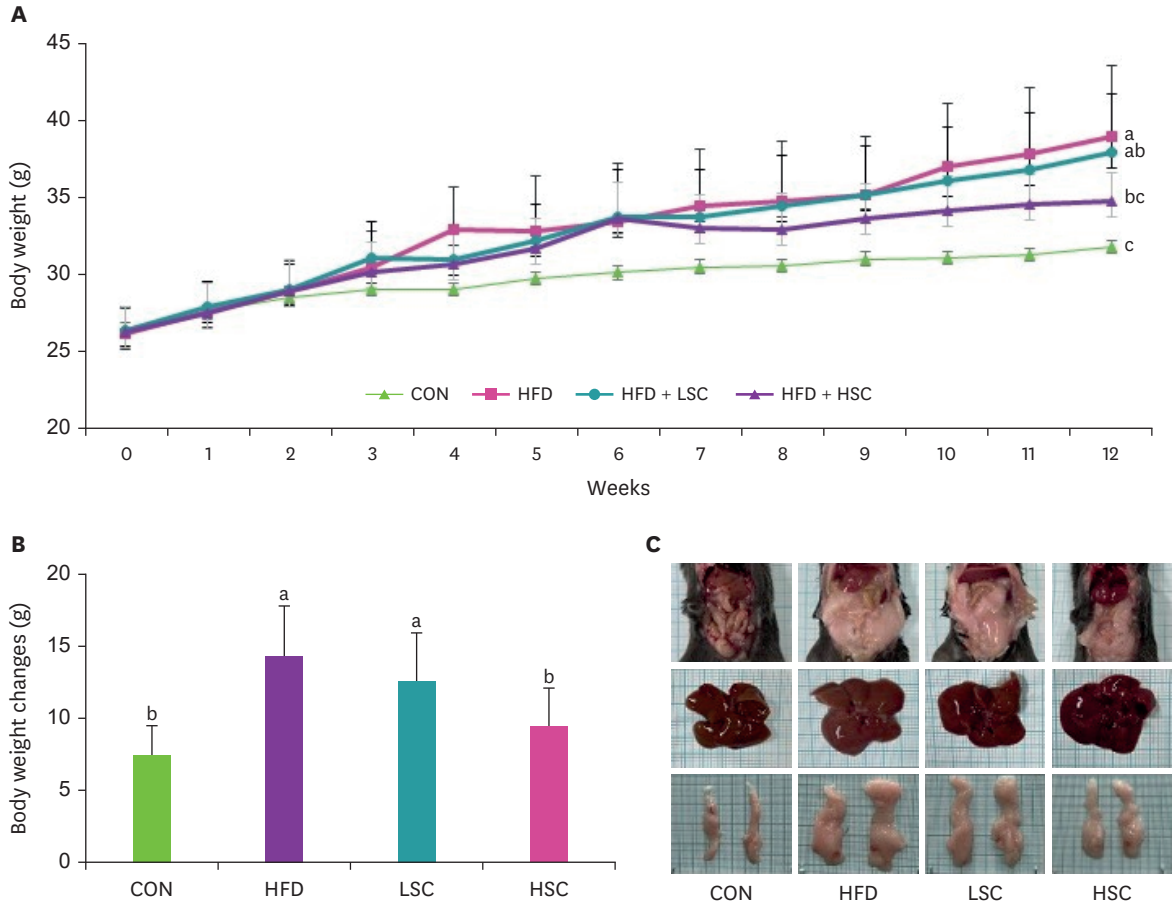

Fig. 1. Effects of sour cherry powder on body and organ weights.
(A) body weight (g), (B) body weight changes (g) during the experimental period, and (C) the representative images of abdominal laparotomy, liver, and visceral fat of each group were presented.
Data are presented as the mean ± standard deviation (n = 14 each). All data from each group were compared using one-way analysis of variance (ANOVA). Different letters (a > b > c) with a column indicate significant differences ($p < 0.05$) as determined by Tukey's post hoc tests.
CON, control normal diet group; HFD, high-fat diet group; LSC, high-fat diet + 1% sour cherry powder group; HSC, high-fat diet + 5% sour cherry powder group.

Table 2. Effects of sour cherry powder on body and organ weights

Variables	CON	HFD	LSC	HSC
Body weight				
Initial body weight (g)	25.46 ± 1.68^{ns}	26.22 ± 1.57	26.36 ± 1.60	26.29 ± 1.63
Final body weight (g)	31.75 ± 2.88^{c}	39.04 ± 4.60^{a}	37.96 ± 3.83^{ab}	34.76 ± 1.91^{bc}
Body weight changes (g)	7.44 ± 2.04^{b}	13.62 ± 3.75^{a}	12.59 ± 3.33^{a}	9.43 ± 2.74^{b}
Organ weight				
Kidney (g)	0.37 ± 0.04^{ns}	0.43 ± 0.08	0.39 ± 0.04	0.40 ± 0.04
Liver (g)	1.06 ± 0.12^{a}	1.19 ± 0.12^{b}	1.12 ± 0.12^{b}	1.08 ± 0.11^{ab}
Visceral fat (g)	0.94 ± 0.28^{c}	2.26 ± 0.57^{a}	2.18 ± 0.52^{a}	1.55 ± 0.58^{b}

Data are presented as the mean ± standard deviation (n = 14 each). All data from each group were compared using one-way analysis of variance (ANOVA). Different letters (a > b > c) with a column indicate significant differences ($p < 0.05$) as determined by Tukey's post hoc tests (ns: not significant).
CON, control normal diet group; HFD, high-fat diet group; LSC, high-fat diet + 1% sour cherry powder group; HSC, high-fat diet + 5% sour cherry powder group.

Effects of sour cherry powder on the visceral coefficient and lipid droplet size

The visceral coefficient (%) showed similar results as visceral weight (g) (**Fig. 2**). The level in the HFD group was higher than that in the CON group, while the level in the HSC group was

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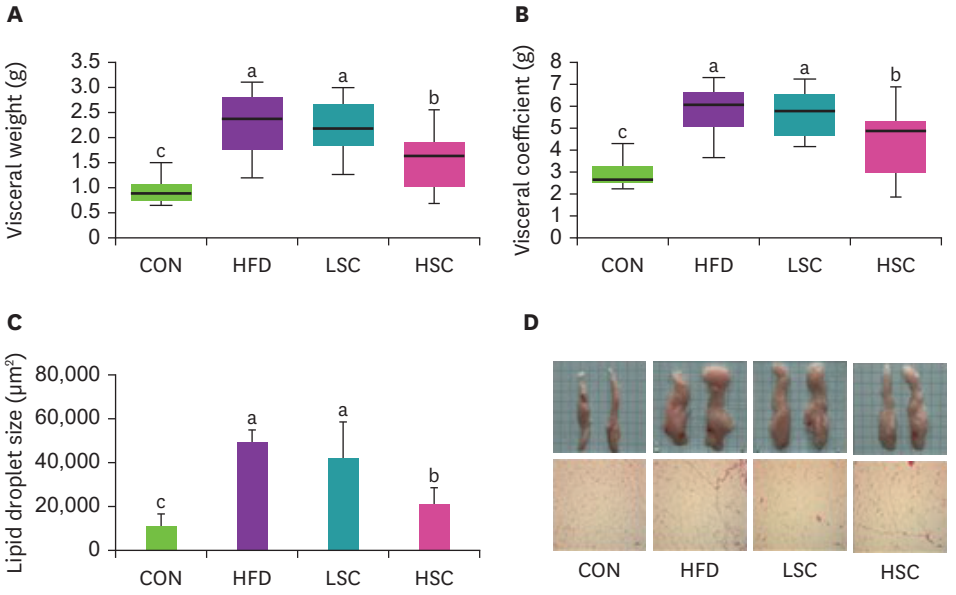

Fig. 2. Effects of sour cherry powder on the visceral coefficient and lipid droplet size.
(A) visceral weight (g), (B) visceral coefficient (%), (C) lipid droplet size (μm²), and (D) the representative images of overall morphology and hematoxylin and eosin staining on adipose tissue were presented.
Data are presented as the mean ± standard deviation (n = 14 each). All data from each group were compared using one-way analysis of variance (ANOVA). Different letters (a > b > c) with a column indicate significant differences ($p < 0.05$) as determined by Tukey's post hoc tests.
CON, control normal diet group; HFD, high-fat diet group; LSC, high-fat diet + 1% sour cherry powder group; HSC, high-fat diet + 5% sour cherry powder group.

lower than that in the HFD group ($p < 0.001$). Furthermore, the lipid droplet size was like the visceral coefficient (%). The HFD group was significantly larger than the CON group, and the HSC group was smaller than the HFD group ($p < 0.001$).

Effects of sour cherry powder on serum and hepatic lipid levels

The serum and hepatic lipid levels are shown in **Fig. 3**. The HFD group showed significantly different serum and hepatic parameters than the CON group ($p < 0.001$). After providing sour cherry for 12 weeks, in the case of the LSC group, serum TG and TC levels tended to decrease, while serum HDL-C levels revealed a significantly increased compared with the HFD group ($p < 0.01$). The HSC group prominently showed a significant decrease in serum TG and TC levels compared with the HFD group ($p < 0.001$), whereas serum HDL-C levels were significantly increased ($p < 0.01$). Hepatic TG, TC were higher in the HFD group than those in the CON group ($p < 0.001$). In contrast, hepatic TG and TC levels in both sour cherry groups were significantly lower than those in the HFD group ($p < 0.001$).

Effects of sour cherry powder on hepatic SREBP-1c and FAS protein expression

The protein expression levels of hepatic SREBP-1c and FAS in the liver were also examined, and the results are presented in **Fig. 4**. SREBP-1c and FAS protein expression in the HFD group was significantly upregulated compared to that in the CON group ($p < 0.001$). Both the sour cherry groups showed significantly suppressed SREBP-1c expression compared with the HFD group (-56 and -59%, respectively) ($p < 0.001$). In addition, FAS expression in the sour cherry groups (LSC, -60% and HSC, -66%) was significantly downregulated compared to that in the HFD group ($p < 0.001$).

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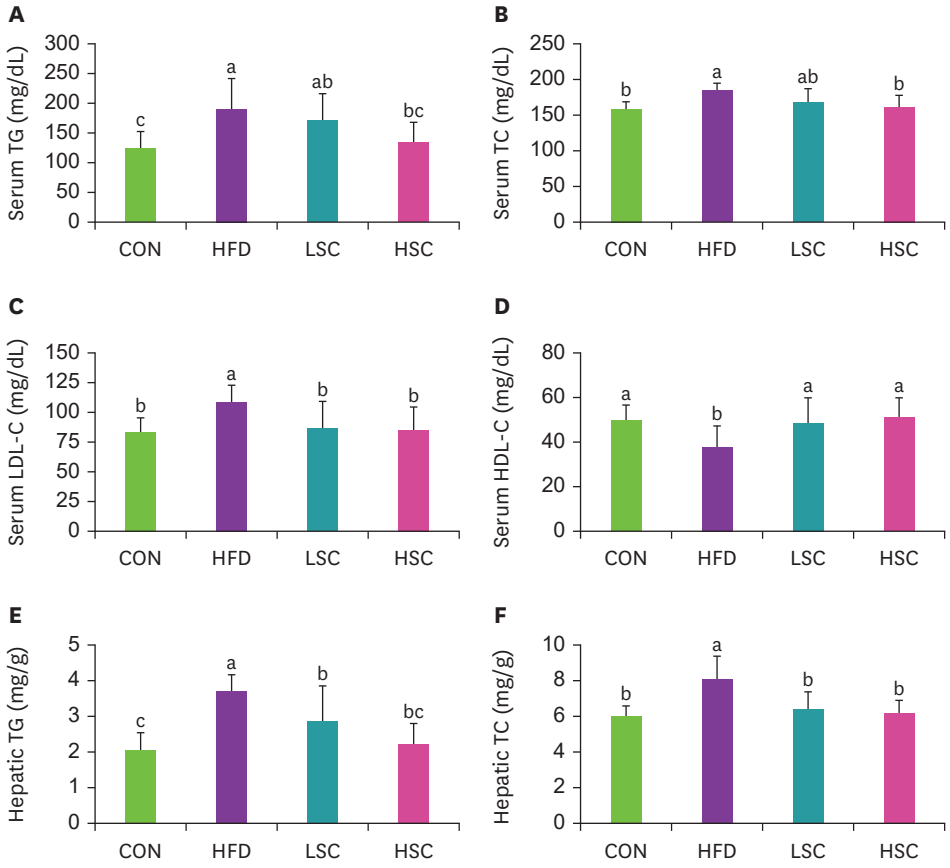

Fig. 3. Effects of sour cherry powder on serum and hepatic lipid levels.
(A) serum TG (mg/dL), (B) serum TC (mg/dL), (C) serum LDL-C (mg/dL), (D) serum HDL-C (mg/dL), (E) hepatic TG (mg/g) and (F) hepatic TC (mg/g) were measured and compared among experimental groups.
Data are presented as the mean ± standard deviation (n = 14 each). All data from each group were compared using one-way analysis of variance (ANOVA).
Different letters (a > b > c) with a column indicate significant differences ($p < 0.05$) as determined by Tukey's post hoc tests.
CON, control normal diet group; HFD, high-fat diet group; LSC, high-fat diet + 1% sour cherry powder group; HSC, high-fat diet + 5% sour cherry powder group; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

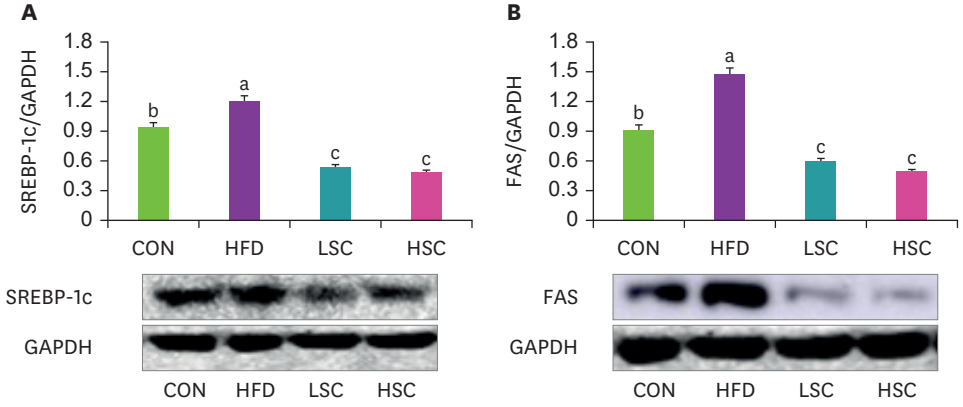

Fig. 4. Effect of sour cherry powder on expression of hepatic lipogenesis-related proteins.
The protein expression of (A) SREBP-1c and (B) FAS were presented.
Data are presented as the mean ± standard deviation (n = 14 each). All data from each group were compared using one-way analysis of variance (ANOVA).
Different letters (a > b > c) with a column indicate significant differences ($p < 0.05$) as determined by Tukey's post hoc tests.
CON, control normal diet group; HFD, high-fat diet group; LSC, high-fat diet + 1% sour cherry powder group; HSC, high-fat diet + 5% sour cherry powder group; SREBP-1c, sterol regulator binding protein-1c; FAS, fatty acid synthase.

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Effects of sour cherry powder on hepatic lipolysis and p-AMPK/p-ACC/CPT-1 protein expression

Hepatic ATGL, and HSL activities were lower in the HFD group than those in the CON group ($p < 0.001$), while in both sour cherry groups were significantly activated than those in the HFD group ($p < 0.001$) (**Fig. 5**). The hepatic p-AMPK, p-ACC, and CPT-1 protein expression were significantly lower in the HFD group than in the CON group ($p < 0.001$). The sour cherry groups showed significantly upregulated p-AMPK/AMPK protein expression ratio in a dose-dependent manner compared with the HFD group ($p < 0.001$). Compared to the HFD group, the ratio of p-ACC/ACC protein expression ratio of the sour cherry group significantly increased to 102% and 183%, respectively ($p < 0.001$). CPT-1 protein expression in sour cherry supplement groups showed the remarkably ascending CPT-1 protein expression in HSC (+613%) and LSC (+363%) groups, respectively ($p < 0.001$) (**Fig. 5**).

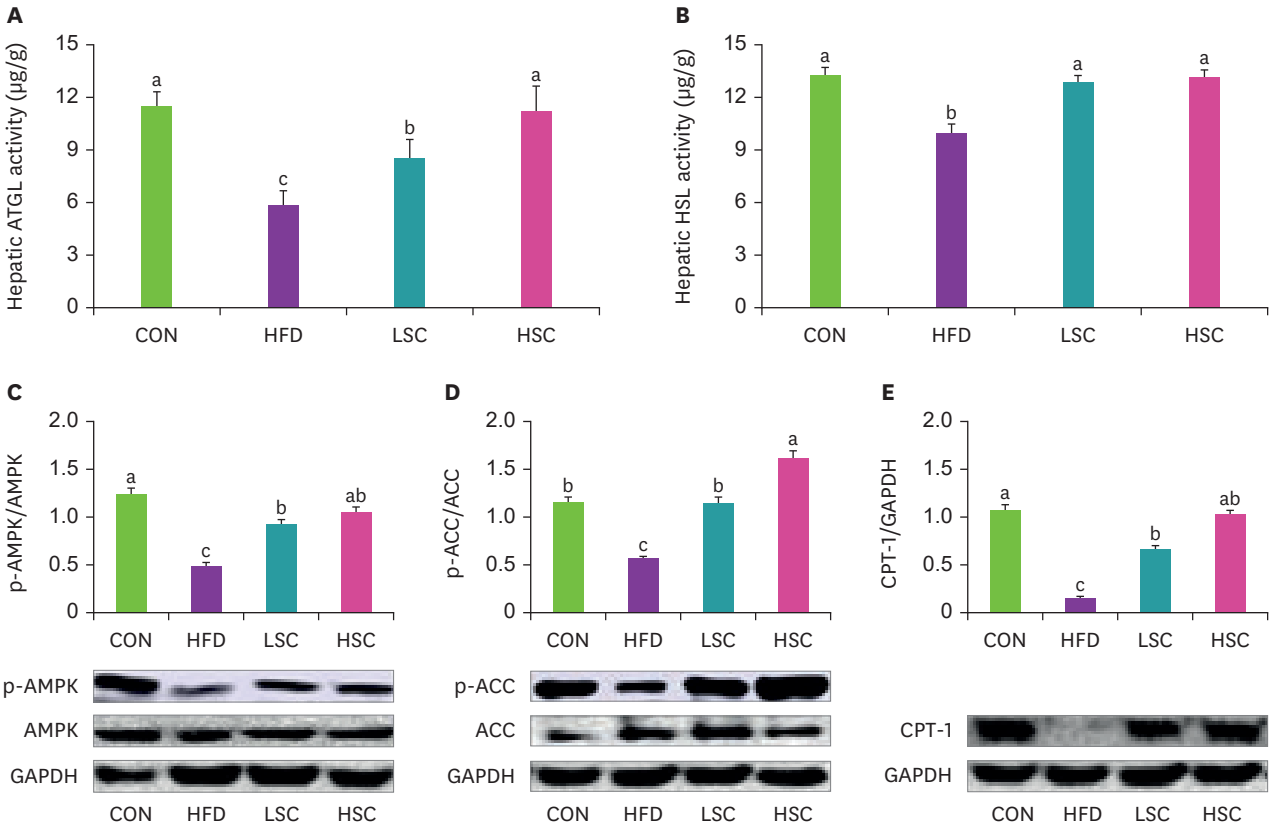

Fig. 5. Effects of sour cherry powder on hepatic lipolysis and ß-oxidation-related proteins.
(A) hepatic ATGL activity (μg/g), (B) hepatic HSL activity (μg/g), the protein expression of (C) p-AMPK, (D) p-ACC and (E) CPT-1 were presented.
Data are presented as the mean ± standard deviation (n = 14 each). All data from each group were compared using one-way analysis of variance (ANOVA). Different letters (a > b > c) with a column indicate significant differences ($p < 0.05$) as determined by Tukey's post hoc tests.
CON, control normal diet group; HFD, high-fat diet group; LSC, high-fat diet + 1% sour cherry powder group; HSC, high-fat diet + 5% sour cherry powder group; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; p-AMPK, phosphorylated adenosine monophosphate-activated protein kinase; AMPK, adenosine monophosphate-activated protein kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; p-ACC, phosphorylated acetyl-CoA carboxylase; ACC, acetyl-CoA carboxylase; CPT-1, carnitine palmitoyltransferase-1.

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DISCUSSION

Our study showed that the intake of sour cherries had a positive impact on the regulation of lipid metabolism in mice with HFD-induced obese mice of a 12-week period. According to chronic disease studies, a HFD or an unbalanced diet impacts chronic diseases, particularly related to the absorption of gut lipids and the formation of adipose tissue [22]. HFD induces excessive energy levels, hence, increased body weight, lipid droplet size, and imbalance in hepatic lipid metabolism. In contrast, this study showed that the phytochemical-rich sour cherry improved the lipid profiles, activated AMPK to increase hepatic lipolysis and fatty acid β-oxidation, and reduced body weight and fatty acid synthesis to prevent fat accumulation in the HFD-fed obese mice.

In vivo and in vitro studies using dietary anthocyanins have confirmed chronic disease improvement by applying a 5–200 mg anthocyanin/kg diet [5,23–25]. Based on the studies, we applied that the LSC group and the HSC group were fed 8.8 mg, and 43.7 mg of cyanidin-3-glucoside, respectively. Moreover, our previous study revealed that sour cherries have antioxidant activities to be 56.95% of 1,1-diphenyl-2-picrylhydrazyl radical scavenging and 30.04% of 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid using two most common radical scavenging assays. Therefore, the experiment was conducted by confirming the possibility of a positive effect on the lipid-associated mechanisms in the body by taking sour cherry.

We verified that the final body weight was approximately 2.8% and 11% lower in taking the 1%, 5% sour cherry groups, respectively ($p < 0.001$) than in the HFD group. Interestingly, body weight changes, visceral weight, and lipid droplet size showed the same tendency. Especially, in the HSC group, significantly decreasing visceral fat weight (g) ($p < 0.01$) as well as the lipid droplet size was significantly reduced ($p < 0.001$). Several studies have reported the relationship between visceral fat, lipid droplet size, and chronic disease risk [26,27]. These results suggest that sour cherry supplementation for 12 weeks in HFD-induced obese mice effectively affected the weight loss rate, decreasing the visceral weight and lipid droplet size. Thus, the intake of sour cherry for 12 weeks positively affected HFD-induced obesity in mice and may positively influence chronic diseases by improving the lipid metabolism in the body.

In the results of the dyslipidemia-related serum biomarkers, the 5% sour cherry intake group showed significantly reduced serum TG and TC levels compared with the HFD group ($p < 0.001$). Serum HDL functions to protect from cardiovascular disease risks and is measured from the cholesterol efflux capacity and HDL enzyme activity; HDL is an essential antioxidant and protects the endothelium [28]. A previous study reported that phytochemical-rich fruits exert anti-atherosclerotic effects by improving HDL function and dyslipidemia [29]. HDL-C is a bottom line for improving lipid metabolism because it can downregulate LDL-C. Our results also showed improved serum lipid levels related to dyslipidemia, particularly improved serum HDL-C levels. The effects of C3G in rich sour cherries are considered to be attributed to its mediation of oxysterol efflux to HDL-C by C3G.

Increased hepatic TG is stored as lipid droplets during these processes, resulting in the development of non-alcoholic fatty liver disease (NAFLD) and other chronic diseases [30]. In our study, the hepatic TG and TC levels were higher in the HFD group than those in the CON group, whereas those in the sour cherry-supplemented groups were significantly lower than those in the HFD group ($p < 0.001$). Some studies have reported that dietary phytochemicals reduce hepatic TG and TC levels, consistent with the results of our research. Based on

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the results, we suggest that sour cherry exerts a positive effect on NAFLD-related chronic diseases by regulating lipid metabolism.

This study showed that sour cherry supplementation significantly suppressed hepatic SREBP-1c and FAS protein expression ($p < 0.001$). SREBP-1c regulates the expression of ACC and synthesis from malonyl-CoA and FAS, increases the condensation of malonyl-CoA, and produces palmitate [11,31]. Palmitate induces synthesizing de novo lipogenesis (DNL) endogenously. DNL is associated with an imbalance of lipid metabolism because DNL synthesizes fatty acids independent of nutrient availability and hormones. Suppression of SREBP-1c and FAS leads to a decline in lipid accumulation and obesity-related metabolic diseases [32]. Similarly, anti-obesity study has reported that the effects of dietary phytochemicals occur via various mechanisms, including a decrease in expression of SREBP-1c, and FAS [33]. Therefore, sour cherry treatment may suppress lipid accumulation by reducing the levels of proteins involved in fatty acid synthesis.

Our results showed both sour cherry groups were significantly upregulated hepatic ATGL and HSL activities compared with the HFD group ($p < 0.001$). ATGL catalyzes the first step of intracellular lipolysis to mobilize triacylglycerol (TAG) stores via catecholamines. HSL catalyzes diacylglycerol to monoacylglycerol. In other words, ATGL and HSL are related to decreasing TAG levels [13,34]. This study confirmed the therapeutic effects of sour cherry supplementation on TAG accumulation in obese mice via ATGL and HSL activity in the liver.

AMPK leads to activating ATGL and HSL, as well as promotes fatty acid β-oxidation by phosphorylating ACC and stimulating the activity of CPT-1. Hepatic p-AMPK activation also inhibits the expression of SREBP-1c, which reduces lipid accumulation and lipogenesis [11-13]. Dietary phytochemicals are potential regulators of AMPK activity [35]. Our results showed that the expression levels of p-AMPK, p-ACC, and CPT-1 were significantly reduced in the HFD group ($p < 0.001$). These results suggest that dyslipidemia and obesity can deteriorate fatty acid β-oxidation. However, the p-AMPK/p-ACC/CPT-1 signal was activated in the sour cherry treatment groups, especially in the 5% sour cherry intake group, showing a significant upregulation ($p < 0.001$). Thus, we can infer that the regular intake of sour cherry positively affected hepatic lipolysis and fatty acid β-oxidation via AMPK signals (**Fig. 6**).

We found that phytochemical-rich sour cherry reduced body weight, visceral weight, and lipid droplet size, improved serum and hepatic lipid concentrations, and activated lipolytic enzymes. These results indicate that activation of AMPK signaling induced hepatic lipolysis and fatty acid β-oxidation and inhibited fatty acid synthesis. Notably, the most significant effect was observed in the 5% sour cherry group containing 43.7 mg of C3G.

Further studies are needed to reveal the positive effects of oxidative stress by detailed mechanisms. Because oxidative stress is strongly related to obesity, dyslipidemia, and other chronic diseases in the body. However, we only examined the hepatic lipid metabolism via AMPK signaling. Also, a more extensive investigation into the various phytochemical components is imperative to gain a coherent understanding of improving the lipid metabolism effects and therapeutic potential of sour cherries.

Our findings highlight the potential of sour cherry in preventing dyslipidemia, obesity, and related chronic diseases via AMPK signaling. These results can be helpful in selecting and developing a variety of fruit forms, such as healthy functional foods, for future studies.

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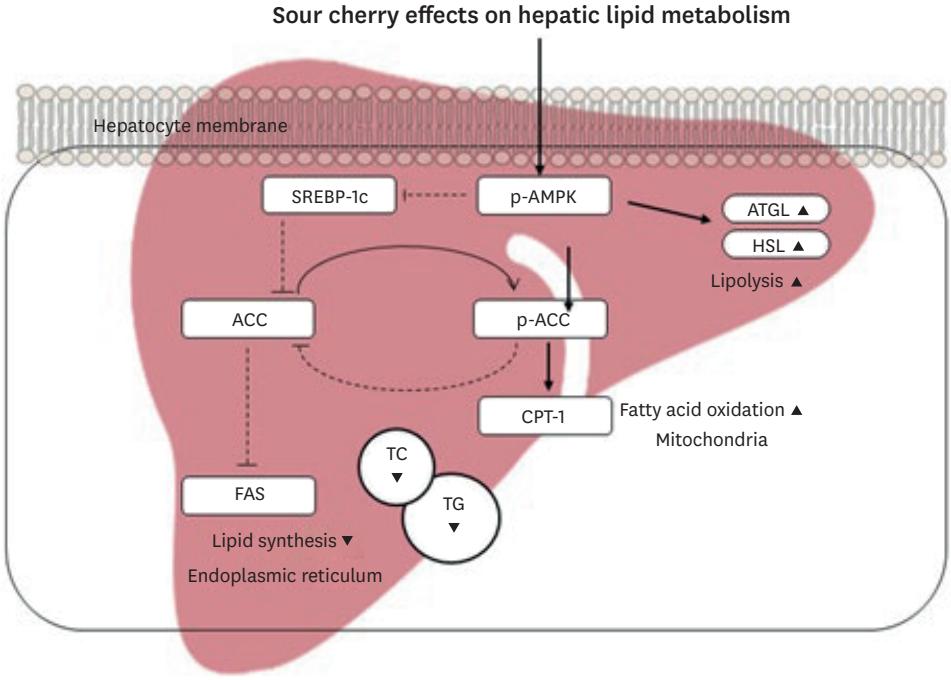

Fig. 6. Effects of sour cherry powder on hepatic lipid metabolism.
SREBP-1c, sterol regulator binding protein-1c; p-AMPK, phosphorylated adenosine monophosphate-activated protein kinase; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; ACC, acetyl-CoA carboxylase; p-ACC, phosphorylation of acetyl-CoA carboxylase; CPT-1, carnitine palmitoyltransferase-1; FAS, fatty acid synthase; TC, total cholesterol TG, triglyceride.

SUMMARY

We confirmed that the treatment of sour cherry powder can alleviate lipid metabolism imbalance and symptoms of dyslipidemia in HFD-induced obese mice. Although we did not assess oxidative stress markers related to chronic diseases such as obesity and dyslipidemia, the reduction in intra-visceral fat weight and lipid concentrations, along with the alleviation of AMPK signaling, suggests potential benefits in mitigating chronic conditions like obesity and dyslipidemia. Further investigation is warranted to examine oxidative stress indicators in these conditions.

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