Hawthorn Fruit Attenuates Atherosclerosis by Improving the Hypolipidemic and Antioxidant Activities in Apolipoprotein E-Deficient Mice

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Aims: The protective effects of hawthorn fruit against atherosclerosis and the potential underlying mechanisms of this fruit in improving the hypolipidemic and antioxidant activities were investigated in apolipoprotein E-deficient (apoE−/−) mice.

Methods: ApoE−/− mice were divided into a control group (n = 10) and hawthorn fruit group (n = 10). The mean size of the lesions in the aortic root was assessed, and the serum glucose levels, insulin levels, lipid profiles, total antioxidant capacity (T-AOC) and superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) activities were measured. The mRNA levels of hepatic genes related to lipid metabolism and antioxidant enzymes were examined.

Results: The hawthorn fruit group mice developed significantly decreased (p < 0.05) atherosclerotic lesions. The levels of serum lipids decreased significantly (p < 0.05) and the levels of cholesterol/triglycerides, including very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), decreased in the hawthorn fruit group. The hawthorn fruit mice exhibited significantly increased T-AOC values and SOD and GSH-PX activities (p < 0.05). The hepatic fatty acid synthase (FAS) and sterol regulatory element binding protein-1c (SREBP-1c) mRNA levels were reduced by 42% (p < 0.05) and 23% (p < 0.05) in the mice fed the hawthorn fruit diet compared with that observed in the mice fed a standard diet. However, the hepatic hydroxymethylglutaryl CoA reductase (HMG-CoAR) mRNA levels showed no significant differences between the two groups. The mRNA expression levels of the antioxidant enzymes (SOD1, SOD2, Gpx3) were higher (p < 0.05) in the livers of the hawthorn fruit diet mice compared with those observed in the control mice.

Conclusions: Hawthorn fruit exerts a protective effect against atherosclerosis in apoE−/− mice by improving the hypolipidemic and antioxidant activities.


Key words: Hawthorn fruit, Atherosclerosis, Apolipoprotein E-deficient mouse
on, hawthorn fruit has been used to treat the early stages of congestive heart failure and angina pectoris. Moreover, the chemical composition of hawthorn fruit has been the subject of extensive studies. Flavonoids and proanthocyanidins, major ingredients of hawthorn fruit, are responsible for the fruit’s pharmacological activity. Nevertheless, the effects of hawthorn fruit in preventing cardiovascular diseases remain unknown.

Studies have demonstrated that the development of atherosclerotic lesions is associated with the serum lipid levels, especially the plasma cholesterol and triglyceride levels. Hawthorn fruit is beneficial to the cardiovascular system, partially due to its effects on serum cholesterol. Previous reports have shown that the consumption of hawthorn fruit decreases the serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels in hyperlipidemic humans and rats fed a hyperlipidemic diet. Experiments using New Zealand white rabbits and hamsters as animal models have also confirmed that hawthorn fruit consumption decreases serum cholesterol. The intake of hawthorn fruit also decreases the serum levels in mice fed a high-fat diet. Therefore, the consumption of hawthorn fruit may reduce the risk of cardiovascular disease.

Atherosclerosis or “hardening of the arteries” is a complex chronic disease that results from the interactions of a variety of factors. An imbalance of oxidation and antioxidation is one of the primary causes of atherosclerosis. LDL present in the vascular endothelium is oxidized to ox-LDL. The oxidative modification of human LDL as an independent risk factor for atherosclerosis is believed to play an important role in the development of atherosclerosis. Decreasing the production of ox-LDL by inhibiting LDL oxidation and taking measures to antagonize ox-LDL may be means of preventing and controlling atherosclerosis. Research has demonstrated the protective effects of blueberries against atherosclerosis in apolipoprotein E-deficient (apoE−/−) mice; the potential mechanisms may involve a reduction in oxidative stress due to both inhibition of lipid peroxidation and the enhancement of antioxidant defense. In recent studies, extracts of hawthorn fruit have been shown to inhibit LDL and liposome oxidation in cell cultures and significantly reduce the levels of lipid hydroperoxides in humans. Analyses of hawthorn fruit extract using chemical methods have indicated that hawthorn is a promising plant due to its high antioxidant activity. These results suggest that antioxidant effects may be one mechanism underlying the potential antiatherogenic effects of hawthorn fruit.

However, the underlying mechanisms of these protective effects remain poorly understood, and whether hawthorn fruit exerts antiatherogenic effects in mouse models of atherosclerosis has not been reported. In the present study, we used the apoE−/− mouse model since its pathogenesis of atherosclerosis resembles that observed in humans and it has been widely applied in cardiovascular research. We investigated the mechanisms of hawthorn fruit in apoE−/− mice from the two aspects of hypolipidemic and antioxidant activities.

### Materials and Methods

**Preparation of the Hawthorn Fruit and Animals**

Fresh hawthorn fruit (Crataegus pinnatifida) was purchased in Shandong, China. After removing the seeds, the fruit flesh was immediately freeze dried by being ground into powder in a coffee grinder. As shown in Table 1, the standard diet was prepared by mixing the indicated ingredients. The hawthorn fruit diet was similar to the standard diet, with the exception that 1% freeze-dried hawthorn fruit was added.

**Table 1. Composition of the diets**

<table>
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<tr>
<th></th>
<th>Hawthorn fruit diet</th>
<th>Standard diet</th>
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<tr>
<td>g/kg dry diet</td>
<td></td>
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<tr>
<td>Hawthorn fruit</td>
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<td>0</td>
</tr>
<tr>
<td>Protein</td>
<td>200</td>
<td>200</td>
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<tr>
<td>Cellulose</td>
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<td>50</td>
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<tr>
<td>Corn starch</td>
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<tr>
<td>Fat</td>
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<td>50</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>40</td>
<td>40</td>
</tr>
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</table>

1. The vitamin mix provided the following amounts (mg/kg dry diet) of nutrients: retinol, 12; thiamine, 40; cholecalciferol, 0.125; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyano-cobalamin, 0.1; ascorbic acid, 1,600; β-α-tocopherol, 340; menadione, 80; nicotinic acid, 200; para-aminobenzoic acid, 100; folic acid, 10; biotin, 0.6.

2. The mineral mix provided the following amounts (mg/kg dry diet) of nutrients: CaCO₃, 12,000; K₂HPO₄, 10,750; CaHPO₄, 10,750; MgSO₄·7H₂O, 4000; ZnSO₄·7H₂O, 350; MnSO₄·H₂O, 100; CuSO₄·5H₂O, 50; Na₂SiO₃·3H₂O, 40; AlK(SO₄)₂·12H₂O, 10; K₂CrO₄, 7.5; NaF, 5; NiSO₄·6H₂O, 5; H₂BO₃, 15; CoSO₄·7H₂O, 2.5; KIO₃, 2; LiCl, 0.75; Na₂SeO₃, 0.75; (NH₄)₂CO₃, 0.5.

Fresh hawthorn fruit (Crataegus pinnatifida) was purchased in Shandong, China. After removing the seeds, the fruit flesh was immediately freeze dried by being ground into powder in a coffee grinder. As shown in Table 1, the standard diet was prepared by mixing the indicated ingredients. The hawthorn fruit diet was similar to the standard diet, with the exception that 1% freeze-dried hawthorn fruit was added.

**Homozgyous apoE−/− mice** with a C57BL/6 genetic background were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). The mice were housed in sterile, filter-top cages (three to four mice per cage). They were provided food and water ad libi-
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Experimental Design

Male apoE<sup>−/−</sup> mice (4 weeks old) were randomly divided into two experimental groups (n = 10 per group). The mice were fed either the standard diet (control group) or the standard diet formulated to contain 1% freeze-dried hawthorn fruit (hawthorn fruit group) for 16 weeks. All foods were prepared in tablets, and the daily portions were packed in zip-locked bags, flushed with nitrogen, sealed and stored at 4°C. The mice were monitored daily for any clinical illnesses.

At the end of the 16 weeks, the mice were fasted overnight then sacrificed via suffocation with CO₂. The liver and serum samples were stored in aliquots at −80°C until then analysis. The heart was removed from the body with aortic root and perfused with cold 10% phosphate-buffered formalin for two minutes, then fixed in 10% phosphate-buffered formalin for 48 hours. The animal studies were approved strictly in accordance with the institutional guidelines of the Animal Care and Use Committee of Shandong Normal University (Jinan, China).

Body Weight and Food Intake Determination

Body weight and food intake were measured using an electronic scale. The body weight was recorded once every three days. The pellet food was first weighed then placed in the cage food container; after 24 hours, the remaining food was weighed. The difference represented the daily food intake. Any unconsumed pellet food was discarded every day and fresh pellet food was provided to ensure consistent food quality to the mice throughout the study.

Atherosclerotic Lesion Analysis

The upper half of the heart was dissected, penetrated overnight in 30% sucrose, then embedded in OCT. Sequential 10-μm frozen sections were cut from the apex towards the base of the heart until the aortic valve leaflets appeared. From this point, 20 sections representing every second serial section over a distance of 200 μm were collected and stained with Sudan IV. Images were captured of each Oil red O-stained cross-section using an Olympus DP71 camera mounted on a microscope (Olympus IX-71, Japan). In these sections, atherosclerotic lesions were analyzed using the Image Pro-Plus 6.0 software program (Media Cybernetics, USA). The results are reported as the average of all 20 aortic sections. In order to minimize artificial error, all analyses were performed by an identical operator.

Biochemical Assays of Serum

The serum glucose levels were determined using an Accu-Chek Active Glucometer (Roche Applied Science, Penzberg). The serum insulin levels were measured using the Ultra Sensitive Mouse Insulin ELISA kit (KYM, Biotechnology company, China) using murine insulin as the standard. The serum total cholesterol (TC) and triglyceride (TG) levels were measured with enzymatic assays using kits obtained from Wako Chemicals USA (Richmond, VA) in accordance with the manufacturer’s instructions. A lipoprotein analysis of fasting serum samples was performed using fast protein liquid chromatography (FPLC). Pooled serum samples (100 μL) were loaded onto the Superose 6 column (Amersham Pharmacia). Sixty 0.5-mL fractions were collected to measure the cholesterol and TG levels. Fractions 6 to 13 contain very-low-density lipoprotein (VLDL), fractions 14 to 30 contain intermediate-density lipoprotein/low-density lipoprotein (IDL/LDL) and fractions 31 to 45 contain high-density lipoprotein (HDL).

T-AOC and Antioxidant Enzymes Activities in the Serum Analysis

The T-AOC values in the serum was evaluated using the Quantitative Assay for total antioxidant potential kit (Jiancheng, Bioengineering institute, China) according to the manufacturer’s instructions. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) were measured in serum samples using an SOD assay kit and GSH-PX assay kit purchased from Cayman, following the procedures provided by the manufacturer. The data are expressed in units per milliliter of serum.

Quantitative RT-PCR Analysis

Total RNA was extracted from the liver using TRlzol reagent (Invitrogen, USA) in accordance with the manufacturer’s instructions and treated with DNase I (Takara, Japan). The quality and integrity of the RNA were determined using absorbance at 260 and 280 nm and gel electrophoresis to confirm the presence of strong and intact ribosomal 28 S and 18 S bands. Reverse transcription of RNA into cDNA was performed using Superscript II reverse transcriptase (Invitrogen, USA) primed with Olig(dT)₁₈ according to the manufacturer’s instructions. The quality and integrity of the cDNAs were tested by initially amplifying the housekeeping gene β-actin. Quantitative real-time PCR was performed using the Rotor-Gene 3000 Real-time PCR System (Corbett Research, Syd-
The sequences of the forward and reverse primers used are listed in Table 2. Amplification was detected according to the Sybergreen (Molecular Probes, USA) method. The specificity of the amplified PCR products was determined after the final cycle by generating a melting curve with a heating rate of 1℃/s between 72 and 99℃. The relative expression values of the target gene mRNAs were normalized to that of β-actin mRNA in each sample. Amplification of the target genes and β-actin was performed using separate tubes. All reactions were performed in triplicate.

**Statistical Analysis**

The data are expressed as the mean ± SD. Student's t-test was used to analyze differences between the groups. A value of *p* < 0.05 was considered to indicate a significant difference. The statistical analyses were performed using a standard software package (SPSS for Windows 18.0).

### Results

#### Body Weight and Food Intake

During the course of the study, the hawthorn fruit diet was well tolerated by the mice. There were no significant differences in body weight or food intake between the hawthorn fruit diet group and the standard diet control group (Table 3).

**Table 3. Changes in body weight and food intake in the mice supplemented with hawthorn fruit**

<table>
<thead>
<tr>
<th></th>
<th>Hawthorn fruit</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Initial body (g)</td>
<td>19.66 ± 1.85</td>
<td>19.55 ± 2.27</td>
</tr>
<tr>
<td>Final body (g)</td>
<td>33.40 ± 2.40</td>
<td>35.50 ± 4.12</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>3.73 ± 0.17</td>
<td>3.70 ± 0.21</td>
</tr>
</tbody>
</table>

The values are presented as the mean ± SD, and the numbers in parentheses indicate the number of mice used.

#### Development of Atherosclerotic Lesions

The consumption of hawthorn fruit effectively prevented the development of atherosclerotic lesions in the apolipoprotein E-deficient mice. As shown in Fig. 1, the apoE−/− mice were fed either a standard diet or 1% freeze-dried hawthorn fruit for 16 weeks, and the mean atherosclerotic lesion area in the aortic sinus among the apoE−/− mice fed hawthorn fruit was significantly decreased by 18% compared to that observed in the control mice (*p* < 0.05) (Fig. 1).

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Table 2. Primers used for the real-time RT-PCR analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Oligonucleotide sequence (5’-3’)</th>
<th>Annealing temperature (℃)</th>
<th>Product length</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAS</td>
<td>F</td>
<td>5’GCAGGGTCTATGCCACTATT3’</td>
<td>60</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5’TGTTACACCTTGGCTCTTGCT3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMG-CoAR</td>
<td>F</td>
<td>5’CGAAGCAGGCTCTATGGAAG3’</td>
<td>60</td>
<td>255</td>
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<tr>
<td></td>
<td>R</td>
<td>5’GCTCCAATCACCAAGGGATAAT3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPT-1</td>
<td>F</td>
<td>5’GGCCTTGTTGATGCTGCTT3’</td>
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<td>107</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5’GAACCTTGGCTGCGTAAGAC3’</td>
<td></td>
<td></td>
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<tr>
<td>SREBP-1c</td>
<td>F</td>
<td>5’TGCGGTGTTGATGCTGCTT3’</td>
<td>60</td>
<td>257</td>
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<tr>
<td></td>
<td>R</td>
<td>5’TAAGGGGTGAGGAGTGGAGG3’</td>
<td></td>
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<tr>
<td>PPARα</td>
<td>F</td>
<td>5’CTTATACATCACTTGGCTCTGTCAG3’</td>
<td>60</td>
<td>104</td>
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<tr>
<td></td>
<td>R</td>
<td>5’TCAAATGGCCACTGTCTT3’</td>
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<td></td>
</tr>
<tr>
<td>SOD1</td>
<td>F</td>
<td>5’TGCTGTTGACTTGGGATT3’</td>
<td>60</td>
<td>79</td>
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<tr>
<td></td>
<td>R</td>
<td>5’GCTTTAGGCTGTTGAGTGGAG3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD2</td>
<td>F</td>
<td>5’CCAGACCTGCTTGAGCCTATG3’</td>
<td>60</td>
<td>241</td>
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<tr>
<td></td>
<td>R</td>
<td>5’GCTTTAGGCTGCTTGAGTGGAG3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gpx3</td>
<td>F</td>
<td>5’TCTTCTGAGACCAAGCAGACAA3’</td>
<td>60</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5’GGAGGCCTAAGCCCTGAAAGC3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>F</td>
<td>5’TCTTCCACTGCTTGCTGATC3’</td>
<td>59</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5’GTACTTCCCTGCTGCTGATC3’</td>
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F, sense primer; R, antisense primer.
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(IDL)/LDL (fractions 14-30) and HDL (fractions 31-45) (Fig. 2). Supplementation with hawthorn fruit decreased both the cholesterol and TG levels of the VLDL and IDL/LDL peaks. As shown in Fig. 2, the changes in the areas under the curve indicated that the numbers of VLDL, IDL, and LDL particles decreased, consistent with the decrease in the serum TC and TG levels. There was a similar amount of HDL particles in both the hawthorn fruit diet and standard diet groups.

T-AOC and Antioxidant Enzymes Activities in the Serum

The serum T-AOC values were higher in the hawthorn fruit dietary group than in the control group (p < 0.05) (Fig. 3A). A further analysis revealed that the serum SOD and GSH-PX activities in the mice fed the hawthorn fruit diet were also remarkably higher than those observed in the mice fed the standard diet (p < 0.05) (Fig. 3B and C).

Gene Expressions of Related Lipid Metabolism and Antioxidant Enzymes in the Liver

In an attempt to elucidate the mechanisms by which the hawthorn fruit diet alters lipid metabolism and antioxidant enzymes, the mRNA levels of hepatic genes involved in fatty acid and cholesterol metabolism and antioxidant enzymes were assessed using real-time RT-PCR (Fig. 4). As shown in Fig. 4, following the consumption of the hawthorn fruit diet, the levels of genes for fatty acid synthase (FAS) and sterol regulatory element binding protein-1c (SREBP-1c) in the liver were markedly decreased compared with those observed in the control diet mice. The hepatic FAS and SREBP-1c mRNA levels were significantly reduced by 42% (p < 0.05) and 23% (p < 0.05), respectively, in the mice fed a hawthorn fruit diet compared with those observed in the mice fed the standard diet. However, the mRNA levels of hepatic hydroxymethyl-

Serum Glucose and Insulin Levels

As shown in Table 4, no significant differences (p > 0.05) were observed in the levels of serum glucose or insulin between the hawthorn fruit group and the standard diet control mice at week 16 (70.84 ± 15.84 versus 78.32 ± 14.42 mg/dL, 0.45 ± 0.11 versus 0.61 ± 0.09 ng/mL, respectively).

Serum Lipid Profiles

The levels of serum TG and TC were significantly decreased in the hawthorn fruit diet mice compared with those observed in the standard diet group. As shown in Table 4, the serum TC and TG levels in the hawthorn fruit treated-mice were significantly decreased by 27.7% and 43.3%, respectively, compared to those observed in the standard diet control mice.

The serum lipoproteins were separated using FPLC, and the lipoprotein peaks included VLDL (fractions 6-13), intermediate-density lipoprotein (IDL)/LDL (fractions 14-30) and HDL (fractions 31-45) (Fig. 2). Supplementation with hawthorn fruit decreased both the cholesterol and TG levels of the VLDL and IDL/LDL peaks. As shown in Fig. 2, the changes in the areas under the curve indicated that the numbers of VLDL, IDL, and LDL particles decreased, consistent with the decrease in the serum TC and TG levels. There was a similar amount of HDL particles in both the hawthorn fruit diet and standard diet groups.

Table 4. Metabolic characteristics of the mice fed the hawthorn fruit diet and the standard diet

<table>
<thead>
<tr>
<th></th>
<th>Hawthorn fruit (n = 10)</th>
<th>Control (n = 10)</th>
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</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>546.30 ± 56.97*</td>
<td>755.33 ± 65.93</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>303.08 ± 70.51*</td>
<td>534.42 ± 67.47</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>70.84 ± 15.84</td>
<td>78.32 ± 14.42</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>0.45 ± 0.11</td>
<td>0.61 ± 0.09</td>
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The data are expressed as the mean ± SD, and the numbers in parentheses indicate the number of mice used. The asterisks indicate differences from the control group, *p < 0.05.

Fig. 1. The protective effect of hawthorn fruit on the development of early atherosclerotic fatty streak lesions in the apoE−/− mice fed a hawthorn fruit diet or a standard diet for 16 weeks. (A) Representative photomicrographs of the aortic sinus stained with Oil red O. (a) hawthorn fruit group; (b) control group. (B) The mean atherosclerotic lesion area in the aortic root. The values are presented as the mean ± SD, n = 10. *Difference from the control group, p < 0.05.

Fig. 2. The changes in the areas under the curve indicated that the numbers of VLDL, IDL, and LDL particles decreased, consistent with the decrease in the serum TC and TG levels. There was a similar amount of HDL particles in both the hawthorn fruit diet and standard diet groups.

Fig. 3A. The serum T-AOC values were higher in the hawthorn fruit dietary group than in the control group (p < 0.05). A further analysis revealed that the serum SOD and GSH-PX activities in the mice fed the hawthorn fruit diet were also remarkably higher than those observed in the mice fed the standard diet (p < 0.05) (Fig. 3B and C).

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</tbody>
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The data are expressed as the mean ± SD, and the numbers in parentheses indicate the number of mice used. The asterisks indicate differences from the control group, *p < 0.05.
glutaryl CoA reductase (HMG-CoAR) did not show any significant differences between the mice fed hawthorn fruit and those fed the standard diet. The hepatic carnitine palmitoyl transferase 1 (CPT-1) and peroxisome proliferator-activated receptor alpha (PPARα) mRNA levels were strongly increased by 125% ($p<0.05$) and 62% ($p<0.05$), respectively, in the mice fed the hawthorn fruit diet compared with those observed in the mice fed the standard diet.

The mRNA expression levels of the antioxidant enzymes, including SOD1, SOD2 and glutathione peroxidase-3 (Gpx3), were higher in the livers of the mice fed the hawthorn fruit diet than in the mice fed the control diet. The mRNA levels of SOD1 and SOD2 in the mice fed the hawthorn fruit diet were increased by 25% ($p<0.05$) and 42% ($p<0.05$), respectively, compared with those observed in the mice fed the standard diet. The mRNA levels of Gpx3 were 29% ($p<0.05$) higher in the hawthorn fruit treated-mice than in the standard control mice.

**Discussion**

The use of natural plant compounds with atheroprotective effects has been proven to have clinical relevance. The present study indicated that apoE $^{-/-}$ mice fed a diet containing 1% freeze-dried hawthorn...
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Hawthorn fruit possesses hypolipidemic activity in atherogenic apoE<sup>−/−</sup> mice. The apoE<sup>−/−</sup> mice fed hawthorn fruit exhibited not only lower serum TC and TG levels, but also lower levels of cholesterol and TG consisting of VLDL and LDL particles (Table 4). Hawthorn fruit may play a role in decreasing the blood lipid levels and enhancing the antioxidant activity.

The present study clearly demonstrated that hawthorn fruit developed fewer atherosclerotic lesions (Fig. 1). Hawthorn fruit may play a role in decreasing the blood lipid levels and enhancing the antioxidant activity.

Fig. 4. Gene expression of lipid metabolism and antioxidant enzymes in the livers of the mice fed the hawthorn fruit diet or the control diet for 16 weeks. The analyses were carried out using real-time RT-PCR. The values are presented as the mean ± SD, n = 10. The asterisks indicate differences from the control group, *p < 0.05.
Fig. 2). However, hawthorn fruit supplementation did not affect the levels of serum HDL particles, consistent with the findings of several published reports.

Recent research has demonstrated that hawthorn fruit methanol extract (80% in water) effectively improves insulin resistance in C57BL/6J mice fed a high-fat diet. In the present study, we demonstrated that the levels of serum glucose and insulin were slightly decreased in the hawthorn fruit diet mice compared with those observed in the standard diet mice, although the differences were not significant. This finding may be related to the dose or extract of hawthorn fruit or the mice model used in this study. A previous study revealed that consumption of hawthorn fruit extract increased the GLUT4 expression in skeletal muscle, suggesting that the effects of hawthorn fruit on glucose homeostasis caused an improvement in muscle glucose uptake. In the present study, in order to understand the mechanisms of fatty acid and triglyceride as well as glucose and insulin lowering, the mRNA levels of FAS and key transcription factors that induce lipogenic SREBP-1c genes were assessed. We found that the expression of FAS in the hawthorn fruit group was significantly decreased compared to that observed in the control group. In addition, the SREBP-1c mRNA levels exhibited the same tendency (Fig. 4), suggesting that hawthorn fruit downregulates the expression of FAS by reducing the SREBP-1c mRNA level. The SREBP-1c activity is considered to be associated with insulin resistance. The decrease in the insulin levels observed in the hawthorn fruit diet mice may have been related to the reduction in the SREBP-1c mRNA levels. A reduction in the FAS expression directly affects the synthesis of triglycerides. Therefore, the TG levels of VLDL in the hawthorn fruit-treated group were obviously lower than those observed in the control group (Fig. 2B).

A previous study showed dysregulation of SREBP-mediated lipogenic genes in PPARα-deficient mice, suggesting that PPARα plays a role in the SREBP-mediated regulation of lipogenic genes in mouse models of dyslipidemia. Other reports have revealed that ethanol or methanol extracts of hawthorn fruit significantly increase the mRNA expression levels of PPARα and that hawthorn flavonoids improve lipid metabolism by regulating the PPAR expression. We examined the mRNA levels of PPARα and found that they were significantly increased in the hawthorn fruit group compared with those observed in the control group. Therefore, we speculate that hawthorn flavonoids play a role in the downregulation of the SREBP-1c gene expression by regulating PPARs. The mRNA levels of CPT-1, a downstream gene of PPARα that plays a role in the oxygenolysis of fatty acids, were higher in the hawthorn fruit diet mice than in the standard diet mice. These results suggest that hawthorn fruit activates PPARα to facilitate the actions of β-oxidation-related enzymes in the liver for lipid degradation and blood lipid decrement.

Nevertheless, there were no significant differences in the expression of HMG-CoAR between the hawthorn fruit diet group and the control group in the present study (Fig. 4). In addition, the decrease in the cholesterol levels of VLDL in the hawthorn fruit diet group was not significantly lower than that observed in the control group (Fig. 2A). In accordance with the findings of other reports, cholesterol synthesis in the liver cells was not inhibited by the consumption of hawthorn fruit, suggesting that the inhibition of cholesterol synthesis is unlikely to be a component of the hypocholesterolemic mechanisms of hawthorn fruit. In addition (Fig. 2A), we clearly found that the cholesterol levels of IDL/LDL in the hawthorn group were lower than those observed in the control group. Although the reduction of the TC levels induced by the consumption of hawthorn fruit is a complex process involving multifaceted interactions of cholesterol metabolism, we speculate that the reduction of the cholesterol levels of IDL/LDL was associated with the activation of LDL receptors (LDLR) and LDL receptor-related proteins (LRP). Research has shown that the oral administration of hawthorn extract in rats significantly suppresses the increase in the serum cholesterol levels and upregulates the effects of LDLR on the cell surface. Therefore, increased LDLR activation accelerates the elimination of serum LDL, resulting in lower blood cholesterol.

Hawthorn fruit possesses both hypolipidemic and antioxidant activities. Generally, flavonoids and proanthocyanidins are responsible for hawthorn fruit’s pharmacological activity. Phenolic compounds extracted from the hawthorn plant exhibit strong antioxidant activity. A previous study indicated that the ethyl acetate fraction obtained from hawthorn extract effectively protects human LDL from Cu²⁺-mediated oxidation and prevents the peroxyl free radical-induced oxidation of α-tocopherol in human LDL. Tadic et al. reported that Crataegus pinnatifida ethanolic extract (CPEE) possesses free radical scavenging activity and reduces paw edema in a carrageenan-induced paw edema model. Therefore, the protective effects of hawthorn fruit on the cardiovascular system may also be attributed to these antioxidants because they reduce the production of free radicals. We evaluated genes associated with fatty acid oxida-
tion, such as SOD1, SOD2 and Gpx3, and found that their mRNA levels were increased in the hawthorn fruit diet group compared to that observed in the control group. To further determine whether hawthorn fruit consumption improves the levels of antioxidant enzymes, we measured the serum T-AOC levels and the activities of two enzymes, including SOD and GSH-PX. The results showed that the serum T-AOC, SOD and GSH-PX levels were significantly elevated in the hawthorn fruit treated-mice (Fig.3), suggesting that hawthorn fruit boosts the activities of antioxidant enzymes and alleviates subsequent damage to arteries by decreasing the oxidative modification of LDL.

In conclusion, our study provided evidence that dietary intake of hawthorn fruit protects mice from atherosclerosis. The consumption of hawthorn fruit decreased the levels of blood lipids and increased the antioxidant activity in the apoE/−/− mouse model. Hawthorn fruit may developed as a health food to provide protection against the development of atherosclerosis.

Acknowledgements

This study was supported in part by funds from the National Natural Science Foundation of China (81100193) and the Research Fund for the Doctoral Program of Higher Education of China (20113704120006) to Liang Zhang.

Conflicts of Interest

None.

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