Effects of Pectin Pentaoligosaccharide from Hawthorn (*Crataegus pinnatifida* Bunge. var. Major) on the Activity and mRNA Levels of Enzymes Involved in Fatty Acid Oxidation in the Liver of Mice Fed a High-Fat Diet

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ABSTRACT: The regulatory effects of haw pectin pentaoligosaccharide (HPPS) on fatty acid oxidation-related enzyme activities and mRNA levels were investigated in the liver of high fat diet induced hyperlipidemic mice. Results showed that HPPS (150 mg/kg for 10 weeks) significantly suppresses weight gain (32.3 ± 0.26 and 21.1 ± 0.14 g for high-fat diet and HPPS groups, respectively), decreases serum triacylglycerol levels (1.64 ± 0.09 and 0.91 ± 0.02 mmol/L, respectively), and increases lipid excretion in feces (55.7 ± 0.38 and 106.4 ± 0.57 mg/g for total lipid, respectively), compared to high-fat diet as control. HPPS significantly increased the hepatic fatty acid oxidation-related enzyme activities of acyl-CoA oxidase, carnitine palmitoyltransferase I, 3-ketoacyl-CoA thiolase, and 2,4-dienoyl-CoA reductase by 53.8, 74.2, 47.1, and 24.2%, respectively. Meanwhile, the corresponding mRNAs were up-regulated by 89.6, 85.8, 82.9, and 30.9%, respectively. Moreover, HPPS was able to up-regulate the gene and protein expressions of peroxisome proliferator-activated receptor α. Results suggest that continuous HPPS ingestion may be used as dietary therapy to prevent obesity and cardiovascular diseases.

KEYWORDS: hawthorn, pectin pentasaccharide, enzyme activity, mRNA level, fatty acid oxidation

■ INTRODUCTION

Metabolic syndromes, including obesity, dyslipidemia, and insulin resistance, are some emergent human health problems worldwide.[1] Although the pathogenesis of metabolic syndromes is complicated and the mechanism details remain unknown, lipid abnormality is now being proposed as a distinct metabolic syndrome feature.[2] Hyperlipidemia, a condition wherein blood lipid levels are abnormally elevated, is well established as one of the major causes of atherosclerotic cardiovascular disease development, which is becoming a major health problem worldwide.[3] Furthermore, nonalcoholic fatty liver disease has also emerged as a serious common feature associated with metabolic disorders and obesity. A dietary excess of saturated fat was shown to contribute significantly to tissue and organ (e.g., hepatic) lipid accumulation.[4] Obesity increases the risk of chronic diseases such as diabetes and other cardiovascular ailments.[5]

The liver plays an important role in regulating interorgan lipid metabolism.[6] Studies have shown that when blood fatty acid (FA) and cholesterol levels are elevated for prolonged periods by excessive energy intake, triglyceride (TG) and total cholesterol can accumulate in nonadipose tissues, including liver and muscle, which can lead to pathological consequences, such as the development of fatty liver or ketosis.[7−10] To prevent this undesirable situation, the body employs a FA oxidation mechanism that allows FA to break down into CO_2 and its ketone body and be released into the blood.[11,12] In this lipolytic process, many enzymes, such as acyl-CoA oxidase

(ACO), carnitine palmitoyltransferase I (CPT-I), CPT-II, 3-ketoacyl-CoA thiolase (3KCT), and 2,4-dienoyl-CoA reductase (DCR), are involved; their gene expression is known to be modulated by the nuclear orphan receptor known as peroxisome proliferator-activated receptor α (PPARα).[13−16]

Pectin is a widely used food additive that functions as a thickener and gelling agent. This polysaccharide is also used as a food fiber human health supplement. Previously, we isolated pectin from the hawthorn (*Crataegus pinnatifida* Bunge) fruit, which belongs to the rose family, and found that this particular fruit possesses higher pectin content than other cultivated fruits.[17] This haw pectin also exhibits superior viscosity, which is 4−6-fold higher than that of commercially available lemon and apple pectins,[17] and its molecular structure was confirmed to contain homogalacturonan and rhamnogalacturonan main chains.[18] Moreover, crude haw pectin hydrolysates derived from high molecular weight haw pectin can significantly lower the total serum cholesterol and TG levels in hyperlipidemic mice.[19] These results revealed great prospects for the application of haw pectin oligosaccharides in the development of functional foods that will benefit human health. Therefore, in this study, the effects of a pure and high-yield fraction of haw pectin pentaoligosaccharide (HPPS), isolated from haw pectin

hydrolysates, on the activity and mRNA levels of FA oxidation-related enzymes in the liver of model mice fed a high-fat diet were investigated to provide sufficient evidence for the broad potential applications of haw pectin and hawthorn fruit in the treatment of metabolic syndromes.

■ MATERIALS AND METHODS

Chemicals. HPPS was prepared using previously described methods with some modifications.[20] Briefly, haw pectin was obtained from haw fruit through hot water extraction and subjected to enzymolysis (pectinase, DSM Food Specialties, China) in 0.02 mmol/L acetate buffer at pH 3.5 and 50 °C with 1% substrate concentration.[18] Twenty grams of haw pectin enzyme lysate was subjected to a larger DEAE-Sephadex A-25 column chromatograph (5.0 cm × 40 cm, HCO_3^- form). The column was directly eluted with 1 bed volume of 0.25 mol/L NH_4HCO_3, followed by elution with 1 bed volume of 0.3 mol/L NH_4HCO_3 to obtain the HPPS fraction. The HPPS fraction was then deionized and freeze-dried for use in subsequent experiments. Total sugar and uronic acid contents were determined using the phenol−H_2SO_4[21] and m-hydroxydiphenol methods,[22] respectively. HPPS purity was analyzed by a high-performance liquid chromatography (HPLC) using a Shimadzu SPD-20A system (Shimadzu, Japan). An Asahipak NH_2 P-50 column (4.6 mm × 250 mm, Japan) was used for the separation at 40 °C with 0.3 mol/L sodium phosphate buffer (pH 4.4) as the mobile phase. The flow rate was 0.6 mL/min, and the detector was RI. The molecular weight of HPPS was analyzed by HPLC-ESI-MS (Finnigen-LCQ Advantage MAX, USA) at a capillary voltage of 4 kV in positive ion mode. The HPLC condition was as mentioned above. The sugar composition of HPPS was analyzed by thin-layer chromatography (TLC) and gas−liquid chromatography (GLC). Silica gel plate (Qingdao Haiyang Chemical, China) was used for TLC analysis with the developing solvents of n-butanol, acetic acid, and H_2O (4:6:3, v/v/v) and spotting reagent of 50% sulfuric acid. The glycosyl residue of HPPS was analyzed by GLC as alditol acetates after complete reduction and acid hydrolysis.[23,24] In the acid hydrolysis, the sample (3 mg) was heated in 1.0 mol/L H_2SO_4 (1 mL) at 100 °C for 16 h. GLC was performed on an Agilent-7890A GC (USA) apparatus. A CBP-10-M25-025 capillary column (0.22 mm × 25 m) was used and operated at 220 °C. Methylation analysis of HPPS was carried out using previously described procedures.[20]

Animals and Diets. Sixty 6-week-old male Kunming mice, with weights ranging from 25 to 30 g, were purchased from HFK Bioscience Co., Ltd. (Beijing, China). Upon arrival at the animal laboratory, the animals were housed individually in a room with controlled temperature (23 ± 1 °C) and humidity (60% ± 5%), under a 12 h light−dark cycle and fed a standard commercial diet (Beijing HFK Bioscience Co., Ltd.). After 1 week of acclimatization, the animals were randomly divided into normal control (NC), high-fat control (HFC), and three HPPS groups (Table 1, n = 12). The NC group was fed a standard diet, and the other two groups were fed a high-fat diet. The composition of the experimental diet was based on our previous study.[19] All groups were treated by oral infusion with the same volume of water (NC and HFC groups) or HPPS daily. During the experimental period, food and drinking water were supplied ad libitum. Food intake and body weight were recorded once per day.[16]

Table 1. Experimental Group of Animals

group[a]	dose (mg/kg body wt)	n	diet[b]
HPPS	50	12	HF
	150	12	
	300	12	
HFC	0	12	HF
NC	0	12	standard

[a]HPPS, haw pectin pentaoligosaccharide; HFC, high-fat control; NC, normal control. [b]HF, high-fat.

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Shenyang Pharmaceutical University (SYXK-L-2010-0009).

Sample Collection and Processing. At the end of the experimental period, animals were anesthetized by isoflurane (H20050501, Manrid Inc., USA) after 12 h of fasting. Blood was collected from the orbital sinus, and serum was collected by blood centrifugation at 1500g for 30 min at 4 °C for biochemical analysis. After laparotomy, the liver samples were removed, weighed, frozen with liquid nitrogen, and stored at −80 °C. Feces were collected daily from individual mice and freeze-dried into powder form for further analysis.[25,26]

Measurement of Serum and Fecal Lipids. Fecal lipids in the lyophilized fecal samples were extracted with a 2:1 (v/v) chloroform/methanol mixture using the method described by Folch et al.[27] The TG contents in the serum and fecal samples were determined using an enzymatic colorimetric kit (code 290-63701, Wako, Japan). Free fatty acid (FFA) in the serum samples was determined using the NEFA-C-test (code 294-63601, Wako). Fecal total lipids were determined gravimetrically by evaporation of the organic solvent in the fecal and serum lipid extracts.

Liver Histology. Liver tissues were fixed in 10% buffered formalin at room temperature. Formalin-fixed livers were embedded in paraffin, sectioned, stained with hematoxylin and eosin (H&E), and analyzed by photomicroscopic observation.

Enzyme Analyses. Enzyme source fractions in the liver were prepared according to methods developed by Cho et al.[28] and Gotoh et al.[29] A 10% (w/v) homogenate was prepared in a buffer (0.1 M triethanolamine; 0.02 M EDTA; and 2 mM dithiothreitol, pH 7.0) and then centrifuged for 10 min at 600g to discard any cellular debris. The supernatant was then centrifuged for 30 min at 12000g and 4 °C to remove the mitochondrial pellet. Subsequently, the supernatant was ultracentrifuged twice at 100000g for 60 min at 4 °C to obtain the cytosolic supernatant, and protein content was determined using the Bradford assay with bovine serum albumin as standard.[30]

Enzyme activities of ACO, CPT-I, CPT-II, and 3KCT were measured spectrophotometrically as described previously.[31−33] DCR activity was measured as described by Clejan and Schulz.[34] Results were expressed as nanomoles per minute per milligram protein.

The protein content of PPARα in the liver was determined using a commercial ELISA kit (code ab133113, ABCAM, U<K) according to the manufacturer's instructions. Results were expressed as nanograms per milligram protein.

RNA Preparation and Real-Time PCR. Total RNA was isolated from the liver using TRIZOL (catalog no. 15596-026, Invitrogen, USA) and was reverse-transcribed into cDNA with random hexamers using the PrimeScript RT reagent kit with gDNA Eraser (code D2639A, Takara, China). Quantitative real-time PCR was performed using SYBR Premix Ex Taq (Tli RNaseH Plus) (code DRR081S, Takara) and the $2^{-\Delta\Delta Ct}$ method on the iQ5 Optical System (Bio-Rad, USA). Primer sequences used in the study are shown in Table 2. Real-time PCR was performed using the following conditions: 95 °C for 30 s, followed by 40 cycles at 95 °C for 10 s, and 50−54 °C for 30 s. The expression of Actb (a housekeeping gene, AK147787.1) was determined as an internal control. The expression of all genes was normalized by Actb and the experimental controls, and the results were expressed as $2^{-\Delta\Delta Ct}$.

Statistical Analysis. Results are given as the mean ± the standard error of the mean (SEM). Significant differences among the groups were determined by analysis of variance (SAS Institute, Cary, NC, USA), and group means were considered to be significantly different at $p < 0.05$ or $p < 0.01$ as determined by Duncan' s multiple-range test.

■ RESULTS

Chemical Analysis of HPPS. To prepare large amounts of the experimental sample in a simple manner, HPPS was recovered through DEAE-Sephadex A-25 column chromatography with 0.3 mol/L NH_4HCO_3. The total sugar and uronic acid contents in the recovered sample were 99.7 and 99.1%,

Table 2. Primer Sequences for Real-Time Polymerase Chain Reaction

gene	forward primer (5′→3′)	reverse primer (5′→3′)
Aco	TTTGTTGTCCCTATCCGTGAGA	CCGATATCCCCAACAGTGATG
Cpt-I	CTGTTAGGCCTCAACACCGAAC	CACAGTGTCCTGTCTCCGTGT
Cpt-II	ATCCCCTGGATATGTCCCAATA	CATCACGACTGGGTTTGGGTAT
3kct	GCGGTTTGGCATTTCACG	TTGTCACCCTTGTCATCCAG
Dcr	TAGTTCTCAATGGCACAGC	CATTACAAAGCCTGATACACT
Pparα	CCTCAGGGTACCACTACGGAGT	GCCGAATAGTTCGCCGAA
Actb	GTTGCCAATAGTGATGACCT	GGACCTGACAGACTACCTCA

respectively. The purity of HPPS in HPLC was 98.6% (Figure 1A). The sugar composition of HPPS was only galacturonic
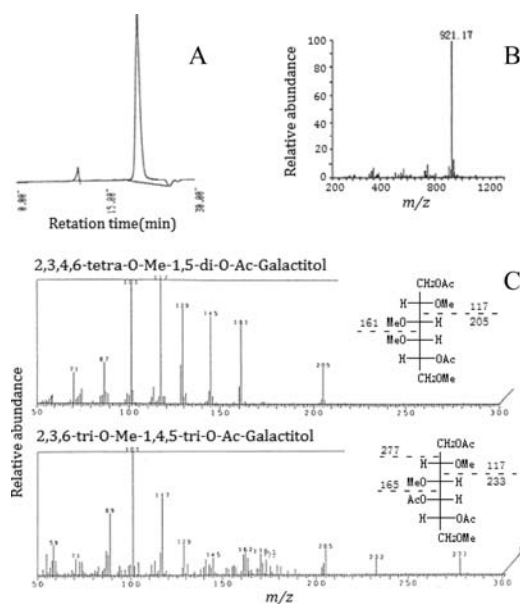

Figure 1. Chemical analysis of HPPS by HPLC (A), ESI-MS (B), and GC-MS (C).

acid based on TLC analysis. In the GLC, a single peak of galactose was detected for reduced HPPS, which further supported the result of TLC. These results indicated that the recovered sample was pure galacturonan. In the mass spectrum (Figure 1B), a fragment of m/z 898 (m/z 921−Na$^+$) was equivalent to the molecular weight of pentaoligogalacturonide. From the GC-MS spectra (Figure 1C), the glycosidic linkages of (1→4)-galactose were also observed, with an approximately

1:4 molar ratio between (1→)-galactose and (→4)-galactose. Given that the source of HPSS was haw pectin, the main chain of haw pectin being composed of α-1,4-linked galacturonic acid,[18] the results confirmed that the haw pectin enzymatic hydrolysate recovered from DEAE-Sephadex A-25 column chromatography with 0.3 mol/L NH$_4$HCO$_3$ was a pentaoligogalacturonic acid with α-(1→4) linkages, which is an objective product (HPSS) of this study.

Food Intake, Body Weight, Serum and Fecal Lipid Concentrations, and Liver Histology. As shown in Tables 3 and 4, the high-fat diet significantly increased the body weight and serum lipid levels of mice after both 4 and 10 week treatment periods compared with the standard diet ($p < 0.05$). Thus, this model has successfully induced hyperlipidemia in mice. However, no significant difference was detected in the amount of food intake during the experiment.

HPPS supplementation significantly ($p < 0.01$) alleviated the high fat diet induced increase in body weight and serum lipid levels and significantly ($p < 0.01$) stimulated lipid secretion in feces, compared with HFC after 10 weeks of administration. At 4 weeks, the body weight gain was also significantly ($p < 0.01$) lower and the fecal total lipid level was significantly ($p < 0.01$) higher in the HPPS group than in the HFC group.

Liver histology (Figure 2) revealed that either 4 or 10 weeks of the high-fat diet (HFC) caused a high accumulation of microvesicular-type fat in the hepatocytes. HPPS administration (150 mg/kg) clearly improved high fat diet induced hepatic fat accumulation and microvesicular hepatic steatosis.

Effect of HPPS on the Activity of Hepatic Enzymes Involved in FA Oxidation. HPPS supplementation significantly ($p < 0.05$) increased the enzyme activities of peroxisomal 3KCT and mitochondrial CPT-I in the livers of mice after 4 and 10 weeks of administration, compared with that of HFC (Figure 3B,C). Moreover, compared with HFC, the enzyme activities of peroxisomal ACO and DCR in the HPPS group

Table 3. Food Intake, Body Weight, and Serum and Fecal Lipid Levels in Mice Fed Control and HPPS-Supplemented Diets at 4 Weeks[a]
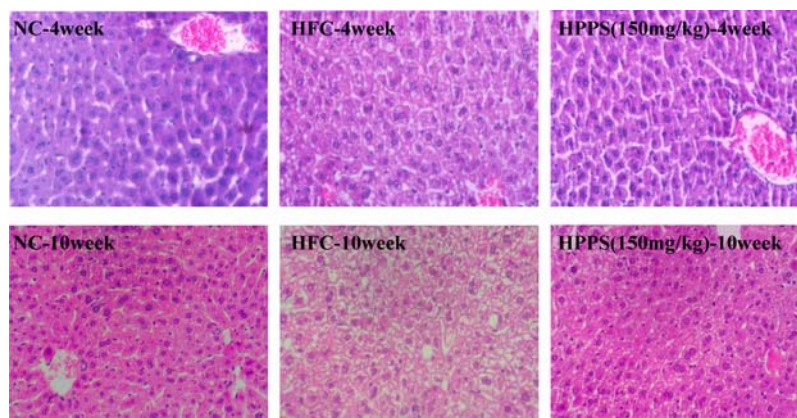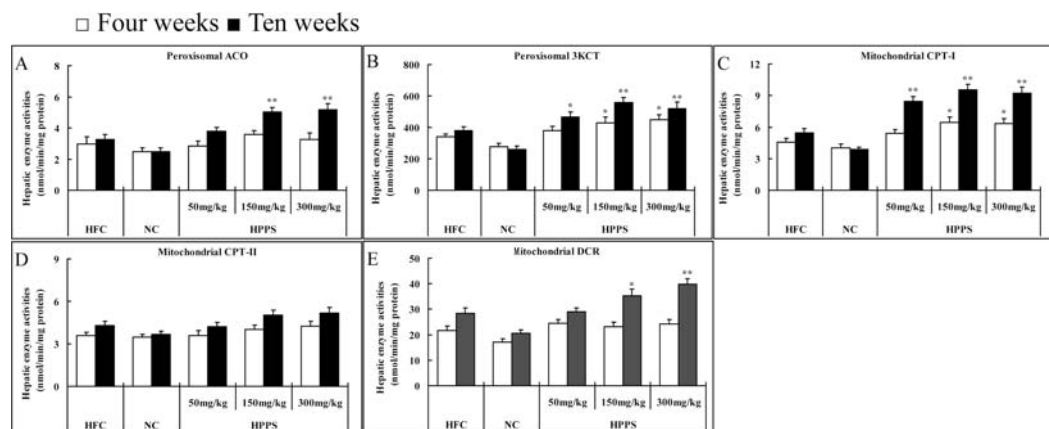
			HPPS		
item	HFC	NC	50 mg/kg body wt	150 mg/kg body wt	300 mg/kg body wt
food intake (g/day)	2.17 ± 0.09	2.25 ± 0.17	2.12 ± 0.11	2.22 ± 0.21	2.30 ± 0.24
wt gain (g)	20.3 ± 0.38	14.7 ± 0.37**	15.4 ± 0.52**	15.9 ± 0.55**	17.9 ± 0.55**
serum lipid (mmol/L)					
TG	1.51 ± 0.04	1.22 ± 0.08*	1.41 ± 0.06	1.40 ± 0.03	1.44 ± 0.07
FFA	0.58 ± 0.05	0.31 ± 0.04**	0.55 ± 0.02	0.52 ± 0.06	0.56 ± 0.03
fecal lipids					
TG (μmol/g)	3.04 ± 0.04	2.27 ± 0.08	3.25 ± 0.14	3.54 ± 0.24	3.19 ± 0.16
total lipids (mg/g)	38.7 ± 0.32	26.2 ± 0.23*	42.4 ± 0.33	50.9 ± 0.45*	60.4 ± 0.28**

[a]HPPS, haw pectin pentaoligosaccharide; HFC, high-fat control; NC, normal control; TG, triglyceride; FFA, free fatty acid. Values represent the mean ± SEM ($n = 12$). *, $p < 0.05$, and **, $p < 0.01$, compared with the HFC group.

Table 4. Food Intake, Body Weight, and Serum and Fecal Lipid Levels in Mice Fed Control and HPPS-Supplemented Diets at 10 Weeks[a]

item	HFC	NC	HPPS		
			50 mg/kg	150 mg/kg	300 mg/kg
food intake (g/day)	2.37 ± 0.14	2.28 ± 1.11	2.32 ± 0.21	2.29 ± 0.17	2.36 ± 0.18
wt gain (g)	32.3 ± 0.26	22.6 ± 0.18**	22.9 ± 0.16**	21.1 ± 0.14**	20.9 ± 0.11**
serum lipid (mmol/L)					
TG	1.64 ± 0.09	1.14 ± 0.07**	1.14 ± 0.08**	0.91 ± 0.02**	1.10 ± 0.02**
FFA	0.84 ± 0.09	0.41 ± 0.02**	0.62 ± 0.07**	0.45 ± 0.06**	0.67 ± 0.06**
fecal lipids					
TG (μmol/g)	3.69 ± 0.04	2.54 ± 0.21**	4.14 ± 0.17*	5.36 ± 0.15**	4.96 ± 0.22**
total lipids (mg/g)	55.7 ± 0.38	38.2 ± 0.45**	77.5 ± 0.58**	106.4 ± 0.57**	92.2 ± 0.64**

[a]Values represent the mean ± SEM ($n = 12$). *, $p < 0.05$, and **, $p < 0.01$, compared with the HFC group.

Figure 2. Histological analysis of mouse livers.

Figure 3. Effect of HPPS on the activities of hepatic fatty acid oxidation enzymes in mice. Enzyme activities were measured after 4 weeks (□, white histogram) and 10 weeks (■, black histogram) of administration. Values represent the mean ± SEM ($n = 12$). *, $p < 0.05$, and **, $p < 0.01$, compared with the HFC group. HFC, high-fat control; NC, normal control; HPPS, haw pectin pentasaccharide.

(150−300 mg/kg) were also significantly ($p < 0.01$) higher at 10 weeks of treatment but were not statistically different at 4 weeks (Figure 3A,E). However, the change in CPT-II activity was not significant for either treatment period, as shown in Figure 3D.

Effect of HPPS on mRNA Levels of Hepatic Enzymes Involved in FA Oxidation. To investigate whether the changes in hepatic enzyme activity are due to alterations in the expression of genes controlling FA oxidation, we analyzed the mRNA levels of ACO, CPT-I, CPT-II, 3KCT, and DCR in the livers of mice supplemented with HPPS.

As shown in Figure 4, compared with HFC, HPPS consumption significantly ($p < 0.01$) increased the mRNA levels of ACO by 41.2, 89.6, and 55.4%; those of CPT-I by 58.4, 115.8, and 44.5%; those of 3KCT by 54.4, 82.9, and 84.9% for three doses; and those of DCR by 30.9 and 42.8% for 150 and 300 mg/kg doses, respectively, in the livers of mice after 10 weeks of administration. By contrast, at 4 weeks after HPPS supplementation, the mRNA levels of CPT-I (65.7% for 300 mg/kg, $p < 0.01$) and 3KCT (37.5 and 40.5% for 150 and 300 mg/kg, respectively, $p < 0.05$) were significantly up-regulated, compared with that of HFC. The mRNA levels of ACO, CPT-II, and DCR tended to be higher (6.1−28.9%) in the HPPS

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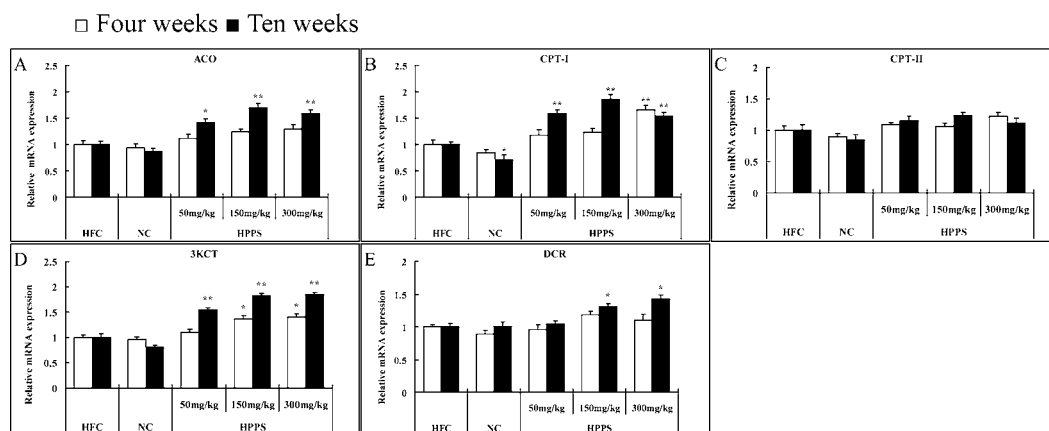
Figure 4. Effect of HPPS on mRNA levels of hepatic fatty acid oxidation enzymes in mice. Measurements were conducted after 4 weeks (□, white histogram) and 10 weeks (■, black histogram) of administration. Values represent the mean ± SEM ($n = 12$). *, $p < 0.05$, and **, $p < 0.01$, compared with the HFC group.

groups than in the HFC groups after 4 weeks of administration. However, no statistical significance was observed.

Effect of HPPS on Hepatic Protein Content and PPARα mRNA Level. PPARα is mainly expressed in the liver. The activation of PPARα induces the mRNA expression of several genes involved in FA oxidation to reduce circulating lipid level. Figure 5B shows that the expression of PPARα was activated by
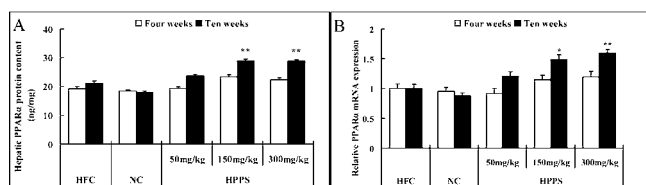

Figure 5. Effect of HPPS on hepatic protein content and mRNA level of PPARα in mice. Measurements were conducted after 4 weeks (□, white histogram) and 10 weeks (■, black histogram) of administration. Values represent the mean ± SEM ($n = 12$). *, $p < 0.05$, and **, $p < 0.01$, compared with the HFC group.

the addition of HPPS to the high-fat diet. Compared with HFC, HPPS-treated mice had significantly ($p < 0.01$) higher protein content (37.4% for 150 mg/kg, 36.5% for 300 mg/kg) and PPARα mRNA levels (48.4% for 150 mg/kg, 59.3% for 300 mg/kg) in the liver after 10 weeks of administration (Figure 5). After 4 weeks of administration, the protein content and PPARα mRNA level in mice liver were 0.5−22.4% higher in the test subjects fed HPPS supplement than in those fed the high-fat diet. However, the differences were not significant.

■ DISCUSSION

Pectin is known as a soluble food fiber that can reduce the levels of fat and cholesterol in the liver and blood.[35] This polysaccharide could also be used as diet food for body weight reduction.[36] However, as a functional food material, the use of pectin is restricted because of difficulty in handling due to its high viscosity and low dispersion capacity. In addition, abnormal lipid metabolism, which is a major risk factor for developing dyslipidemic diseases, may arise from improper lifestyle in most cases, including excess energy intake.[37] High levels of dietary fat could result in the accumulation of TG throughout many tissues, especially in adipose and certain nonadipose tissues,[38] subsequently causing lipid metabolic

disorder-related diseases and obesity, among others. In this study, a high-fat diet significantly increased the body weight of mice. However, pure HPPS, which is nearly nonviscous, exhibited strong suppressive effects on the weight gain of mice fed a high-fat diet, in agreement with our previous results that confirmed the inhibitory effects of crude haw pectin hydrolysate on the weight gain of mice fed a high-fat diet.[19] A similar beneficial effect of oligofructose on weight reduction was also reported in mice.[39] The present results revealed a potential benefit of dietary supplement for the prevention and inhibition of obesity. Thus far, a number of studies have reported on food fiber and its effect on body weight gain/loss in animals and humans. However, early response and constancy remain unclear. Our present experimental data showed that HPPS significantly suppressed body weight gain of mice treated with a high-fat diet at both early (4 weeks) and prolonged (10 weeks) administration periods. This suppressive effect seemed more efficient at 10 weeks (the suppression ratios were 29.3−35.2% for doses of 50−300 mg/kg, respectively, compared with HFC) than at 4 weeks (suppression ratios of 11.7−24.4%, compared with HFC). The results are useful for understanding the biological functions of food fiber, especially food oligosaccharides, to develop and enhance their application strategies.

Dietary food carbohydrates are beneficial as they improve lipid metabolism, which provides protection from hyperlipidemic-related diseases.[40,41] Fiordaliso et al.[42] reported that dietary oligofructose could significantly reduce the serum and hepatic levels of TG in rat. However, there were few evidentiary reports on oligosaccharide affecting the fatty acid oxidation in animals or human. Our present data showed that HPPS markedly decreased the serum TG levels of mice fed a high-fat diet, in line with our previous study on the influence of crude haw pectin hydrolysates in the serum TG of mice subjected to a high-fat diet.[19] HPPS markedly increased hepatic FA oxidation-related enzyme activities, including those of ACO, CPT-I, 3KCT, and DCR along with the increase in their corresponding mRNA levels. Alterations in hepatic FA oxidation modify the availability of FA for TG synthesis, which then affect serum lipid levels.[14,32] The present results on hepatic FA oxidation enzymes might account for the mechanisms of serum TG lowering in HPPS-treated mice. Moreover, HPPS significantly increased the activity and mRNA levels of peroxisomal 3KCT, ACO, DCR, and mitochondrial CPT-I in the livers of mice after

both 4 weeks (150−300 mg/kg) and 10 weeks of administration. However, the observed variations in mitochondrial CPT-II activity and mRNA level were not significant. These results indicate that the up-regulation of peroxisomal enzymes might be primarily responsible for the HPPS-induced increase in the activity and mRNA levels of enzymes involved in FA oxidation.

Among the hepatic nuclear receptors, PPARα is a ligand-activated transcription factor, the activation of which induces the mRNA expression of several genes involved in FA oxidation to reduce the circulating lipid level.[43,44] Woo et al.[45] reported that fucoxanthin supplementation improves plasma and hepatic lipid metabolism by up-regulating PPARα and its target genes, such as ACOX1 and CPT. In the present study, HPPS supplementation for 10 weeks significantly up-regulated PPARα mRNA levels by 48.4 and 59.3% at doses of 150 and 300 mg/kg along with the up-regulation of PPARα protein expression by 37.4 and 36.5% for 300 mg/kg, respectively, compared with those of control mice fed a high-fat diet. The present results on PPARα and hepatic FA oxidation-related enzyme up-regulation suggest that HPPS might be a natural ligand and activator of PPARα, which in turn up-regulates the expression of downstream genes involved in hepatic FA oxidation.

Studies have demonstrated that an increased release of FFAs from adipose to other tissues, mainly to the liver, would cause hepatic esterification of FFA to TGs, which results in the formation of fatty liver and consequent reduction in FFA oxidation.[46] Our present experiment demonstrated that a high-fat diet reduced FA oxidation in the liver of mice while increasing serum FFA contents. By contrast, HPPS significantly lowered serum FFA contents in mice fed a high-fat diet. Similar observations have also been reported previously, wherein short-chain oligosaccharide supplementation in rats resulted in reduced plasma FFAs and TG, as well as hepatic FA synthase activity.[47]

The consumption of HPPS significantly increased fecal lipids, which might be due to the interruption of digestion and absorption of fat in mice. These observations were similar to the behavior of chitooligosaccharides in rats subjected to a high-fat diet.[48] Overall, considering the results mentioned above, HPPS was able to decrease body weight gain and serum TG contents in mice fed a high-fat diet through two potential mechanisms: (1) promoting FA degradation through oxidation pathways in liver and (2) interrupting pathways involved in fat digestion and absorption. However, the underlying details of such mechanisms still need further investigations.

In addition, the observed increase in the activities and gene expression of FA oxidation-related enzymes, as well as the decrease in weight gain and serum lipid levels after HPPS administration, which tended to be more effective at 10 weeks than at 4 weeks of treatment, revealed the constant influence of HPPS on the said biological activities.

In conclusion, the present experiments demonstrated that HPPS can suppress body weight gain and serum TG levels possibly by improving hepatic lipid metabolism through the activation of PPARα, which in turn up-regulates the expression of lipid oxidation-related enzymes, and by interrupting fat digestion and absorption. The results revealed the biomedical potential of pectin molecules for human and animal health benefits, particularly the possibility of creating functional dietary supplements to prevent and improve obesity cases and lipid metabolism disorder-related diseases.

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Notes
The authors declare no competing financial interest.

■ ABBREVIATIONS USED

HPPS, haw pectin pentasaccharide; NC, normal control; HFC, high-fat control; HF, high-fat; TG, triglyceride; TC, total cholesterol; FA, fatty acid; FFA, free fatty acid; ACO, acyl-CoA oxidase; CPT-I, carnitine palmitoyltransferase I; CPT-II, carnitine palmitoyltransferase II; 3KCT, 3-ketoacyl-CoA thiolase; DCR, 2,4-dienoyl-CoA reductase; PPARα, peroxisome proliferator-activated receptor α

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