Effect of Dietary Purified Xanthohumol from Hop (Humulus lupulus L.) Pomace on Adipose Tissue Mass, Fasting Blood Glucose Level, and Lipid Metabolism in KK-Ay Mice

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Abstract: We previously showed that xanthohumol-rich hop extract (XRHE, ~18% xanthohumol) exerts anti-obesity effects in rats fed a high-fat diet through regulation of fatty acid metabolism. In this study, we examined the effects of dietary purified xanthohumol from XRHE (PX, ~91.9% xanthohumol) in KK-Ay mice in order to understand the anti-obesity effects of xanthohumol alone because XRHE contains 82% unknown compounds. Dietary consumption of PX significantly inhibited an increase in the visceral fat weight of mice compared to those fed control diet without PX. Plasma leptin level was significantly lower in the PX-fed group than in the control group. Dietary PX lowered hepatic fatty acid synthesis by down-regulation of SREBP1c mRNA expression in the liver. On the other hand, fatty acid β-oxidation in the liver was promoted by dietary PX through the up-regulation of PPARα mRNA expression. Moreover, the fecal levels of fatty acids and carbohydrates increased by dietary PX. PX inhibited lipase or α-amylase activity in vitro. Thus, we found that PX may exert anti-obesity effects through the regulation of lipid metabolism and inhibition of intestinal fat and carbohydrate absorption, and that xanthohumol alone may exert anti-obesity effects.

Key words: hop, xanthohumol, obesity, lipid metabolism, mouse

1 INTRODUCTION

Increase in lifestyle-related disease patients, including diabetes and cardiovascular diseases, with diversification of eating habits has become a social problem in developed countries. In obesity, excessive accumulation of visceral adipose contributes more strongly to the incidence of lifestyle-related diseases. Development of obesity increases the risk for diseases such as dyslipidemia, hypertension, and type 2 diabetes. Therefore, prevention or improvement of obesity will reduce the risk of these diseases.

Various food ingredients attract attention because of their functionality in obesity management. Among these, the anti-obesity effect of polyphenols, including tea catechins, has been well studied. Polyphenols are components contained in a wide range of food products of plant origin such as vegetables, fruits, and coffee.

In recent study, dietary coffee polyphenols, peanut skin-derived polyphenols, or cacao liquor procyanidins inhibited the increase in adipose tissue mass and lipids levels, including triacylglycerol or cholesterol, in high-fat diet-fed C57BL/6J mice. Thus, dietary polyphenols may show beneficial physiological effects on the modulation of lipid metabolism accompanying obesity.

We focused on the functionality of hop plant (Humulus lupulus L.) extracts, including high-molecular-weight procyanidins or xanthohumol in previous studies. Xanthohumol is a prenylated chalconoid and non-hydrophilic flavonoid unlike other polyphenols. Several studies have reported that xanthohumol have bioactivities such as anticancer activity, anti-oxidant activity, and estrogenic activity.

Dietary xanthohumol-rich hop extract (XRHE, ~18% xanthohumol) suppressed the increase in body weight, mesenteric white adipose tissue, liver weight, and triacylglycerol levels in the plasma and liver through regulation of hepatic fatty acid metabolism and inhibition of intestinal
fat absorption in Wistar rats fed a high-fat diet. In addition, XRHE inhibited the intracellular fat droplets in and the differentiation of 3T3L1 cells by regulating adipogenic factors.

Xanthohumol inhibits the development of obesity and hepatic steatosis through down-regulation of hepatic SREBP1. Moreover, xanthohumol acts on farnesoid X receptor (FXR), suppresses white adipose tissue weight, liver weight, plasma triacylglycerol, and glucose levels through the regulation of lipid and glucose metabolism. Thus, the anti-obesity effect exerted by xanthohumol is expected; however, it is dependent on the experimental conditions and/or xanthohumol purity.

A major limitation of our studies is that the XRHE used contains unknown compounds in addition to xanthohumol, although these unknown compounds were not flavonoids. Therefore, we must use purified xanthohumol from XRHE to show the anti-obesity effects of xanthohumol alone. In this study, we examined the effects of dietary purified xanthohumol from XRHE (PX, ~91.9% xanthohumol) on growth, diabetes, adipocytokine levels, hepatic fatty acid metabolism, and fecal excretion in KK-Ay mice, which is an animal model of obesity-diabetes.

2 EXPERIMENTAL PROCEDURES

2.1 Reagents
XRHE was purchased from Asama Chemical Co., Ltd., (Tokyo, Japan). In this study, we purified XRHE to elucidate the biological activities of xanthohumol. All other chemicals were of analytical grade.

2.2 Purification of xanthohumol
We purified XRHE according to the method described by Ono et al. Around 2 g of XRHE was washed thrice with 200 mL of acetone. The filtrates were collected and concentrated to dryness. The concentrated extract was then mixed with 130 mL of ethanol and 70 mL of distilled water. Subsequently, the solution was gradually mixed with a suitable amount of 12 N NaOH until its pH reached 11. Then, the solution was mixed with 320 mL of distilled water and 19.4 g of KCl, and stirred for 30 min. After readjusting its pH to 11, the resulting mixture was subjected to filtration to remove precipitate, if any. The filtrate was collected, mixed with an appropriate amount of 25% H2SO4 to reach a pH value of 8.0, and stirred very slowly for 30 min to form a yellow precipitate. This precipitate was collected by filtration, washed with ~30 mL of distilled water, and lyophilized. The resultant powder was purified xanthohumol (PX).

2.3 Analysis of PX
The level of phenolic compounds in PX was found to be 919.3 mg/g equivalent of xanthohumol by Folin-Ciocalteu method. These compounds were analyzed using the Shimadzu LCMS 2010A series HPLC-PDA-ESI-MS system (Shimadzu Co., Kyoto, Japan) with YMC-Pack Pro C18 RS column (5 µm, 150 mm × 2.0 mm I.D.; YMC Co., Ltd., Kyoto, Japan). Phenolic compounds were eluted using the mobile phase consisting of 0.1% (v/v) aqueous acetic acid (phase A) and 0.1% acetic acid in methanol (phase B) at a flow rate of 0.2 mL/min. The linear gradient of phase B was 0% for the first 1 min, increased from 0% to 100% from 1 to 55 min, and maintained at 100% from 55 to 70 min. The detector wavelength was set at 280–370 nm. The mass spectra (MS) were acquired in the positive ion mode for analysis of phenolic compounds. Other conditions were set as follows. Ions were scanned from 100 to 600 m/z, with a scan speed of 1,000 amu/s. Nitrogen was used as the nebulizing gas at a flow rate of 1.5 L/min. The drying gas pressure was 0.1 MPa. The HPLC max plot chromatograms of XRHE(A) and PX(B) at 280–370 nm are shown in Fig. 1. The m/z (H+) values of xanthohumol (peak 2) were 355, 377, and 409.

2.4 Animals and diet
All animal experiments were conducted according to the guidelines provided by the Ethical Committee of Experimental Animal Care at the Meiji University (approval code: IACUC 10-0008). KK-Ay/TaJc1 mice (3-week-old males; CLEA Japan, Inc., Tokyo, Japan) were housed individually in a temperature (22–24°C) and light-controlled (7:00–19:00) room. After a 1-week-long acclimatization period, 13 KK-Ay/TaJc1 mice were divided into 2 groups; one group (6 mice) was fed the control diet (C group; 2% lard and 5% corn oil), and the other group (7 mice) was fed control diet supplemented with 0.2% PX (PX group). The diets were prepared according to the AIN-93G recommendations; the detailed composition of the diets is shown in Table 1. The mice were pair-fed by measuring their daily food consumption. Body weight was measured every 7 days. After 81 days, the mice were anesthetized using diethyl ether and bled from the fundus of the eye; various tissues were then quickly excised. Plasma was prepared by centrifugation after allowing blood to clot at room temperature. These samples were kept at ~80°C until analysis.

2.5 Plasma and liver lipid analyses
The levels of plasma triacylglycerols, free fatty acids, total cholesterol, and high-density lipoprotein (HDL) cholesterol were measured using commercial kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan) after the mice were killed. Liver lipids were extracted by the method described by Folch et al. The levels of liver triacylglycerols and cholesterol were measured according to the methods of Ide et al., Burchard et al., and Rouser et al., re-
Effect of xanthohumol on obesity in mice

Table 1  Diet composition.

<table>
<thead>
<tr>
<th>Component(%)</th>
<th>C</th>
<th>PX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>36.8</td>
<td>36.6</td>
</tr>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Pregelatinized Cornstarch</td>
<td>13.2</td>
<td>13.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Lard</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral mix(AIN93)</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mix(AIN93)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Purified xanthohumol from XRHE</td>
<td>0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

XRHE, xanthohumol-rich hop extract; C, KK-Ay mice fed control diet without purified xanthohumol from XRHE; PX, KK-Ay mice fed test diet with purified xanthohumol from XRHE.

2.6 Plasma levels of glucose, HbA1c, and insulin

The levels of plasma glucose (Glucose CII-Test Wako; Wako Pure Chemical Industries, Ltd., Osaka, Japan) and HbA1c (Diazyme Laboratories, Inc., CA, USA) were measured using kits. Plasma insulin levels were measured by using a Mercodia Mouse Insulin ELISA Kit (Mercodia, Uppsala, Sweden).

2.7 Plasma adipokine levels

The plasma levels of adiponectin and leptin were measured using an Adiponectin ELISA Kit (Assaypro Co., MO, USA) and Leptin (Mouse/Rat) ELISA Kit (AVIS-CERA BIOSCIENCE, Inc., CA, USA), respectively.

2.8 RNA extraction

Total RNA was extracted from mice liver tissue by using Sepasol-RNA I super G (Nacalai Tesque, Inc., Kyoto, Japan). RNA concentration was determined by measuring the absorbance at 260 nm by using a BioSpec-nano (SHIMADZU, Co., Kyoto, Japan).

2.9 Oligonucleotide primer sequences

The primers for reverse-transcription polymerase chain reaction (RT-PCR) amplification of the mouse sterol regulatory element-binding protein 1c (SREBP1c), fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME), peroxisome proliferator-activated receptor alpha (PPARα), acyl-CoA oxidase (ACOX), and carnitine palmitoyltransferase II (CPTII) gene were designed using Primer3Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus.cgi). The primers, synthesized by Operon Biotechnologies (Tokyo, Japan), were designed to flank known or putative introns of this gene, thereby preventing the amplification of any contaminating genomic DNA. The primer sequences were as follows: SREBP1c (Gene ID: 78968), forward 5′-GGGACCACTG-GATTGCACATT-3′ and reverse 5′-AGGAAGGCTTCC-GAGAGAGA-3′; FAS (Gene ID: 50671), forward 5′-TG-TGAGACACATCGTTTGA-GC-3′ and reverse 5′-CCCGAGGTTGGTTT-TAGA-3′; G6PDH (Gene ID: 14380), forward 5′-CTGTCGACACGGCCTCC-TCTCTCTT-3′ and reverse 5′-GACCATCTCTTTGGCACAAGGTATG-3′; ME (Gene ID: 17436), forward 5′-TGGATGGCCAGAGGTGT-GTC-3′ and reverse 5′-CATTACAGCCCAAGGTCTCAGCA-3′; PPARα (Gene ID: 25747), forward 5′-GGTTGATGACAGCT-GCAAAG-3′ and reverse 5′-GCCAGAGTTGAGGCTTC-GC-3′; ACOX (Gene ID: 50681), forward 5′-TGCTTTGTTT-CTCCCTTTCCATC -3′ and reverse 5′-GGTCCATAATGCTGGTCTGT-TG-3′; CPTII (Gene ID: 25413), forward 5′-CTCTGTCAAGATGGGAAAC-3′ and reverse 5′-GATGCTCTATCGGAGATC-3′; and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Gene ID: 24383), forward 5′-CTCATGACCACAGTCCATGC-3′ and reverse 5′-TTCAGCTCTGGGATGACCTT-3′, which was used as a control.

2.10 Real-time quantitative polymerase chain reaction

One microgram of RNA was incubated at 65°C for 5 min, and then quickly cooled on ice. Reverse transcription of RNA was performed using a ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo Co., Ltd., Osaka, Japan) and by heating the sample to 37°C for 15 min, followed by heating at 98°C for 5 min. An aliquot of the generated cDNA samples was mixed with 5 μL of THUNDERBIRD SYBR qPCR MIX (Toyobo Co., Ltd., Osaka, Japan) in the presence of 0.3 μmol of each of the forward and reverse primers for GAPDH and SREBP1c. This reaction mix was then subjected to the following cycling conditions in a Chromo 4 Sequence Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA): 1 cycle at 95°C for 1 min, followed by 40 cycles each of 95°C for 15 s and 58.5°C for 1 min. The results (fold-changes) were expressed as relative folds by comparing the amount of RNA of the target gene to that of GAPDH as an internal control, as determined by the equation 2^ΔΔCt [target + Ct/GAPDH].

2.11 Fatty acid and carbohydrate levels in feces

Fatty acids in feces were extracted by the method described by Jeejeebhoy et al.190, and analyzed using the Shimadzu gas chromatograph GC-14B (Shimadzu Co., Kyoto, Japan) with ZB-WAX column (0.25 mm I.D. × 30 m; Phenomenex, CA, United States). Carbohydrates in feces were extracted by the method described by Okuma et al.195.

Fecal glucose levels were measured using a commercial kit (Glucose CII-Test Wako; Wako Pure Chemical Industries, Ltd., Osaka, Japan).

2.12 Inhibition of lipase or α-amylase activity by PX in vitro

The inhibition of lipase activity by PX was analyzed according to the method of Kurihara et al.200. After the hydrolysis of triolein with or without PX, the level of free fatty acids was measured using a commercial kit (NFEF C-test Wako; Wako Pure Chemical Industries, Ltd., Osaka, Japan). The inhibition of α-amylase activity by PX was analyzed according to the method of Yoshikawa et al.201. The concentration of remainder starch, after hydrolysis of soluble starch, was also measured by iodo-starch reaction.

2.13 Statistical analysis

The data are expressed as the mean ± standard deviation (SD). The data were analyzed statistically using the Student’s t-test to evaluate significant differences between the values of the 2 groups.
3 RESULTS

3.1 Effects of dietary PX on growth parameters

Body weight was not significantly different between the 2 groups from days 0 to 81. There were no significant differences in the final body weight, waist size, and liver weight at 81 days between the 2 groups (Table 2). Cecal weight was significantly higher in the PX group, compared to the C group. However, the weight of epididymal, perirenal, or mesenteric white adipose tissues was significantly lower in the PX group than in the C group. Thus, the total weight of white adipose tissues was significantly lower in the PX group than in the C group.

3.2 Effects of dietary PX on plasma and liver lipid levels

Plasma triacylglycerol, free fatty acids, total cholesterol, and HDL cholesterol levels were not significantly different between the 2 groups (Table 3).

Liver triacylglycerol level did not significantly different between the groups. However, total liver cholesterol level was significantly lower in the PX group, compared to that in the C group.

3.3 Effects of dietary PX on fasting blood glucose, HbA1c, and insulin levels

Plasma fasting blood glucose, HbA1c, and insulin levels were not significantly different between the 2 groups (Fig. 2). However, HbA1c levels in the PX group tended to be lower than in the C group (p = 0.07). Furthermore, the fasting plasma glucose and insulin levels in the PX group were lower, compared to that in the C group.

3.4 Effects of dietary PX on plasma adiponectin and leptin levels

Plasma adiponectin level was not significantly different between the 2 groups. However, plasma leptin level was significantly lower in the PX group than in the C group (Fig. 3).

3.5 Effects of dietary PX on mRNA expression of SREBP-1c and fatty acid synthesis enzymes in the liver

The hepatic mRNA expression of SREBP1c was significantly lower in the PX group than in the C group (Fig. 4). Moreover, mRNA expression of FAS and ME was lower in the PX group than in the C group, although the former was not significant (p = 0.05).

3.6 Effects of dietary PX on mRNA expression of PPARα and fatty acid β-oxidation enzymes in the liver

The hepatic mRNA expression of PPARα was significantly higher in the PX group than in the C group (Fig. 5). The mRNA expression of ACOX and CPT II was higher in the

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**Table 2** Effect of dietary PX on growth parameters.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>481.5±52.2</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>25.3±1.9</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>44.0±3.4</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>18.7±2.7</td>
</tr>
<tr>
<td>Final waist (cm)</td>
<td>11.8±1.2</td>
</tr>
<tr>
<td>Liver weight (g/100g body weight)</td>
<td>4.47±0.37</td>
</tr>
<tr>
<td>Cecum weight (g/100g body weight)</td>
<td>0.83±0.20</td>
</tr>
<tr>
<td>WAT weight</td>
<td></td>
</tr>
<tr>
<td>Epididymal WAT (g/100g body weight)</td>
<td>4.03±0.84</td>
</tr>
<tr>
<td>Perirenal WAT (g/100g body weight)</td>
<td>1.85±0.31</td>
</tr>
<tr>
<td>Mesenteric WAT (g/100g body weight)</td>
<td>2.14±0.64</td>
</tr>
<tr>
<td>Total WAT (g/100g body weight)</td>
<td>8.02±1.15</td>
</tr>
</tbody>
</table>

Data are presented as the mean±SD of 6 or 7 mice in each group. *Significantly different from C group at p < 0.05 (Student’s t-test). WAT, white adipose tissue. The other abbreviations are the same as in Table 1.

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**Table 3** Effect of dietary PX on plasma and liver lipid levels.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Plasma triacylglycerol (mg/dL)</td>
<td>109.4±47.6</td>
</tr>
<tr>
<td>Free fatty acid (mEq/L)</td>
<td>3.19±0.89</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>175.6±27.1</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>158.0±17.1</td>
</tr>
<tr>
<td>Liver triacylglycerol (mg/g)</td>
<td>122.4±29.4</td>
</tr>
<tr>
<td>Cholesterol (mg/g)</td>
<td>32.8±6.7</td>
</tr>
</tbody>
</table>

Data are presented as the mean±SD of 6 or 7 mice in each group. *Significantly different from C group at p < 0.05 (Student’s t-test). Abbreviations are the same as in Table 1.
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PX group.

3.7 Effects of dietary PX on fecal excretion of fatty acid or carbohydrates
Fecal weight was not significantly different between the 2 groups (Fig. 6). However, fecal levels of fatty acids and carbohydrate were significantly higher in the PX group than in the C group.

3.8 Inhibitory action of PX on lipase or α-amylase activity
Lipase activity was inhibited by PX at 0.25–2.5 mg/mL (Fig. 7); however, it was not dose-dependent. Contrary to this observation, α-amylase activity was inhibited in a dose-dependent manner, and the half-maximal inhibitory concentration of PX was 10 mg/mL.

4 DISCUSSION
Increasing cases of obesity due to overnutrition or lack of exercise have become a serious social problem in developed countries because obesity is closely related to the development of various lifestyle diseases\textsuperscript{22, 23}. Therefore, the anti-obesity effects of some food ingredients have been attracting additional attention. The anti-obesity effect of tea catechins, including (-)-epigallocatechin-3-gallate, is widely accepted\textsuperscript{24}; it is a government-approved food for specified healthcare-related use in Japan. Many other polyphenols play regulatory roles in lipid metabolism. For example, quercetin reduced plasma and liver lipid levels in C57BL/6J OlaHsd mice by regulating lipid metabolism\textsuperscript{25}.
Procyanidins also exert hypolipidemic effects in various animal models\textsuperscript{5, 26}.

In this study, we focused on the anti-obesity effects of polyphenols obtained from hop extract. In another recent study, we observed that dietary XRHE strongly suppressed body weight gain and increase in liver weight and triacylglycerol levels in the plasma and liver, through the regulation of hepatic fatty acid metabolism and inhibition of intestinal fat absorption in Wistar male rats fed a high-fat diet\textsuperscript{7}. However, it was not clear whether those effects were exerted by xanthohumol alone because XRHE has 82 unknown non-flavonoid compounds. Here, we studied the anti-obesity effects of PX containing 91.9xanthohumol in order to elucidate the action of xanthohumol alone.

We observed that dietary PX significantly inhibited the
weight gain of epididymal, perirenal, and mesenteric white adipose tissues, although the final body weight did not change between the 2 groups. These observations were different from those of the previous study using XRHE in rats fed a high-fat diet\(^7\). This discrepancy can be attributed to the differences in species or dietary conditions. However, we expect xanthohumol to inhibit the increase in adipose tissue mass. Further studies are necessary to clarify this discrepancy.

Dietary PX reduced the levels of fasting blood glucose and insulin in male KK-Ay mice, although not significantly. HOMA-IR level also tended to be lower in the PX group (42.28 ± 8.65), compared to the C group (21.9 ± 8.08). We found that dietary hop pomace polyphenols significantly reduced the fasting blood glucose and HbA1c levels in Otsuka Long Evans Tokushima Fatty (OLETF) rats at 40 days\(^5\). These effects may be exerted by the inhibition of carbohydrate digestion because PX inhibited α-amylase activity and promoted carbohydrate excretion into feces as described later. Some polyphenolics inhibited carbohydrate digestion\(^27, 28\). Dietary PX may show anti-hyperglycemic effects by inhibiting glucose absorption, exerted by the inhibition of carbohydrate-hydrolyzing enzymes.

Liver is an essential organ that plays a key role in the metabolism of fatty acids, cholesterol, and carbohydrates. Hepatic fatty acid level is mainly regulated by fatty acid synthesis and fatty acid β-oxidation.

However, the triacylglycerol levels in the plasma and liver are not modulated by dietary PX. These observations differ from those of a previous study involving rats fed a
high-fat diet containing XRHE\textsuperscript{71}. We inferred that the present results might be affected by the non-fasting condition or differences among species. Dietary PX suppressed FAS and ME mRNA expression through reduction of SREBP1c mRNA expression. These data agreed with those from a previous study involving XRHE-fed rats. Other researchers reported the anti-obesity and anti-diabetes effect of xanthohumol. For example, Miyata \textit{et al.} reported that dietary xanthohumol reduced body weight, liver weight, white adipose tissue weight, and triacylglycerol levels in the liver through the regulation of hepatic SREBP1c mRNA expression and promotion of fecal excretion of triacylglycerols in C57BL/6J mice fed a high-fat diet\textsuperscript{85}. LeeCole \textit{et al.} reported that xanthohumol lowered the body weight and fasting plasma glucose levels in obese male Zucker rats\textsuperscript{39}. Prakash \textit{et al.} reported that dietary xanthohumol markedly reduced plasma cholesterol levels and hepatic triacylglycerol and cholesterol levels by suppressing hepatic lipid metabolism via activation of AMP-activated protein kinase in ApoE-deficient mice\textsuperscript{30}. Thus, xanthohumol and its metabolites exert regulatory effects on hepatic lipid metabolism.

In the present study, we found that dietary PX significantly increased hepatic fatty acid β-oxidation activities, involving ACOX and CPTII, by upregulating hepatic PPARα.

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**Fig. 5** Effect of dietary PX on hepatic fatty acid β-oxidation.
Data are presented as the mean ± SD of the data for 6 or 7 mice in each group. *Significantly different from C group at $p<0.05$ (Student’s $t$-test). PPAR, peroxisome proliferator-activated receptor alpha; ACOX, acyl-CoA oxidase; CPT II, carnitine palmitoyltransferase II. Other abbreviations are the same as in Table 1.

**Fig. 6** Effect of dietary PX on the fecal levels of fatty acids and carbohydrates.
Data are presented as the mean ± SD of the data for 6 or 7 mice in each group. *Significantly different from C group at $p<0.05$ (Student’s $t$-test). Abbreviations are the same as in Table 1.
mRNA expression. The effect of XRHE on fatty acid β-oxidation was not clarified in previous studies. Dietary xanthohumol may regulate fatty acid metabolism by promoting hepatic fatty acid β-oxidation in addition to suppressing fatty acid synthesis, although this needs to be verified in other animal models.

Hepatic cholesterol levels were lowered by dietary PX. These results are consistent with those of a previous study using XRHE\textsuperscript{7}. Other polyphenols also exerted hypocholesterolemic effect in various animal models\textsuperscript{31, 32}. Metabolites from xanthohumol such as 8-prenylnaringenin may modulate hepatic cholesterol metabolism because of estrogen activity\textsuperscript{33}.

Adipocytokines are endocrine factors secreted by adipocytes; they play important roles in homeostasis maintenance by being involved in carbohydrate and lipid metabolism\textsuperscript{34}. Leptin, mainly produced by the white adipose tissue, regulates energy level, food intake, and various inflammatory mediators. We observed that dietary PX reduced plasma leptin levels, along with a decrease in white adipose tissue mass, in KK-Ay mice. It tended to increase plasma adiponectin levels in Wistar rats in a previous study\textsuperscript{7}, although this effect was not observed in the present study. Therefore, dietary PX may improve plasma adipocytokine levels, especially leptin, by decreasing visceral fat weight in mice.

The fecal fatty acid and carbohydrate levels in the PX group significantly increased, as observed in case of XRHE consumption. PX inhibited the activity of a pancreatic lipase and α-amylase in our in vitro experiment (IC\textsubscript{50} of PX on α-amylase was 10 mg/mL). Thus, dietary PX may exert anti-obesity effects through the suppression of lipid and carbohydrate absorption from the small intestine by inhibiting pancreatic lipase and α-amylase activities. These effects may be related to the above-mentioned results.

Cecal weight significantly increased by dietary PX. The final body weight without cecal weight tended to be lower in the PX group (42.2 ± 2.8 g) than in the C group (43.6 ± 3.4 g). Moreover, the total WAT weight was significantly lower in the PX group (8.08 ± 0.47 g/100 g body weight) than in the C group (6.1 ± 0.2 g body weight). The effect of short-chain fatty acids (SCFAs) will be examined in the next study because intestinal bacterial flora may be altered by the antibiotic activity of PX and because SCFA may affect lipid metabolism.

Xanthohumol is a major prenylated flavonoid (chalcone) found in the hop plant. Approximately 1000 prenylated flavonoids have been identified from plants. The hydrophobic prenyl chain can affect cellular uptake and biological functions by improving the affinity for biological membranes\textsuperscript{35}. Chalcones and prenylated flavonoids were reported to exert anti-obesity effects, except for the physiological effects such as anti-inflammatory and anti-diabetes activities. For example, phlorizin, one of the dihydroxychalcones typically contained in apples, ameliorated lipid metabolism and plasma glucose levels in streptozotocin-induced diabetic rats\textsuperscript{36}. In addition, licochalcone A, a major prenylated flavonoid constituent of the Glycyrrhiza plant, prevented adipocyte differentiation and lipogenesis through the suppression of PPAR\textgamma and SREBP1 in ICR mice and 3T3-L1 cells\textsuperscript{37}. Moreover, 4-hydroxyderricin (4-HD) and xanthoangelol (XAG), the major prenylated chalcones present in

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig7.png}
\caption{Inhibitory action of PX on lipase or α-amylase activity. Data are presented as the mean ± SD of 3 analyses.}
\end{figure}
Ashitaba, prevented adiposity by modulating lipid metabolism via phosphorylation of AMP-activated protein kinase in the adipose tissue and liver\(^9\). Moreover, 8-prenyl quercetin, 4-HD, and XAG were found to be absorbed and distributed to various tissues such as the liver and white adipose tissue, in recent studies\(^9\). Therefore, we inferred that xanthohumol, a prenylated chalcone, may ameliorate lipid and glucose metabolism by affecting various tissues, like other chalcones and prenylated flavonoids.

This study was performed to elucidate the anti-obesity effects of xanthohumol alone, by using PX. We observed that dietary PX exerted anti-obesity effects by regulating lipid metabolism and inhibiting intestinal fat absorption in KK-A\(y\) mice; this was observed in previous experiments with XRHE-fed rats too. PX contains \(\sim 91.9\%\) xanthohumol. Thus, we suggested that xanthohumol may exert anti-obesity effects. However, dietary PX did not exert significantly different effects on the plasma lipid and liver triacylglycerol levels in KK-A\(y\) mice, which is an animal model of obesity-diabetes. On the other hand, these parameters changed in a previous study involving rats. This discrepancy can be attributed to species difference and dietary conditions. Dietary PX significantly lowered hepatic cholesterol levels. However, because few studies focused on xanthohumol-induced changes in cholesterol metabolism, little is known about the effect of xanthohumol on cholesterol metabolism. In future studies, we will examine in detail the effects and mechanism of the cholesterol-lowering effect of PX by using healthy mice.

5 CONCLUSION

We observed that dietary PX inhibited the increase in visceral fat weight in KK-A\(y\) mice by regulating hepatic fatty acid metabolism and leptin secretion from adipocytes. Dietary PX also promoted fecal excretion of fatty acids and carbohydrates. Thus, it can be said that xanthohumol alone exerts anti-obesity effects because PX contains \(\sim 91.9\%\) xanthohumol.

References

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