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Short communication

Polysaccharide gel coating of the leaves of *Brasenia schreberi* lowers plasma cholesterol in hamstersHyunsook Kim<sup>a, b</sup>, Qian Wang<sup>c</sup>, Charles F. Shoemaker<sup>d</sup>, Fang Zhong<sup>c</sup>, Glenn E. Bartley<sup>e</sup>, Wallace H. Yokoyama<sup>e, \*</sup><sup>a</sup> Department of Nutrition, University of California, Davis, CA, USA<sup>b</sup> Department of Physiology, College of Veterinary Medicine, Konkuk University, Seoul, South Korea<sup>c</sup> Department of Food Science, Jiangnan University, Wuxi, China<sup>d</sup> Department of Food Science and Technology, University of California, Davis, CA, USA<sup>e</sup> USDA, ARS, Western Regional Research Center, Albany, CA, USA

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## ABSTRACT

*Brasenia schreberi* (蓴菜 chún cài) is an invasive aquatic weed found in the USA, but the plant has economic value in Asia where it is cultivated for food. The young leaves of *B. schreberi* are coated with gelatinous water-insoluble mucilage. This mucilage is a polysaccharide composed of galactose, mannose, fucose, and other monosaccharides. Because some carbohydrate gels are hypocholesterolemic, we evaluated their cholesterol-lowering properties in male hamsters fed hypercholesterolemic diets containing 2% gel coat from *B. schreberi* (GEL), or 1% cholestyramine (CA), or 5% hydroxypropyl methylcellulose (HPMC), and compared them to 5% microcrystalline cellulose (control) for 3 weeks. We found that very-low-density lipoprotein-, low-density lipoprotein-, and total-cholesterol concentrations in plasma were significantly lowered by GEL, CA, and HPMC compared to control. High-density lipoprotein-cholesterol concentration was lowered by CA and HPMC. Body weights and abdominal adipose tissue weight of GEL and control group animals were greater than those of the CA and HPMC groups. Fecal lipid excretion was greater in the CA and HPMC groups than in the control group. Expression of hepatic CYP51 and CYP7A1 mRNA was upregulated by CA, HPMC, and GEL, indicating increased hepatic cholesterol and bile acid synthesis. Expression of low-density lipoprotein receptor mRNA was upregulated by all treatments. These results suggest that modulation of hepatic expression of cholesterol and bile acid metabolism-regulated genes contributes to the cholesterol-lowering effects of GEL.

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## 1. Introduction

The perennial water plant *Brasenia schreberi* (蓴菜 chún cài), called the watershield in the USA and junsai in China and Japan, produces a mucilage coating that covers the underwater leaves and stems. In the USA, *B. schreberi* blocks waterways and is considered a nuisance invasive species, although plant specialists believe that it is not an imported or exotic plant.<sup>1</sup> In Asia, it is considered a vegetable, and is cultivated and traded. The mucilage coating has been reported to have antialgal and antibacterial properties, although studies of its allelopathic, antialgal, and antibacterial

properties were conducted on extracts from the whole plant.<sup>1</sup> The mucilage consists of a gelatinous polysaccharide coating composed of about 32–40% D-galactose, 19–29% D-glucuronic acid, 13–16% L-fucose, 10–14% D-mannose, and other monosaccharides.<sup>2</sup> Mannose is believed to form the backbone of the polymer, and galactose forms its side chains. The acidic polymer can be extracted with hot water or alkali, but subsequently loses its ability to form gels. When used as a food, the leaves with the gel coating are served in cool soups to preserve its gel texture. Mannan-based polysaccharides such as glucomannan (konjac root) and galactomannan (fenugreek, guar gum, and locust bean gum) have been shown to lower blood cholesterol,<sup>3</sup> suggesting that *B. schreberi* polysaccharide may also lower plasma cholesterol.

Syrian hamsters are widely used as animal models of plasma and liver cholesterol modulation by diet.<sup>4</sup> Plasma cholesterol lowering by dietary intake of psyllium,<sup>5</sup> cholestyramine (CA),<sup>6</sup>

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guar,<sup>7</sup> oat and barley beta-glucans,<sup>8</sup> and hydroxypropyl methylcellulose (HPMC)<sup>9</sup> have been studied in hamsters. Cholesterol synthesis in the livers of male hamsters and male humans are low compared to other rodents<sup>10</sup> or female hamsters and humans. The sensitivity to dietary cholesterol-lowering foods or food components is facilitated by high initial plasma cholesterol levels. Dietary cholesterol can increase plasma cholesterol levels but also lowers hepatic cholesterol synthesis. Decreasing hepatic cholesterol synthesis in male hamster has little effect on plasma cholesterol because of the lower hepatic cholesterol synthesis, whereas more dietary cholesterol is required to increase plasma cholesterol in rats or mice. Hamsters also have identical primary bile acids to humans. Therefore, we selected a male Syrian hamster fed a hypercholesterolemic diet as the animal model to determine the effects of feeding the gel from *B. schreberi*.

## 2. Materials and methods

### 2.1. Animal care

Male golden Syrian hamsters (50–60 g, LVG strain; Charles River, Wilmington, MA, USA) were housed individually in wire-bottomed cages in an environmentally controlled room (20–22°C, 60% relative humidity, 12-hour alternating light/dark cycle). The hamsters were fed 5001 rodent diet (LabDiet; PMI International, Redwood, CA, USA; protein, 239 g/kg; fat, 50 g/kg; non-nitrogenous substances, 487 g/kg; crude fiber, 51 g/kg; ash, 70 g/kg; energy, 17 MJ/kg; and sufficient amount of minerals and vitamins for healthy maintenance) *ad libitum* for 1 week prior to feeding the experimental diets to acclimatize them to the new environment. Hamsters were weighed and randomized into four groups of nine to 10 hamsters each and were fed high-fat diets *ad libitum* for 4 weeks. The total dietary fiber content and cholesterol content of all diets were 5% and 0.1%, respectively. Dietary fiber composition of the diets were as follows: 5% microcrystalline cellulose (MCC) in the control diet, 5% HPMC (The Dow Chemical Company, Midland, MI, USA) in the HPMC diet, 1% CA and 4% MCC in the CA diet, and 2% *B. schreberi* polysaccharide and 3% MCC in the *B. schreberi* polysaccharide (GEL) diet. Diets contained 17% of energy as protein, 43% as carbohydrate, and 39% as fat (Table 1). Body weights were recorded weekly, and food intake was monitored twice per week. The study was approved by the Animal Care and Use Committee, Western Regional Research Center, USDA, Albany, CA, USA.

### 2.2. Polysaccharide composition of *B. schreberi* gel

*B. schreberi* leaves were collected from a lake in the Sierra Nevada foothills of California. The gel coating surrounding the

leaves was stripped off by hand, centrifuged to separate leaf fragments from the gel and freeze-dried for the feeding study. The monosaccharide composition of the gels was analyzed by the method of gas chromatography. Briefly, a sample of the freeze-dried gel was hydrolyzed by 2M trifluoroacetic acid for 1.5 hours at 115°C. The hydrolysate was dried, and water was added to drive off any acidic residues once more. Inositol was added as the internal standard. The aldehyde hydrolysates were reduced to their respective alcohols with hydroxylamine in pyridine and reacted with acetic anhydride to form the acetate derivative. The derivatized monosaccharides were analyzed by injecting 0.8 µL into a 30M Agilent J&W DB-1701 column (Santa Clara, CA, USA) on a gas chromatograph (GC-14A; Shimadzu, Kyoto, Japan). The temperature program was 120°C (2 minutes), ramped to 175°C at 10°C/minute, and ramped to 240°C at 3°C/minute and held for 10 minutes. The flame ionization detector (FID) was kept at 260°C. Standard curves of peak area and monosaccharide standards were determined for quantitation. Molecular weight of the watergel polymer was about  $1.7 \times 10^6$  Da, as determined by multiple-angle laser light scattering (Wyatt Technologies, Santa Barbara, CA, USA). Alduronic acids were determined by the sulfuric acid carbazole method. The dried gel contained 55.2% polysaccharides, 7.34% H<sub>2</sub>O, 7.26% protein, and 13.94% ash.

### 2.3. Plasma and tissue collection

Hamsters were feed deprived for 12 hours and anesthetized with a mixture of Isoflurane (Phoenix Pharmaceutical, St. Joseph, MO, USA) and oxygen. Blood was collected by cardiac puncture with syringes previously rinsed with potassium EDTA solution (15% w/v), and plasma was separated after centrifugation at 2000 × g for 30 minutes at 4°C. Livers were excised, weighed, immediately frozen in liquid nitrogen, and stored at –80°C for analysis.

### 2.4. Plasma and liver lipid analysis

Cholesterol in plasma lipoproteins was determined by size-exclusion chromatography as previously described.<sup>11</sup> Plasma triglycerides were determined by enzymatic colorimetric assays (Genzyme Diagnostics PEI Inc., Charlottetown, PE, Canada). Lyophilized, ground liver samples were extracted using an automated solvent extractor (Dionex, Sunnyvale, CA, USA) at 100°C and ~13.8 MPa with 3:1 hexane:2-propanol (method A) or at 125°C and 6.7 MPa with 3:2 hexane:2-propanol (method B). The lipid extracts were analyzed for hepatic triglycerides (Genzyme Diagnostics PEI Inc.), total cholesterol, and free cholesterol by enzymatic methods using kits (Wako Chemicals, Richmond, VA, USA). Feces were collected for 3 consecutive days immediately prior to sacrifice, lyophilized, milled, and stored at –20°C. Fecal lipids were determined by weighing after solvent extraction at 100°C and 13.8 MPa with 3:1 hexane:2-propanol.

### 2.5. Real-time polymerase chain reaction

Total RNA from livers was extracted using a TRIzol Plus RNA purification kit (Invitrogen, Life Technologies, Carlsbad, CA, USA), and cDNA was synthesized using GeneAmp RNA polymerase chain reaction (PCR) kit (Applied Biosystems, Foster City, CA, USA) as per manufacturer's protocol. Approximately 1 µL of diluted cDNA (1:10) was used in each real-time PCR reaction carried out using SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) with an Mx3000P instrument (Stratagene, Cedar Creek, TX, USA). The cycle conditions were as follows: 5 minutes at 95°C, followed by 20–35 cycles of incubation at 94°C for 15 seconds, and then at 55–60°C for 1 minute and 72°C for 30 seconds. Sequences of the primers used for

**Table 1**  
Diet composition.<sup>a</sup>

Ingredients/diet type	Control	Gel	CA	HPMC
MCC	52.6	31.6	42.1	0
HPMC	0	0	0	52.6
Watergel	0	21.0	0	0
Cholestyramine	0	0	10.5	0
Total fat (%)	20.0	20.0	20.0	20.0
Total protein (%)	20.0	20.0	20.0	20.0
Total carbohydrate (%)	49.8	49.8	49.8	49.8
HPMC (%)	0	0	0	5.0
Total dietary fiber (%)	5.0	5.0	5.0	5.0

CA = cholestyramine; HPMC = hydroxypropyl methylcellulose; MCC = microcrystalline cellulose.

<sup>a</sup> All diets contained anhydrous butter fat, 80.0 g; corn oil, 100.0 g; fish oil, 20.0 g; cholesterol, 1.0 g; casein, 221.9 g; DL-methionine, 3.0 g; choline bitartrate, 3.0 g; AIN-93 mineral mix, 35.0 g; AIN-93 vitamin mix, 10.0 g; and corn starch, 553.3 g.

**Table 2**  
Sequences of PCR primers.

Gene	Product size (bp)	Primer pair	5' Primer sequence 3'
18S	86	Forward	GGTCATAAGCTTGCCTTGAT
		Reverse	GAGGGCCTCACTAAACCATC
$\beta$ -ACTIN	96	Forward	ACGTTCGACATCCGCAAGACCTC
		Reverse	TGATCTCCTTCTGCATCCGGTCA
SCD1	127	Forward	GCCACCTGGCTGGTGAACAGTG
		Reverse	GGTGGTAGTTGTGGAAGCCCTCG
ABCG5	131	Forward	CCCTCACTTAATTGGAGAAT
		Reverse	GTTTCTGATAAATCCAGTCCAA
CYP7A1	154	Forward	ACTGCTAAGGAGGATTTCACTCT
		Reverse	CTCATCCAGGTATCGATCATATT
CYP51	195	Forward	GAGAGAAGTTTGCCTATGTGCC
		Reverse	TGTAACGGATTACTGGGTTTTCT
FXR $\alpha$	100	Forward	CCAAAATGAATCTGATTGAGCA
		Reverse	GCGCCTGCCTCTTGTCTGTTA
LDLR	128	Forward	TGAGGAACATCAAGCATAAAC
		Reverse	ATCCTCAGGCTGACCATCTGT
PPAR $\alpha$	133	Forward	CTCCACCTGCAGAGCAACCA
		Reverse	CGTCAGACTCGGTCTTCTTGAT

PCR = polymerase chain reaction.

this study are shown in Table 2. The primers were validated by assessing the size and sequencing of PCR products. No accumulation of nonspecific products and primer–dimers was observed in a gel electrophoresis test of the PCR reaction products. Results were analyzed using the software provided with the Stratagene Mx3000P QPCR system. Differences in mRNA expression were calculated after normalizing to 18S or  $\beta$ -actin expression.

## 2.6. Statistical analysis

All data are expressed as means  $\pm$  standard error. Differences between control and HPMC groups were determined by two-tailed Student *t* tests. When variances of each group were unequal, significance of differences was determined using Welch's test. Pearson's correlation coefficients were calculated for investigating relationships of plasma total cholesterol, plasma adiponectin concentrations, hepatic cholesterol, and triglyceride concentrations with the expression of hepatic genes (JMP 7 statistical program; SAS Institute, Cary, NC, USA). Significance was defined as  $p < 0.05$ .

## 3. Results

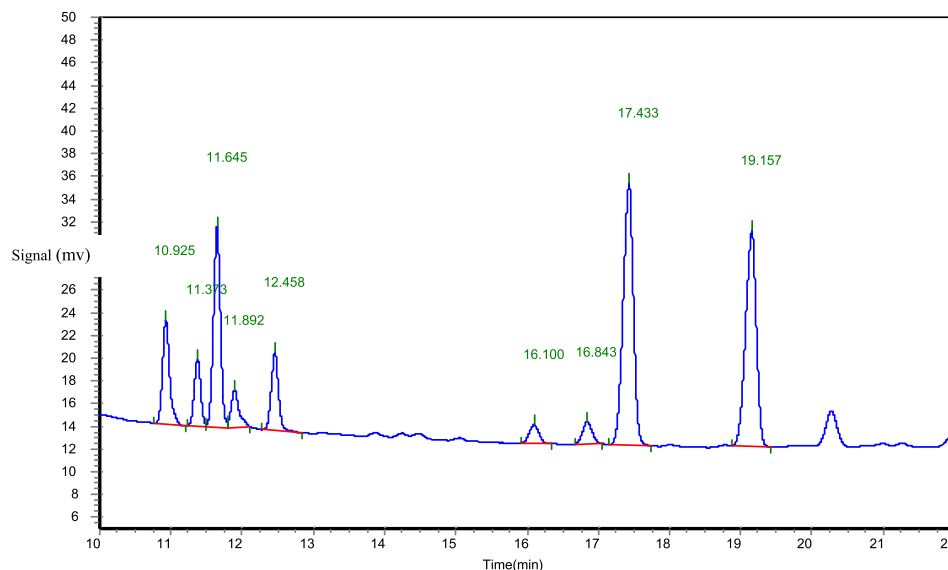
### 3.1. Metabolic effects of water gel supplemented with high-fat diets

Monosaccharide composition and retention times of the exopolysaccharide coating the leaf of *B. schreberi* determined by gas chromatography are shown in Fig. 1; the exopolysaccharide coating contained galactose (25.2%; peak at 17.4 minutes), mannose (1.6%; peak at 16.1 minutes), fucose (6.9%; peak at 11.6 minutes), rhamnose (7.6%; peak at 10.9 minutes), arabinose (3.1%; peak at 11.9 minutes), xylose (5.5%; peak at 12.5 minutes), glucose (2.1%; peak at 17.8 minutes), and alduronic acids (19.6%).

Weight gain of the hamsters fed high-fat diets supplemented with CA and HPMC was significantly lower by 22% and 27%, respectively, compared with the hamsters on the control diet ( $p < 0.05$ ; Table 3). By contrast, hamsters on the GEL diet gained 14% more weight, but the weight gain did not reach statistical significance compared to the control (Table 3). The GEL diet group had 13% greater food intake compared to the control group (Table 3). Feed efficiency (feed intake/weight gain) was significantly greater by 37% and 51% for animals on the CA and HPMC diets, respectively (Table 3). Liver and abdominal adipose weights were, respectively, 40% and 36% lower for the CA diet, and 38% and 29% lower for the HPMC diet (Table 3). Intestinal weights were significantly greater for the CA and HPMC diets by 14% and 38%, respectively (Table 3).

Compared to the control diet, all other diets had a significantly lower percentage of hepatic lipid content. However, only the HPMC diet had a lower percentage of hepatic triglyceride content than the control diet. Hepatic total and free cholesterol contents were significantly lower for all diets compared to control. Percent fecal lipid excretion of the GEL diet was similar to that of the control diet, but both the CA and HPMC diets had significantly greater, about 161%, fecal lipid excretion (Table 3).

Compared to the control diet, all diets had significantly reduced plasma total cholesterol, very-low-density lipoprotein (VLDL) cholesterol, and low-density lipoprotein (LDL) cholesterol. The CA diet was the most effective at lowering plasma total, VLDL, and LDL cholesterol by 76%, 92%, and 91%, respectively, compared to the control diet (Fig. 2). The HPMC diet also lowered plasma total, VLDL, and LDL cholesterol significantly (50%, 73%, and 56%, respectively;



**Fig. 1.** Gas chromatogram of elution of acetylated glucitol derivative of acid-hydrolyzed polysaccharide from *Brasenia schreberi*. The peaks correspond to galactose (17.4 minutes), mannose (16.1 minutes), fucose (11.6 minutes), rhamnose (10.9 minutes), arabinose (11.9 minutes), xylose (12.5 minutes), and glucose (17.8 minutes).

**Table 3**

Anthropometric data, and liver and fecal lipid contents in male hamsters fed control, GEL, CA, or HPMC diet for 3 weeks.

	Control	GEL	CA	HPMC
Weight gain (g/3 wk)	51.7 ± 2.4 <sup>a</sup>	59.0 ± 2.9 <sup>a</sup>	40.4 ± 4.0 <sup>b</sup>	37.8 ± 4.4 <sup>b</sup>
Feed intake (g/3 wk)	178.3 ± 6.0 <sup>b</sup>	208.9 ± 9.1 <sup>a</sup>	184.7 ± 8.7 <sup>b</sup>	201.5 ± 13.1 <sup>b</sup>
Feed efficiency ratio, (g feed/g gain)	3.53 ± 0.24 <sup>a</sup>	3.56 ± 0.19 <sup>a</sup>	4.82 ± 0.59 <sup>b</sup>	5.35 ± 0.35 <sup>b</sup>
Liver (g)	6.99 ± 0.21 <sup>a</sup>	6.52 ± 0.23 <sup>a</sup>	4.19 ± 0.12 <sup>b</sup>	4.36 ± 0.09 <sup>b</sup>
Intestine (g)	2.58 ± 0.08 <sup>a</sup>	2.88 ± 0.11 <sup>a</sup>	2.93 ± 0.11 <sup>b</sup>	3.56 ± 0.16 <sup>b</sup>
Epididymal adipose tissue (g)	4.44 ± 0.28 <sup>a</sup>	5.41 ± 0.45 <sup>a</sup>	2.86 ± 0.15 <sup>b</sup>	3.15 ± 0.15 <sup>b</sup>
Kidney (g)	0.49 ± 0.01 <sup>a</sup>	0.49 ± 0.01 <sup>a</sup>	0.48 ± 0.01 <sup>a</sup>	0.44 ± 0.1 <sup>a</sup>
Hepatic lipids				
Percent total lipid (g/100 g)	17.3 ± 0.5 <sup>a</sup>	9.9 ± 0.8 <sup>b</sup>	11.2 ± 0.3 <sup>b</sup>	14.4 ± 1.0 <sup>b</sup>
Triglyceride (mg/g)	13.3 ± 0.8 <sup>a</sup>	14.1 ± 2.3 <sup>a</sup>	11.3 ± 1.1 <sup>a</sup>	10.9 ± 0.4 <sup>b</sup>
Total cholesterol (mg/g)	30.7 ± 2.0 <sup>a</sup>	15.9 ± 3.1 <sup>b</sup>	5.5 ± 0.3 <sup>b</sup>	9.4 ± 0.9 <sup>b</sup>
Free cholesterol (mg/g)	8.1 ± 0.3 <sup>b</sup>	10.5 ± 1.0 <sup>a</sup>	4.2 ± 0.1 <sup>a</sup>	5.7 ± 0.3 <sup>a</sup>
Fecal lipids				
Percent total fecal lipid (g/100 g)	3.68 ± 0.28 <sup>b</sup>	3.44 ± 0.35 <sup>b</sup>	9.61 ± 1.1 <sup>a</sup>	9.63 ± 0.54 <sup>a</sup>

Data are presented as means ± SE,  $n = 10$ .

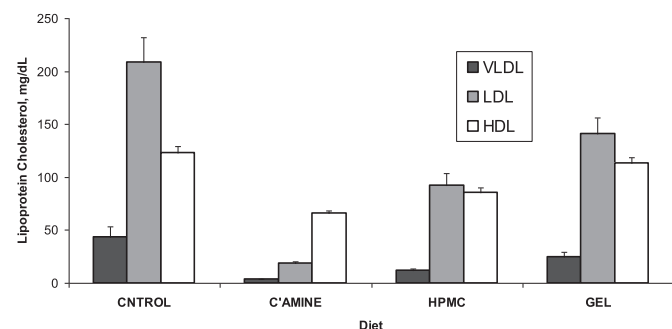
Different letters indicate significant difference at  $p < 0.05$ .

CA = cholestyramine; GEL = gel coat from *Brasenia schreberi*; HPMC = hydroxypropyl methylcellulose; SE = standard error.

**Fig. 2).** The GEL diet lowered plasma total, VLDL, and LDL cholesterol by 26%, 44%, and 33%, respectively (Fig. 2). The GEL diet did not significantly change high-density lipoprotein (HDL) cholesterol, but the CA and HPMC diets significantly lowered HDL cholesterol, by 46% and 31%, respectively. However, the HDL/LDL ratio was significantly increased by all diets, compared to the control diet, in the order CA > HPMC > GEL.

### 3.2. Levels of mRNA expression of hepatic genes related to bile acid and cholesterol metabolism

Expression of hepatic genes related to bile acid synthesis including *CYP7A1* (cholesterol 7 $\alpha$ -hydroxylase) and *FXR $\alpha$*  (farnesoid X receptor) was upregulated by 16- and 1.5-fold, respectively, by feeding the GEL diet (Fig. 3). The mRNA level of the hepatic gene that regulates cholesterol synthesis, *CYP51* (lanosterol 14 $\alpha$ -demethylase), was upregulated by 2.6-fold by GEL compared to control (Fig. 3). The expression level of LDL receptor was upregulated by



**Fig. 2.** Effect of control, GEL, CA, or HPMC diets on concentration of plasma lipids. Male golden Syrian hamsters were fed high-fat diets containing 5% (w/w) microcrystalline cellulose (control), 2% *Brasenia schreberi* polysaccharide and 3% MCC (GEL), 1% CA and 4% MCC (CA), and 5% hydroxypropyl methylcellulose (HPMC) for 3 weeks, and blood was collected in the fasting state. Data are expressed as mean ± SE;  $n = 8–10$ /group. Different letters indicate significant difference at  $p < 0.05$ . CA = cholestyramine; GEL = gel coat from *B. schreberi*; HPMC = hydroxypropyl methylcellulose; MCC = microcrystalline cellulose; SE = standard error.

1.8-fold, whereas mRNA level of the sterol transporter protein, *ABCG5* [ATP binding cassette (ABC) half-transporter], in the GEL diet was downregulated by 3.3-fold compared to the control diet (Fig. 3). Hepatic mRNA profiles of the CA and HPMC diets showed a similar pattern of gene expression to that of the GEL diet (Fig. 3). The expression level of toll-like receptor-4 in the adipose tissue was downregulated by the GEL and CA diets by 0.4- and 0.6-fold, respectively, compared to the control diet (data not shown) (Fig. 4).

## 4. Discussion

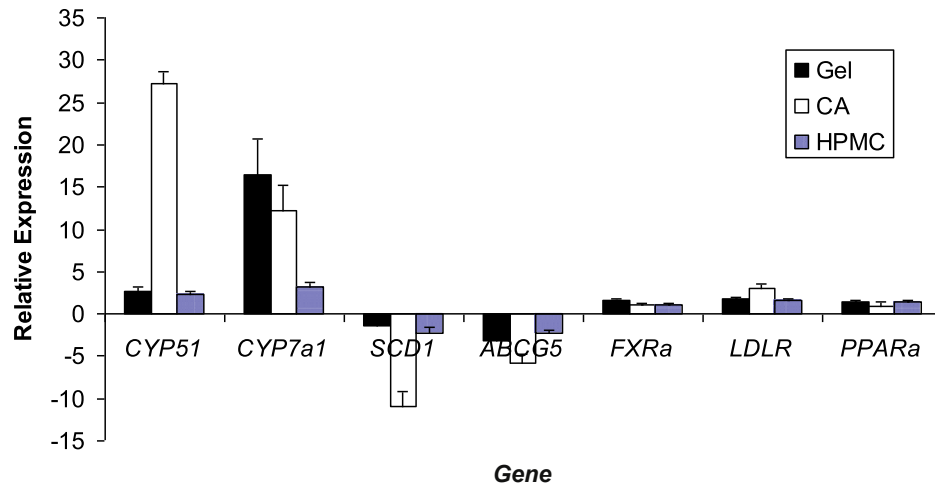
The polysaccharide gel coating the leaves of *B. schreberi* was shown to lower plasma VLDL, LDL, and total cholesterol in male hamsters fed a hypercholesterolemic high-fat diet. Although we have hypothesized that the polysaccharide component of the mucilage coating is responsible for the cholesterol-lowering effect, and only fed the polysaccharide coating, it is also important to note that extracts of the whole plant have been shown to have allelopathic, antialgal, and antibacterial properties,<sup>1</sup> and may possibly be present in the polysaccharide coating and may affect hepatic cholesterol metabolism.

The gelatinous coating surrounding the young leaves of the aquatic plant, *B. schreberi*, is a galactomannan polysaccharide and, like other galactomannans such as guar gum or fenugreek mucilage, may reduce plasma cholesterol.<sup>3,12</sup> *B. schreberi* polysaccharide, unlike guar or fenugreek, is highly branched, contains other neutral sugars besides galactose and mannose, and contains a significant amount (19–29%) of glucuronic acid<sup>2</sup> (19.6% in this study). The high glucuronic acid content and cross-linking with Ca<sup>2+</sup> or other cations may be responsible for the stability of the gel in water, while other galactomannans form dispersible viscous solutions.

Various physiological properties of *B. schreberi* have been reported, but only one alluded to hypocholesterolemic properties: “Hypoglycemic and antilipidemic effects of plant polysaccharides,” by Luo et al<sup>13</sup> in a short Chinese language abstract. The title suggests that they might have observed effects on cholesterol or triglyceride. The total plant extract of *B. schreberi* administered to HepG2 cell culture showed a reduction of secreted total, VLDL, LDL, and HDL cholesterol<sup>14</sup> and suggests the presence of hypocholesterolemic compounds. However, in a study on mice on high-fat diets supplemented with ethanol extracts of *Brasenia*, Evans et al<sup>12</sup> reported lowered mesenteric and epididymal adipose, lowered serum TG and glucose, but not lowered cholesterol. In this study, potential hypocholesterolemic properties of the *B. schreberi* polysaccharide gel leaf coating were determined in male hamsters, an animal model widely used for studies of dietary components and cholesterol metabolism, after being fed a high-fat and also a high-saturated-fat diet supplemented with cholesterol. It is possible that bioactive compounds, to suppress environmental pathogens, may also be secreted into the gel layer. The physiological effects and expression of genes related to cholesterol, bile acid, and fat metabolism were compared with those of two other well-studied polymers, CA and HPMC, whose cholesterol-lowering mechanisms are known.<sup>15,16</sup> CA is a cationic polystyrene divinylbenzene polymer that binds negatively charged bile acids. Binding of bile acids can precipitate cholesterol from mixed micelles as well as reduce reabsorption of bile acids necessary to maintain homeostasis of the hepatobiliary pool of bile acids. HPMC is a non-fermentable, viscous polymer that also increases excretion of bile acids. Different doses of CA, HPMC, and GEL were used, so inferences about relative activity should not be made.

In contrast to the CA and HPMC diets, the GEL diet had little effect on the final body weight, weight gain, or feed efficiency, although the intake of GEL food was significantly greater (Table 2). This difference with CA and HPMC may be due to the greater dose





**Fig. 3.** Hepatic mRNA expression of lipid metabolism-related genes, including cholesterol 7 $\alpha$ -hydroxylase (*CYP7A1*), farnesoid X receptor (*FXR*)  $\alpha$ , lanosterol 14R-demethylase (*CYP51*), LDL receptor (*LDLR*), ATP binding cassette (ABC) half-transporter (*ABCG5*), peroxisome proliferator-activated receptor (*PPAR*)  $\alpha$ , and stearoyl-coenzyme A desaturase (*SCD*)-1 in male golden Syrian hamsters fed high-fat diets containing 5% (w/w) microcrystalline cellulose, 2% *Brasenia schreberi* polysaccharide and 3% MCC (GEL), 1% cholestyramine and 4% MCC (CA), and 5% HPMC for 3 weeks. Each mRNA was normalized to 18S and is expressed relative to the control level. Data are expressed as mean  $\pm$  SE;  $n = 8$ –9/group. CA = cholestyramine; GEL = gel coat from *B. schreberi*; HPMC = hydroxypropyl methylcellulose; LDL = low-density cholesterol; MCC = microcrystalline cellulose; SE = standard error.

of HPMC or higher efficacy of CA, and the greater excretion of fecal lipids by these polymers compared to GEL. Correspondingly, liver and adipose weights of the hamsters on the CA and HPMC diets were lower, but intestinal weights were greater compared to the control hamsters. Viscous polymers produce higher intestinal weights, suggesting that GEL polysaccharides had lower viscosity than the HPMC or CA diets due to lower concentrations or inherent low viscosity. There were no differences in liver, intestinal ( $p = 0.07$ ) or adipose, and kidney weights between the GEL and control groups.

All polymer diets reduced total-, VLDL-, and LDL-cholesterol levels compared to the control (Table 2). The CA diet had the largest effect in reducing the total cholesterol (76%) compared to the control diet. Total cholesterol reduction by HPMC (50%) was greater than the reduction by GEL (25%), but the concentration of HPMC (5%) was also much higher compared to GEL polysaccharide (2%). The relative ratios of VLDL, LDL, and HDL cholesterol of GEL and HPMC are similar and suggest that their polysaccharides act similarly in the gut. HPMC is a viscous, nonfermentable polysaccharide; the GEL polysaccharide was isolated as a gel, but it may become a viscous polymer since extraction with hot water or alkali

results in viscous solutions.<sup>17</sup> Ingestion, gastric contractions, and acidic conditions may also break down the gel structure.

The expressions of hepatic genes related to cholesterol and bile acid synthesis by the GEL, CA, and HPMC diets were similar. Upregulation of *CYP51* and *CYP7A1* mRNA was observed in all GEL, CA, and HPMC diets (Fig. 3), indicating that both hepatic cholesterol and bile acid synthesis were increased.<sup>18</sup> Plasma cholesterol (Fig. 2) and hepatic gene expression (Fig. 3) results are consistent with our previous observations, which showed that dietary supplementation with HPMC<sup>16</sup> upregulated both *CYP7A1* and *CYP51* mRNA when VLDL and LDL cholesterol were lowered. These studies have found that increased excretion of fecal bile acid was associated with enhanced hepatic cholesterol and bile acid synthesis. In this study, fecal lipids were increased by about three-fold (Table 3), and in previous studies bile acid and total fecal lipid excretion increases were accompanied by decreased plasma cholesterol. In the current study, compared to the control diet, the GEL diet did not change fecal lipid excretion, while the CA and HPMC diets significantly increased fecal lipids. Therefore, the mechanism for cholesterol lowering by GEL appears to be different from those of HPMC and CA, and may be due to other bioactive components than viscous polymer coating. Downregulation of *ABCG5* gene in all three diets would limit cholesterol efflux to the bile duct in order to conserve cholesterol for bile acid synthesis.

## 5. Conclusion

In summary, plasma VLDL-, LDL-, and total-cholesterol concentrations were significantly lowered by GEL, CA, and HPMC compared to the control. Fecal lipid excretion was greater in the CA and HPMC groups than in the control group, but was not changed in the GEL group. Expressions of hepatic *CYP51* and *CYP7A1* mRNA were upregulated by CA, HPMC, and GEL, indicating increased hepatic cholesterol and bile acid synthesis. Expression of LDL receptor mRNA was upregulated by all treatments. The cholesterol-lowering effect of GEL was comparable with that of CA and HPMC, although the former appears to be mediated via a different mechanism. These results suggest that modulation of hepatic expression of genes related to cholesterol and bile acid metabolism contributes to the cholesterol-lowering effects of GEL.



**Fig. 4.** Table of Contents (TOC) graphic. Note. The TOC graphic was obtained from Robert H. Mohlenbrock (USDA-NRCS PLANTS Database/USDA NRCS. Northeast Wetland Flora: Field Office Guide to Plant Species. Chester: Northeast National Technical Center; 1995).

## Author disclosure statement

The authors have no conflicts of interest to declare.

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## References

1. Elakovich SD, Wooten JW. An examination of the phytotoxicity of the water-shield, *Brasenia schreberi*. *J Chem Ecol*. 1987;13:1935–1940.
2. Kakuta M, Misaki A. Polysaccharide of "Junsai (*Brasenia schreberi* J.F. Gmel)" mucilage: constitution and linkage analysis. *Agric Biol Chem*. 1979;43:993–1005.
3. Boban PT, Nambisan B, Sudhakaran PR. Hypolipidaemic effect of chemically different mucilages in rats: a comparative study. *Br J Nutr*. 2006;96:1021–1029.
4. Zhang Z, Wang H, Jiao R, et al. Choosing hamsters but not rats as a model for studying plasma cholesterol-lowering activity of functional foods. *Mol Nutr Food Res*. 2009;53:921–930.
5. Turley SD, Daggy BP, Dietschy JM. Cholesterol-lowering action of psyllium mucilloid in the hamster: sites and possible mechanisms of action. *Metabolism*. 1991;40:1063–1073.
6. Daggy BP, O'Connell NC, Jerdack GR, Stinson BA, Setchell KD. Additive hypocholesterolemic effect of psyllium and cholestyramine in the hamster: influence on fecal sterol and bile acid profiles. *J Lipid Res*. 1997;38:491–502.
7. Trautwein EA, Kunath-Rau A, Erbersdobler HF. Effect of different varieties of pectin and guar gum on plasma, hepatic and biliary lipids and cholesterol gallstone formation in hamsters fed on high-cholesterol diets. *Br J Nutr*. 1998;79:463–471.
8. Delaney B, Nicolosi RJ, Wilson TA, et al. Beta-glucan fractions from barley and oats are similarly antiatherogenic in hypercholesterolemic Syrian golden hamsters. *J Nutr*. 2003;133:468–475.
9. Kim H, Bartley GE, Young SA, Davis PA, Yokoyama W. HPMC supplementation reduces abdominal fat content, intestinal permeability, inflammation, and insulin resistance in diet-induced obese mice. *Mol Nutr Food Res*. 2012;56:1464–1476.
10. Dietschy JM, Spady DK, Stange EF. Quantitative importance of different organs for cholesterol synthesis and low-density-lipoprotein degradation. *Biochem Soc Trans*. 1983;11:639–641.
11. German JB, Xu R, Walzem R, et al. Effect of dietary fats and barley fiber on total cholesterol and lipoprotein cholesterol distribution in plasma of hamsters. *Nutr Res*. 1996;16:1239–1249.
12. Evans AJ, Hood RL, Oakenfull DG, Sidhu GS. Relationship between structure and function of dietary fibre: a comparative study of the effects of three galactomannans on cholesterol metabolism in the rat. *Br J Nutr*. 1992;68:217–229.
13. Luo Z-Y, Hu X-B, Wu M-C. Hypoglycemic and antilipemic effects of plant polysaccharides. *Food Sci*. 2007;28:596–599.
14. Takahashi J, Toshima G, Matsumoto Y, et al. *In vitro* screening for anti-hyperlipidemic activities in foodstuffs by evaluating lipoprotein profiles secreted from human hepatoma cells. *J Nat Med*. 2011;65:670–674.
15. Stedronsky ER. Interaction of bile acids and cholesterol with non-systemic agents having hypocholesterolemic properties. *Biochim Biophys Acta*. 1994;1210:255–287.
16. Bartley GE, Yokoyama W, Young SA, et al. Hypocholesterolemic effects of hydroxypropyl methylcellulose are mediated by altered gene expression in hepatic bile and cholesterol pathways of male hamsters. *J Nutr*. 2010;140:1255–1260.
17. Kakuta M, Misaki A. Structural characterization of water-shield mucilage of "Junsai (*Brasenia schreberi* J.F. Gmel)" polysaccharide. *Foods Food Ingredients J Japan*. 2004;209:298–304.
18. Kim H, Bartley GE, Rimando AM, Yokoyama W. Hepatic gene expression related to lower plasma cholesterol in hamsters fed high-fat diets supplemented with blueberry peels and peel extract. *J Agric Food Chem*. 2010;58:3984–3991.