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Supplementation of Hydroxypropyl Methylcellulose into Yeast Leavened All-Whole Grain Barley Bread Potentiates Cholesterol-Lowering Effect

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ABSTRACT: We investigated in Syrian Golden hamsters the biological impact and its underlying mechanism of single whole grain breads supplemented with 2−3% hydroxypropyl methylcellulose (HPMC), a semisynthetic viscous soluble dietary fiber (SDF) as a substitute for gluten. Hamsters were fed high-fat diets supplemented with 48−65% (w/w) differently ground, freeze-dried single grain breads including whole grain wheat, barley, barley supplemented with HPMC, debranned oat, and oat supplemented with HPMC which were compared to a diet containing microcrystalline cellulose (control). All single grain breads significantly lowered plasma LDL-cholesterol concentrations compared to the control. Enrichment with HPMC further lowered plasma and hepatic cholesterol concentrations. Despite the reduced molecular weight of naturally occurring soluble (1→3),(1→4)-β-D-glucan (β-glucan) caused by the bread-making process, whole grain barley breads downregulated hepatic expression of *CYP7A1* and *HMG-CoAR* genes that are responsible for bile acid and cholesterol synthesis, suggesting a possible role of bioactive compounds such as short-chain fatty acids and phenolic compounds from barley bread. Barley bread enriched with HPMC downregulated expression of *ABCG5* gene. Taken together, it appears that distinctive modulation of synthesis and excretion of hepatic cholesterol and bile acid contributes to the cholesterol-lowering properties of whole grain barley breads and breads enriched with HPMC. These data suggests that alternative whole grain breads supplemented with HPMC may provide consumers with a staple food that can assist in cholesterol management.

KEYWORDS: whole grain, barley, viscous soluble fiber, cholesterol, HMG-CoAR, CYP7A1, HPMC, hamster

INTRODUCTION

Consumption of diets rich in whole grains has been associated with lower risk of chronic diseases such as cardiovascular disease (CVD)[1,2] and diabetes.[3] Health benefits for CVD are attributed to various bioactive components such as dietary fiber, trace minerals, vitamins, lignans, and phytochemicals in the whole grains.[4] The FDA defines whole grains as the bran, germ, and starchy endosperm of amaranth, barley, buckwheat, bulgur, corn, millet, quinoa, rice, rye, oats, sorghum, teff, triticale, wheat, and wild rice.[5] Americans consumed only 26.6 g of whole grains per day[6] in 2003. Although whole grain intake is low, Americans have increased daily caloric intake from cereals from 450 to 600 calories between 1980 and 2005. This intake is mainly white wheat flour since wheat makes up 80% of all U.S. grain consumption and other grains such as barley and oat make up a mere 4% of consumption.[7] This low level of whole grain consumption results in an overall inadequate intake of health promoting fiber and other phytonutrients.

The large numbers of multi-whole grain breads in the marketplace reflect the increasing interest of health-conscious consumers in the added nutritional value of whole grains. Consumers recognize that other grains such as oat and barley contain components such as β-glucan, a soluble dietary fiber (SDF), that lower plasma cholesterol and limit the rise of postprandial blood glucose and insulin. Consumption of β-glucan from whole grain barley or oat was more beneficial compared to insoluble fiber from whole grain wheat.[8,9] This interest has prompted manufacturers to include oat and barley flours into their formulations but only at low levels since nonwheat flours can decrease loaf volume. Flour from hard wheat is the predominant component of most bread available to consumers because it exclusively contains the level and quality of gluten necessary for the volume expansion and crumb texture in baked bread.[10]

The effects of β-glucans depend on their polymeric structure; the glycan polymer can be degraded by endogenous cereal glucanases, yeast enzymes, and shear during the bread-making process.[11,12] Other viscous soluble fibers such as hydroxypropyl methylcellulose (HPMC), xanthan gum, pectin, and guar gum also lower plasma cholesterol.[13] HPMC is a well-characterized, nonfermentable, semisynthetic cellulose derivative with physiological properties of a SDF. Previous studies in animals and humans have linked increasing HPMC viscosity with hypocholesterolemic effects and increased fecal excretion of bile acid and

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Table 1. Diet Composition

ingredients[a]	g/kg					
	control	wheat	barley	barley-HPMC	oat	oat-HPMC
butter fat	74.1	72.4	72.2	72.2	71.2	71.2
corn oil	92.6	56.9	51.1	51.1	43.4	43.4
fish oil	18.5	18.1	18.0	18.0	17.8	17.8
cholesterol	0.9	0.9	0.9	0.9	0.9	0.9
MCC[b]	48.7	0	0	0	12.6	12.6
bread[c]	0	482.2	561.1	561.1	654.2	654.2
casein	205.5	175.5	156.9	156.9	154.4	154.4
DL methionine	2.8	2.7	2.7	2.7	2.7	2.7
choline bitartrate	2.8	2.7	2.7	2.7	2.7	2.7
AIN-93 mineral mix	32.4	31.7	31.6	31.6	31.2	31.2
AIN-93VX vitamin mix	9.3	9.1	9.0	9.0	8.9	8.9
corn starch	512.4	147.8	93.9	93.9	0	0
fat % of total energy	36.6	36.6	36.7	37.0	39.6	40.0
protein % of total energy	18.3	18.3	18.3	18.5	17.7	17.9
carbohydrate % of total energy	45.1	45.1	45.0	44.4	42.7	42.0
total HPMC %	0	0	0	2.3	0	2.7
total dietary fiber %	4.9	4.5	4.5	6.6	4.5	7.0

[a] All the ingredients were purchased from Dyets (Bethlehem, PA) except the fish oil (menhaden oil, #F8020, Sigma-Aldrich, St. Louis, MO). According to the manufacturer the 18 g of fish oil used in the diets contributes 1.8-2.5 g of eicosapentaenoic acid and 1.4−2.7 g of docosahexaenoic acid. [b] MCC (microcrystalline cellulose, control): nonviscous water-insoluble fiber. [c] Breads containing whole grain wheat, barley, barley with 5% HPMC, oat, oat with 5% HPMC, respectively.

cholesterol.[14−19] These effects are similar to those of fermentable SDF such as psyllium[20] and β-glucan.[21] Furthermore, HPMC-supplemented diets have shown a positive effect in regulating adipocytokine production in addition to improving lipid and glucose homeostasis.[22] A recent study[23] from our laboratory has demonstrated that the improvements in plasma lipid profiles seen in hamsters fed high fat (HF) diets supplemented with HPMC are linked to altered hepatic gene expression of bile acid and cholesterol as well as fatty acid metabolism-related genes, possibly due to modulation by fecal bile acid excretion and intestinal cholesterol absorption.

HPMC is a hydrocolloid frequently used as a gluten substitute in gluten-free bread,[24,25] and was used as early as 1976 to make rice breads for those who have gluten intolerance or celiac disease.[26] The breads described in this study are intended to improve the nutrition and health of the general population and not for individuals with gluten intolerance. In order to develop whole grain breads from a single grain source such as barley or oat flours that would impart additional significant health benefits complementary to whole wheat bread, HPMC or other gluten replacers are necessary. In our previous study, all-barley and all-oat breads enriched with 5% soluble fiber (HPMC), substitution for gluten, resulted in lower but reasonable loaf volumes, a darker crust, lighter crumb color, and acceptable textures. The overall acceptability of the barley bread was significantly higher than the whole wheat breads with or without HPMC.[27]

The Syrian Golden hamster (*Mesocricetus auratus*) has been extensively used as a model to study cholesterol metabolism because of its similarity to humans in terms of lipid profiles[28,29] and high sensitivity to dietary cholesterol resulting in hypercholesterolemic plasma profiles.[30] In order to determine the hypocholesterolemic effect due to the composition of three different single-grain breads alone or enriched with HPMC and the changes in β-glucan by the bread-making process, male hamsters were fed HF diets containing freeze-dried and ground breads produced from all-whole grain wheat, debranned-oat, whole grain barley, HPMC-supplemented debranned-oat (oat-HPMC) and whole grain barley (barley-HPMC) breads. The profiles of plasma and hepatic lipids and the expression of selected hepatic genes relating to cholesterol and bile acid metabolism were investigated.

MATERIALS AND METHODS

Animals and Diets. Male Syrian Golden hamsters (approximately 80 g, LVG strain) were purchased from Charles River Laboratories (Wilmington, MA). The hamsters were housed individually in an environmentally controlled room (20−22 °C, 60% relative humidity, 12 h alternating light:dark cycle). Hamsters were acclimatized and given water and a rodent diet (LabDiet #5001, PMI International, Redwood, CA; protein, 239 g/kg; fat, 50 g/kg; nonnitrogenous substances, 487 g/kg; crude fiber, 51 g/kg; ash, 70 g/kg; energy, 17 MJ/kg; and sufficient amounts of minerals and vitamins for healthy maintenance) *ad libitum* for 1 wk prior to the initiation of the experimental diets. Hamsters were weighed and randomized into 6 groups of 10 hamsters each and fed high-fat diets *ad libitum* containing 48−65% (w/w) ground, freeze-dried breads (whole grain wheat, whole grain barley, debranned-oat, barley +5% HPMC (The Dow Chemical Company), oat +5% HPMC and a control diet containing 5% microcrystalline cellulose (MCC; Dyets Inc. Bethlehem, PA) for 3 wk (Table 1). MCC is an insoluble fiber that has little effect on sterol metabolism.[20] Molecular weights of β-glucan were determined by size-exclusion chromatography and multiple angle laser light scattering detection as reported earlier.[31,32] β-Glucan content was determined enzymatically (AACC method 32-23, Megazyme, Wicklow, Ireland).

Table 2. Sequences of PCR Primers

gene	product size (bp)	primer pair	5′ primer sequence 3′
18S	86	forward	GGTCATAAGCTTGCGTTGAT
		reverse	GAGGGCCTCACTAAACCATC
ABCB11	136	forward	AACAACGCATTGCTATTGCTC
		reverse	GTCCGACCCTCTCTGGCTTT
ABCG5	131	forward	CCCCTCACTTAATTGGAGAAT
		reverse	GTTTCTGATAAATCCAGATCCAA
CYP7A1	154	forward	ACTGCTAAGGAGGATTTCACTCT
		reverse	CTCATCCAGGTATCGATCATATT
HMG-CoAR	164	forward	GGGGAGTTCAAACTGTATTACTT
		reverse	ACGCTCCTTGAACACCTAGCATC
		reverse	GTTACCTGCGCAAGCTTCTCTGA
SREBP-2[a]	93	forward	AGCTGGCAAATCAGAAAAACAACAAG
		reverse	GATTAAAGTCTTCAATCTTCAAGTCCAC

[a] Reference 52.

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.

Plasma and Liver Collection. Hamsters were feed-deprived for 12 h and anesthetized with isoflurane (Phoenix Pharmaceutical, St. Joseph, MO). Blood was collected by cardiac puncture with syringes previously rinsed with potassium EDTA solution (15 wt %/v). The plasma was separated after centrifugation at 2000*g* for 30 min at 4 °C. Livers were collected, weighed, and immediately frozen in liquid nitrogen for analysis.

Plasma Lipids, Hepatic Lipids, and Glucose Analysis. Cholesterol in plasma lipoproteins was determined by size-exclusion chromatography as previously described.[33] Briefly, an Agilent 1100 HPLC chromatograph was employed with a postcolumn derivatization reactor, consisting of a mixing coil (#1615-50 Bodman, Aston, PA) in a temperature-controlled water jacket (Aura Industrials, Staten, NY). A Hewlett-Packard (Agilent, Palo Alto, CA) HPLC pump 79851-A was used to deliver cholesterol reagent (Roche Diagnostics, Indianapolis, IN) at a flow rate of 0.2 mL/min. Bovine cholesterol lipoprotein standards (Sigma Aldrich, St. Louis, MO) were used to calibrate the signal on the basis of peak areas. Fifteen microliters of plasma was injected onto a Superose 6HR HPLC column (Pharmacia LKB Biotechnology, Piscataway, NJ). The lipoproteins were eluted with a pH 7.0 buffer solution containing 0.15 M NaCl and 0.02% sodium azide at a flow rate of 0.5 mL/min. Plasma triglyceride level was determined by an enzyme assay kit (Genzyme Diagnostics PEI Inc., PE, Canada).

Hepatic triglycerides, total cholesterol, and free cholesterol were determined by enzymatic colorimetric assays using assay kits (Genzyme Diagnostics PEI Inc., PE, Canada, Roche Diagnostics, Indianapolis, IN, and Wako Chemicals, Richmond, VA).

Blood glucose concentration in feed-deprived hamsters was measured in tail vein samples using an OneTouch Ultrameter (LifeScan Inc., Milpitas, CA).

Real-Time RT-PCR. Total RNA from livers was extracted using TRIzol plus RNA purification kit (Invitrogen, Life Technologies, Carlsbad, CA), and cDNA was synthesized using GeneAmpRNA PCR kit (Applied Biosystems, Foster City, CA) per the manufacturer's protocol. One microliter of diluted cDNA (1:10) was used in each real-time RT-PCR using SYBR Green Supermix (Bio-Rad, Hercules, CA) with an Mx3000P instrument (Stratagene, Cedar Creek, TX). The cycle conditions were as follows: 5 min at 95 °C followed by 20−35 cycles of incubation at 94 °C for 15 s, then 55−60 °C for 1 min and 72 °C for 30 s. The sequences of the primers used for this study are shown in Table 2. The primers were validated by size and sequencing of PCR products. No accumulation of nonspecific products and primer−dimers was observed in a gel electrophoresis test of the PCR products. The results were analyzed using the software provided with the Stratagene Mx3000P QPCR system. Differences in mRNA expression were calculated after normalizing to *18S* mRNA expression.

Fecal Bile Acids and Total Lipid Analysis. Feces were collected during the last 3 consecutive days of the feeding period and were lyophilized, milled, and stored at −20 °C. Bile acids were determined by HPLC, and fecal total lipid contents were determined gravimetrially after solvent extraction (ASE 200, Dionex Corp., Sunnyvale, CA) as described previously.[34]

Statistical Analysis. All data are expressed as means ± SE. One-way analysis of variance (ANOVA) was performed to examine the effect of treatment on plasma biomarkers, lipid levels, and body and tissue weights using the JMP7 statistical program (SAS Institute, Cary, NC). Tukey−Kramer HSD (honestly significant difference) tests were used to determine the significant differences in group means. Pearson correlation coefficients were calculated for investigating relationships between hepatic expression of *CYP7A1* and *HMG-CoA* genes. Significance was defined at $P < 0.05$.

RESULTS

Metabolic Effects of Single-Grain Breads with HPMC Supplementation on Hamsters. Plasma total-cholesterol concentrations were 16, 41, and 31% lower in hamsters fed the wheat, barley-HPMC, and oat-HPMC bread diets than in the control diet, respectively, for 3 wk ($P < 0.05$) (Figure 1A). Although not statistically significant, reductions of total cholesterol in hamsters fed the barley and oat bread diets were also lower, 13% and 12%, respectively, than in the control (Figure 1A). All of the bread diets showed 30−50% lower plasma low density lipoprotein (LDL)-cholesterol concentrations compared with the control diet ($P < 0.05$) (Figure 1A). Plasma high density lipoprotein (HDL)-cholesterol concentrations were 21% and 6% lower in barley-HPMC and oat-HPMC, respectively, than in the control diet (Figure 1A). Although HDL concentrations were lower in the barley-HPMC and oat-HPMC diets, the LDL/HDL ratio was substantially lower in all diets containing bread than those in the control diet (Figure 1B).

Hepatic total lipid concentrations were lower in hamsters fed the barley, barley-HPMC, oat, and oat-HPMC diets than those in the control diet ($P < 0.05$) (Figure 2A). Hepatic triglyceride concentrations were also lower in barley-HPMC and oat-HPMC diets than in the wheat diet ($P < 0.05$) (Figure 2B). Barley-HPMC and oat-HPMC diets lowered the total hepatic cholesterol concentrations by 81% compared with the control diet ($P < 0.05$) (Figure 2B). The oat diet also lowered total hepatic cholesterol concentrations by 42% (Figure 2B). Barley-HPMC and oat-HPMC diet significantly lowered hepatic free cholesterol concentrations compared with the control diet ($P < 0.05$) (Figure 2B).

Food intake in the barley-HPMC diet was 15% greater than those in the control diet, although liver weight in the barley-HPMC diet was 23% lower than in the wheat and oat diets (Table 3). Feed efficiency was higher for the wheat, oat, and oat-HPMC diets compared to the control. In addition, the oat-HPMC diet lowered liver weight by 22% compared with the wheat diet. Final body weights, retroperitoneal adipose weights, and blood glucose of the bread diets did not differ from the control diet. Hamsters fed the barley and barley-HPMC diets excreted significantly more bile acids than did control diet ($P < 0.05$) (Table 3). Moreover, greater fecal total lipid excretion by 142% was observed in the barley-HPMC diets than in the control diet ($P < 0.05$) (Table 3).

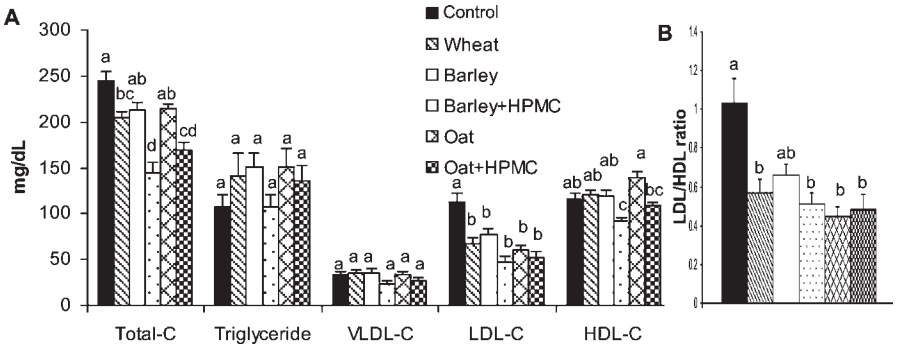

Figure 1. Effect of bread diets containing whole grain wheat, barley, barley with HPMC, oat, oat with HPMC on concentration of plasma lipids (A) and LDL/HDL ratio (B) in male Golden Syrian hamsters fed diets containing breads of whole grain wheat, whole grain barley, debranned-oat and/or enriched with HPMC for 3 wk; blood was collected in a food-deprived state. Data are expressed as means ± SE; $n = 8-10$/group. Different letters indicate significant difference at $P < 0.05$.

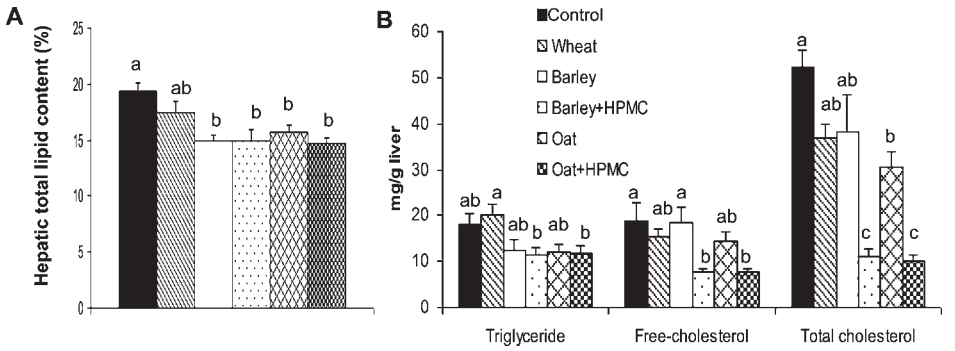

Figure 2. Effect of breads containing whole grain wheat, barley, barley with HPMC, oat, oat with HPMC on hepatic total lipid content (A) and hepatic triglyceride and cholesterol contents (B). Data are expressed as means ± SE; $n = 8-10$/group. Different letters indicate significant difference at $P < 0.05$.

Table 3. Anthropometrics, Fecal Lipid, and Plasma Glucose Concentration in Male Hamsters Fed Breads Containing Whole Grain Wheat, Barley, Barley with HPMC, Oat, Oat with HPMC for 3 Wk[a]

	control	wheat	barley	barley-HPMC	oat	oat-HPMC
			Anthropometric Data			
body wt, g	118.9 ± 2.6	128.3 ± 2.6	126.8 ± 3.4	128.2 ± 4.5	127.7 ± 4.9	125.4 ± 3.6
food intake, g/d	8.2 ± 0.2 a	8.3 ± 0.2 ab	8.2 ± 0.3 ab	9.6 ± 0.5 b	8.2 ± 0.3 ab	8.5 ± 0.4 ab
liver wt, g	5.7 ± 0.3 ab	6.1 ± 0.3 a	6.0 ± 0.3 ab	4.7 ± 0.2 b	6.1 ± 0.4 a	4.9 ± 0.3ab
RA[b] wt, g	1.8 ± 0.1	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.1	2.4 ± 0.2	2.3 ± 0.2
feed efficiency, wt gain/food intake	0.18 ± 0.01 a	0.24 ± 0.01 b	0.22 ± 0.01 ab	0.22 ± 0.02 ab	0.24 ± 0.01 b	0.27 ± 0.02b
			Fecal Bile Acids and Total Lipid			
fecal bile acids, μg/g	2100 ± 98a	2600 ± 120 ab	2800 ± 120 b	2900 ± 147 b	ND[c]	ND
% total lipid, g/100 g	3.1 ± 0.5a	5.6 ± 0.4 ab	5.3 ± 0.2 ab	7.5 ± 1.8 b	ND	ND
blood glucose, mg/dL	89.4 ± 7.0	111.9 ± 13.1	98.6 ± 7.8	127.5 ± 16.5	120.8 ± 1.9	103.8 ± 5.7

[a] Values are means ± SE, $n = 9-12$. Not sharing the same letters indicates significant difference at $P < 0.05$. [b] RA: Retroperitoneal adipose tissue. [c] ND: Not determined.

Reduced Average Molecular Weight of β-Glucan in Barley-Grain Bread. The amount and molecular weight (MW) of the β-glucan were determined in the barley flour and bread. The barley diet had 3.4% β-glucan, but the wheat and oat diets had lower levels, 0.6% and 0.7%, respectively. The average MW of the β-glucan in barley flour and bread was determined by size exclusion chromatography and multiple angle laser light scattering detection. The bread making process lowered the average MW of barley β-glucan by 43% from 796,000 Da in the flour to 452,000 Da. The HPMC used in this study has a MW of 390,000[35] and is not fermented[36] in passage through the digestive system.

Effects of Single-Grain Bread with HPMC Supplementation on Expression of Hepatic Genes Related to Cholesterol and Bile Acid Metabolism. Hepatic expression of genes regulating the major cholesterol and bile acid metabolism was determined in order to understand the molecular basis for the cholesterol lowering by single grain breads (wheat, oat, and barley) and HPMC supplementation. The mRNA levels of

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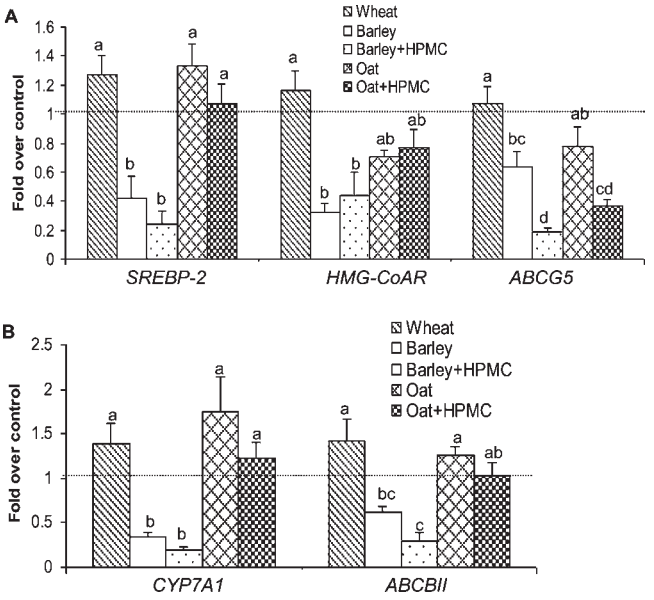

Figure 3. Hepatic mRNA expression of cholesterol-related genes (A) including sterol responsive element binding protein-2 (*SREBP-2*), 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMG-CoA*R), ATP binding cassette (ABC) half-transporter (*ABCG5*) and bile acid synthesis-related genes (B) including cytochrome P450 7A1 (*CYP7A1*), and ATP-binding cassette, subfamily B member 11 (*ABCB11*) in male Golden Syrian hamsters fed breads containing whole grain wheat, barley, barley with HPMC, oat, oat with HPMC for 3 wk. Each mRNA was normalized to *18S* mRNA and is expressed as a relative level to MCC group. Data are expressed as means ± SE; $n = 8-9$/group. Different letters indicate significant difference at $P < 0.05$.

hepatic genes involved in cholesterol synthesis, *SREBP-2* (sterol response element binding protein-2) and *HMG-CoAR* (3-hydroxy-3-methylglutaryl CoA reductase), were 0.42- and 0.24-fold lower, respectively in the barley diet, and 0.3- and 0.4-fold lower in the barley-HPMC diet, respectively, than in the control diet (Figure 3A). The mRNA level of *ABCG5* (ATP-binding cassette subfamily G member 5) was 0.19- and 0.36-fold lower, respectively, in barley-HPMC and oat-HPMC diets than in the control diet (Figure 3A).

The barley and barley-HPMC diets lowered expression of *CYP7A1* (cytochrome P450 7A1) mRNA by 0.34- and 0.19-fold, respectively, than in the control diet (Figure 3B). In addition, the mRNA levels of *ABCB11* (ATP-binding cassette, subfamily B member 11) were lower in the barley and barley-HPMC by 0.62- and 0.3-fold, respectively, compared with the control diet (Figure 3B).

DISCUSSION

The availability of bread, formulated entirely with whole grain barley or oat flours, and supplemented with HPMC, may promote the additional consumption of whole grains and the health benefits for CVD attributed to whole grains. The present study showed that all breads containing whole grain wheat and barley, or debranned oat were capable of significantly lowering plasma LDL-cholesterol compared with the control diet.

Enrichment of the barley and oat breads with HPMC (2.3−2.7%), as a substitute for gluten, resulted in additional lowering of plasma total- and LDL-cholesterol concentrations compared to the control diet. HPMC supplementation maintained the favorable LDL/HDL ratio. Moreover, hepatic cholesterol concentrations were significantly lower in both barley- and oat-HPMC diets compared to their respective barley and oat diets. Although the HPMC supplemented diets further decreased cholesterol concentrations, in our experience feeding less than 3% highly viscous HPMC itself does not significantly lower plasma cholesterol concentrations in hamsters. These data collectively suggest that HPMC and other bioactive components present in the flours may act additively or synergistically to contribute to the overall cholesterol-lowering effect.

In this study, we found that, compared with the 5% HPMC in a previous study,[23] the combination of 2% HPMC and 5% cereal dietary fibers had a similar efficacy for lowering plasma total- and LDL-cholesterol and hepatic cholesterol concentrations. However, the underlying mechanisms appear to be different. The expression of critical regulatory genes for hepatic cholesterol and bile acid metabolism such as *SREBP-2, HMG-CoAR, CYP7A1* and *ABCB11* was upregulated by 5% HPMC in the previous study but downregulated by the all-barley and barley-2% HPMC bread diets in the present study. Components of barley have been shown previously to inhibit hepatic HMG-CoAR enzyme activity.[37,38] Short chain fatty acids (SCFA), generated by intestinal bacterial fermentation, could be contributors to these modulations of hepatic genes and enzymes related to cholesterol metabolism. Male Wistar rats fed a SCFA diet had lowered hepatic and intestinal cholesterol synthesis rates and concomitantly lowered plasma cholesterol concentrations.[39] Direct treatment of human enterocyte cells by SCFA, such as propionate and butyrate, downregulated expression of gene related cholesterol synthesis.[40]

Several previous studies[20,41,42] including our earlier hamster study[23] have suggested that increased hepatic cholesterol and bile acid synthesis are the result of a compensatory mechanism for fecal bile acid loss. Rats fed guar gum or psyllium husk and hamsters fed 5% HPMC for 2 or 3 weeks had increased fecal bile acid excretion, hepatic expression of *CYP7A1* and *HMG-CoA* mRNA and decreased plasma total cholesterol levels.[20,23,42] In our earlier hamster study, 5% HPMC supplementation induced 63% greater fecal bile acid loss.[23] In contrast, in the present study, total bile acid excretion was only 38% greater with barley-HPMC diet than those in the control diet. Therefore, the levels of HPMC (2.3−2.7%), supplemented into 5% whole grain barley bread, may not have been sufficient to maximize bile acid excretion nor upregulation of hepatic expression of *SREBP-2* and *CYP7A1* genes.

Studies in humans and rats[43,44] have suggested that there is a significant link between cholesterol synthesis and activity of CYP7A1, a key enzyme for the initial rate-limiting step in bile acid synthesis. In this study, there was a strong positive correlation ($r = 0.66$, $P < 0.001$) between *CYP7A1* and *HMG-CoA* mRNA expression in the whole grain barley and barley-HPMC bread diets. This correlation suggests that decreases in hepatic bile acid and cholesterol synthesis by whole grain barley bread are connected. While there is always a limitation of relying on mRNA expression levels as a measure of pathway flux, previous studies have shown a linear relationship between enzyme activity and gene expression for these enzymes. For example, dietary supplementation with psyllium or cholestyramine upregulates both *CYP7A1* mRNA and CYP7A1 enzyme activity.[20,45]

The fold-change of *CYP7A1* mRNA expression was comparable to the fold-change of *ABCB11* mRNA compared to the control, gene encoding a bile acid transporter, in whole grain barley and barley-HPMC bread diets. This reduction of *ABCB11* gene expression may prevent the loss of hepatic bile acids when hepatic bile acid synthesis is low as indicated by *CYP7A1* gene

expression. *ABCG5*, a cholesterol transporter, in the liver mediates cholesterol secretion into the bile duct, and its upregulation is presumed to increase biliary cholesterol excretion resulting in lower plasma cholesterol levels.[46] HPMC supplementation both into barley and oat breads downregulated levels of hepatic *ABCG5* mRNA by ~80% compared to the control diet, indicating the decreased rate of cholesterol secretion may be necessary to compensate for the low hepatic cholesterol content.

While heat processing may inactivate β-glucanases that can hydrolyze β-glucan,[47] the bread-making process is known to result in β-glucan hydrolysis.[48] However, the effectiveness of β-glucan for cholesterol lowering has been shown to be variable and dependent on a wide range of β-glucan MW or viscosity.[12,49,50] Polymer size reduction of 85−90% by enzymatic hydrolysis or mechanical shear from 1,000 to 2,000 kDa to 100−200 kDa range did not eliminate the hypocholesterolemic properties of β-glucan.[11,32] In the present study, average MW of β-glucan in the barley bread diet was observed to decrease by ~400 kDa and 43%, similar to earlier studies of enzymatic degradation of β-glucan in the oat bread,[51] indicating cholesterol lowering efficacy of the barley bread β-glucan.

In conclusion, we have demonstrated that consumption of whole grain breads reduces plasma cholesterol concentrations in male hamsters; particularly LDL-cholesterol concentration. These hypocholesterolemic effects of whole grain barley and wheat breads appear to be mediated by distinctive modulation of hepatic genes related to cholesterol and bile acid synthesis. The supplementation of breads with HPMC, as a substitute of gluten, further enhances the hypocholesterolemic properties of whole grain barley breads, particularly for plasma total and hepatic cholesterol concentrations, possibly via increased bile acid excretion. This study also suggests that in addition to dietary fiber the complex compositions of whole grains may result in multiple means of regulating the synthesis and excretion of cholesterol and bile acids in the liver.

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