

Barley Intake Induces Bile Acid Excretion by Reduced Expression of Intestinal ASBT and NPC1L1 in C57BL/6J Mice

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ABSTRACT: To investigate the hypocholesterolemic mechanism of barley *in vivo*, six-week-old C57BL/6J mice were fed a high-fat diet (HFD) or high-fat diet containing barley (HFD-B) for seven weeks. Total and LDL cholesterol concentrations were significantly reduced in the HFD-B group while fecal cholesterol and bile acid was increased. Real-time PCR and immunoblot analysis revealed the induction of FXR expression, which in turn suppressed the expression of ASBT and NPC1L1 in the HFD-B group compared with the controls. In the liver, the expression of HMG-CoA reductase was significantly reduced while LDL receptor expression was unaltered in the HFD-B group compared with the controls. Our data suggest that the hypocholesterolemic effects of barley are primarily the result of reduced dietary cholesterol uptake and bile acid resorption. Reduced expression of intestinal ASBT and NPC1L1 may play a key role in the regulation of dietary cholesterol and bile acid metabolism in mice consuming a diet containing barley.

KEYWORDS: barley, cholesterol, bile acid, ASBT, NPC1L1

INTRODUCTION

Hypercholesterolemia is a key risk factor for the development of coronary heart disease, which is a major cause of death in developed societies. Based on a meta-analysis, a 1% decrease in one's plasma cholesterol level can lower the risk of coronary events up to 3%, and this level of cholesterol reduction can be achieved through the intake of a low-cholesterol, low-fat diet¹ and dietary fiber.²

Barley is an excellent grain food source of dietary fiber. It contains significant amounts of the soluble dietary fiber β -glucan, which is found in insignificant quantities in major staples like rice and wheat. Barley intake has been shown to reduce plasma cholesterol concentrations in numerous animal^{3–5} and human studies,^{6–9} and its hypocholesterolemic effects are comparable to those found in oatmeal.¹⁰ Several human studies showed hypocholesterolemic effects of barley.^{6,7,11,12} Based on these and other studies, the Food and Drug Administration allowed the labels of foods containing soluble fiber from barley products to claim that consumption of these foods may reduce plasma cholesterol levels and reduce the risk of heart disease.¹³

Several reports have been proposed regarding hypocholesterolemic mechanisms of barley β -glucan. Delaying bile acid resorption and inhibiting dietary cholesterol uptake in the intestinal epithelium have been suggested as the major mechanisms^{17,18} underlying the increased delivery of bile acid in the feces.¹⁴ Reduction of hepatic cholesterol synthesis¹⁴ and the fermentation of barley β -glucan by human fecal microbiota to produce short-chain fatty acids, which have hypocholesterolemic effects in circulation, have been suggested as well.¹⁵ However, the molecular mechanism of barley in cholesterol metabolism is still not completely understood. Therefore, we investigated the

hypocholesterolemic mechanism of barley *in vivo* focusing on the effect on the intestine and the liver, the two major tissues for the regulation of cholesterol and bile acid metabolism. The C57BL/6J mice were fed either a high-fat diet (HFD) or a high-fat diet with barley containing 9.2% β -glucan (HFD-B) for seven weeks, after which the expression of key genes involved in cholesterol and bile acid metabolism was assessed.

MATERIALS AND METHODS

Reagents. A Total Bile Acids Assay Kit (Enzymatic Cycling) was purchased from BioQuant, Inc. (San Diego, CA, USA). Total RNA extraction reagent (RNAiso Plus) and real-time PCR premix (SYBR Premix Ex Taq) were obtained from Takara (Otsu, Japan). Oligo (dT)15 primer was obtained from Promega (Madison, WI, USA). PowerOpti-ECL Western blotting detection reagent was purchased from Amersham-Pharmacia Korea (Seoul, Korea). Primary (anti-HMG-CoA reductase, -LDL receptor, -CYP7A1, -NPC1L1, -FXR, -FGF15, -apical sodium-dependent transporter [ASBT], and - α -tubulin) and secondary antibodies (anti-rabbit, -mouse, and -goat immunoglobulin G) were acquired from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA).

Animals and Diets. Six-week-old C57BL/6J mice were purchased from Samtako Co. Kyunggido, Korea. All mice were maintained at a controlled temperature of 21–25 °C and 50–60% humidity under a 12 h light/dark cycle and fed a diet of commercial chow for one week.

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Table 1. Composition of HFD and HFD-B Diets

| formulation | HFD | | HFD-B | |
|-----------------|------|--------|-------|--------|
| | g % | kcal % | g % | kcal % |
| protein | 24 | 20 | 27 | 20 |
| carbohydrate | 41 | 35 | 39 | 33 |
| fat | 24 | 45 | 25 | 47 |
| β -glucan | 0 | | 2.2 | |
| total | | 100 | | 100 |
| kcal/kg | 4776 | | 4899 | |

| ingredient | HFD | | HFD-B | |
|--------------------|-------|------|-------|------------------|
| | g | kcal | g | kcal |
| casein | 200 | 800 | 200 | 800 |
| corn starch | 155 | 620 | | |
| sucrose | 50 | 200 | 50 | 200 |
| dextrose | 132 | 528 | 132 | 528 |
| cellulose | 50 | 0 | | |
| barley fraction | | | 200 | 700 ^a |
| soybean oil | 25 | 225 | 25 | 225 |
| lard | 175 | 1575 | 175 | 1575 |
| mineral mix | 35 | 0 | 35 | 0 |
| vitamin mix | 10 | 40 | 10 | 40 |
| L-cystine | 3 | 12 | 3 | 12 |
| choline bitartrate | 2.5 | 0 | 2.5 | 0 |
| total | 837.6 | 4000 | 832.5 | 4078 |

^aA total kilocalorie of barley was calculated based on the amounts of kilocalories in carbohydrates (136 g), fats (6.4 g), and proteins (24.4 g) in barley fraction as multiplying the amount of protein grams by four; multiplying the carbohydrate grams by four; and multiplying fat grams by nine.

The mice were then randomly divided into two groups ($n = 9-10$ mice each) and fed either a modified high fat diet (HFD) based on AIN-93G (Feedlab Korea Co., Ltd., Korea) or a HFD diet containing barley (HFD-B). In the HFD-B diet, all of the corn starch (18.5% w/w) and cellulose (6% w/w) in HFD were replaced with barley fraction. The compositions of the HFD and HFD-B diets are shown in Table 1. The barley (*Hordeum vulgare*) used in the study was a newly bred strain by National Institute of Crop Science, Rural Development Administration (Iksan, Korea) that contained 8–10% of β -glucans. The barley was dehulled, ground, and passed through a 100-mesh sieve. The mice were fed the diets for seven weeks. At weeks 0 and 4, blood samples were collected by retro-orbital bleeding and kept in K₃EDTA tubes after a 12–14 h of fasting. Plasma samples were obtained by centrifugation at 4 °C and 12,000 rpm for 15 min and stored at –80 °C for lipid analysis. After the treatment ended, the mice were fasted overnight (12–14 h) and the mice were weighed and sacrificed at 10:00 a.m. on the next day. After 7 weeks, blood samples were obtained by cardiac puncture under general anesthesia prior to mouse sacrifice. Liver and small intestine tissues were collected and stored at –80 °C for total RNA and protein extraction. All animal procedures were performed in accordance with the approved protocols of the Animal Experimentation Committee of Korea University (Protocol No. KUIACUC-20090420–4).

Plasma and Hepatic Lipid Concentrations. Liver lipids were extracted as described by Chung et al.¹⁸ Total, high-density lipoprotein (HDL), and LDL cholesterol and triglyceride (TG) levels in the plasma and the hepatic TG level were quantified with enzymatic methods using a Cobas C111 automatic analyzer (Roche, Basel, Switzerland). All of the

reagents were purchased from Roche, and the measurements were performed according to the manufacturer's instructions. The hepatic cholesterol level was measured using an Amplex Red Cholesterol Assay Kit (Invitrogen, Foster City, CA, USA) according to the manufacturer's instructions.

Determination of Bile Acid Excretion. Stools from individual mice were collected during the final five days of the study and then dried, weighed, and ground. Bile acids were extracted as described by Jeun et al.,¹⁹ with modifications. Briefly, 0.2 g of fecal material was soaked in 100% methanol for seven days. The methanol extracts were centrifuged and concentrated to a 0.5 mL final volume. The total bile acid concentration was analyzed using a Total Bile Acid Assay Kit according to the manufacturer's instructions. All assays were performed in triplicate.

Isolation of Total RNA and Real-Time Quantitative (q)PCR Analysis. Total RNA from mouse liver or small intestine (ileum) was isolated using an RNAiso Plus kit according to the manufacturer's instructions. To generate cDNA, 2 μ g of total RNA was reverse-transcribed with oligo (dT)15 using PrimerScript Reverse Transcriptase according to the manufacturer's protocol, resulting in 20 μ L of cDNA. Mouse gene-specific primers were designed using the published nucleotide sequences for LDL receptor from Kim et al.,²⁰ HMG-CoA reductase, CYP27, and CYP7A1 from Chung et al.,²¹ FXR from Wolters et al.,²² ASBT, ileal BA binding protein (IBABP), FGF15, and cyclophilin from Rao et al.,²³ and GAPDH from Barber et al.²⁴ Real-time qPCR was performed using Bio-Rad iQ SYBR Green Supermix reagent (Bio-Rad, Hercules, CA, USA) with the Bio-Rad iQ5 Cycler System. The reaction conditions were as follows: 95 °C for 3 min followed by 50 cycles of 95 °C for 10 s, 60 °C for 15 s, and 72 °C for 20 s. A melting curve of 71 cycles, starting at 55 °C and increasing by 0.5 °C every 10 s, was done to determine primer specificity. The expression levels were normalized to the levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or cyclophilin and analyzed using iQ5 System Software (version 2) with the normalized expression (CT) method according to the manufacturer's guidelines. The values are expressed as percentages, with the values in the control group defined as 100.

Immunoblot Analysis. Mouse liver or small intestine (ileum) was homogenized in a buffer containing 10 mM Tris-HCl (pH 7.4), 0.1 M EDTA, 10 mM NaCl, 0.5% Triton X-100, and one tablet of protease inhibitor cocktail (Roche, Mannheim, Germany). The lysate was clarified by centrifugation at 14,000 rpm for 10 min at 4 °C. The protein concentration was determined using a Bio-Rad protein kit with bovine serum albumin (Sigma, St. Louis, MO, USA) as the standard. Equal amounts of protein were boiled in a sample buffer (5% β -mercaptoethanol) for 5 min. The samples were separated by SDS-PAGE and blotted onto a nitrocellulose membrane (0.45 μ m; Schleicher & Schuell BioScience, Keene, NH, USA). Nonspecific protein binding was blocked by incubation in Tris-buffered saline (TBS; pH 7.4) containing 0.1% Tween 20 and 5% nonfat dry milk. Primary antibodies were used at the following dilutions: anti-HMG-CoA reductase, 1:2000; anti-CYP7A1, 1:1000; anti-LDL receptor, 1:1000; anti-NPC1L1, 1:1000; anti-FXR, 1:200; anti-ASBT, 1:200; and anti-FGF15, 1:1000. The dilutions were made in fresh blocking buffer containing 5% nonfat dry milk. The membranes were incubated with the primary antibodies overnight at 4 °C. After washing several times with TBS-T (0.1% Tween 20 in TBS), the membranes were incubated with 1:1000 anti-rabbit (HMGCoA reductase, CYP7A1, and LDL receptor), 1:1000 anti-goat (NPC1L1, FXR, ASBT, and FGF15), or 1:1000 anti-mouse (α -tubulin) immunoglobulin G. Immunoreactive bands were detected using an ECL Western blot detection reagent (Amersham-Pharmacia, Seoul, Korea) and exposed to high performance chemiluminescence film for 30 s. The immunoblots were scanned using a ChemiDoc XRS+ System (Bio-Rad, Hercules, CA, USA), and quantified with Gel-Pro Analyzer software (Media Cybernetics, Bethesda, MD, USA).

Statistical Analysis. All data are expressed as the mean \pm SEM. Two groups were compared using Student's *t* test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Plasma and Hepatic Lipid Levels and Bile Acid Excretion. The barley fraction contained 9.2% β -glucan, 57.0% starch and 12.2% protein. The percentage of fat and ash was low (3.2 and 1.9%, respectively). To determine the hypocholesterolemic effect of barley, mice were fed an HFD (control) or HFD-B for seven weeks. There were no significant differences in food intake during feeding period among the groups (data not shown). Plasma lipid levels in the control and barley groups were measured at weeks 0, 4, and 7. The levels of plasma total and LDL cholesterol were significantly lower in mice fed the diet containing barley than in those fed the HFD control diet (Figure 1A,B). The plasma cholesterol level decreased by 14 and 19% ($P < 0.01$) at weeks 4 and 7, respectively, in the HFD-B group. The LDL cholesterol level was dramatically reduced at week 4 by 41% and at week 7 ($P < 0.05$) by 23% compared to the control group.

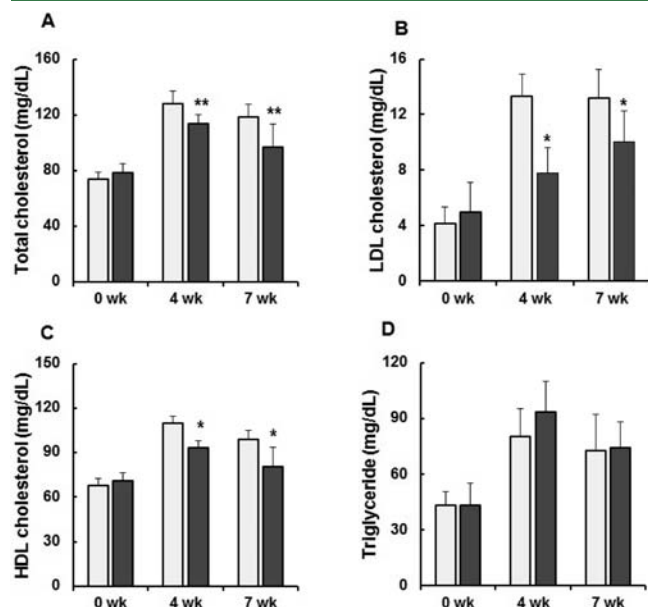


Figure 1. Effect of HFD-B on total plasma cholesterol (A), LDL cholesterol (B), HDL cholesterol (C), and triglyceride (D) levels in C57BL/6J mice. The values represent the mean \pm SEM of eight to nine mice. * $P < 0.05$ compared with the controls, ** $P < 0.01$ compared with the controls. (light bars) HFD, high-fat diet group; (dark bars) HFD-B, high-fat diet containing a barley with 9.2% β -glucan group; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

The HDL cholesterol level was reduced (Figure 1C) compared with the HFD control group; however, atherogenic indices were not different between the groups (Table 2). No significant differences in the ratios of total cholesterol:HDL and LDL cholesterol:HDL were seen among the groups. No significant differences in TG levels were observed among the groups (Figure 1D). The hepatic lipid concentrations were unaltered between the groups after barley feeding (Figure 2A). However, the barley group showed a significant increase in fecal bile acids and cholesterol (+36%, $P < 0.05$ and +207%, $P < 0.01$, respectively) compared with the control group (Figures 2B and 2C).

Gene Expression in Cholesterol Metabolism. Because the plasma cholesterol levels were reduced significantly in the HFD-B group, we investigated the expression of key genes in cholesterol metabolism. Expression of the genes encoding HMG-CoA reductase, CYP7A1, CYP27, and LDL receptor in the liver and NPC1L1 in the intestine was assessed using real-time qPCR (Table 3). The HFD-B diet caused a slight but significant reduction in the expression of HMG-CoA reductase ($-16%$, $P < 0.05$) and NPC1L1 ($-30%$, $P < 0.05$), but did not affect LDL receptor or CYP27 expression compared with the controls. The expression of CYP7A1, which encodes cholesterol 7- α hydroxylase, was reduced ($-57%$, $P < 0.05$) in the livers of the HFD-B mice compared with the controls.

Gene Expression in Bile Acid Metabolism in the Intestine. Interestingly, compared with controls, the mRNA level of ASBT was significantly reduced by 51% in mice fed the barley diet (Table 2, $P < 0.05$). No significant difference in the mRNA level of IBABP was detected among the groups. As mentioned previously, the expression of CYP7A1 was downregulated and CYP27 expression was unaltered while fecal bile acid excretion was markedly induced in the barley group compared with the control group (Table 3). FGF15 expression was somewhat induced by barley feeding ($+8%$, $P < 0.05$). FXR expression in the intestine was slightly but significantly increased by 13% in the barley group compared with the control group, however, its expression in the liver was unaltered in the barley group.

Protein Expression in Cholesterol and Bile Acid Metabolism in the Intestine. Immunoblotting results showed similar trends to the real-time qPCR analysis results, and the ASBT level was significantly reduced by 30% (Figure 3). Interestingly, the NPC1L1 protein level was significantly reduced in the barley group by 44% compared with the controls. The levels of FXR and FGF15 were significantly elevated ($+260$ and $+90%$, respectively).

Protein Expression in Cholesterol and Bile Acid Metabolism in the Liver. The protein expression of HMG-CoA reductase, CYP7A1, LDL receptor, and FXR in the liver was examined by immunoblotting (Figure 4). The HMG-CoA reductase level was strongly suppressed to 57% in the HFD-B

Table 2. Effect of Barley on Atherogenic Indices of C57BL/6J Mice^a

| | 0 wk | | 4 wk | | 7 wk | |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | HFD | HFD-B | HFD | HFD-B | HFD | HFD-B |
| TC/HDL-C | 1.08 \pm 0.06 | 1.10 \pm 0.03 | 1.17 \pm 0.08 | 1.26 \pm 0.08 | 1.20 \pm 0.08 | 1.20 \pm 0.09 |
| LDL/HDL-C | 0.06 \pm 0.01 | 0.07 \pm 0.02 | 0.13 \pm 0.04 | 0.08 \pm 0.04 | 0.13 \pm 0.02 | 0.13 \pm 0.02 |

^a Values represent mean \pm SEM of eight to nine mice. HFD, high fat diet group; HFD-B, high fat diet containing barley with 9.2% of β -glucan; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

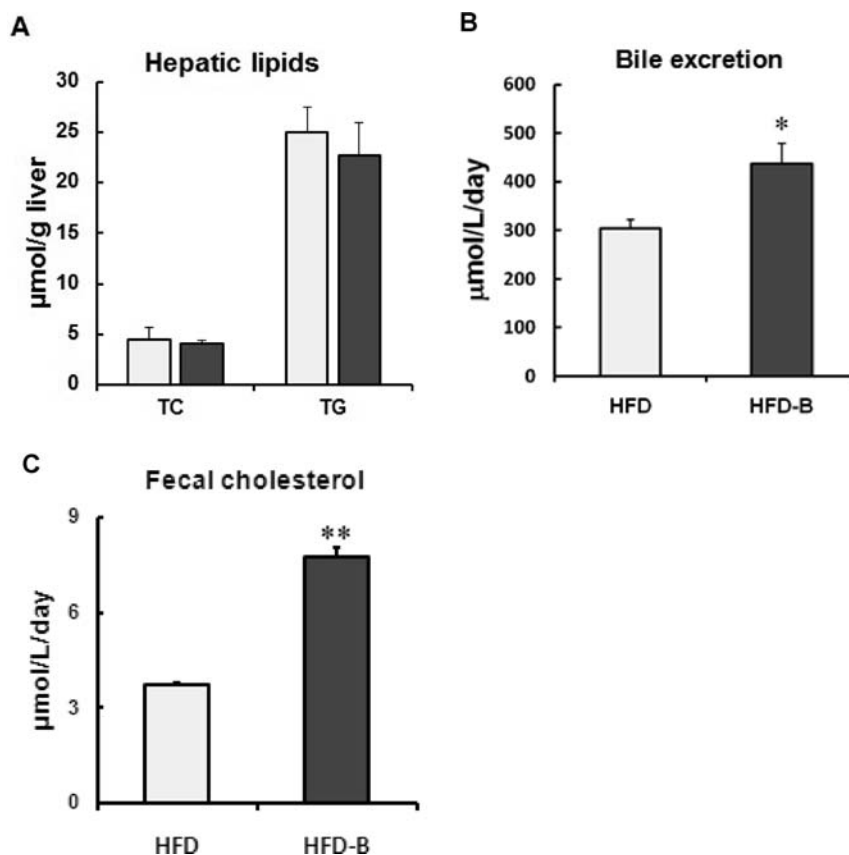


Figure 2. Effect of barley on hepatic lipid levels (A), fecal bile acid (B) and cholesterol excretion (C) in C57BL/6J mice. TC, total cholesterol; TG, triglyceride levels. (light bars) HFD, high-fat diet group; (dark bars) HFD-B, high-fat diet containing a barley with 9.2% β -glucan group. The values represent the mean \pm SEM of eight to nine mice. $P < 0.05$ compared with the controls.

group; similarly, CYP7A1 expression was significantly decreased by 44% in HFD-B compared to the HFD control group. Similar to our real-time qPCR results, the level of LDL was not altered among the groups. FXR expression was markedly induced in the HFD-B group (+150%, $P < 0.01$), although its gene expression was unaltered.

DISCUSSION

Barley is one of the most effective grain foods at reducing LDL cholesterol concentrations in animals and humans.^{6,28} The hypocholesterolemic effect of barley has been largely attributed to its high content of β -glucan, a polysaccharide consisting of glucose residues joined by (1–3), (1–4)- β -glucosidic linkages. Our previous experiments with several barley strains showed that β -glucan content in barley diet (0.4–2.2%, w/w) was significantly correlated with total and LDL-cholesterol reduction in mice (unpublished data), thus, in this study, we fed mice an HFD containing high β -glucan containing barley (2.2% β -glucan in HFD-B diet) to investigate the hypocholesterolemic mechanisms of barley at work in the intestine and liver. In this regard, the HFD-B group showed reduced plasma total and LDL cholesterol concentrations compared with the controls, which is in line with previously reported data.^{6,7,11,12} The hypocholesterolemic mechanisms of barley in mice found in the present study are summarized in Figure 5.

The reduction in total and LDL cholesterol was 19 and 23% after 7 weeks of barley diet, which is also comparable to results

from other groups.^{3,4,10,16} Plasma TG and hepatic lipids were unaltered in the HFD-B group compared with the controls while plasma cholesterol levels were significantly reduced. Since we fed normolipidemic mice a barley diet, it is reasonable that hepatic lipid levels were maintained within a normal range after barley feeding. The slight decrease in HDL cholesterol observed in our study was also found in other studies using barley or oat preparations in hamsters,^{10,27,28} however, the LDL:HDL cholesterol ratio was not significantly altered. In mice, HDL particles carry >70% of plasma cholesterol; thus, a slight reduction in HDL cholesterol is sometimes found following treatment with hypocholesterolemic agents. Reportedly, this effect has only been observed in rodents but does not appear to occur in humans,^{6,29–31} which carry most of plasma cholesterol in LDL particles. Barley intake is well-known to induce hepatic cholesterol gene expressions. In our study, consumption of the HFD-B diet significantly decreased the mRNA and protein levels of HMG-CoA reductase in agreement with the results from Qureshi et al.³² It has been shown that barley phytochemicals, especially phenolic compound abundant in barley (0.2–0.4%),³³ may affect hepatic cholesterol metabolism.

Through gene and protein expression studies, we found that the expression of two key transporters for dietary cholesterol and bile acids in the intestine, the NPC1L1 and ABST, were significantly reduced in the barley group compared with the controls. NPC1L1 has been identified and characterized as an essential protein in the intestinal cholesterol absorption process.^{34,35} NPC1L1 localizes to the brush border membrane of

absorptive enterocytes in the small intestine. Mice deficient in NPC1L1 exhibited a significant reduction in the intestinal uptake and absorption of cholesterol and phytosterols.³⁴ Characterization of the NPC1L1 in cholesterol uptake revealed that cholesterol absorption inhibitor ezetimibe specifically binds to an extracellular loop of NPC1L1 and inhibits its sterol transport function.³⁵ We found that HFD-B feeding significantly suppressed NPC1L1 protein and mRNA levels, which could significantly contribute to lower plasma and LDL cholesterol levels. One study showed that β -glucan consumption decreased cholesterol absorption by

Table 3. Effect of Barley on mRNA Levels of Key Genes Involved in Cholesterol and Bile Acid Metabolism in the Liver and Intestine

| | mRNA level ^a (%) | |
|----------------------------|-----------------------------|----------------------|
| | HFD | HFD-B |
| Hepatic Gene Expression | | |
| HMG-CoA reductase | 100 ± 6 | 84 ± 17 ^b |
| CYP7A1 | 100 ± 22 | 43 ± 14 ^b |
| CYP27 | 100 ± 9 | 96 ± 10 |
| LDL receptor | 100 ± 13 | 91 ± 3 |
| FXR | 100 ± 7 | 104 ± 4 |
| Intestinal Gene Expression | | |
| NPC1L1 | 100 ± 17 | 70 ± 3 ^b |
| FXR | 100 ± 5 | 113 ± 3 ^b |
| ASBT | 100 ± 31 | 49 ± 15 ^b |
| IBABP | 100 ± 13 | 99 ± 8 |
| FGF-15 | 100 ± 9 | 108 ± 7 ^b |

^a Values represent mean ± SEM of eight to nine mice. ^b $P < 0.05$ compared with control. HFD, high fat diet group; HFD-B, high fat diet containing barley with 9.2% of β -glucan; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoprotein; CYP7A1, a gene encoding cholesterol-7 α -hydroxylase; CYP 27, a gene encoding sterol 27-hydroxylase; NPC1L1, Niemann–Pick C1 like 1; FXR, farnesoid X receptor; SHP, small heterodimer partner; ASBT, ileal apical sodium-dependent bile acid transporter; FGF 15, fibroblast growth factor 15.

44% compared with subjects who did not receive β -glucan,³⁶ and another study of nine subjects with ileostomies who were administered 11.6 g of native or hydrolyzed β -glucan showed cholesterol absorption was decreased by 19%.³⁷ The reduction of NPC1L1 gene expression may provide a mechanism for a reduced dietary cholesterol uptake in the previous studies.

Cholesterol is mainly eliminated from the body via conversion to bile acids, and we demonstrate that dietary HFD-B was able to increase the excretion of bile acids significantly by 36%. Intestinal bile acid absorption is an essential step in the enterohepatic circulation. Ileal bile acid transport is mediated by ASBT and has been shown to mediate approximately 75% of the bile acids recycled in the human body.³⁸ Consequently, the inhibition of ASBT activity reduces the amount of circulating bile acids, leading to the stimulation of their synthesis and a reduction in cholesterol levels.³⁹ Specific inhibitors of ASBT function have been developed for the treatment of cardiovascular heart disease and atherosclerosis such as S-8921, 264W90, and PR835.^{40–42} Furthermore, the molecular mechanisms responsible for the regulation of ASBT, particularly those involving FXR, an orphan nuclear transcription factor, are beginning to be understood.^{25,43,44} In mice and rabbits, bile acid feeding represses ASBT expression by acting through FXR.^{43,44} Our results suggest that barley may, at least in part, act through FXR to suppress the expression of ASBT, resulting in a reduction in total plasma and LDL cholesterol levels via a significant decrease in bile acid absorption and increase in fecal bile acid excretion. This together with reduced expression of NPC1L1 may contribute to the reduction of plasma cholesterol levels in mice.

Bile acid formation from cholesterol in the liver is a major pathway for cholesterol degradation, and it involves CYP7A1, which encodes cholesterol 7- α hydroxylase, the rate-limiting enzyme in the bile acid biosynthetic pathway, and CYP27 encodes the rate-limiting enzyme in alternative bile acid synthesis.⁴⁵ However, surprisingly, the HFD-B did not change the expression of CYP27 and decreased both the protein and mRNA levels of hepatic CYP7A1 in the barley group compared with the controls. Several reports showed increased expression of hepatic CYP7A1 after fiber-rich diets including barley or oatmeal,^{14,46,47} and they suggested that the induction of CYP7A1 compensated the reduced bile acid resorption from the intestine during fiber rich diet,

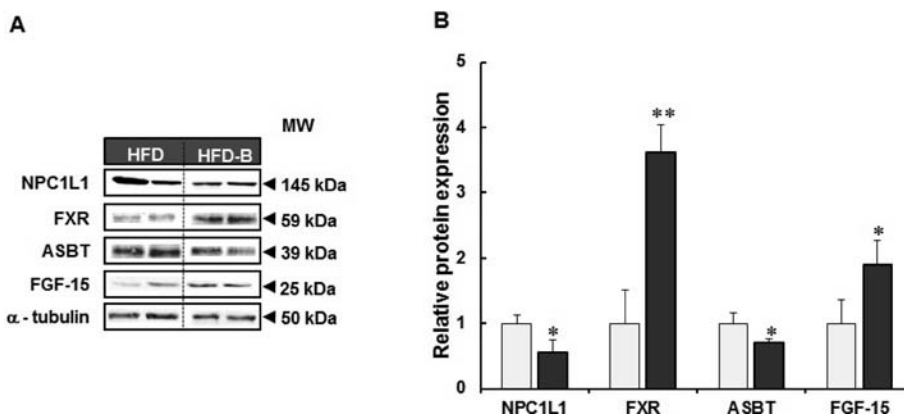


Figure 3. Effect of barley on the intestinal expression of proteins related to mouse cholesterol metabolism. NPC1L1, FXR, ASBT, and FGF15 expression was normalized to α -tubulin. The values represent the mean ± SEM for eight to nine mice. * $P < 0.05$ compared with the control, ** $P < 0.01$ compared with the control. (light bars) HFD, high-fat diet group; (dark bars) HFD-B, high-fat diet containing a barley fraction with 9.2% β -glucan group; NPC1L1, Niemann–Pick disease type C1 gene-like 1 protein; FXR, farnesoid X receptor; ASBT, ileal-specific bile acid transporter; FGF15, fibroblast growth factor 15.

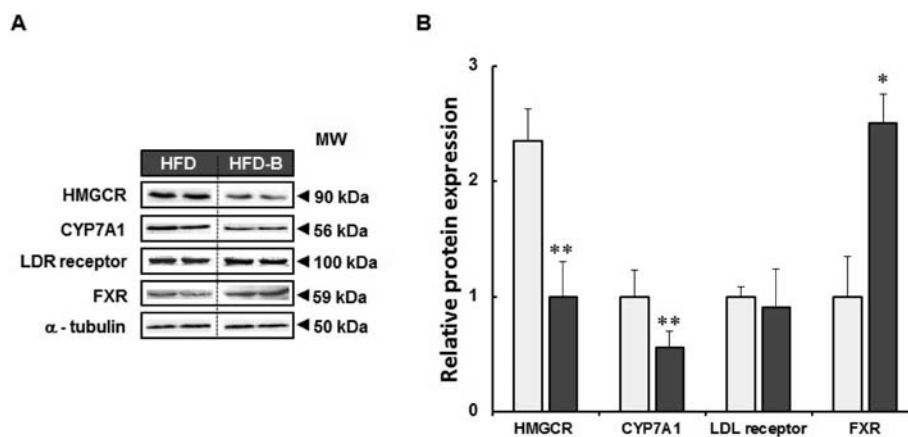


Figure 4. Effect of barley on the hepatic expression of proteins related to mouse cholesterol metabolism. Protein expression was normalized to α -tubulin. The values represent the mean \pm SEM of eight to nine mice. * $P < 0.05$ compared with the controls, ** $P < 0.01$ compared with the controls. (light bars) HFD, high-fat diet group; (dark bars) HFD-B, high-fat diet containing a barley with 9.2% β -glucan group; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low-density lipoprotein; CYP7A1, cholesterol 7 α -hydroxylase; FXR, farnesoid X receptor.

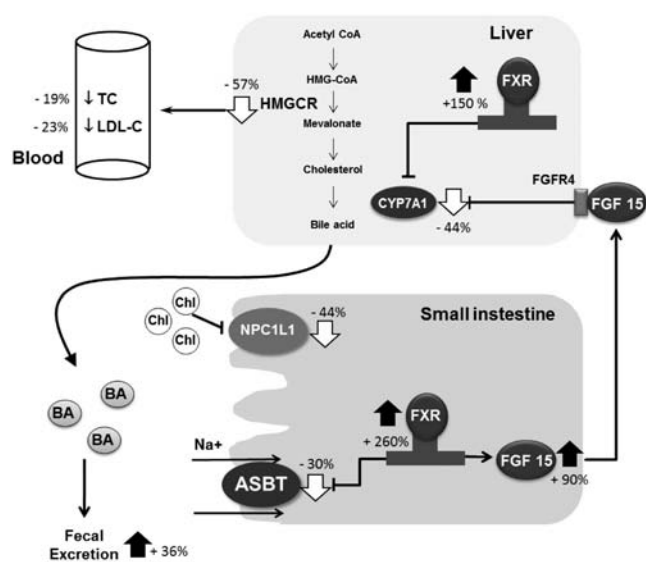


Figure 5. Schematic model depicting the mechanisms whereby barley regulates cholesterol and bile acid metabolism. Values indicate the percent changes of the protein expression in mice after HFD-B feeding for 7 weeks. TC, total cholesterol; LDL-C, LDL cholesterol; Chl, dietary cholesterol; BA, bile acids; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; FXR, farnesoid X receptor; CYP7A1, cholesterol 7 α -hydroxylase; FGFR4, fibroblast growth factor receptor 4; NPC1L1, Niemann–Pick disease type C1 gene-like 1 protein; ASBT, ileal apical sodium-dependent bile acid transporter; FGF15, fibroblast growth factor 15.

however, some studies did not find CYP7A1 induction especially during high protein high fiber or barley diet, and reported reduced expression of CYP7A1⁴⁸ comparable to our data. They reported that barley with high protein suppressed the HMG-CoA reductase expression, with a lowering of serum cholesterol level, but CYP7A1 expression was also reduced to compensate hepatic cholesterol reduction. Our data showed similar results, and the unexpected reduction of the CYP7A1 expression, potentially by upregulation of hepatic FXR, may be either due to compensate the HMG-CoA reduction or due to counterbalance cholesterol reduction from the decreased intestinal bile acid resorption.

Bile acid homeostasis is regulated via enterohepatic circulation and nuclear hormone receptor, FXR, which plays a key role as a bile acid sensor and is expressed at high levels in the liver and intestine. Activation of FXR downregulates CYP7A1 in the liver.²⁵ The action of FXR has been studied intensively. In the intestine, FXR activates SHP, a transcription factor, and upregulates the expression of FGF15, a hormone secreted from the intestine, and travels to the liver to bind fibroblast growth factor receptor 4 (FGFR4). High levels of FGF15 could eventually suppress the classical bile acid synthetic pathway by the downregulation of CYP7A1.^{25,49,50} It has been shown that, in the intestine, FXR is coupled to reduce ASBT and increase IBABP expression resulting in reduced intestinal absorption of bile acids and the prevention of intracellular bile acid accumulation.²⁵ It is thought to be the major transporter for apical bile salt reabsorption in these tissues.³⁸ We found that barley feeding increased FXR mRNA and protein expression in the intestine, and this could suppress ASBT expression in the intestine. FXR protein expression was also induced in the liver. It has been reported that the induction of FGF15 and SHP is required for the FXR-mediated repression of CYP7A1 and bile acid synthesis in the liver.²⁵ In our study, HFD-B increased the expression of FGF15 at the mRNA and protein levels in the intestine. It appears that the net hepatic effects of increased FXR and FGF15 in circulation could sufficiently downregulate CYP7A1 expression. Although there was no observed difference in the expression of IBABP between the groups, both the mRNA and protein levels of ABST in the intestine were significantly reduced in the HFD-B group compared with the controls.

In conclusion, barley showed hypocholesterolemic effects in high-fat fed mice. The suppression of dietary cholesterol uptake and enhancement of fecal bile acid excretion may be major mechanisms underlying this effect (Figure 5). Barley markedly increased the fecal excretion of bile acids by the FXR-mediated repression of ASBT and bile acid reabsorption and reduced dietary cholesterol uptake by NPC1L1.

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ABBREVIATIONS USED

ASBT, ileal apical sodium-dependent bile acid transporter; BA, bile acids; CYP7A1, cholesterol 7 α -hydroxylase gene and protein; CYP27, sterol 27-hydroxylase gene and protein; FGF15, fibroblast growth factor 15; FXR, farnesoid X receptor; FGFR4, fibroblast growth factor receptor 4; HFD, high-fat diet; HFD-B, high-fat diet containing a barley; HDL, high-density lipoprotein; IBABP, ileal BA binding protein; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; NPC1L1, Niemann–Pick disease type C1 gene-like 1 protein; TC, total cholesterol; TG, triglyceride

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