Barley β-Glucan Lowers Serum Cholesterol Based on the Up-Regulation of Cholesterol 7α-Hydroxylase Activity and mRNA Abundance in Cholesterol-Fed Rats

Jeong-Lye Yang1, Young-Hwa Kim1, Hyun-Sook Lee2, Mak-Soon Lee1 and Yangha Kim Moon2,*

1Research Institute of Human Ecology, Changwon University, Changwon 641-773, Korea
2Department of Food and Nutritional Sciences, Ewha Womans University, Seoul 120-750, Korea
(Received January 20, 2003)

Summary Barley, which is high in soluble dietary fiber (particularly β-glucan), is thought to have hypocholesterolemic effects. The present study was performed to further elucidate the hypocholesterolemic actions of waxy barley, and the extent to which they can be attributed to β-glucan. Male Sprague-Dawley rats (n=21) were fed control (fiber-free) diets, diets with the addition of 2.5% refined β-glucan or 30% waxy barley that provides approximately 2.5% β-glucan in the diet for 2 wk. Body weight gain and food efficiency of rats were unaffected by diet. β-glucan or waxy barley decreased serum levels of total cholesterol (p<0.05) by 13.5% or 18.9%, and also decreased LDL-cholesterol 19.4% or 24.3%, respectively. Addition of refined β-glucan or waxy barley to the diet resulted in greater bile acid excretions (p<0.05) compared to the control group. The waxy barley diet up-regulated by 2.3 times and the β-glucan diet by 1.5 times the activity of cholesterol 7α-hydroxylase (CYP7A1). Hepatic CYP7A1 mRNA level paralleled the increases in enzyme activity. The results of this study suggest that the hypocholesterolemic effects of both β-glucan and a waxy barley diet may be due to the enhancement of CYP7A1 expression resulting from increased fecal excretion of bile acids.

Key Words Barley β-glucan, CYP7A1, mRNA, hypocholesterolemic, fecal bile acids

The hypocholesterolemic properties of barley have attracted much interest in recent years and have been the subject of several studies (1-4). Barley, which is high in soluble dietary fiber, has been demonstrated to be hypocholesterolemic in chicks (5), rats (6) and humans (7, 8). β-glucans are found in cereal grains, located primarily in the endosperm cell walls of both oats and barley, and are actively involved in the metabolic response to barley products (2, 9). Waxy barley contains about 8% of β-glucan, an amount which is 50 times more than that in rice and 7 times more than in wheat, making it an important source of dietary fiber (10). Some barley cultivars, notably those of the hullless type, having waxy-type starch, have high concentrations of β-glucan (11). β-glucans are homopolysaccharides composed of glucopyranosyl units with (1-3) and (1-4) linkages in a ratio of 1:2.5 (12). Based on their extractability in water, β-glucans may be divided into soluble and insoluble fractions. An aqueous solution of β-glucans extracted from barley flour is viscous and the degree of viscosity is attributed to the molecular weight and concentration of β-glucans (13). Although the soluble fiber β-glucan appears to be the major hypocholesterolemic ingredient in barley, its mode of action is not fully understood. In addition to β-glucans, barley has various other bioactive components including tocotrienols, α-sitosterol, oryzanols, and unsaturated fatty acids that are known to have various physiological effects (14, 15).

The ability of β-glucan as a soluble fiber to improve cholesterol metabolism might have several explanations (5, 16, 17). First, soluble fiber increases the viscosity of the digesta and increases the thickness of the unstirred layer in the small intestine. It might, therefore, be expected to inhibit uptake of cholesterol and bile acids (18). Second, having passed through the small intestine, soluble fiber is an excellent substrate for fermentation by the microorganisms in the large bowel. The volatile fatty acids produced by fermentation enter the blood stream and appear to suppress hepatic cholesterol synthesis, specifically HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis (19). Third, because bile acids are the main excretory route for cholesterol from the body, changes in bile acid metabolism in response to certain dietary fibers have been implicated in their hypocholesterolemic action (20).

Investigations of the hypocholesterolemic effect of fibers have focused on greater excretion of bile acids and total steroids leading to an up-regulation of bile acid biosynthesis. Synthesis of bile acids from cholesterol is regulated by feedback inhibition of the rate-limiting enzyme, cholesterol 7α-hydroxylase (CYP7A1), by bile acids returning to the liver via the enterohepatic circu-

* To whom correspondence should be addressed.
E-mail: yhmoon@ewha.ac.kr

381
lation. Experiments with dietary fiber sources that result in lower cholesterol levels tend to support this increased excretion/increased synthesis hypothesis (13, 14, 16). Hepatic CYP7A1 activity, protein mass, mRNA levels and the rate of transcription are all higher in rats fed soluble dietary fiber (21, 22) and lower in rats fed bile acids (15, 17-19). Similar effects have been observed in guinea pigs (23, 24).

The purpose of this study was to understand more fully the role of these potential mechanisms in the hypocholesterolemic action of waxy barley, and the extent to which they can be attributed to β-glucan, by measuring fecal bile acid excretion and CYP7A1 at both the activity and mRNA expression levels in rats fed diets containing 0.5% cholesterol.

MATERIALS AND METHODS

Animals and diets. Three groups of seven male Sprague-Dawley rats (initial weights, 150±5 g, SLC, Japan) were housed individually in a temperature (22±2°C), relative humidity (55±5%), and light (dark, 06:00-18:00 h) controlled room. The rats were fed a non-purified diet (Rodent Laboratory Chow, Ralston Purina, St. Louis, MO) for a 7 d stabilization period. Experimental diets employed were a modification of the AIN76 (25) purified rodent diet (Dyets, Bethlehem, PA) (Table 1). Rats were fed control (fiber-free) diets, diets with the addition of 2.5% refined β-glucan (Megazyme, Australia) or 30% waxy barley that provides approximately 2.5% β-glucan in the diet (10). The waxy barley flour was obtained by grinding with a 2-mm grating. All diets contained 0.5% cholesterol and were iso-nitrogenous. The rats were given free access to food and water for 2 wk, and food intake and body weight gain were monitored twice per week. During the last 5 d of the experimental period, all feces were collected from each rat and weighed. The feces were dried for 10 h at 60°C, pulverized, and stored at -20°C until analysis for bile acid concentration. At the end of the experiment, rats were deprived of food for 16 h and then anesthetized using CO2. A central longitudinal incision was made into the abdominal wall, and blood samples were collected by cardiac puncture. Blood samples were centrifuged at 4°C for 20 min at 1500×g, and the serum was separated and stored at -20°C until analyzed. Liver samples were excised, immediately frozen in liquid nitrogen and stored at -70°C until analyzed. All animal procedures described conformed to NIH guidelines (26).

Cholesterol and triglyceride concentrations. The concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL), and triglyceride in the serum were determined by an enzymatic colorimetric method (27). Liver cholesterol and triglyceride concentrations were determined by enzymatic colorimetric method (27) after extraction with chloroform/methanol (2:1, v/v) (28). Serum LDL cholesterol was calculated by the formula of Friedewald and Levey (29).

Fecal bile acid measurement. Fecal lipid was extracted by the method of Tokunaga et al. (30), and the

| Table 1. Composition of experimental diets, |
| Ingredients | Control1 | β-glucan | Waxy barley2 |
| Casein | 200 | 200 | 182.03 |
| Soybean oil | 100 | 100 | 96.04 |
| Mineral mix3 | 35 | 35 | 35 |
| Vitamin mix4 | 10 | 10 | 10 |
| D. l-methionine | 3 | 3 | 3 |
| Choline bitartrate | 2 | 2 | 2 |
| Cholesterol | 5 | 5 | 5 |
| Corn starch | 150 | 150 | 150 |
| β-glucan | 25 | 25 | 25 |
| Waxy barley | — | 300 | 300 |
| Sucrose | 495 | 470 | 219.54 |

1 Fiber-free diet. 2 30% waxy barley provides approximately 2.5% β-glucan in the final diet (10). The amounts of casein and soybean oil were adjusted to provide constant levels of protein and lipid. 3 AIN-76 mineral mixture (25). 4 AIN-76 vitamin mixture (25).

extracted solution was used to determine bile acid concentration enzymatically by the method of Kim et al. (31).

Assay of microsomal cholesterol 7α-hydroxylase activity. Assay of CYP7A1 activity was performed with the procedure of Van Cantfort et al. (32) as modified by Oda et al. (33). About 2 g of liver was homogenized in 4 volumes of cold 0.1 m potassium phosphate buffer (pH 7.4) containing 1 mmol/L EDTA and 50 mmol/L NaF. The homogenates were centrifuged at 12,000×g for 15 min, and the supernatant was recentrifuged at 100,000×g for 60 min. Microsomal pellets were resuspended in the same buffer and stored at -70°C. The protein content in microsomes was measured according to Bradford (34).

The microsomal pellet was resuspended in 2 ml of 0.1 m potassium phosphate buffer (pH 7.4) containing 0.1 mmol/L EDTA, 50 mmol/L NaF, 2 mmol/L NADPH, 20 mmol/L cysteamine, 200 μmol/L cholesterol, 1.5 g/L Tween 80, 6 μCi [7n]-3H cholesterol (Amersham, UK). Incubation was carried out at 37°C for 30 min. The reaction was stopped by the addition of 6 ml of 20% trichloroacetic acid. Then the reaction mixture was centrifuged at 1,500×g for 10 min. Chloroform was added to the supernatant to extract the unreacted radiolabeled cholesterol. After the second extraction with chloroform, 1 ml of the upper aqueous phase was transferred to a counting vial, and the radioactivity was quantified by liquid scintillation counter.

RNA extraction and Northern blot analysis. Total RNA was isolated from 0.5 g liver according to Chomczynski and Sacchi (35). For Northern blot analysis, 20
### Hypcholesterolemic Effects of Barley β-Glucan

**Table 2.** Body weight and food intake of rats fed control, β-glucan or waxy barley diets.

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Initial body weight</th>
<th>Final body weight</th>
<th>Body weight gain</th>
<th>Food Intake</th>
<th>Food efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>157.1±5.0 g</td>
<td>249.9±16.8 g</td>
<td>6.6±0.8 g/d</td>
<td>27±1.1 g/d</td>
<td>0.24±0.03 g/g</td>
</tr>
<tr>
<td>β-glucan</td>
<td>156.7±4.2 g</td>
<td>244.8±8.1 g</td>
<td>6.3±0.6 g/d</td>
<td>26.8±1.1 g/d</td>
<td>0.24±0.01 g/g</td>
</tr>
<tr>
<td>Waxy barley</td>
<td>157.5±4.5 g</td>
<td>238.8±13.1 g</td>
<td>5.5±0.5 g/d</td>
<td>25.6±1.2 g/d</td>
<td>0.22±0.02 g/g</td>
</tr>
</tbody>
</table>

Values are means±SD, n=7. Means in a column with different superscripts are significantly different, p<0.05.

**Table 3.** Serum lipoprotein cholesterol and triglyceride concentrations of rats fed control, β-glucan or waxy barley diets.

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Total cholesterol</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
<th>HDL/LDL</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.85±0.15 mmol/L</td>
<td>0.47±0.10</td>
<td>1.03±0.11</td>
<td>0.45±0.09</td>
<td>0.77±0.16</td>
</tr>
<tr>
<td>β-glucan</td>
<td>1.60±0.19 mmol/L</td>
<td>0.39±0.04</td>
<td>0.83±0.13</td>
<td>0.48±0.11</td>
<td>0.89±0.13</td>
</tr>
<tr>
<td>Waxy barley</td>
<td>1.50±0.17 mmol/L</td>
<td>0.41±0.06</td>
<td>0.78±0.10</td>
<td>0.53±0.08</td>
<td>0.67±0.15</td>
</tr>
</tbody>
</table>

Values are means±SD, n=7. Means in a column with different superscripts are significantly different, p<0.05.

**Table 4.** Liver cholesterol and triglyceride concentrations in rats fed control, β-glucan or waxy barley diets.

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Lipid mg/g wet liver</th>
<th>Cholesterol μmol/g wet liver</th>
<th>Triglyceride μmol/g wet liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58.4±7.82</td>
<td>31.19±5.25</td>
<td>22.34±6.98</td>
</tr>
<tr>
<td>β-glucan</td>
<td>55.2±6.38</td>
<td>27.71±6.09</td>
<td>21.99±7.82</td>
</tr>
<tr>
<td>Waxy barley</td>
<td>58.9±8.41</td>
<td>27.00±6.77</td>
<td>18.37±5.47</td>
</tr>
</tbody>
</table>

Values are means±SD, n=7. Means in a column with different superscripts are significantly different, p<0.05.

μg of RNA was electrophoresed in a 1% agarose gel containing 2.2 M formaldehyde. The integrity of total RNA was routinely checked by agarose gel electrophoresis and ethidium bromide staining of the two ribosomal RNA bands. The gel was blotted by capillary action onto a nylon membrane filter (Hybond-N+, Amersham, UK), and then hybridized with the labeled probe for 16–20 h under identical conditions with the addition of at least 10⁶ cpm/ml of 32P random primed cDNA probe (36). The blots were washed twice sequentially in 0.2× SSC and 0.1% SDS, 0.1× SSC and 0.1% SDS for 20 min each at 65°C. Membranes were exposed to X-ray film at -70°C with an intensifying screen. Levels of 18S rRNA were used to normalize for differences in loading.

**cDNA probes.** The plasmid pCR10000 (TA3) containing a 1.6 kb EcoRI/HindIII fragment that contained the entire coding region of the rat CYP7A1 gene, was kindly provided by Dr. Hans M.G. Princen (Gaubius Laboratory, TNO-PG). The excised insert was labeled with [32P]-dCTP using a DNA labeling system (Takara, Japan).

**Statistical analysis.** Data for the control, β-glucan or waxy barley groups were analyzed by one-way ANOVA; p≥0.05 was taken as indicating no significant difference. Where ANOVA showed significance, differences among groups were evaluated by Duncan’s multiple range test (37).

**RESULTS**

Random assignment of rats to the three experimental groups resulted in initial body weights that were not different (Table 2). Rates of body weight gain did not differ among the rats fed control, β-glucan or waxy barley diets and there were no significant differences in final body weights. The barley diet did reduce food intake in comparison to the control diet, with rats fed β-glucan consuming intermediate amounts. Food efficiency was not affected by diet.

Consumption of β-glucan or waxy barley significantly decreased serum cholesterol concentrations by 13% and 19%, respectively (Table 3). LDL cholesterol concentrations were 24% lower in barley-fed rats, though an apparently similar reduction in β-glucan fed rats resulted in levels that did not differ statistically from either control or barley-fed animals. There were no significant effects of diet on HDL cholesterol or triglyceride concentrations in serum. β-glucan or waxy barley did not affect total lipids, cholesterol or triglycerides in liver in comparison to the control diet (Table 4). Fecal bile acid excretions were elevated 61% in rats fed β-glucan and almost doubled in the waxy barley group (Fig. 1).

Consumption of β-glucan or waxy barley resulted in elevated activity (Fig. 2) and mRNA levels (Fig. 3) of
Fig. 1. Effects of β-glucan or waxy barley diets on fecal bile acid excretions in rats. Rats were fed the fiber-free control, 2.5% refined β-glucan or 30% waxy barley diets, all with 0.5% cholesterol for 2 wk. Feces were collected for the last 5 d of the experimental period and assayed for bile acids as described in Materials and Methods. Values are means±SD, n=7 for all groups. Bars with different letters are significantly different, p<0.05.

Fig. 2. Effects of β-glucan or waxy barley diets on hepatic cholesterol 7α-hydroxylase (CYP7A1) activity in rats. Rats were fed the fiber-free control, 2.5% refined β-glucan or 30% waxy barley diets, all with 0.5% cholesterol for 2 wk. Livers were removed, microsomes prepared and assayed for CYP7A1 activity as described in Materials and Methods. Values are means±SD, n=7 for all groups. Bars with different letters are significantly different, p<0.01.

CYP7A1 compared with rats fed the control diet. β-glucan had a greater effect on both activity (160% increase, relative to controls) and mRNA level (126%) than waxy barley (activity, 68%; mRNA level 84% greater than controls). Measurements of CYP7A1 mRNA and activity were quite concordant during these dietary intervention.

DISCUSSION

While waxy barley did result in a small reduction in food intake, there was no effect of diet on food efficiency and rates of weight gain were not significantly different among groups. A reduction in food intake and body weight gain was reported by Martinez et al. (11) in chicks fed barley, with or without β-glucanase, but in that case barley comprised 70% of the diet. Vachon et al. (38) fed rats 2.5% β-glucan from oats for 2 wk and found lowered food intake and growth. However, the reduction in food intake and growth was not a major role in their hypocholesterolemic effects. The hypocholesterolemic effects resulting from barley feeding were consistent with previous studies in this area. Ranhotra et al. (39) found that serum total cholesterol concentrations were decreased by 16.4% in rats fed 25% finely ground barley for 4 wk. Newman et al. (40) found that chicks fed barley diets containing 0.5% cholesterol with or without β-glucanase had lower total serum cholesterol and LDL cholesterol concentrations than those fed corn diets. Furthermore, lesser reductions in total and LDL cholesterol concentrations were found with barley diets including β-glucanase, suggesting that the active involvement of β-glucan in the hypocholesterolemic effects of barley products. Sixty percent hull-less barley-fed chicks had lower total plasma cholesterol concentration than those fed wheat regardless of dietary fat
In this study, β-glucan or waxy barley did not affect liver lipids. Oda et al. (41) reported that there was no significant effect on hepatic cholesterol concentrations in rats fed diets containing 2% barley gum compared with 5% cellulose-fed controls. However, Anderson et al. (42) observed that diet-induced hypercholesterolemic rats fed 6% soluble fibers such as oat gum and pectin had significantly lower liver cholesterol concentrations. Also, hepatic cholesterol was dose-dependently decreased in rats fed a 12.5% citrus pectin diet containing 0.25% cholesterol (43). The different responses in hepatic lipid content to similar dietary fibers among laboratories may be due to variations in the percentage of added dietary cholesterol, the presence or absence of cholic acid, the level of dietary fiber, the experimental period and the animal species used.

To evaluate effects of dietary fiber on cholesterol metabolism, dietary cholesterol concentrations usually employed ranges from 0.2% to 1%. Our diet included 0.5% cholesterol and no added cholic acid. Many investigators have generally chosen 5–10% of dietary fibers for various nutritional studies (44, 45). We added 2.5% β-glucan or 30% waxy barley contributing approximately 2.5% β-glucan to the diet (10).

The amounts of β-glucan or waxy barley used in this study are more reasonable than those of some others: 2.5% β-glucan or 30% waxy barley would be equivalent to a daily consumption of about 40–45 g dietary fiber in humans. At any rate, 2.5% β-glucan as the dietary fiber source used in our study did not significantly suppress liver cholesterol and triglyceride accumulation in rats fed 0.5% cholesterol.

Historically, the hypocholesterolemic effect of dietary fiber has been attributed to its ability to inhibit intestinal absorption of bile acids and neutral steroids, resulting in greater fecal bile acid and total steroid excretions. These effects vary with type of fiber and are also species dependent. García et al. (46) reported that fecal bile acid excretion was significantly higher in 7% pectin-fed rats than fiber-free controls, suggesting that pectin, by enhancing fecal bile acid excretion, may cause up-regulation of bile acid biosynthesis, resulting in reduced serum cholesterol concentrations. Anderson et al. (42) also observed that oat bran intake increased fecal bile acid excretion, probably contributing to its cholesterol-lowering effect. In our study, the addition of 2.5% refined β-glucan or 30% waxy barley to the diet led to an increase in bile acid excretion compared with control rats.

A reduction in absorbed bile acids would be expected to increase the expression and activity of hepatic CYP7A1, and this was exactly the effect seen in β-glucan or waxy barley-fed rats. Roy et al. (23) reported similar effects from experiments feeding soluble dietary fiber to guinea pigs. Greater effects on this enzyme were found in the current study for rats fed β-glucan than the rats fed waxy barley. The β-glucan in the diet may lead to greater viscosity in the intestine, diminishing absorption of bile acids, thereby resulting in up-regulation of hepatic of CYP7A1 mRNA abundance and activity. However, feeding waxy barley to rats, while stimulating CYP7A1 to a lesser extent than β-glucan alone, resulted in a greater hypocholesterolemic effect. This suggests that barley may possess additional hypocholesterolemic factors, possibly tocotrienol or soluble dietary fibers other than β-glucan (40), which operate in a manner independent of CYP7A1.

In conclusion, the addition of β-glucan or waxy barley to rat diets had a beneficial effect on cholesterol metabolism. Both β-glucan and waxy barley were associated with lower serum and LDL cholesterol and also with higher fecal bile acid excretions. CYP7A1 activity was greater in fiber-fed rats and this appeared to result from increased expression of mRNA. While waxy barley had less of an effect than refined β-glucan on CYP7A1, it had a greater effect on serum cholesterol. Thus β-glucan appears to account for some, but not all of the hypocholesterolemic actions of waxy barley. Further research is required to fully delineate the mechanisms that contribute to the hypocholesterolemic effects of waxy barley and its constituents.

Acknowledgments
We gratefully thank Dr. Hedley Freake (University of Connecticut, Storrs) for reading the manuscript and giving helpful suggestions. This work was supported by the Korean Research Foundation Grant (KRF-2002-075-E00002).

REFERENCES

9) Bourdon I, Yokoyama W, Davis P, Hudson C, Backus R, Richter D, Knuckles B, Schneeman BO. 1999. Post-


Hypocholesterolemic Effects of Barley β-Glucan


