# Cholesterol-Lowering Effects in Hamsters of $\beta$ -Glucan-Enriched Barley Fraction, Dehulled Whole Barley, Rice Bran, and Oat Bran and Their Combinations

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#### **ABSTRACT**

Cereal Chem. 70(4):435-440

The cholesterol-lowering activity of a  $\beta$ -glucan-enriched barley fraction (GEB) (fed at several levels), milled dehulled whole barley, stabilized rice bran, oat bran, and 1:1 dietary fiber combinations of GEB with rice bran or oat bran, and rice bran with oat bran was investigated in 0.25% cholesterol-fed hamsters. All diets contained 10% total dietary fiber (TDF), 9.0% fat, and 3.1% N, except one GEB diet with 13.9% TDF.  $\beta$ -Glucan levels of the cereal diets ranged from 0.8 to 6.0%. After 21 days, total plasma cholesterol was significantly reduced by oat bran, the

rice bran-oat bran combination, and the 13.9% TDF GEB diets as compared with the cellulose control diet. The ratio of high-density lipoprotein cholesterol to total plasma cholesterol was maintained. Liver cholesterol was significantly reduced in hamsters by all test diets, except dehulled whole barley. The viscous property of diets containing cereal fractions suggests that plasma cholesterol-lowering by oat bran and GEB (13.9% TDF) may result in reduced absorption or reabsorption of lipids, bile acids, and their metabolites.

In both human (Anderson et al 1984, Davidson et al 1991) and animal studies (Chen and Anderson 1979; Chen et al 1981; Klopfenstein and Hoseney 1987; Qureshi et al 1987; Ranhotra et al 1987, 1991; Jennings et al 1988; Shinnick et al 1988; Kahlon et al 1990; Newman et al 1992), oat and barley fiber, or their fractions, have lowered elevated blood cholesterol, a risk factor in coronary heart disease (Lipid Research Clinics Program 1984). Several workers have attributed the hypocholesterolemic effects of barley and oats to  $\beta$ -glucan content (Chen et al 1981, Fadel et al 1987, Klopfenstein and Hoseney 1987). The concentration of  $\beta$ -glucan ranges from 3.0 to 6.9% in barley and from 2.2 to 4.2% in whole oats (Aman and Graham 1987). Recently, a fractionation procedure was developed for concentrating  $\beta$ -glucans from 6% in dehulled whole barley to 19% in a  $\beta$ -glucan-enriched fraction (GEB) (Knuckles et al 1992).

Hypocholesterolemic effects of rice bran and some of its fractions (neutral detergent fiber, hemicellulose, rice bran oil, and unsaponifiable matter) have been observed (Suzuki and Oshima 1970; Ayano et al 1980; Ishibashi and Yamamoto 1980; Suzuki 1982; Sugano et al 1984; Sharma and Rukumini 1986, 1987; Seetharamaiah and Chandrasekhara 1988, 1989; Hegsted et al 1990; Kahlon et al 1990, 1992a,b; Nicolosi et al 1991). In contrast to barley and oat bran, rice bran is very low in  $\beta$ -glucans and soluble dietary fiber (1.8 and 1.4%, respectively).

In this study, hamsters fed 0.25% cholesterol diets were used to investigate the cholesterol-lowering effect of a GEB fraction in comparison with milled dehulled whole barley, oat bran, stabilized rice bran, and paired combinations of these cereals (1:1, total dietary fiber [TDF]).

## **MATERIALS AND METHODS**

Male, 22-day-old, weanling Syrian golden hamsters (Sasco, Inc. Omaha, NE) were kept individually in wire-bottom cages in a controlled environment (20–22°C, 60% rh, 12-hr light and dark cycles). After one week of being fed a basal diet containing 10% cellulose and no cholesterol, the animals were assigned by selective randomization (blocked by weight, one animal per treatment from each block) to 10 groups of 10 animals each. Treatment diets and water were provided ad libitum for a 21-day feeding period. Feed consumption was measured twice weekly, and animals were weighed once a week. All the animal procedures were approved by the Animal Care and Use Committee of the Western Regional

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Research Center, USDA, Albany, CA, and conformed to the principles in *Guide for the Care and Use of Laboratory Animals* (Committee on Care and Use of Laboratory Animals 1985).

The GEB fraction was obtained from Steptoe barley by a threestep grinding and sieving procedure (Knuckles et al 1992). Coarse material after the final sieving was the GEB fraction with a  $\beta$ glucan content of 18.9%. Rice bran, stabilized by extrusion at 130°C, was obtained from a local milling company. Oat bran was oat bran cereal (Quaker Oats Co., Chicago, IL). Dietary fiber and  $\beta$ -glucan composition of the cereals is shown in Table I. Diet ingredients were analyzed for TDF and soluble dietary fiber (Prosky et al 1988),  $\beta$ -glucans (McCleary and Glennie-Holmes 1985, McCleary and Nurthen 1986), crude fat (AOAC 920.39C, 1990), and nitrogen (Kjeldahl procedure).

All experimental diets contained 0.25% cholesterol, 9.0% fat, and 3.1% nitrogen. Composition of the diets is given in Table II. Corn oil and casein (401150 and 400625, respectively, Dyets, Inc., Bethlehem, PA) were supplemental sources of fat and nitrogen, respectively. The control diet contained 10% cellulose (CC). GEB-fraction diets contained 3.3 (GEB3), 4.3 (GEB4), or 6.0% (GEB6) β-glucans. Remaining diets contained either milled dehulled whole barley (WB), stabilized rice bran (SR); oat bran (OT); or combinations of 1:1 TDF from GEB+SR, GEB+OT, or SR+OT. All diets contained 10% TDF, except GEB6, which contained 13.9% TDF.

After 21 days, the animals were fasted for 16 hr and anesthetized with  $CO_2$  for sample collection. Blood was drawn by cardiac puncture into plastic tubes containing anticoagulant (ethylene-diamine tetraacetic acid, dipotassium salt, 0.8 mg/ml of blood) and centrifuged at  $1,500 \times g$  for 30 min at 4°C to obtain plasma. Livers were excised, rinsed, blotted, weighed, and kept on dry ice. Livers and plasma aliquots were stored at  $-70^{\circ}$ C. Plasma samples were analyzed by enzymatic colorimetric procedures for cholesterol (Sigma diagnostic kit 352, Sigma Chemicals, St. Louis, MO) and triglycerides (Gilford diagnostic kit 23422, Gilford Systems, Oberlin, OH). Plasma cholesterol (PC) values were determined using standard curves obtained by running several concentrations of standards provided with the diagnostic kits.

Pooled, fresh plasma samples were prepared from three or four animals per pool (three pools per treatment) using 0.4 ml of plasma from each animal. Protease inhibitor (epsilon-amino caproic acid, 1.3 mg/ml of plasma) (ICN Biomedicals, Inc., Costa Mesa, CA) and antimicrobial agent (garamycin 50 mg/ml,  $10 \mu l/ml$  of plasma) (Schering Corp., Kenilworth, NJ) were added to the pooled plasma samples as preservatives. Lipoproteins were fractionated from 1 ml of pooled plasma using density-gradient ultracentrifugation (Havel et al 1955). After plasma background density was adjusted to 1.019 with NaCl, plasma was centrifuged at 40,000 rpm for 18 hr at 17°C in 2-ml tubes with adapters in a Beckman 50.3 rotor (Beckman Instruments, Palo Alto, CA). The top 0.34-ml layer, containing the very low-density lipoprotein fraction, was removed with a Pasteur pipette. The next 0.34-

ml layer was removed as background, the subnatant density was adjusted to 1.063, and centrifuged at 40,000 rpm for 24 hr at 17°C. The top 0.34-ml layer was removed as the low-density lipoprotein fraction; another 0.34-ml layer was removed as background. The subnatant contained high-density lipoprotein. Lipoprotein fractions were analyzed for cholesterol using the procedure described for plasma.

Each liver was individually thawed, minced, and thoroughly mixed to obtain a homogeneous 1-g sample for extraction of total lipids using the procedure of Folch et al (1957). Liver cholesterol was determined in aliquots (30  $\mu$ l) of extract after evaporation under nitrogen and solubilization with Triton X-100 (Carlson and Goldfarb 1977), using the same enzymatic kits as for PC. Liver triglycerides were determined by a colorimetric method (Sigma kit 405), after aliquots were evaporated under nitrogen and redissolved in chloroform-methanol, 2:1 ( $\nu$ / $\nu$ ). Liver

TABLE I
Percent Composition (db) of Whole Barley,
β-Glucan-Enriched Barley Fraction (GEB),
Rice Bran, and Oat Bran<sup>a</sup>

Cereal	Total Dietary Fiber	Soluble Dietary Fiber	β-Glucan	Fat	Nitrogen	Dry Matter
Dehulled whole						
barley	17.2	6.0	5.7	3.1	2.2	91.0
GEB	43.8	19.8	18.9	3.7	2.1	90.2
Rice bran	22.9	1.4	1.8	20.6	2.4	92.2
Oat bran	18.6	8.0	8.3	7.7	3.6	89.7

<sup>&</sup>lt;sup>a</sup>Total and soluble dietary fiber analyzed by the method of Prosky et al (1988),  $\beta$ -glucans by McCleary and Glennie-Holmes (1985), fat by AOAC method 920.39C (1990), and nitrogen by Kjeldahl method.

values were determined from standard curves obtained by running National Bureau of Standards reference material for cholesterol (SRM 911b) and triglycerides (tripalmitin, SRM 1595) through the procedure described for the plasma samples.

In vitro relative viscosities for experimental diet slurries (159 g of diet in 400 ml of phosphate buffer, pH 6.5, 37° C) were measured with spindle T-A, inverted T (1.894-in. crosspiece length) at 20 rpm using a Helipath stand with a Brookfield Synchro-Lectric viscometer (Brookfield Engineering, Stoughton, MA). All samples were analyzed in triplicate.

Data were statistically analyzed using analysis of variance and Duncan's new multiple range test (Steel and Torrie 1960). A value of P < 0.05 was considered the criterion of significance.

#### RESULTS AND DISCUSSION

## Weight Gain, Feed Intake

Animal weights on arrival were  $39.5 \pm 0.3$  (mean  $\pm$  SEM). After one week of basal diet, selectively randomized initial animal weights were similar for all treatments ( $59.9 \pm 0.8$  g). Significantly higher feed intake was observed in hamsters fed GEB4, GEB+SR, SR, and SR+OT diets. Significantly higher final weights were observed with GEB4, GEB+OT, SR, OT, and SR+OT diets after 21 days, compared with those fed CC diet (Table III).  $\beta$ -Glucan content of CC, GEB4, GEB+SR, GEB+OT, SR, OT, and SR+OT diets was 0.0, 4.3, 2.6, 4.4, 0.8, 4.5, and 2.6%, respectively. This suggests that feed intake and final weights were not related to the  $\beta$ -glucan content of the diets. Feed-gain ratio was significantly lower in hamsters fed GEB+OT, SR, and OT diets than in those fed CC diet. This also suggests that feed-gain ratios were not related to the  $\beta$ -glucan content of the diets.

TABLE II Composition of Diets (% Dry Matter)

Diets <sup>a,b</sup>	Cellulose	Whole Barley	β-Glucan-Enriched Barley Fraction (GEB) <sup>c</sup>	Rice Bran	Oat Bran	Corn Oil	Corn Starch	Casein
Cellulose basal	10.0		• • •			9.0	56.0	20.0
Cholesterol (0.25%)								
Cellulose control	10.0		• • •			9.0	55.7	20.0
Dehulled whole barley		58.2	• • •			7.2	17.5	11.8
GEB3	2.3		17.6			8.4	48.9	17.7
GEB4			22.8			8.2	46.8	17.0
GEB6	• • •		31.7			7.8	39.4	15.8
GEB + rice bran	• • •		11.4	21.8		4.1	42.3	15.1
GEB + oat bran			11.4		26.9	6.5	37.7	12.2
Rice bran	• • •		• • •	43.7			37.9	13.2
Oat bran	• • •		• • •		53.7	4.9	28.7	7.5
Rice bran + oat bran				21.8	26.9	2.4	33.3	10.4

<sup>&</sup>lt;sup>a</sup> All diets contained 3.5% mineral mix (AIN 1980), 1.0% vitamin mix (AIN 1980), 0.3% methionine, and 0.2% choline bitartrate.

TABLE III

Effect of Barley,  $\beta$ -Glucan-Enriched Barley Fraction (GEB), Rice Bran (SR), and Oat Bran (OT) Diets

Containing 0.25% Cholesterol on Body Weight, Weight Gain, Feed Intake and Feed-Gain in Hamsters (Mean  $\pm$  SEM)

Diet, % Dry Matter <sup>a</sup>	β-Glucan (%)	Feed Intake (g/d)	Final Weight (Fasting)	Feed-Gain (g)
Cellulose control, 10%	0.0	$8.5 \pm 0.2 \text{ d}$	99.1 ± 2.0 c	$3.9 \pm 0.1 \text{ ab}$
Dehulled whole barley, 58.2%	3.3	$8.8 \pm 0.2 \text{ cd}$	$102.4 \pm 2.3 \text{ bc}$	$3.7 \pm 0.1 \ bc$
GEB3, 17.6%	3.3	$9.0 \pm 0.1 \text{ b-d}$	$102.0 \pm 1.5 \text{ bc}$	$3.7 \pm 0.1 \text{ bc}$
GEB4, 22.8%	4.3	$9.4 \pm 0.1 \text{ ab}$	$106.4 \pm 2.2 \text{ ab}$	$3.7 \pm 0.1 \text{ b-d}$
GEB6, 31.7%	6.0	$9.0 \pm 0.1 \text{ b-d}$	$98.7 \pm 1.0 \text{ c}$	$4.1 \pm 0.1 a$
GEB, $11.4\% + SR$ , $21.8\%$	2.6	$9.2 \pm 0.2 \text{ a-c}$	$103.7 \pm 1.9 \text{ a-c}$	$3.9 \pm 0.1$ ab
GEB, $11.4\% + OT$ , $26.9\%$	4.4	$9.1 \pm 0.2 \text{ a-d}$	$107.8 \pm 1.6 \text{ ab}$	$3.5 \pm 0.1 \text{ cd}$
SR. 43.7%	0.8	$9.7 \pm 0.2 a$	$109.3 \pm 1.6 a$	$3.5 \pm 0.1 \text{ cd}$
OT, 53.7%	4.5	$9.0 \pm 0.2 \text{ b-d}$	$109.2 \pm 2.7 a$	$3.4 \pm 0.1 d$
SR, 21.8% + OT, 26.9%	2.6	$9.2 \pm 0.3 \text{ a-c}$	$106.3 \pm 2.7 \text{ ab}$	$3.6 \pm 0.1 \text{ b-d}$

<sup>&</sup>lt;sup>a</sup>All diets contained 9.0% fat, 3.1% N, and 10% total dietary fiber, except GEB6, which contained 13.9% total dietary fiber. Values within a column with different letters are significantly different ( $P \le 0.05$ ). Initial weight was similar for all treatments 59.9  $\pm$  0.8 g.

<sup>&</sup>lt;sup>b</sup> Diets were equal in fat (9.0%), nitrogen (3.1%), and total dietary fiber (10%), except GEB6, which contained 13.9% total dietary fiber.

<sup>&</sup>lt;sup>c</sup>18.9% β-glucans. Coarse fraction resulting from a three-step pin milling and sieving of dehulled barley (Steptoe variety).

#### **Total PC**

Total PC values were significantly lower in hamsters fed GEB6, OT, or SR+OT diets than in those fed CC or GEB+SR diets (Table IV). Significant PC reductions in hamsters fed OT diet (10% TDF) is in agreement with our previous report (Kahlon et al 1990) and with observations by Shinnick et al (1990) in rats. Significant PC reductions in animals fed the 13.9% TDF GEB6 diet is in agreement with Newman et al (1991), who observed that a 14% TDF barley diet significantly reduced PC in 0.5% cholesterol-fed chicks. Previously, we reported significant PC reductions in hamsters fed 10% TDF SR diets containing 0.5 or 0.3% cholesterol compared with those fed CC diets (Kahlon et al 1990, 1992a,b). In the current study, PC reductions were not significant in hamsters fed 10% TDF SR diet containing 0.25% cholesterol. This apparent discrepancy may be due, in part, to the lower dietary cholesterol level in the current experiment, which was used to reduce fatty infiltration of tissues associated with high dietary cholesterol (Chanutin and Ludewig 1933, Beynen et al 1986). Cholesterol-lowering effect of a test substance is related to the degree of hypercholesterolemia in the test subjects or animals (Shinnick et al 1988; Newman et al 1992; Kahlon et al 1992a,b).

Among diets GEB4, GEB+OT, and OT, with similar total dietary fiber (10%) and  $\beta$ -glucan levels (4.3, 4.4, and 4.5%, respectively), only OT-fed hamsters exhibited significant PC reductions compared with animals fed the CC diet. Significant PC reductions with barley-containing diets were observed only with diet GEB6, containing 14% TDF and 6% β-glucans. If the cholesterol-lowering activity of oats and barley is assumed to be caused by  $\beta$ -glucans (Chen et al 1981, Kirby et al 1981, Newman et al 1992), then the data suggest that oat bran  $\beta$ -glucans are more hypocholesterolemic than processed barley  $\beta$ -glucans. However, Klopfenstein and Hoseney (1987) reported significantly lower serum cholesterol in rats fed bread containing 7% barley  $\beta$ -glucans but not 7% oat  $\beta$ -glucans. Their results may have been influenced by the 9% higher cholesterol intake and 10% lower  $\beta$ -glucan intake of the oat-fed group. Contradictory results between the studies may be due, in part, to solubility differences between oat and barley  $\beta$ -glucans. Discrepancies also may be related to variable cholesterol-lowering effects of  $\beta$ -glucans from different barley varieties (Bengtsson et al 1990).

PC reductions were positively correlated to the level of  $\beta$ -glucans in the diet when GEB was fed as the sole source of dietary fiber, although significant effects were observed only with 31.7% GEB (6%  $\beta$ -glucans) in the diet. Barley diets WB, GEB3, GEB4, and GEB6 ( $\beta$ -glucan content of 3.3, 3.3, 4.3, and 6.0%, respectively) resulted in PC reductions of 10, 1, 6, and 15%, respectively. Greater PC reductions occurred in animals consuming WB diet than in those consuming GEB3 or GEB4 diets with similar or

higher  $\beta$ -glucan content. This suggests that, in addition to  $\beta$ -glucans, there may be other components, such as protein, starch, specific lipid fractions, or as yet unidentified components, with potential hypocholesterolemic properties in whole barley. Qureshi et al (1987) reported that  $\alpha$ -tocotrienols of barley oil have hypocholesterolemic activity.

Significant PC reductions were observed during the current study in hamsters fed SR+OT diet with 2.3% B-glucans from oat bran and 0.3%  $\beta$ -glucans from rice bran. This suggests that any contribution of rice bran in lowering PC is likely to be due to components other than  $\beta$ -glucans. Cholesterol-lowering activity has been reported with rice bran fractions (Avano et al 1980. Suzuki 1982), rice bran oil (Suzuki and Oshima 1970, Nicolosi et al 1991), unsaponifiable matter (Ishibashi and Yamamoto 1980; Sharma and Rukmini 1986, 1987), oryzanol (Seetharamaiah and Chandrasekhara 1988, 1989), and rice protein (Sugano et al 1984). Some of the cholesterol-lowering effects of these cereal fractions may be caused by replacement of part of the casein in the diet with plant protein. Casein has been shown to be hypercholesterolemic relative to plant proteins (Sugano et al 1984, Terpstra et al 1991). Previously, we found that intact, stabilized rice bran is more cholesterol-lowering than its recombined fractions (Kahlon et al 1992b).

### Lipoprotein Cholesterol Values

Very low-density lipoprotein cholesterol (VLDLc) values were similar for all treatments, suggesting that PC reductions were not due to changes in production and clearance or uptake of VLDLc (Table IV). Low-density lipoprotein cholesterol (LDLc) values were significantly lower in hamsters fed GEB6, GEB+OT, or OT diets, compared with those in hamsters fed the CC diet. In addition, the LDLc-PC ratio was significantly lower in hamsters fed the OT diet than in hamsters fed CC, GEB3, or GEB4 diets. Reduction of LDLc is considered beneficial in lowering coronary heart disease risk (Lipid Research Clinics Program 1984). Highdensity lipoprotein cholesterol (HDLc) values were significantly lower in hamsters fed GEB4, GEB6, GEB+OT, OT, or SR+OT diet but not in hamsters fed WB, GEB3, GEB+SR, or SR diet compared with those fed CC diet. However, HDLc-PC ratios were similar for all treatments. HDLc transports cholesterol from tissues to the liver for excretion into bile; therefore, maintaining a higher proportion of HDLc is positively correlated with reduced coronary heart disease risk (Keys 1980). LDLc-HDLc ratios were similar for all treatment groups. Significant reductions in LDLc without affecting HDLc have been reported with oat bran diets (Anderson et al 1984, Van Horn et al 1986, Kestin et al 1990, Davidson et al 1991). Significant LDLc reductions have been observed with barley diets, but HDLc was either not influenced or was significantly elevated (Fadel et al 1987; Newman et al

TABLE IV

Effect of Barley, β-Glucan-Enriched Barley Fraction (GEB), Rice Bran, and Oat Bran Diets Containing 0.25% Cholesterol on Plasma Cholesterol (PC), Very Low Density Lipoprotein Cholesterol (VLDLc), Low Density Lipoprotein Cholesterol (LDLc), High Density Lipoprotein Cholesterol (HDLc), and Triglycerides in Hamsters\*-b

Diets, % Dry Matter	β-Glucan (%)	Total Cholesterol (mg/dl)	VLDLc (mg/dl)	LDLc (mg/dl)	HDLc (mg/dl)	LDLc/HDLc (mg/dl)	LDLc/PC (mg/dl)	HDLc/PC (mg/dl)	Triglycerides (mg/dl)
Cellulose control, 10%	0.0	$302 \pm 10 \text{ a}$	55 ± 9 a	76 a	144 a	53 ab	29.2 a	55 a	$288 \pm 59 \text{ ab}$
Dehulled whole barley, 58.2%	3.3	$270 \pm 10 \text{ ab}$	$45 \pm 4 a$	$66 \pm 3 \text{ a-d}$	$137 \pm 2$ ab	$48 \pm 2 ab$	$27 \pm 1$ ab	$55 \pm 1 a$	$216 \pm 22 \text{ b}$
GEB3, 17.6%	3.3	$298 \pm 20 \text{ ab}$	$59 \pm 8 a$	$74 \pm 8 a-c$	$137 \pm 5 \text{ a-c}$	$55 \pm 7 ab$	$27\pm2$ a	$51 \pm 3 a$	$332 \pm 43 a$
GEB4, 22.8%	4.3	$285 \pm 11 \text{ ab}$	$59 \pm 1a$	$72 \pm 11 \text{ a-c}$	$126 \pm 6 \text{ bc}$	$58 \pm 12 a$	$28 \pm 4 a$	$49 \pm 3 a$	$342 \pm 35 a$
GEB6, 31.7%	6.0	$258 \pm 8 b$	$48 \pm 5 a$	$58 \pm 3 \text{ cd}$	$123 \pm 6 c$	$47 \pm 2 ab$	$25 \pm 1$ ab	$54 \pm 1$ a	$212 \pm 16 \text{ b}$
GEB, $11.4\%$ + rice bran, $21.8\%$	2.6	$298 \pm 1 a$	$62 \pm 14 a$	$76 \pm 9 ab$	$140 \pm 3$ ab	$54 \pm 7 ab$	$26 \pm 1$ ab	$49 \pm 4 a$	$313 \pm 65 \text{ ab}$
GEB, $11.4\%$ + oat bran, $26.9\%$	4.4	$266 \pm 11 \text{ ab}$	$51 \pm 5 a$	$58 \pm 2 \text{ b-d}$	$128 \pm 3 \text{ bc}$	$45 \pm 1 ab$	$25 \pm 1$ ab	$54 \pm 1 a$	$246 \pm 36 \text{ ab}$
Rice bran, 43.7%	0.8	$276 \pm 6 ab$	$52 \pm 3 a$	$68 \pm 3 \text{ a-d}$	$136 \pm 2 \text{ a-c}$	$50 \pm 2 ab$	$27 \pm 1$ ab	$53 \pm 1 a$	$274 \pm 16 \text{ ab}$
Oat bran, 53.7%	4.5	$260 \pm 9 b$	$55 \pm 5 a$	$53 \pm 2 d$	$130 \pm 4 \text{ bc}$	$41 \pm 2 b$	$22 \pm 1 \text{ b}$	$55 \pm 1 a$	$266 \pm 37 \text{ ab}$
Rice bran, 21.8%									
+ oat bran, 26.9%	2.6	$263 \pm 8 b$	$48 \pm 7 a$	$60 \pm 1 \text{ a-d}$	$129 \pm 4 \text{ bc}$	$46 \pm 2 ab$	$25 \pm 1$ ab	$55 \pm 1 a$	$273 \pm 41 \text{ ab}$

<sup>&</sup>lt;sup>a</sup> All diets contained 9.0% fat, 3.1% N, and 10% total dietary fiber, except GEB6, which contained 13.9% total dietary fiber. Values (mean  $\pm$  SEM) within a column with different letters are significantly different ( $P \le 0.05$ ).

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bVLDLc, LDLc, and HDLc were determined on pools of three or four animal plasma samples (three pools per treatment) after density-gradient ultracentrifugal separation of lipoproteins.

1989; Qureshi et al 1980, 1987). Full-fat rice bran diet fed to chicks significantly lowered serum LDLc while increasing HDLc (Newman et al 1992). The ratio of HDLc to PC was significantly raised in subjects consuming rice bran (Kestin et al 1990). Differences in HDLc response may relate to the species studied or the methodology used for HDLc quantitation (precipitation vs. ultracentrifugation).

#### Plasma Triglycerides

Compared with the CC diet, there were no significant differences in plasma triglyceride levels (Table IV) in animals fed any of the treatment diets. This is in agreement with findings by Chochi et al (1984) and Fadel et al (1987). Other studies with barley diets have reported lowering of serum triglycerides in swine (Qureshi et al 1987) or raising of blood triglyceride levels in chicks (Burger et al 1982), depending on the variety or fraction of barley used. Studies with oat bran either have shown no significant effect in laboratory animals (Shinnick et al 1988, Kahlon et al 1990) or humans (Kestin et al 1990, Davidson et al 1991), or they have shown a significant decrease in human serum triglyceride levels (Anderson et al 1984). Rice bran consumption resulted in significant reductions in plasma triglycerides in some animal studies (Suzuki 1982, Newman et al 1992, Kahlon et al 1992b), but studies with humans (Kestin et al 1990, Hegsted et al 1990) or hamsters (Kahlon et al 1990, 1992a) have shown no significant effect. Inconsistent responses in plasma triglycerides may be partly attributable to variability in the cereals or fractions tested as well as the test animals, test diets, or subjects studied.

# Liver Weight

In hamsters fed GEB4, GEB6, or GEB+SR diet, liver weight per 100 g of fasting body weight values were significantly lower than in those fed CC, WB, or OT diets (Table V). Significantly lower liver weight per 100 g of body weight in animals fed GEB6 diet may be due to the higher dietary fiber content and lower caloric density of the GEB6 diet. However, significantly lower liver weights in hamsters fed GEB4, GEB6, and GEB+SR diets, as compared with those fed OT diet, is difficult to explain. It is apparently not associated with fatty infiltration, as animals fed the OT diet had significantly lower liver cholesterol than the animals fed the GEB6 or GEB+SR diets. Animals fed the OT diet also had significantly lower liver triglycerides than those fed GEB4 or GEB+SR diets. In our previous studies (Kahlon et al 1992a,b), apparent fatty infiltration of the liver was reduced in hamsters fed rice bran and several of its fractions, but we did not observe a similar effect with rice bran in the present study.

TABLE V Effect of Barley,  $\beta$ -Glucan-Enriched Barley Fraction (GEB), Rice Bran, and Oat Bran Diets Containing 0.25% Cholesterol on Liver Weight, Liver Cholesterol, and Triglycerides in Hamsters<sup>a</sup>

Diets, % Dry Matter	β-Glucan (%)	Liver Weight/ 100 g Fasting Body Weight (g)	Cholesterol (mg/g)	Triglycerides (mg/g)
Cellulose	0.0	$5.1 \pm 0.1 \text{ ab}$	54 ± 1 a	$12 \pm 1$ ab
Dehulled whole	0.0	3.1 ± 0.1 ab	34 ± 1 a	12 1 1 40
barley, 58.2%	3.3	$5.1 \pm 0.1 a$	$49 \pm 3 \text{ ab}$	$13 \pm 3 \text{ b-d}$
GEB3, 17.6%	3.3	$4.9 \pm 0.1 \text{ bc}$	$38 \pm 2 de$	$14 \pm 1$ a
GEB4, 22.8%	4.3	$4.8 \pm 0.1 c$	$32 \pm 2 e$	$12 \pm 1 \text{ a-c}$
GEB6, 31.7%	6.0	$4.7 \pm 0.1 \text{ c}$	$42 \pm 2 \text{ cd}$	$15 \pm 4 \text{ a-d}$
GEB, 11.4% +				
rice bran, 21.8%	2.6	$4.8 \pm 0.1 c$	$43 \pm 2 \text{ cd}$	$14 \pm 2 ab$
GEB, 11.4% +				
oat bran, 26.9%	4.4	$4.9 \pm 0.1 \text{ a-c}$	$35 \pm 2 e$	$9 \pm 1 \text{ cd}$
Rice bran, 43.7%	0.8	$4.9 \pm 0.1 \text{ a-c}$	$43 \pm 1 \text{ cd}$	$12 \pm 1 \text{ a-c}$
Oat bran, 53.7%	4.5	$5.1 \pm 0.1 \text{ ab}$	$34 \pm 2 e$	$9\pm1$ d
Rice bran, 21.8% +				
oat bran, 26.9%	2.6	$4.9 \pm 0.1 \text{ a-c}$	$45 \pm 2 \text{ bc}$	$12 \pm 1 \text{ a-c}$

<sup>&</sup>lt;sup>a</sup> All diets contained 9.0% fat, 3.1% N, and 10% total dietary fiber, except GEB6, which contained 13.9% total dietary fiber. Values (mean  $\pm$  SEM) within a column with different letters are significantly different ( $P \le 0.05$ ).

This discrepancy may relate to the lower cholesterol level used in this study compared with that of the previous studies.

#### Liver Cholesterol

Liver cholesterol values (mg/g) in hamsters fed all treatment diets, except WB, were significantly lower than those in animals fed the CC diet. Significant reductions in liver cholesterol in hamsters fed any of the oat, rice, or barley diets, except WB, is in agreement with other reports on barley (Qureshi et al 1980, Klopfenstein et al 1987, Ranhotra et al 1991), oat bran (Welch et al 1986, Shinnick et al 1988, Kahlon et al 1990), and rice bran (Kahlon et al 1990, 1992a,b). Lack of liver cholesterol reduction with milled dehulled barley is in agreement with observations reported by Chochi et al (1984) in chicks.

#### Liver Triglycerides

Liver triglyceride concentrations (mg/g) in GEB+OT or OT diet groups were significantly lower than those in CC, GEB3, or GEB+SR diet groups. Significant reductions in liver triglycerides in animals fed GEB+OT or OT diet may suggest reduced uptake, synthesis, or storage of liver triglycerides with these diets. Similar observations have been reported in rats fed barley diets (Klopfenstein and Hoseney 1987) or oat bran diets (Shinnick et al 1990). Multiple regression analysis, using feed intake and final body weight as covariates, showed small differences, but the significant effects of plasma and liver lipids did not change.

### In Vitro Viscosity

Relative viscosities of the various treatment diet slurries are presented in Figure 1. Diet GEB6, with 13.9% TDF, was excluded from the comparisons; its viscosity was much higher than all other diets because of its greater TDF and  $\beta$ -glucan content. Values (cP) for GEB6 at 30-min intervals were >500 (30 min), 301 (60 min), 220 (90 min), 192 (120 min), 176 (150 min), and 163 (180 min). GEB3 and GEB4 diets had parallel viscosity curves, with highest viscosity at 30 min, that decreased abruptly and continued to decrease with time. With the OT diet, in vitro viscosity continued to increase from 63 to 104 cP over the 3-hr measuring period. The cholesterol-lowering effect of the OT diet may be related to its viscosity-raising properties, which could reduce fat and cholesterol absorption or bile acid reabsorption from the gastrointestinal tract. The barley diet slurry viscosity patterns we observed were similar to those reported by Bengtsson et al (1990), where the highest viscosity was observed initially, then decreased with time with the barley variety Arizona. In barley

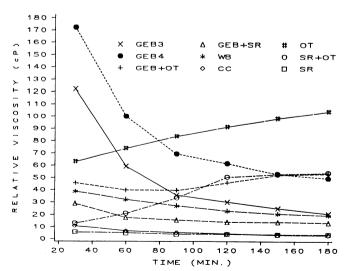


Fig. 1. Relative in vitro viscosities (cP) of slurries of control diet containing 10% cellulose (CC); whole barley (WB);  $\beta$ -glucan-enriched barley (GEB3, GEB4) fraction containing 3.3 and 4.3  $\beta$ -glucans, respectively; oat bran (OT); stabilized rice bran (SR); and combinations of these diets at 30-min intervals measured with a viscometer at 20 rpm for 159 g of diet in 400 ml of phosphate buffer (pH 6.5, 37°C).

diets, reduced viscosity with time may relate to endogenous  $\beta$ glucanase or amylase content. A viscosity pattern similar to that seen with OT was observed by Bengtsson et al (1990) with the barley variety Washonupana, GEB4, GEB+OT, and SR+OT (\betaglucan contents of 4.3, 4.4 and 2.6%, respectively) exhibited similar viscosities (52-53 cP) at 150 min. The variable response in PC levels but similar viscosity characteristics of GEB4, GEB+OT, and SR+OT slurries after 150 min suggests that multifactorial mechanisms may be regulating cholesterol levels and metabolism. SR diet resulted in a low-viscosity pattern similar to that of the CC diet, whereas SR+OT diet, which resulted in significant PC reductions, exhibited a viscosity about 50% of that of OT diet. The data suggest that the contribution of rice bran to PC reductions may relate to mechanisms other than sequestering or entrapment of lipids or bile acids in the gastrointestinal tract. Significant liver cholesterol reductions by all of the treatment diets (except WB) exhibiting wide variability in slurry viscosities, suggests that liver cholesterol levels may be regulated by receptor, synthesis, or catabolic mechanisms in addition to reduced absorption from the gastrointestinal tract.

In conclusion, under the conditions of this study, the ratio of HDLc to total PC was maintained; total PC was significantly lowered in hamsters fed OT diet, a diet with 1:1 TDF combination of SR+OT, or a GEB6 diet. Significant liver cholesterol reductions were observed with SR, OT, and GEB fraction and their combinations. Viscous properties of cereal fiber or fraction diets appear to be a mechanism for reduced absorption or reabsorption of lipid, bile acids, and their metabolites, influencing PC levels.

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[Received September 16, 1992. Accepted February 15, 1993.]