

In Vitro Binding of Bile Acids by Rice Bran, Oat Bran, Barley and β -Glucan Enriched Barley

T. S. Kahlon^{1,2} and C. L. Woodruff¹

ABSTRACT

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The in vitro bile acid binding by rice bran, oat bran, dehulled barley, and β -glucan enriched barley was determined using a mixture of bile acids at a duodenal physiological pH of 6.3. Six treatments and two blank incubations were conducted testing substrates on an equal protein basis. The relative in vitro bile acid binding of the cereal brans on an equal total dietary fiber (TDF) and insoluble dietary fiber (IDF) basis considering cholestyramine as 100% bound was rice bran 45 and 49%; oat bran 23 and 30%; dehulled barley 33 and 57%; and β -glucan enriched barley 20 and 40%, respectively. Bile acid bindings on equal protein basis for the respective cereals were 68, 26, 41, and 49%. Bile acid binding by rice bran may account to a great extent for its cholesterol-lowering properties, while bile acid binding by oat bran suggests that the primary mechanism

of cholesterol lowering by oat bran is not due to the bile acid binding by its soluble fiber. Bile acid binding was not proportional to the soluble fiber content of the cereal brans tested. Except for dehulled barley, bile acid binding for rice bran, oat bran, and β -glucan enriched barley appear to be related to their IDF content. Highest relative bile acid binding values for rice bran and β -glucan enriched barley were observed on an equal protein basis, whereas highest values for dehulled barley were based on IDF. Data suggest that of all four cereals tested, bile acid binding may be related to IDF or protein anionic, cationic, physical and chemical structure, composition, metabolites, or their interaction with active binding sites.

Cereal brans are considered to be desirable for human consumption due to their reported health benefits. Extensive research reviewed by Kahlon and Chow (1997) has shown that incorporating rice bran, oat bran, or barley fractions in the diet results in plasma cholesterol reductions, which lower the risk of cardiovascular disease. Bile acids are acidic steroids synthesized in the liver from cholesterol. After conjugation with glycine or taurine, they are secreted into the duodenum. Bile acids are actively reabsorbed by the terminal ileum and undergo an enterohepatic circulation (Hofmann 1977). Binding of bile acids and increasing their fecal excretion has been hypothesized as a possible mechanism for lowering cholesterol by dietary fiber (Trowell 1975; Lund et al 1989; Anderson and Siesel 1990). By binding bile acids, cereal fibers prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids (Eastwood and Hamilton 1968; Balmer and Zilversmit 1974; Kritchevsky and Story 1974). The healthful or cholesterol-lowering properties of cereal brans could be predicted by evaluating their in vitro bile acid binding, based on positive correlation found between in vitro and in vivo studies showing that cholestyramine binds bile acids and cellulose does not (Suckling et al 1991; Nakamura and Matsuzawa 1994; Daggy et al 1997; Kahlon and Chow 2000). The relative in vitro bile acid binding of the cereal brans on an equal total dietary fiber (TDF) basis, considering cholestyramine as binding 100%, was rice bran 51%; wheat bran 31%; oat bran 26%; and corn bran 5% (Kahlon and Chow 2000). Significant reductions (45%) in aortic plaque were observed in six weeks when soy isolate replaced casein in the diet as a source of protein (Kahlon and Chow 1999), suggesting that plant protein may be associated with bile acid binding. The objective of this study was to evaluate in vitro bile acid binding by rice bran, oat bran, dehulled barley, and a β -glucan enriched barley fraction using an equal amount of

protein from each cereal source in a bile acid mixture under duodenal physiological pH of 6.3.

MATERIALS AND METHODS

Rice bran, oat bran, and barley (food-grade, national brands, obtained from regional mills) were ground in a Thomas-Wiley mill No. 1 (Arthur Thomas, Philadelphia, PA) to pass a 2-mm screen. β -Glucan enriched barley fraction was prepared in house as described previously (Knuckles and Chiu 1995). Samples were analyzed for insoluble and soluble dietary fiber (Prosky et al 1988), nitrogen by combustion with a (Leco FP-428, St. Joseph, MI), ether-extracted crude fat by method 920.39C (AOAC 1990), and moisture by method 935.29 (AOAC 1990). Samples were heated to 130°C for 30 min to deactivate the endogenous enzymes (Randall et al 1985). Composition of the cereal fractions tested is given in Table I. Eight replicate incubations, six with bile acid mixture and two substrate blanks without bile acid mixture were run for each cereal and each control. Cellulose, a nonbile acid binding fiber, was the negative control and cholestyramine, a bile acid binding anionic resin was the positive control. All treatments used a 25-mg protein content for each cereal ($N \times 5.7$) per incubation. Dry matter weights for incubations were 140, 147, 196, and 199 mg for rice bran, oat bran, barley, and β -glucan enriched barley, respectively, and 25 mg each for cholestyramine and cellulose. The total dietary (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) contents in each incubation for rice bran, oat bran, barley, and β -glucan enriched barley were 38, 4, 34; 29, 7, 22; 31, 13, 18; and 61, 30, 31 mg, respectively.

Bile Acid Binding Procedure

The in vitro bile acid binding procedure was a modification of that by Camire et al (1993) as previously reported (Kahlon and Chow 2000). Six replicates with 25 mg of protein per test sample and two individual substrate blanks of rice bran, oat bran, barley, enriched barley, cholestyramine, and cellulose, as well as a positive blank (2.88 μ mol, bile acid mixture/incubation) were weighed into 12 \times 125 mm glass, screw-capped tubes. Samples were digested in 1 mL 0.01N HCl for 1 hr in a 37°C shaker bath. After this acidic digestion, which simulated gastric digestion, the sample pH was adjusted to 6.3 with 0.1 mL of 0.1N NaOH. To each test sample was added 4 mL of bile acid mixture solution (0.72 μ mol/mL) in a 0.1M phosphate buffer, pH 6.3. The stock solution of bile acid mixture contained taurocholic acid (9 mmol/L),

¹ Western Regional Research Center, USDA-ARS, Albany, CA 94710. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

² Corresponding author. Phone: 510-559-5665, Fax 510-559-5777. E-mail: tsk@pw.usda.gov.

RESULTS AND DISCUSSION

taurochenocholic acid (9 mmol/L), taurodeoxycholic acid (9 mmol/L), glycocholic acid (3 mmol/L), glycochenocholic acid (3 mmol/L) and glycodeoxycholic acid (3 mmol/L). This bile acid mixture was formulated with taurine-conjugated bile acids providing 75% and glycocholic bile acids 25% of the bile acids. A phosphate buffer (4 mL, 0.1M, pH 6.3) was added to the individual substrate blanks. After the addition of 5 mL of porcine pancreatin (5x, 10 mg/mL, in a 0.01M phosphate buffer, pH 6.3; providing amylase, protease, and lipase digestion of samples), tubes were incubated for 1 hr in a 37°C shaker bath. Mixtures were transferred to 10-mL centrifuge tubes (Oak Ridge 3118-0010 Nalgene, Rochester, NY) and centrifuged at an average 99,000 × g in a 75-Ti rotor at 39K for 18 min at 25°C in an ultracentrifuge (model L-60, Beckman, Palo Alto, CA). Supernatant was removed into a second set of labeled tubes. An additional 5 mL of phosphate buffer was used to rinse out the incubation tube and was added to the centrifuge tube which was vortexed and centrifuged as before. Supernatant was removed and combined with the previous supernatant tube. Aliquots of pooled supernatant were frozen at -20°C for bile acids analysis. Bile acids were analyzed using Sigma bile acids procedure No. 450 (Sigma, St. Louis, MO) using a Ciba-Corning Express Plus analyzer (Bayer, Tarrytown, NY).

Statistical Analysis

Each sample was analyzed in triplicate. Unbound bile acid values were determined in treatment samples relative to no sample controls (bile acid positive blanks) after subtracting no bile acid cereal blanks. Relative recoveries of bile acids were determined from the standard curve and performance of analysis was monitored by bile acid calibrators (Sigma 450-11) at 5, 25, 50, 100, and 200 µmol/L. The effect of treatment was tested using Lavene's test for homogeneity. Least square means were calculated. Dunnett's one-tailed test was used for comparison of cholestyramine as well as cellulose against all treatments. Differences among cereals were tested for significance with Tukey's test for comparison of all possible pairs of means (SAS Institute, Cary, NC). A value of $P \leq 0.05$ was considered the criterion of significance.

On an equal dry matter basis, bile acid binding was significantly higher with cholestyramine (10.91 µmol/100 mg) and significantly lower with cellulose (-0.11 µmol/100 mg) than all other treatments (0.48–1.32 µmol/100 mg) (Table II). There were significant differences in bile acid binding among all four cereal treatments (rice bran > β-glucan enriched barley > barley > oat bran). Cholestyramine bound 95% of the bile acids. Similar bile acid binding (96%) under similar in vitro conditions was observed previously (Kahlon and Chow 2000). Story and Kritchevsky (1976) reported 81% bile acid binding by cholestyramine using 50 mg of substrate and 50 µmol of bile acids. Higher bile acid binding by cholestyramine in our studies may be due to the use of physiological pH or a higher substrate to bile acid ratio. Assigning bile acid binding to cholestyramine as 100%, the relative bile acid binding for the test samples was rice bran 12%; oat bran 4%, barley 5%; and β-glucan enriched barley 6%. Similar bile acid binding on a dry matter basis for rice bran (13%) as well as oat bran (5%) have been reported (Kahlon and Chow 2000). Similar amounts of dry matter were used for rice and oat bran per incubation, however their bile acid binding values varied by threefold. Also, similar amounts of dry matter were used for the two barley treatments, which resulted in significant differences in their bile acid binding. Thus, data suggest that bile acid binding by cereals tested was not proportional to their dry matter content. The amount of starch used per incubation for rice bran, oat bran, barley, and β-glucan enriched barley was 35, 78, 131, and 103 mg, respectively. Considering starch as the bile acid binding component, rice bran, oat bran, barley, and β-glucan enriched barley treatments bound 5.3, 0.9, 0.9, and 1.3 µmol/100 mg of starch, respectively. Rice bran with the lowest amount of starch per incubation resulted in highest values, and oat bran and barley treatments with starch amounts 2.2- and 3.7-fold higher than rice bran resulted in similar lowest bile acid binding values. Data suggest that bile acid binding does not appear to be related to the starch content of the cereal fractions tested. A lack of correlation between starch content and bile acid binding was expected because all samples were

TABLE I
Composition of Cereal Brans, Cholestyramine, and Cellulose (% dry matter basis)

Source	Moisture	Dietary Fiber			Fat	Nitrogen ^a	Ash	Starch ^b
		Total	Insoluble	Soluble				
Rice bran	5.4	27.0	24.5	2.5	23.7	2.5	9.9	25.1
Oat bran	8.9	19.5	14.5	5.0	7.5	2.9	2.9	53.6
Barley (dehulled)	10.2	15.8	9.1	6.7	2.8	2.4	1.1	66.6
Enriched barley	9.0	30.8	15.5	15.3	3.2	1.9	3.4	51.8
Cholestyramine	9.6	100	100
Cellulose	5.4	100	100

^a N × 5.7.

^b 100 - (TDF + fat + protein + ash)%.

TABLE II
In Vitro Bile Acid Binding by Rice Bran, Oat Bran, Barley, and Enriched Barley^{a,b}

Treatment	Bile Acid Binding (µmol/100 mg, dry matter)	Binding Relative to Cholestyramine (%)
Rice bran	1.32 ± 0.02b	12.1 ± 0.2b
Oat bran	0.48 ± 0.02e	4.4 ± 0.2e
Barley (dehulled)	0.57 ± 0.02d	5.2 ± 0.2d
Enriched barley	0.67 ± 0.02c	6.1 ± 0.2c
Cholestyramine	10.91 ± 0.02a	100.0 ± 0.2a
Cellulose	-0.11 ± 0.02f	-1.0 ± 0.2f

^a Equal weight, dry matter basis. Pooled values (means ± SEM) within a column with different letters differ significantly ($P \leq 0.05$), $n = 6$.

^b Rice bran, oat bran, barley, enriched barley, cholestyramine, and cellulose treatments contained 140, 147, 196, 199, 25, and 25 mg of dry matter, respectively.

TABLE III
In Vitro Bile Acid Binding by Rice Bran, Oat Bran, Barley, and Enriched Barley^{a,b}

Treatment	Bile Acid Binding (µmol/100 mg of TDF)	Binding Relative to Cholestyramine (%)
Rice bran	4.88 ± 0.05b	44.7 ± 0.4b
Oat bran	2.46 ± 0.05d	22.6 ± 0.4d
Barley (dehulled)	3.60 ± 0.05c	33.0 ± 0.4c
Enriched barley	2.17 ± 0.05e	19.9 ± 0.4e
Cholestyramine	10.91 ± 0.05a	100.0 ± 0.4a
Cellulose	-0.11 ± 0.05f	-1.0 ± 0.4f

^a Equal total dietary fiber (TDF) basis. Pooled values (means ± SEM) within a column with different letters differ significantly ($P \leq 0.05$), $n = 6$.

^b Rice bran, oat bran, barley, enriched barley, cholestyramine, and cellulose treatments contained 38, 29, 31, 61, 25 and 25 mg of TDF, respectively.

digested with pancreatin which contained α -amylase. Previously, bile acid binding of peanut oil was evaluated at $\approx 4\%$ (Kahlon and Chow 2000). Rice bran oil has a higher unsaponifiable fraction than peanut oil but similar fatty acid composition. Rice bran unsaponifiable fraction is partly responsible for its cholesterol-lowering properties (Seetharamaiah and Chandrasekhara 1989; Kahlon et al 1996). The observations reported here suggest that rice bran oil sterol fraction may be responsible for only a minor portion of its bile acid binding. The variability in bile acid binding between various cereal fractions may relate to differences in anionic, cationic, physical, and chemical structure.

Significantly higher bile acid binding with rice bran suggests that a possible mechanism for its cholesterol-lowering ability includes binding bile acids and increasing neutral sterol excretion. This is in agreement with previous animal feeding studies with rice bran (Kahlon et al 1996, 1999). Minimal binding of bile acids by oat bran is consistent with low neutral sterol excretion reported with an oat bran diet in hamsters (Kahlon et al 1999). Significantly higher bile acid binding by β -glucan enriched barley compared with oat bran suggest that β -glucan enriched barley may have higher healthful potential than oat bran. These observations suggest the need to conduct human studies feeding barley and its fractions to validate their health-promoting potential.

In vitro bile acid binding by rice bran, oat bran, barley, and β -glucan enriched barley on equal total dietary fiber (TDF) basis is shown in Table III. Cholestyramine and cellulose are 100% TDF on a dry matter basis (Table I), so data for these two substrates are the same as in Table II. There were significant differences in bile acid binding between the cereals tested on a TDF basis. Values were rice bran > barley > oat bran > β -glucan enriched barley (4.88, 3.60, 2.46, and 2.17 $\mu\text{mol}/100$ mg of TDF, respectively). Relative bile acid binding on an equal TDF basis considering cholestyramine as 100% bound was rice bran 45%; oat bran 23%; dehulled barley 33%; and β -glucan enriched barley 20%. Similar bile acid binding by rice bran (48%) and oat bran (23%) on a TDF basis was observed previously (Kahlon and Chow 2000). Bile acid binding of dehulled barley obtained in the current study are similar to those reported for wheat bran (Kahlon and Chow 2000). Wheat bran has been shown to prevent colon cancer in rats (Pajari et al 2000; Reddy et al 2000) by improving colon health, binding toxic metabolites, bile acids, and cancer-causing agents. Similar bile acid binding of dehulled barley and higher bile acid binding of rice bran compared with wheat bran suggests that dehulled barley and rice bran should be evaluated for their colon cancer prevention potential. Significantly lower bile acid binding by β -glucan enriched barley compared with other cereals is due to higher TDF content used (61 vs. 29–38 mg) per incubation to obtain 25 mg of protein. It further suggests that bile acid binding of β -glucan enriched barley is not related to its TDF content. The amount of soluble dietary fiber (SDF) used per incubation for rice bran, oat bran, barley, and β -glucan enriched barley was 3.5, 7.4, 13.1, and 30.4 mg, respectively. Among the cereals tested, rice

bran had the highest bile acid binding with the lowest amount of SDF, and β -glucan enriched barley had the lowest bile acid binding with the highest amount of SDF per incubation. There was a possibility that SDF present in the supernatant may have bound bile acids. In three supernatants each of oat bran and β -glucan enriched barley samples, SDF was precipitated by 4 volumes of 95% ethanol (Proskey et al 1988), and clear supernatant was obtained by centrifugation at $99,000 \times g$ for 18 min. Analysis of the SDF-removed supernatants revealed that all the unbound bile acids were quantitatively present in the clear supernatant. Data suggest that bile acid binding in the cereals tested was not related to the SDF level. These data confirmed our previous observations (Kahlon and Chow 2000) that the primary mechanism of cholesterol lowering by cereal brans is not bile acid binding by SDF. These observations disagree with those of Anderson and Siesel (1990), who reported bile acid binding with oat β -glucans (SDF). However, in vivo sterol excretion data in hamster studies (Kahlon et al 1996, 1999) are in agreement with in vitro data of the current study and with our previous report (Kahlon and Chow 2000). There was a possibility that affinity of bile acids for SDF may have changed in the presence of ethanol, which would result in quantitative recovery of unbound bile acids in the supernatant.

In vitro bile acid binding by rice bran, oat bran, dehulled barley, and β -glucan enriched barley on an equal insoluble dietary fiber (IDF) basis is shown in Table IV. Cholestyramine and cellulose are 100% IDF on a dry matter basis (Table I), so data for these two substrates are the same as in Table II. There were significant differences in bile acid binding between the cereals tested on a IDF basis. Values were barley > rice bran > β -glucan enriched barley > oat bran (6.23, 5.38, 4.31, and 3.31 $\mu\text{mol}/100$ mg of IDF, respectively). Relative bile acid binding on an equal IDF basis considering cholestyramine as 100% bound was rice bran 49%, oat bran 30%, dehulled barley 57%, and β -glucan enriched barley 40%. The amount of IDF used per incubation for rice bran, oat bran, barley, and β -glucan enriched barley was 34, 22, 18, and 31 mg, respectively. Relative bile acid binding values per mg of IDF for rice bran, oat bran, dehulled barley, and β -glucan enriched barley were 1.4, 1.4, 3.2, and 1.3, respectively. Except for dehulled barley, the data for rice bran, oat bran, and β -glucan enriched barley suggest that bile acid binding may be related to their IDF content.

In vitro bile acid binding by rice bran, oat bran, barley, and β -glucan enriched barley on an equal protein basis is shown in Table V. The values for cholestyramine and cellulose are listed for comparison and are the same as in Table II. There were significant differences among in vitro bile acid binding by the cereals tested on an equal protein basis. Each incubation used 25 mg of protein for each cereal tested. Values were rice bran > β -glucan enriched barley > barley > oat bran (7.4, 5.3, 4.4, and 2.8 $\mu\text{mol}/100$ mg of protein, respectively). Considering cholestyramine as 100% bound, relative binding values on a protein basis were rice bran 68%, β -glucan enriched barley 49%, dehulled barley 41%, and oat bran

TABLE IV
In Vitro Bile Acid Binding by Rice Bran, Oat Bran, Barley,
and Enriched Barley^{a,b}

Treatment	Bile Acid Binding ($\mu\text{mol}/100$ mg of IDF)	Binding Relative to Cholestyramine (%)
Rice bran	5.38 \pm 0.07c	49.3 \pm 0.7c
Oat bran	3.31 \pm 0.07e	30.3 \pm 0.7e
Barley (dehulled)	6.23 \pm 0.07b	57.1 \pm 0.7b
Enriched barley	4.31 \pm 0.07e	39.5 \pm 0.7d
Cholestyramine	10.91 \pm 0.07a	100.0 \pm 0.7a
Cellulose	-0.11 \pm 0.07f	-1.0 \pm 0.7f

^a Equal insoluble dietary fiber (IDF) basis. Pooled values (means \pm SEM) within a column with different letters differ significantly ($P \leq 0.05$), $n = 6$.

^b Rice bran, oat bran, barley, enriched barley, cholestyramine, and cellulose treatments contained 34, 22, 18, 31, 25, and 25 mg of TDF, respectively.

TABLE V
In Vitro Bile Acid Binding by Rice Bran, Oat Bran, Barley,
and Enriched Barley^{a,b}

Treatment	Bile Acid Binding ($\mu\text{mol}/100$ mg, protein)	Binding Relative to Cholestyramine (%)
Rice bran	7.36 \pm 0.07c	67.5 \pm 0.6b
Oat bran	2.81 \pm 0.07e	25.8 \pm 0.6e
Barley (dehulled)	4.44 \pm 0.07b	40.7 \pm 0.6d
Enriched barley	5.31 \pm 0.07e	48.7 \pm 0.6c
Cholestyramine	10.91 \pm 0.07a	100.0 \pm 0.6a
Cellulose	-0.11 \pm 0.07f	-1.0 \pm 0.6f

^a Equal protein basis. Pooled values (means \pm SEM) within a column with different letters differ significantly ($P \leq 0.05$), $n = 6$.

^b All treatments contained 25 mg of protein.

26%. Randomly selected supernatants of the treatments were tested for nitrogen to evaluate whether partially digested soluble protein or free amino acids may be present in the supernatant and responsible for binding bile acids. No nitrogen was found in the supernatant of any of the treatments by combustion (Leco FP-428). All the values were similar to the blank values obtained for the tin foil used for wrapping the sample (<0.005% N). However, randomly selected supernatants (two samples per treatment) were tested with 0.35% ninhydrin in absolute alcohol with sample to ninhydrin reagent ratio of 5:1 in a boiling water bath for 7 min. Then absorbance was measured at 570 nM (Hwang and Ederer 1975). Similar absorbance values of a deep purple color were observed in all the treatments, indicating the presence of amino acids, possibly from undigested pancreatin, that did not pellet from centrifugation at 99,000 × g for 18 min. Replacing casein with soy protein resulted in a 45% reduction in the plaque in the aortic arch in hamsters (Kahlon et al 1999). In the current study, if protein was the bile acid binding component, data suggest that a variability exists of 39–216% in bile acid binding among the cereals tested. The difference in bile acid binding among the cereals tested may relate to the variability in their protein composition, structure, anionic or cationic nature of the metabolites produced during digestion, or their interaction with active binding sites. Significantly higher bile acid binding by rice bran than barley, enriched barley and oat bran on dry matter, TDF, and protein bases suggests that health-promoting properties of a rice bran diet may include lowering cholesterol and plaque formation as a result of binding of bile acids and increasing sterol excretion.

In conclusion, the relative in vitro bile acid binding on an equal protein basis for the respective cereals were 68, 26, 41, and 49%. These results point to bile acid binding by rice bran as the primary mechanism responsible for its cholesterol-lowering properties. Additionally, bile acid binding by oat bran suggests a mechanism of cholesterol lowering by oat bran other than bile acid binding by its soluble fiber. Bile acid binding was not proportional to the soluble fiber content of the cereal brans tested. Except for dehulled barley, bile acid binding for rice bran, oat bran, and β-glucan enriched barley appear to be related to their IDF content. Highest relative bile acid binding values for rice bran and β-glucan enriched barley were observed based on an equal protein basis, whereas highest values for dehulled barley were on an IDF basis. Data suggest that, of all four cereals tested, bile acid binding may be related to IDF or protein anionic, cationic, physical and chemical structure, composition, metabolites, and their interaction with active binding sites. Complete elucidation of cholesterol-lowering mechanisms of cereal dietary fibers warrants further investigation.

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