

In Vitro Binding of Bile Acids by Rice Bran, Oat Bran, Wheat Bran, and Corn Bran

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ABSTRACT

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The in vitro bile acid binding by rice, oat, wheat, and corn brans was determined using a mixture of bile acids normally secreted in human bile at a physiological pH of 6.3. The objective of the study was to relate bile acid binding of cereal brans to health promoting properties. Three experiments were conducted testing substrates on an equal weight (dry matter) basis, an equal total dietary fiber (TDF) basis, and an equal TDF and equal fat basis. Each experiment was repeated to validate the results (for a total of six experiments). The relative in vitro bile acid binding of the cereal

brans on an equal TDF basis considering cholestyramine as 100% bound was rice bran 51%, wheat bran 31%, oat bran 26%, and corn bran 5%. The data suggest that cholesterol lowering by rice bran appears to be related to bile acid binding. The primary mechanism of cholesterol lowering by oat bran may not be due to bile acid binding by soluble fiber. Bile acid binding did not appear to be proportional to the soluble fiber content of the cereal brans tested. Bile acid binding by wheat bran may contribute to cancer prevention and other healthful properties.

Cereal brans are considered to be desirable for human consumption according to reported health benefits. Extensive research reviewed by Kahlon and Chow (1997) has shown that incorporating rice bran or oat bran in the diet results in plasma cholesterol reductions that lower the risk of cardiovascular disease. Fecal bulking and improved regularity have been associated with wheat bran and other insoluble dietary fibers. 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 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 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 in vitro bile acid binding based on positive correlation between in vitro and in vivo studies showing that cholestyramine binds bile acids and cellulose does not. The objective of this study was to evaluate in vitro bile acid binding by rice bran, oat bran, wheat bran, and corn bran using a bile acid mixture similar to that found in human bile (Carey and Small 1970, Rossi et al 1987) at a physiological pH of 6.3, approximating that of the duodenum.

MATERIALS AND METHODS

Rice, oat, wheat, and corn brans (obtained from local mills) were ground in a Thomas-Wiley mill No. 1 (Arthur Thomas, Philadelphia, PA) to pass a 2-mm screen. Rice bran was stabilized by extrusion; the other brans were received unprocessed. Samples were analyzed for insoluble and soluble dietary fiber (Prosky et al 1988), nitrogen (Kjeldahl method), ether extracted for crude fat by method 920.39C (AOAC 1990), and moisture by method 935.29 (AOAC 1990). Composition of the cereal brans is given in Table I.

In all six experiments, cellulose, a nonbile acid binding negative control, and cholestyramine, a bile acid binding anionic resin positive control were included. Experiments 1 and 2 were conducted using 30 mg of dry matter of each bran per incubation. For Experiments 3 and 4, treatment samples were based on an equal amount (27 mg) of total dietary fiber (TDF) for each bran. Dry matter weights for each incubation were 100, 139, 52, and 31 mg for rice, oat, wheat, and corn brans, respectively, and 27 mg each for cholestyramine and cellulose. Experiments 5 and 6 were conducted with test brans in equal amounts of TDF (27 mg) and fat (24 mg). Peanut oil was used as a supplemental source of fat due to its similarity in fatty acid composition to rice bran oil. No fat was added to the rice bran samples. Dry matter weight for each incubation was 101, 153, 75, 55, 51, and 51 mg for rice, oat, wheat, and corn bran, cholestyramine, and cellulose, respectively, which included 0–24 mg of peanut oil. In Experiment 6, one treatment using only peanut oil was also included.

Bile Acid Binding Procedure

The in vitro bile acid binding procedure was a modification of that by Camire et al (1993). Triplicate 30-mg test samples and one individual substrate blank of rice, oat, wheat, and corn bran cholestyramine, and cellulose, as well as a bile mixture (2.88 μmol /incubation, positive blank) were weighed into 12- \times 125-mm glass, screw-capped tubes. Samples were digested in 1 mL of 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 solution mixture (0.72 μmol /mL) in a 0.1M phosphate buffer, pH 6.3. The stock solution of bile acid mixture contained taurocholic acid (9 μmol /mL), taurochenocholic acid (9 μmol /mL), taurodeoxycholic acid (9 μmol /mL), glycocholic acid (3 μmol /mL), glycochenocholic acid (3 μmol /mL), and glycodeoxycholic acid (3 μmol /mL). This bile acid mixture was formulated based on human bile composition, with taurine-conjugated bile acids providing 75% and glycocholic bile acids providing 25% of the bile acids (Carey and Small 1970, Rossi et al 1987). Bile acid stock solution was diluted to 0.72 μmol /mL before use. Phosphate buffer (4 mL, 0.1M, pH 6.3) was added to the individual substrate blank. After the addition of 5 mL of porcine pancreatin (5 \times , 10 mg/mL, in a 0.01M phosphate buffer, pH 6.3), tubes were incubated for 1 hr in a 37°C shaker bath. Mixtures were transferred to 10-mL Oak Ridge centrifuge tubes (3118-0010 Nalgene, Rochester, NY) and centrifuged at 30,000 \times g in a 75-Ti rotor at 39,000 rpm/min 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 added to the centrifuge tube, which was vortexed and then centrifuged as before. Supernatant was removed

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

Source	Moisture	Dietary Fiber			Fat	Nitrogen
		Total	Insoluble	Soluble		
Rice bran	5.4	27.0	24.5	2.5	23.7	2.5
Oat bran	8.9	19.5	14.5	5.0	7.5	2.9
Wheat bran	14.6	52.2	48.4	3.8	3.8	2.7
Corn bran	4.9	86.5	86.3	0.2	1.4	0.5
Cholestyramine	9.6	100	100
Cellulose	5.4	100	100

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). Each sample was analyzed in triplicate. Values were determined from a standard curve obtained by analyzing Sigma bile acid calibrators (Sigma 450-11; 5, 25, 50, 100, and 200 $\mu\text{mol/L}$). Individual substrate blanks were subtracted, and bile acid concentrations were corrected based on the mean recoveries of bile acid mixture (positive blank). Because each experimental design was repeated twice, statistical analysis was performed combining identical experiments. The term experiment and its interaction with treatment were handled as random effects using PROC MIXED (SAS Institute, Cary, NC) and, within experiment error, was modeled individually when the estimate differed significantly between experiments. The effect of treatment was tested using the Satterthwaite approximation to estimate appropriate degrees of freedom. Least squares means were calculated and differences tested for significance with Tukey's test for comparison of all possible pairs of means. Data were analyzed by Bonferroni test for multiple comparisons using SAS software. A value of $P \leq 0.05$ was considered the criterion of significance.

RESULTS AND DISCUSSION

In Experiments 1 and 2, bile acid binding on an equal dry matter basis (30 mg of substrate/incubation) was significantly higher with cholestyramine (8.94 $\mu\text{mol}/100$ mg) than all other treatments (0.13–2.23) (Table II). There was significantly higher bile acid binding with rice bran and wheat bran than with corn bran and cellulose. Values with rice bran were also significantly higher than with oat bran. Cholestyramine bound 93% of the bile acids. 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 the current study may be due to the use of physiological pH or a higher ratio of substrate to bile acid. Assigning bile acid binding to cholestyramine as 100%, the relative bile acid binding for the test brans was rice bran 25%, wheat bran 20%, oat bran 5%, and corn bran 3%.

Cholestyramine is an anion exchange resin that binds cholesterol and is recommended for lowering cholesterol. Comparing bile acid binding of cereal brans with cholestyramine is very appropriate. Significantly higher bile acid binding with rice bran suggests that a possible mechanism for cholesterol lowering 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). The results do not support the suggestion that a mechanism for cholesterol lowering with oat bran is through binding bile acids with soluble fiber (Anderson and Siesel 1990). The relationship between cholesterol lowering and increased bile acid excretion in rats (Schrijver et al 1992) and increased bile acid excretion in ileostomy patients consuming a high-fiber oat-based diet (Zhang et al 1992) may be explained as a

TABLE II
In Vitro Bile Acid Binding by Rice Bran, Oat Bran, Wheat Bran,
and Corn Bran (Experiments 1 and 2)^{a,b}

Treatment	Bile Acid Binding ^c ($\mu\text{mol}/100$ mg, dry matter)	Binding Relative to Cholestyramine ^d (%)
Rice bran	2.23b	25.0b
Oat bran	0.45cd	5.0c
Wheat bran	1.80bc	20.0b
Corn bran	0.26d	2.9c
Cholestyramine	8.94a	100.0a
Cellulose	0.13d	1.6c

^a On an equal weight, dry matter basis. All treatments contained 30 mg of dry matter.

^b Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^c Pooled \pm standard error of the mean values: ± 0.25 ($n = 6$).

^d Pooled \pm standard error of the mean values: ± 1.2 ($n = 6$).

result of the higher bacterial content with diets high in fermentable fiber, because bacteria may bind bile acids in the cecum (Lund et al 1989). Wheat bran has limited potential to lower cholesterol (Truswell and Beynen 1992, Anderson et al 1994, Trautwein et al 1998). The moderate level of bile acid binding by wheat bran (20%) may be associated with healthful effects such as diluting toxic metabolites, improving gastrointestinal mucosal health, preventing constipation, and reducing the risk of cancer. Marcus and Heaton (1986) and Alberts et al (1996) have reported that wheat fiber and wheat bran bind bile acids, reduce transit time, and lower bile acid concentration by fecal bulking, thereby preventing colon cancer. Story and Kritchevsky (1976) observed 11% binding (relative to cholestyramine as 100%) of bile acids by wheat bran in contrast to Vahouny (1978), who found little or no bile acid sequestering activity with wheat bran. The higher bile acid binding with wheat bran in the current report may be explained by the use of a bile acid mixture with pH 6.3, which is similar to that secreted in the human duodenum (Carey and Small 1970, Rossi et al 1987), as well as simulated gastric and pancreatin digestion steps.

To determine whether the bile acid binding was related to the TDF level of the cereal brans, Experiments 3 and 4 were conducted using an equal amount (27 mg) of TDF in each of the treatments. The dry matter needed for each incubation of rice, oat, wheat, and corn bran was 100, 139, 52, and 31 mg, respectively. Because cholestyramine and cellulose are 100% TDF on a dry matter basis (Table I), Experiments 3 and 4 were essentially a repeat of Experiments 1 and 2 for these two substrates (Table III), except that a 10% lower amount (27 mg) of substrate was used in these experiments. Bile acid binding values on a dry matter basis for rice bran and wheat bran were significantly higher than those for oat bran, corn bran, and cellulose. Relative bile acid binding for rice bran was 48% lower compared with the binding in Experiments 1 and 2 (Table II). On an equal TDF basis, rice bran bound significantly more bile acids than oat bran, wheat bran, corn bran, and cellulose. Bile acid binding by oat bran and wheat bran was similar, and values were significantly higher than corn bran and cellulose. With higher amounts of dry matter used in Experiments 3 and 4 (rice bran, oat bran, and wheat bran 3.3-, 4.6-, and 1.7-fold, respectively) compared with Experiments 1 and 2, bile acid binding relative to cholestyramine decreased (48, 8, and 20%, respectively). Bile acid binding by corn bran (Table III vs. II) increased (34%) without much change in the dry matter used. This high variability in values is due to low bile acid binding by corn bran. Relative bile acid binding on an equal TDF basis, considering cholestyramine as 100% bound, was rice bran 48%, wheat bran 30%, oat bran 23%, and corn bran 5%. The bile acid binding relative to cholestyramine, with the increased amount of dry matter used to equalize the TDF content of these brans was 1.9-, 4.7-, 1.5-, and 1.6-fold higher than that obtained on a dry matter basis in Experiments 1 and 2. The data suggest that bile acid binding by rice bran

TABLE III
In Vitro Bile Acid Binding by Rice Bran, Oat Bran, Wheat Bran, and Corn Bran (Experiments 3 and 4)^a

Treatment ^b	Dry Matter Basis		Total Dietary Fiber Basis	
	Bile Acid Binding ^c ($\mu\text{mol}/100\text{ mg}$)	Binding Relative to Cholestyramine ^d (%)	Bile Acid Binding ^e ($\mu\text{mol}/100\text{ mg}$)	Binding Relative to Cholestyramine ^f (%)
Rice bran	1.33b	13.0b	4.94b	48.3b
Oat bran	0.47c	4.6c	2.39c	23.4c
Wheat bran	1.62b	15.9b	3.11c	30.4c
Corn bran	0.40c	3.9c	0.46d	4.5d
Cholestyramine	10.24a	100.0a	10.24a	100.0a
Cellulose	0.29c	2.8c	0.29d	2.8d

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^b All treatments were equal in total dietary fiber (27 mg). Dry matter weights for each incubation were 100, 139, 52, and 31 mg for rice, oat, wheat and corn bran, respectively, and 27 mg each for cholestyramine and cellulose.

^c Pooled \pm standard error of the mean values: ± 0.13 ($n = 6$).

^d Pooled \pm standard error of the mean values: ± 1.2 ($n = 6$).

^e Pooled \pm standard error of the mean values: ± 0.18 ($n = 6$).

^f Pooled \pm standard error of the mean values: ± 1.9 ($n = 6$).

TABLE IV
In Vitro Bile Acid Binding by Rice Bran, Oat Bran, Wheat Bran, and Corn Bran (Experiments 5 and 6)^a

Treatment ^b	Dry Matter Basis		Total Dietary Fiber Basis	
	Bile Acid Binding ^c ($\mu\text{mol}/100\text{ mg}$)	Binding Relative to Cholestyramine ^d (%)	Bile Acid Binding ^e ($\mu\text{mol}/100\text{ mg}$)	Binding Relative to Cholestyramine ^f (%)
Rice bran	1.44b	28.0b	5.341b	53.4b
Oat bran	0.49c	9.5c	2.771c	27.7c
Wheat bran	1.12b	21.9b	3.28c	32.9c
Corn bran	0.21c	3.9c	0.44d	4.3d
Cholestyramine	5.14a	100.0a	10.01a	100.0a
Cellulose	0.26c	5.1c	0.50d	5.1d

^a Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^b All treatments were equal in total dietary fiber (27 mg) and crude fat (24 mg) per incubation. Dry matter weight for each incubation was 101, 153, 75, and 55 mg for rice, oat, wheat, and corn brans, respectively, and 51 mg each for cholestyramine and cellulose, which included 0–24 mg of peanut oil.

^c Pooled \pm standard error of the mean values: ± 0.09 ($n = 6$).

^d Pooled \pm standard error of the mean values: ± 1.4 ($n = 6$).

^e Pooled \pm standard error of the mean values: ± 0.21 ($n = 6$).

^f Pooled \pm standard error of the mean values: ± 1.9 ($n = 6$).

is not related to dry matter, as dry matter increased over threefold, but there was only a twofold increase in binding on a TDF basis. With oat bran and wheat bran, bile acid binding appears to be related to dry matter content. Soluble dietary fiber (SDF) does not appear to be the main bile acid binding component as relative binding by wheat bran was 30% with 2 mg of SDF/test and was 23% by oat bran with 7 mg of SDF/test. These observations disagree with those of others (Anderson and Siesel 1990), who reported bile acid binding with oat β -glucans (SDF). This discrepancy may be explained by in vivo and in vitro differences, as soluble fibers ferment and increase microbial mass in the gut, and these microbes bind bile acids (Lund et al 1989). There is minimal bile acid binding with corn bran, which resulted in values similar to those of cellulose.

Bile acids solubilize fat for absorption by forming micelles. The dry matter (mg) contributed by each substrate for incubation and the supplemental peanut oil added to each was cellulose 27, 24; cholestyramine 27, 24; rice bran 101, 0; oat bran 140, 13; wheat bran 52, 22; and corn bran 32, 23 mg, respectively. On a dry matter basis, cholestyramine bound 5.14 $\mu\text{mol}/100\text{ mg}$ (Table IV). This value was 54% of the binding in Experiments 1–4 (9.59 $\mu\text{mol}/100\text{ mg}$). Because added fat constituted 46% of the dry matter in Experiments 5 and 6, there appears to be negligible bile acid binding by the added peanut oil. Relative bile acid binding and statistical differences by various brans on a dry matter basis were similar to those observed in Experiments 1–4. Relative bile acid binding on an equal TDF basis with the various brans is similar to that observed in Experiments 3 and 4, suggesting that added peanut oil did not significantly influence the bile acid binding capacity of brans.

Relative binding by peanut oil was 9.4% compared with cholestyramine. Because cholestyramine values were lower due to 46%

of dry matter being composed of peanut oil, the real bile acid binding value for peanut oil is $\approx 4\%$. On an equal TDF basis, rice bran bile acid binding values were significantly higher than those of oat bran, wheat bran, and corn bran. Values for oat bran and wheat bran were similar and significantly higher than those for corn bran.

In conclusion, the relative in vitro bile acid binding on an equal TDF basis (mean of Experiments 3–6) for rice bran was 51%, wheat bran 31%, oat bran 26%, and corn bran 5% of that by cholestyramine. Bile acid binding by rice bran may account to a great extent for cholesterol lowering properties while bile acid binding by wheat bran may be related to cancer prevention and other healthful properties. The data suggest that the primary mechanism of cholesterol lowering by oat bran is not due to bile acid binding by soluble fiber. Bile acid binding did not appear to be proportional to the soluble fiber content of the cereal brans tested. Oat, wheat, and corn brans used in this study were unprocessed, and further work would be needed to determine bile acid binding capacity of these brans after processing. The in vitro findings in this study need to be validated in an animal study.

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