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In vitro binding of bile acids by soy protein, pinto beans, black beans and wheat gluten☆

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Abstract

The in vitro bile acid binding by soy protein, pinto beans, black beans and wheat gluten was determined using a mixture of bile acids secreted in human bile at a duodenal physiological pH of 6.3. Six treatments and two blank incubations were conducted, testing substrates on an equal protein basis. Considering cholestyramine as 100 bound, the relative in vitro bile acid bindings for the soy protein, pinto beans, black beans and wheat gluten, on equal protein basis, were 17, 23, 30 and 12%, respectively. Bile acid binding by soy protein, pinto beans and black beans may influence cholesterol lowering, lipid and lipoprotein metabolism, and reduction of plaque formation in the aortic arch. Higher bile acid binding by black beans and pinto beans than soy protein is encouraging; it suggests that there may be components, other than the bean protein, with the desired health-promoting properties. As for wheat gluten, bile acid binding may relate to its potential for improving gastrointestinal health and reduction of the risk of cancer. These results point to bile acid binding by soy protein, pinto beans, black beans and wheat gluten as indicative of their health-promoting potential. Published by Elsevier Science Ltd.

Keywords: Bile acid binding; Soy protein; Pinto beans; Black beans; Wheat gluten

1. Introduction

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 by which dietary fibre lowers cholesterol (Anderson & Siesel, 1990; Lund, Gee, Brown, Wood, & Johnson, 1989; Trowell, 1975). By binding bile acids, food fractions prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids (Balmer & Zilversmit, 1974; Eastwood & Hamilton, 1968; Kritchevsky & Story, 1974). The healthful or cholesterol-lowering properties of food fractions could be predicted by evaluating their in vitro bile acid binding, based on positive correlations found between in vitro and in vivo studies, showing that cholestyramine binds bile acids and cellulose does not (Daggy, O'Connell, Jerdack, Stinson, & Setchell, 1997; Kahlon & Chow, 2000; Nakamura & Matsuzawa, 1994; Suckling et al., 1991).

Considerably lower risk of coronary heart disease in Asian countries is believed to be due to a higher intake of soy protein and soy-based foods. Soy protein has been shown to lower plasma total and LDL cholesterol in hypercholesterolemic humans and laboratory animals (Anderson, Johnstone, & Cook-Newell, 1995; Anthony, Clarkson, Bullock, & Wagner, 1997; Carroll, 1991; Huff, Roberts, & Carroll, 1982). The proposed mechanisms of action include a decrease in the intestinal absorption of cholesterol or bile acids and changes in hepatic metabolism of cholesterol and lipoproteins (Potter, 1998). Significantly higher bile acid excretion and a reduction in hepatic cholesterol content have been observed in hamsters with soy protein consumption compared with dietary casein (Wright & Salter, 1998). Significant reduction (−45%) in aortic plaque in hamsters was reported when soy protein replaced casein as a source of protein in a 6-week study (Kahlon, Chow, &

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Wood, 1999). Evaluating bile acid binding of soy protein and other beans, such as pinto beans and black beans, would be of interest to explore their health-promoting potential.

Relative to cholestyramine (a bile acid binding and cholesterol lowering drug) and on a dry matter basis, wheat bran has been shown to bind 20% as much bile acids (Kahlon & Chow, 2000). Wheat bran normally does not lower cholesterol (Anderson, Jones, & Riddell-Mason, 1994; Trautwein, Rieckhoff, Kunath-Rau, & Erbersdobler, 1998; Truswell & Beynen, 1992). The authors suggested that bile acid binding by wheat bran may contribute to cancer prevention and other healthful properties. Wheat bran has been shown to prevent colon cancer in rats (Pajari, Oikarinen, Grasten, & Mutanen, 2000; Reddy, Hirose, Cohen, Simi, Cooma, & Rao, 2000) by improving colon health, binding toxic metabolites, and affecting bile acids and cancer-causing agents. Since wheat is the major cereal-consumed world wide, evaluation of bile acid binding of wheat fractions (e.g. wheat gluten) would be desirable to establish the healthful properties of wheat.

The objective of this study was to evaluate in vitro bile acid binding by soy protein, pinto beans, black beans and wheat gluten, based on equal amounts of protein, with a bile acid mixture, observed in human bile, under duodenal physiological pH of 6.3.

2. Materials and methods

2.1. General

Soy protein, pinto beans, black beans and wheat gluten were obtained from local vendors. Soy protein and wheat gluten were in powder form. Pinto beans and black beans were ground in a Thomas-Wiley mill No. 1 (Arthur Thomas, Philadelphia, PA) to pass a 0.6-mm screen. Samples were analyzed for insoluble and soluble dietary fibre (Prosky, Asp, Schweizer, De Vries, & Furda, 1988), for nitrogen by combustion with a Leco FP-428 (Leco Corp., St. Joseph, MI), for ether-extracted crude fat by method 920.39C (AOAC, 1990), and moisture by method 935.29 (AOAC, 1990). Compositions of the soy protein, pinto beans, black beans and wheat gluten are given in Table 1. Cellulose, a non-bile acid binding fiber, was the negative control and cholestyramine, a bile acid binding anionic resin (a drug that lowers cholesterol by binding bile acids), was the positive control. Eight replicate incubations, six with bile acid mixture and two substrate blanks without bile acid mixture, were run for each treatment and each control. All treatments used a 25–27 mg protein content for each treatment (Nitrogen to protein factors used were 6.25 for soy and bean protein and 5.7 for wheat gluten) per incubation.

Table 1
Composition of soy protein, pinto beans, black beans, wheat gluten, cholestyramine and cellulose (dry matter basis,%)

Source	Moisture	Dietary fibre			Fat	Nitrogen[a]
		Total	Insoluble	Soluble		
Soy protein	6.7	5.2	3.7	1.5	0.6	13.5
Pinto beans	8.4	25.4	22.1	3.4	1.1	3.7
Black beans	8.8	25.7	21.8	3.9	1.0	4.3
Wheat gluten	7.4	1.9	1.4	0.5	2.3	13.0
Cholestyramine	9.6	100	100	–	–	–
Cellulose	5.4	100	100	–	–	–

[a] Nitrogen to protein factor used was 6.25 for soy and bean protein, and 5.7 for wheat gluten.

2.2. Bile acid binding procedure

The in vitro bile acid binding procedure was a modification of that by Camire, Zhao, and Violette (1993) as previously reported (Kahlon & Chow, 2000). Six replicates, with 25–27 mg of protein per test sample and two individual substrate blanks as well as a positive blank (2.88 μmol bile acid mixture per incubation), were weighed into 12×125 mm glass, screw-capped tubes. Soy protein, pinto beans, black beans, wheat gluten, cholestyramine and cellulose treatments contained 33, 108, 92, 34, 25 and 25 mg dry matter, respectively. Samples were digested in 1 ml 0.01 N HCl for 1 h in a 37 °C shaker bath. After this acidic incubation which simulated gastric digestion, the sample pH was adjusted to 6.3 with 0.1 ml of 0.1 N NaOH. To each test sample was added 4 ml of bile acid mixture solution (0.72 μmol/ml) in a 0.1 M phosphate buffer, pH 6.3. The stock solution of bile acid mixture contained taurocholic acid (9 mmol/l), 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 mixture was formulated with taurine-conjugated bile acids, providing 75% and glycocholic bile acids 25% of the bile acids. A phosphate buffer (4 ml, 0.1 M, pH 6.3) was added to the individual substrate blanks. After the addition of 5 ml of porcine pancreatin (5×, 10 mg/ml, in a 0.01 M phosphate buffer, pH 6.3; providing amylase, protease and lipase for digestion of samples), tubes were incubated for 1 h in a 37 °C shaker bath. Mixtures were transferred to 10 ml Oak Ridge centrifuge tubes (No. 3118-0010 Nalgene, Rochester, NY) and centrifuged at 63 000×g in a 75-Ti rotor at 39 K for 18 min at 25 °C in an ultracentrifuge (model L-60, Beckman, Palo Alto, CA). Supernatant was removed into a second set of labelled tubes. A further 5 ml of phosphate buffer were used to rinse out the incubation tube and 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 by the Sigma bile acids procedure No. 450 (Sigma, St Louis, MO), using a Ciba-Corning Express Plus analyzer (Polestar Labs, Inc., Escondido, CA). Each sample was analyzed in triplicate. Values were determined from a standard curve obtained by analyzing Sigma bile acid calibrators (Sigma No. 450-11; 5, 25, 50, 100 and 200 μmol/l). Individual substrate blanks were subtracted, and bile acid concentrations were corrected, based on the mean recoveries of bile acid mixture (positive blank). 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, and differences among soy protein, pinto beans, black beans and wheat gluten were tested for significance with Tukey's test for comparison of all possible pairs of means (SAS Institute, Cary, NC). A value of $P \leqslant 0.05$ was considered the criterion of significance.

3. Results and discussion

On an equal dry matter basis, bile acid binding was significantly higher with cholestyramine (10.9 μmol/100 mg) and significantly lower with cellulose (−0.11 μmol / 100 mg) than all other treatments (0.60–1.58 μmol/100 mg; Table 2). Soy protein bound significantly higher amounts of bile acids than did pinto beans, black beans or wheat gluten. Bile acid binding values for black beans and wheat gluten were significantly higher than for pinto beans. Cholestyramine bound 95% of the bile acids. Similar in vitro bile acid binding values (96%) for cholestyramine have previously been observed (Kahlon & Chow, 2000). Cholestyramine bound glycocholate and taurocholate (87 and 93%, respectively; Sugano & Goto, 1990). In our study, cholestyramine binding to the mixture of bile acids was similar to that observed for taurocholate by Sugano and Goto (1990). 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 and/or a higher substrate to bile acid ratio. Assigning a bile acid binding value of 100% to cholestyramine, the relative bile acid binding percentages for the test samples were soy protein, 15%; pinto beans, 6%; black beans, 8%; and wheat gluten, 9%. Similar amounts of dry matter for soy protein and wheat gluten were used per incubation, as these were isolated proteins. However, their bile acid binding values varied by 65% and this difference was significant. A higher amount of dry matter (+17%) was used for pinto beans than for black beans, but it resulted in significantly lower (−33%) bile acid binding. Data suggest that bile acid binding by the bean and wheat proteins tested was not proportional to their dry matter contents. The variability in bile acid binding between various treatments may relate to differences in anionic, cationic, physical and chemical structure. Significantly higher bile acid binding with soy protein suggests that a possible mechanism for its influence on lipid and lipoprotein metabolism and its reduction of aortic plaque includes binding bile acids and increasing neutral sterol excretion. This is in agreement with previous observations showing a significant reduction (−45%) in the aortic plaque in hamsters over a 6-week period in which soy protein isolate replaced casein in the diet as a source of protein (Kahlon et al., 1999). Wheat bran has limited potential to lower cholesterol (Anderson et al., 1994; Trautwein et al., 1998; Truswell & Beynen, 1992). Significant relative bile acid binding of wheat gluten (9%) may be associated with its 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) reported that wheat fibre and wheat bran bind bile acids reduce transit time and lower bile acid concentration by fecal bulking, thereby preventing colon cancer. Pajari et al. (2000), and Reddy et al. (2000), observed that wheat bran prevents colon cancer in rats by improving colon health, binding toxic metabolites, bile acids and cancer causing agents. Relative to cholestyramine, 20% bile acid binding by wheat bran, on a dry matter basis, has been reported (Kahlon & Chow, 2000). This more than two-fold difference in bile acid binding by wheat bran, over that observed for wheat gluten in this study, suggests that in addition to wheat protein there are other components of wheat bran which also bind bile acids. It would be of interest to evaluate bile acid binding of whole wheat flour to explore its health-promoting potential.

In vitro bile acid binding by soy protein, pinto beans, black beans and wheat gluten, on an equal protein

Table 2
In vitro bile acid binding by soy protein, pinto beans, black beans and wheat gluten on equal weight, dry matter (DM) basis[a] [b]

Treatment	Bile acid binding (μ mol/100 mg DM)	Binding relative to cholestyramine,%
Soy protein	1.58±0.03b	14.5±0.3b
Pinto beans	0.60±0.03d	5.5±0.3d
Black beans	0.89±0.03c	8.2±0.3c
Wheat gluten	0.96±0.03c	8.8±0.3c
Cholestyramine	10.91±0.03a	100±0.3a
Cellulose	−0.11±0.03e	−1.0±0.3e

[a] Pooled values (means±S.E.M.) within a column with different letters differ significantly ($P \leqslant 0.05$), $n = 6$.

[b] Soy protein, pinto beans, black beans, wheat gluten, cholestyramine and cellulose treatments contained 33, 108, 92, 34, 25 and 25 mg dry matter, respectively.

Table 3
In vitro bile acid binding by soy protein, pinto beans, black beans and wheat gluten on equal protein basis[a] [b]

Treatment	Bile acid binding (μ mol/100 mg protein)	Binding relative to cholestyramine,%
Soy protein	1.87±0.08d	17.1±0.7d
Pinto beans	2.56±0.08c	23.4±0.7c
Black beans	3.30±0.08b	30.2±0.7b
Wheat gluten	1.29±0.08e	11.9±0.7e
Cholestyramine	10.91±0.08a	100.0±0.7a
Cellulose	−0.11±0.08f	−1.0±0.7f

[a] Pooled values (means±S.E.M.) within a column with different letters differ significantly ($P \leqslant 0.05$), $n = 6$.

[b] Soy protein, pinto beans, black beans, wheat gluten contained 25–27 mg protein, cholestyramine and cellulose treatments contained 25 mg dry matter. Nitrogen to protein factor used was 6.25 for soy and bean protein, and 5.7 for wheat gluten.

basis, is shown in Table 3. The values for cholestyramine and cellulose are listed for comparison and are the same as in Table 2. There were significant differences among in vitro bile acid bindings of the bean proteins and wheat gluten on an equal protein basis. Each incubation used 25–27 mg protein content for each treatment. Bile acid binding values were black beans > pinto beans > soy protein > wheat gluten (3.3, 2.6, 1.9 and 1.3 μ mol/100 mg protein, respectively). Considering cholestyramine as 100% bound, relative binding values, on an equal protein basis, were, black beans, 30%; pinto beans, 23%; soy protein, 17%; and wheat gluten, 12%. If protein was the bile acid binding component, compared with soy protein, bile acid binding by pinto beans and black beans, increased by 37 and 77%, respectively. Soy protein isolate has been shown to lower plasma cholesterol and increase fecal sterol excretion in rabbits (Huff & Carroll, 1980) and in rats and hamsters (Hayashi, Miyazaki, Yamashita, Nakagawa, & Takizawa, 1994). Soy protein has been shown to significantly reduce hepatic cholesterol and increase bile acid excretion (Wright & Salter, 1998); it has been also shown to lower plaque formation in the aortic arch in hamsters (Kahlon et al., 1999). Hydrophobic undigested fractions and high molecular weight undigested fractions of soy protein have been shown to lower cholesterol and bind bile acids even more than soy protein (Iwami, Sakakibara, & Ibuki, 1986; Sugano & Goto, 1990). Thus, these data suggest that pinto beans and black beans may have even higher cholesterol-lowering and plaque reduction potential than soy protein. Since the soy protein used was protein isolate and pinto beans and black beans were used as whole beans, there may be components other than the protein constituents in pinto beans and black beans which also bind bile acids. It would be of interest to evaluate bile acid binding and plaque reduction with whole soy beans as it may have components with desired healthful potential in addition to its protein. The difference in bile acid binding between the wheat and bean proteins tested may relate to the variability in their protein composition and structure, components other than protein (pinto and black beans), hydrophobicity of undigested fractions, anionic or cationic nature of the metabolites produced during digestion and/or their interaction with active binding sites. Significantly higher bile acid binding by black beans, pinto beans and soy protein suggests that diets containing these beans may reduce cholesterol and plaque formation, and may have other health-promoting properties resulting from binding of bile acids and increasing sterol excretion. Human studies need to be conducted using diets containing various beans and wheat fractions to validate their healthful effects from bile acid binding and the probable relation to amelioration of degenerative diseases.

In conclusion, relative to cholestyramine, in vitro bile acid bindings, on an equal protein basis for the soy protein, pinto beans, black beans and wheat gluten were 17, 23, 30 and 12%, respectively. Bile acid binding by soy protein, pinto beans and black beans may relate to their potential influence on cholesterol lowering, lipid and lipoprotein metabolism and lowering the risk of plaque formation in the aortic arch. Higher bile acid binding by black beans and pinto beans than soy protein is encouraging, as it suggests that there may be components in addition to bean protein with the desired health-promoting properties. In the case of wheat gluten, bile acid binding may relate to improving gastrointestinal health and reducing the risk of cancer. These results point to bile acid binding by soy protein, pinto beans, black beans and wheat gluten as indicative of their health-promoting potential.

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