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In vitro binding of bile acids by soy bean (*Glycine max*), black eye bean (*Vigna unguiculata*), garbanzo (*Cicer arietinum*) and lima bean (*Phaseolus lunatus*) ☆

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Abstract

The in vitro binding of bile acids by soy bean (*Glycine max*), black eye bean (*Vigna unguiculata*), garbanzo (*Cicer arietinum*) and lima bean (*Phaseolus lunatus*) was determined, using a mixture of bile acids secreted in human bile at a duodenal physiological pH 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 bean, black eye bean, garbanzo and lima bean, on equal protein basis, were 6%, 14%, 47% and 17%, respectively. Relative bile acid binding on equal dry matter (DM), total dietary fibre (TDF) and insoluble dietary fibre (IDF) basis were for soy bean, 2%, 10% and 12%, black eye bean, 3%, 21% and 25%, garbanzo 10%, 68% and 80%, and lima bean 4%, 19% and 23%, respectively. Except for garbanzo, where values were much higher, bile acid bindings by soy bean, black eye bean and lima bean appear to be related to their DM content. These results indicate that bile acid binding by garbanzo > black eye beans = lima beans > soy beans shows their relative health-promoting potential. Incorporation of garbanzo, black eye and lima bean in diets should be encouraged. Data suggest that, of all four kinds of beans tested, bile acid binding may be related to phytochemical (flavonoid, tannin, estrogenic content), anionic, cationic, physical and chemical structure, composition and metabolites, or their interaction with active binding sites. Animal studies are in progress to validate relationship of in vitro binding of various beans observed herein to lipids, cholesterol-lowering and atherosclerosis amelioration.
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Keywords: Bile acids; Soy bean; Black eye bean; Garbanzo; Lima bean

1. Introduction

The populations of the Asian countries have a notably low risk of coronary heart disease, presumably due to their lower intake of animal protein and higher intake of various beans, including soy protein and soy-based foods. Daily per capita consumption of all bean products in Asia is estimated to be 110 g, whereas in USA it is about 9 g. In USA about 11% of the population is of Hispanic origin and they consume 33% of all the bean products (Lucer, Lin, Allshouse, & Kantor, 2000). Bean consumption of four times or more per week compared with less than once a week have been associated with a 22% lower risk of coronary heart disease (Bazzano et al., 2001). In the western countries, isolated soy bean protein is used in various foods as a substitute for animal protein. Black eye bean, garbanzo and lima bean are used in salads, soups and other food products. Soy protein has been shown to lower plasma total and LDL cholesterol in hypercholesterolemic humans and laboratory animals (Huff, Roberts, & Carroll, 1982; Carroll, 1991; Hayashi, Miyazaki, Yamashita, Nakagawa, & Takizawa, 1994; Anderson, Johnstone, & Cook-Newell, 1995; Anthony, Clarkson, Bullock, & Wagner, 1997). Soy protein has been shown to significantly reduce hepatic cholesterol and increase bile acid excretion (Wright & Salter, 1998); it has also been shown to significantly lower

☆ The mention of firm names or trade products does not imply that they are endorsed or recommended by the US Department of Agriculture over other firms or similar products not mentioned.

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(−45%) plaque formation in the aortic arch in hamsters (Kahlon, Chow, & Wood, 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). Significantly higher bile acid excretion and a reduction in hepatic cholesterol content have been observed in hamsters with soy protein consumption instead of dietary casein (Wright & Salter, 1998). These studies suggest that soy protein and its fractions are responsible for cholesterol lowering properties. However, soy bean fibre has been reported to lower total and LDL cholesterol and atherosclerosis index in rats (Wang, Zhao, & Chen, 1996). Soy protein appears to have a major cholesterol lowering effect. 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).

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 (Trowell, 1975; Lund, Gee, Brown, Wood, & Johnson, 1989; Anderson & Siesel, 1990). By binding bile acids, food fractions prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids (Eastwood & Hamilton, 1968; Balmer & Zilversmit, 1974; 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 (Suckling et al., 1991; Nakamura & Matsuzawa, 1994; Daggy, O'Connell, Jerdack, Stinson, & Setchell, 1997; Kahlon & Chow, 2000). Relative to cholestyramine (a bile acid binding and cholesterol lowering drug) and on an equal protein basis, bile acid binding was soy protein 17%, pinto bean 23% and black bean 30% (Kahlon & Woodruff, 2002). Higher values for pinto bean and black bean suggested that there was a possibility of bile acid binding components in addition to bean protein.

The objective of this study was to determine the health potential of soy bean (*Glycine max*), black eye bean (*Vigna unguiculata*), garbanzo (*Cicer arietinum*) and lima bean (*Phaseolus lunatus*) by evaluating their in vitro bile acid binding on the equal protein basis, with a bile acid mixture observed in human bile at the duodenal physiological pH 6.3.

2. Materials and methods

2.1. General

Food grade soy bean, black eye bean, garbanzo and lima bean were obtained from local vendors. All the 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 total, insoluble and soluble dietary fibre by method 991.43 (AOAC suppl. 3, 1992) for nitrogen by Kjeldahl Method 979.09 (AOAC, 1990), for crude fat with petroleum ether by the accelerated solvent extractor (ASE 200 Dionex Corp., Sunnyvale, CA), as described by Barrios, Camara, Torija, and Alonso (2002), and moisture by method 935.29 (AOAC, 1990). Cellulose, a non-bile acid binding fibre, 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 control. All assays used a 22–23 mg protein content for each substrate (nitrogen to protein factor used for beans was 6.25) or 24 mg dry matter for cholestyramine and cellulose.

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). The stock bile acid mixture was formulated with glycocholic bile acids, providing 75%, and taurine-conjugated bile acids, 25% of the bile acids, based on the composition of human bile (Carey & Small, 1970; Rossi, Converse, & Hoffman, 1987). This mixture contained glycocholic acid (9 mmol/l), glycochenocholic acid (9 mmol/l), glycodeoxycholic acid (9 mmol/l), taurocholic acid (3 mmol/l), taurochenocholic acid (3 mmol/l) and taurodeoxycholic acid (3 mmol/l) in pH 6.3, 0.1 M phosphate buffer. This stock solution of 36 mmol/l was stored in the refrigerator and diluted to the working solution (0.72 μmol/ml) just prior to each assay. Six replicates with 22–23 mg of protein per test sample, one substrate blank and one positive blank (2.88 μmol bile acid mixture per incubation) were weighed into 12×25 mm glass, screw-capped tubes. Soy bean, black eye bean, garbanzo and lima bean, cholestyramine and cellulose assays contained 70, 99, 110, 107, 24 and 24 mg dry matter, respectively. Samples were digested in 1 ml 0.01 N HCl for one hour 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 working solution (0.72 μmol/ml) in a 0.1 M phosphate buffer, pH 6.3. 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 0.1 M phosphate buffer, pH 6.3; providing amylase, protease and lipase for digestion of samples), tubes were incubated for one hour in a 37 °C shaker bath. Mixtures were transferred to 10 ml centrifuge tubes (Oak Ridge 3118–0010 Nalgene, Rochester, NY) and centrifuged at 99000*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. 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 centrifuged as before. Supernatant was removed and combined with that in 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 (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 450–11) at 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 substrate 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 substrates, and differences among soy bean, black eye bean, garbanzo and lima bean 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

Compositions of the soy bean, black eye bean, garbanzo and lima bean are given in Table 1. Both cellulose and cholestyramine were considered as insoluble dietary fibre (IDF) being 100% of dry matter (DM). Dietary fibre contents among these beans were quite similar. Total, insoluble and soluble dietary fibre were for soy bean, 18%, 15% and 3% for black eye bean, 16%, 13% and 3% for garbanzo, 15%, 13% and 2% and for lima bean 19%, 16% and 3%, respectively. Chemical characterization of polysaccharides of various dietary fibre fractions were not conducted; neither were such data found in the literature search. Crude fat varied widely between these beans and soy bean was 17%, black eye bean 2%, garbanzo 5% and lima bean 1%. Protein content was much higher for soy protein (32%) whereas values were quite similar for black eye bean, garbanzo and lima bean (21–23%). Amino acid composition per 100 g of protein are quite similar among these beans except the garbanzo, which has 21–50% higher arginine and 23–34% less tyrosine than the other three beans tested (USDA National Nutrient Database for Standard Reference, Release 16 (July 2003), http://www.nal.usda.gov/fnic/cgibin/nut_search.pl).

On an equal DM basis, bile acid binding was significantly higher with cholestyramine and significantly lower with soy bean and cellulose than black eye bean, garbanzo and lima bean (Table 2). Garbanzo bound significantly more bile acids than black eye bean and lima bean. Cholestyramine bound 96% of the bile acids. Similar in vitro bile acid binding values for cholestyramine have been previously observed (Kahlon & Chow,

Table 2
In vitro bile acid binding by soy bean, black eye bean, garbanzo and lima bean on equal DM basis

Treatment	Bile acid binding (μmol/100 mg DM)	Binding relative to cholestyramine (%)
Soy bean	0.21 ± 0.02^{d}	1.9 ± 0.2^{d}
Black eye bean	0.36 ± 0.02^{c}	3.3 ± 0.2^{c}
Garbanzo	1.11 ± 0.02^{b}	10.0 ± 0.3^{b}
Lima bean	0.41 ± 0.02^{c}	3.7 ± 0.2^{c}
Cholestyramine	11.1 ± 0.09^{a}	100 ± 0.9^{a}
Cellulose	0.17 ± 0.05^{d}	1.5 ± 0.5^{d}

Values (means ± SEM) within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.

Soy bean, black eye bean, garbanzo, lima bean, cholestyramine and cellulose treatments contained 70, 99, 110, 107, 24 and 24 mg dry matter, respectively.

Table 1
Composition of soy bean, black eye bean, garbanzo and lima bean

Source	Moisture as is	Dietary fibre			Fat	Protein ($N \times 6.25$)
		Total percentage of DM	Insoluble	Soluble		
Soy bean	10.7	18.2	15.4	2.8	16.6	31.5
Black eye bean	9.9	15.8	13.1	2.7	1.8	22.9
Garbanzo	9.4	14.7	12.5	2.2	5.3	21.2
Lima bean	11.7	19.2	16.4	2.8	1.2	21.4
Cholestyramine	9.6	100	100	–	–	–
Cellulose	5.4	100	100	–	–	–

2000). Cholestyramine bound glycocholate and taurocholate by 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 bean 2%, black eye bean 3%, garbanzo 10%, and lima bean 4%. Except for garbanzo, where values were much higher, data suggest that bile acid binding by soy bean, black eye bean and lima bean appear to be related to their DM content. Evaluating healthful properties of various foods and fractions would be desirable by testing their bile acid binding on a dry matter basis. Similar observations have been reported for various ready to eat breakfast cereals (Kahlon & Woodruff, 2003a). Soy protein and soy fibre have been shown to lower plasma total and LDL cholesterol, and atherosclerosis index in hypercholesterolemic humans and laboratory animals (Huff et al., 1982; Carroll, 1991; Anderson et al., 1995; Wang et al., 1996; Anthony et al., 1997). Data suggest that animal and human studies should be conducted to explore healthful potential of garbanzo, black eye bean and lima bean.

The bile acid binding on equal total dietary fibre (TDF) basis, is shown in Table 3. Cholestyramine bound bile acids significantly more and cellulose significantly lower than the various beans tested. Considering cholestyramine as 100 bound, on TDF basis, bile acid binding values for soy bean, black eye bean, garbanzo and lima bean were: 10%, 21%, 68% and 19%, respectively. The bile acid binding values for garbanzo were significantly higher and those for soy bean were significantly lower than those for black eye bean and lima bean. The differences in values between beans tested, based on TDF, suggest that their bile acid binding does not appear proportional to their TDF content. The variability in bile acid binding between the beans tested may be related their flavonoid and tannin contents, anionic and cationic, content and physical and chemical structure. Soy bean fibre has been reported to lower total and LDL cholesterol and atherosclerosis index (Wang et al., 1996). Higher (2–7 fold) bile acid binding values, on a TDF basis, by black eye bean, lima bean and garbanzo than soy bean is very encouraging as these beans can be incorporated into foods and their full potential to lower lipids, total and LDL cholesterol, and atherosclerosis index needs to be explored in animal and human studies.

In vitro bile acid binding by soy bean, black eye bean, garbanzo and lima bean on an equal insoluble dietary fibre (IDF) basis is shown in Table 4. Again, bile acid binding by garbanzo was significantly greater and, by soy bean, significantly less than by black eye bean and lima bean. Relative to cholestyramine, bile acid binding, on an equal IDF basis was garbanzo 80%, black eye bean 25%, lima bean 23% and soy bean 12%. Variability in bile acid binding between the treatments may relate to differences in chemical and physical structure. These observations suggest that bile acid binding by the beans tested does not relate their IDF content and conflict with the reported observations that bile acid bindings by rice bran-, oat bran- and β-glucan-enriched barley were related to their IDF content (Kahlon & Woodruff, 2003b). Analysis of the chemical structure of IDF may give more insight of its role in bile acid binding, no such analysis was found in the literature. Previously, bile acid binding of peanut oil was evaluated as about 4% (Kahlon & Chow, 2000). Higher amount of oil present in soy beans would only add a minor contribution to its bile acid binding.

Relating the soluble fibre to the bile acid binding with the beans, studies gave unrealistic values. Previously it has been reported that bile acid binding of high soluble fibre cereals (oat bran- and β-glucan-enriched barley) was not proportional to soluble fibre content (Kahlon and Chow, 2000; Kahlon & Woodruff, 2003b). The

Table 3
In vitro bile acid binding by soy bean, black eye bean, garbanzo and lima bean on equal TDF basis

Treatment	Bile acid binding (μmol/100 mg TDF)	Binding relative to cholestyramine (%)
Soy bean	1.15 ± 0.12^{d}	10.4 ± 1.2^{d}
Black eye bean	2.30 ± 0.12^{c}	20.7 ± 1.2^{c}
Garbanzo	7.55 ± 0.12^{b}	68.1 ± 1.2^{b}
Lima bean	2.14 ± 0.12^{c}	19.3 ± 1.2^{c}
Cholestyramine	11.1 ± 0.12^{a}	100 ± 1.2^{a}
Cellulose	0.17 ± 0.12^{e}	1.5 ± 1.2^{e}

Values (means ± SEM) within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.

Soy bean, black eye bean, garbanzo, lima bean, cholestyramine and cellulose treatments contained 13, 16, 16, 21, 24 and 24 mg TDF.

Table 4
In vitro bile acid binding by soy bean, black eye bean, garbanzo and lima bean on equal IDF basis

Treatment	Bile acid binding (μmol/100 mg IDF)	Binding relative to cholestyramine (%)
Soy bean	1.36 ± 0.14^{d}	12.3 ± 1.4^{d}
Black eye bean	2.77 ± 0.14^{c}	25.0 ± 1.4^{c}
Garbanzo	8.88 ± 0.14^{b}	80.1 ± 1.4^{b}
Lima bean	2.50 ± 0.14^{c}	22.5 ± 1.4^{c}
Cholestyramine	11.1 ± 0.14^{a}	100 ± 1.4^{a}
Cellulose	0.17 ± 0.14^{e}	1.5 ± 1.4^{e}

Values (means ± SEM) within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.

Soy bean, black eye bean, garbanzo, lima bean, cholestyramine and cellulose treatments contained 11, 13, 14, 18, 24 and 24 mg IDF.

chemical composition of TDF and IDF of various beans tested was not found in the literature search nor was it determined; therefore it is not possible to speculate that variability in bile acid may relate to the differences in polysaccharides present in their dietary fibre fractions.

In vitro bile acid binding by soy bean, black eye bean, garbanzo and lima bean on an equal protein basis, is shown in Table 5. Bile acid binding by garbanzo was significantly greater than black eye bean, lima bean and soy bean, whereas values for black eye bean and lima bean were significantly higher than those for soy bean. Variability (2–7 fold) in bile acid binding between treatments, on protein basis, suggests that their bile acid binding is not related to protein content. It was not unexpected that the bile acid binding did not relate to the protein content, since protein was digested with pancreatin. It has been reported that partially digested peptides, or free amino acids, which may have bound the bile acids were not found (by the nitrogen analysis) in the supernatant; only undigested pancreatin was detected by the ninhydrin test (Kahlon & Woodruff, 2003b). 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 et al., 1994). Soy protein has been shown, in hamsters, to significantly reduce hepatic cholesterol and increase bile acid excretion and lower plaque formation in the aortic arch (Wright & Salter, 1998; 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 et al., 1986; Sugano & Goto, 1990). Higher tyrosine (21–50%) and lower arginine (23–34%) in the amino acid composition of garbanzo, compared with the other beans tested, may attribute to its greater bile acid binding. Higher bile acid binding by black eye bean, lima bean and garbanzo, relative to soy bean, suggest that these beans may have even greater health promoting or degenerative disease amelioration potential, which should be explored in animal and human studies. Previously it has been reported that, relative to cholestyramine, black bean, pinto bean and isolated soy protein bound bile acids by 30%, 23% and 17%, respectively (Kahlon & Woodruff, 2002). Lower bile acid binding by whole soy bean in the study reported herein than previously reported values, at similar levels of isolated protein, is difficult explain, but data indicate that bile acid binding is not proportional to the protein content. It has been suggested that various whole beans should be evaluated for their bile acid binding potential. Each variety or cultivar and various beans are unique and should be evaluated for their bile acid binding and health promoting potential.

Table 5
In vitro bile acid binding by soy bean, black eye bean, garbanzo and lima bean on equal protein basis

Treatment	Bile acid binding (μmol/100 mg protein)	Binding relative to cholestyramine (%)
Soy bean	0.67 ± 0.09^{d}	6.0 ± 0.9^{d}
Black eye bean	1.59 ± 0.09^{c}	14.3 ± 0.9^{c}
Garbanzo	5.23 ± 0.09^{b}	47.2 ± 0.9^{b}
Lima bean	1.92 ± 0.09^{c}	17.3 ± 0.9^{c}
Cholestyramine	11.09 ± 0.09^{a}	100.0 ± 0.9^{a}
Cellulose	0.17 ± 0.09^{e}	1.5 ± 0.9^{e}

Values (means ± SEM) within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.

Soy bean, black eye bean, garbanzo and lima bean contained 22, 23, 23 and 23 mg protein; cholestyramine and cellulose contained 24 mg dry matter. Nitrogen to protein factor used for bean protein was 6.25.

In conclusion, relative to cholestyramine, in vitro bile acid binding on an equal TDF, IDF and protein basis had 2–7 fold variability among soy bean, black eye bean, garbanzo, and lima bean. Data suggest that bile acid was not related to TDF, IDF or protein content of the beans tested. Except for garbanzo, where values were very high, bile acid binding by soy bean, black eye bean and lima bean appears to be related to their DM content. Highest bile acid binding, by garbanzo, is very encouraging; various cultivars and varieties of *Cicer arietinum* need to be investigated for their healthful potential. The differences in bile acid binding between various beans tested may relate to the variability of their phytonutrients (flavonoid, tannin, estrogenic contents), non-protein composition, structure, hydrophobicity of undigested fractions, anionic or cationic nature of the metabolites produced during digestion or their interaction with active binding sites. Higher bile acid binding by black eye bean, garbanzo and lima bean than soy beans suggests that animal and human studies should be conducted to explore their potential for lowering blood lipids, lipoprotein and atherosclerosis risk.

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