Gas production in humans ingesting a soybean flour derived from beans naturally low in oligosaccharides[1–3]

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ABSTRACT

Background: Ingestion of soy products may cause excessive intestinal gas. This gas results from colonic bacterial fermentation of the indigestible oligosaccharides raffinose and stachyose, which are present in high concentrations in legumes.

Objective: The objective of the study was to compare gas production and gaseous symptoms in healthy volunteers after ingestion of 34 and 80 g soy flour made from either conventional soybeans or soybeans naturally low in indigestible oligosaccharides.

Design: In a double-blind, randomized, crossover protocol, breath hydrogen (an indicator of carbohydrate malabsorption), flatus frequency, and abdominal symptoms were assessed after subjects ingested the soy products and after 2 control meals (rice or lactose-hydrolyzed milk).

Results: The sum of breath-hydrogen concentrations for 8 h was significantly greater ($P < 0.005$) after 34 g conventional soy (60.4 ± 9.4 ppm) than after low-oligosaccharide soy (34.3 ± 8.1 ppm). Greater differences were observed with 80-g doses: 157.9 ± 19.4 ppm after conventional soy and 50.8 ± 6.8 ppm after low-oligosaccharide soy ($P < 0.001$). Flatus frequency (7.5 ± 1.9 times/12 h) was significantly greater ($P = 0.039$) after ingestion of 80 g conventional soy than after the control, rice meal (3.2 ± 0.8 times/12 h), whereas flatus frequency after the low-oligosaccharide soy meal (3.9 ± 0.7 times/12 h) was comparable with that after the rice meal. There were no significant differences in the severity of other abdominal symptoms.

Conclusion*:* Soy flour derived from low-oligosaccharide soybeans resulted in less gas production than that derived from conventional soybeans. *Am J Clin Nutr* 1999;69:135–9.

KEY WORDS Soy flour, low-oligosaccharide soybeans, breath-hydrogen concentration, gaseous symptoms, humans, raffinose, stachyose

INTRODUCTION

Legumes characteristically contain high concentrations of the indigestible oligosaccharides raffinose [β-D-fructofuranosyl-*O*-α-D-galactopyranosyl-(1→6)-α-D-glucopyranoside] and stachyose [β-D-fructofuranosyl-*O*-α-D-galactopyranosyl-(1→6)-α-D-galactopyranoside-(1→6)-α-D-glucopyranoside]. The indigestibility of these soluble sugars results in their delivery to the colon, where they are rapidly fermented by the colonic flora, resulting in the production of copious gas (1). This gas production may play a role in the acceptability of soy products as a major food source for humans (1–4) and animals (5).

The final steps in the production of raffinose and stachyose in soybeans are catalyzed by the enzymes galactinol–sucrose galactosyltransferase (EC 2.4.1.82, raffinose synthase) and galactinol–raffinose galactosyltransferase (EC 2.4.1.67, stachyose synthetase), respectively (**Figure 1**). Screening of a large number of soy seeds for this enzyme activity resulted in the identification of a seed with very low activity, and this seed was shown to have a low concentration of raffinose and stachyose. The studies described in this report were designed to compare gas production and gaseous symptoms in healthy human volunteers ingesting concentrates derived from conventional and low-oligosaccharide soybeans.

SUBJECTS AND METHODS

Subjects

The soy products were evaluated in 2 double-blind, randomized, crossover studies, each involving 20 healthy volunteers. A total of 28 subjects (11 men and 17 women) aged 29–62 y were studied; 12 individuals took part in both studies. Exclusion criteria included the existence of intercurrent illness, antibiotic ingestion within the previous 2 mo, and allergy to soy products or aspartame. The studies were approved by the Human Subjects Committee of the Institutional Review Board at the Minneapolis Veterans' Affairs Medical Center, and informed consent was obtained from all subjects.

Protocols

Study 1

In random order, 20 subjects ingested each of 3 test meals separated by 1-wk washout periods: *1*) 500 mL soymilk made from

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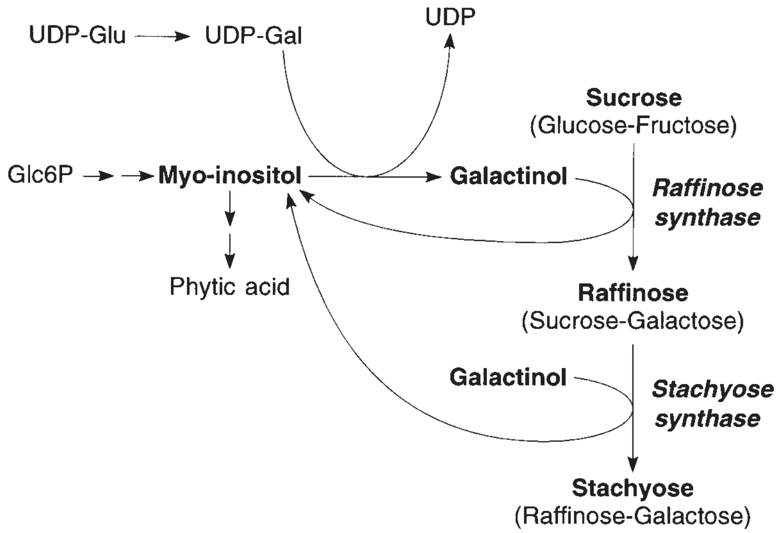

FIGURE 1. Metabolic pathway for the synthesis of raffinose and stachyose from sucrose in soybeans. Galactinol provides galactoses for the conversion of sucrose to raffinose and of raffinose to stachyose. UDP-Glu, uridine diphosphate glucose; UDP-Gal, uridine diphosphate galactose; Glc6P, glucose-6-phosphate.

34 g conventional soy flour in 500 mL water, *2*) 500 mL soymilk made from 34 g low-oligosaccharide soy (DuPont Agricultural Products, Wilmington, DE) in 500 mL water, and *3*) 500 mL lactose-hydrolyzed, 1%-fat cow milk as a negative control. The composition of the conventional and low-oligosaccharide soy flours are shown in **Table 1**. Lactose was hydrolyzed by the addition of 1.07 g of a lactase preparation made from *Kluyveromyces lactis* (Lactaid Inc, Pleasantville, NJ) to 1 L milk, which was then incubated for 48 h at 4 °C (6). An assay using an enzymatic method (Lactose/D-galactose test kit; Boehringer Mannheim Biochemical Inc, Indianapolis) showed negligible lactose at the end of the incubation period. Aspartame (820 mg Equal/L; NutraSweet Co, Deerfield, IL) was added to the 2 soy products to yield a sweetness roughly equivalent to that of the lactose-hydrolyzed milk. All products were ingested with a straw from sealed cardboard containers (Trio Supplies, Minneapolis) to obscure differences in the color of the drinks (6). The taste of the 2 soy products was indistinguishable; however, the lactose-hydrolyzed milk differed in taste from that of the soy products.

Study 2

Twenty subjects were randomly assigned to receive each of 3 test meals over a 3-wk period with a 1-wk washout period between tests: *1*) pudding containing 80 g conventional soy flour, *2*) pudding containing 80 g low-oligosaccharide flour, and *3*) a negative control, 80 g white rice cereal (Kellogg, Battle Creek, MI). (Preliminary studies showed that ingestion of the rice pudding resulted in minimal breath-hydrogen production.) The puddings were produced by mixing the flours with 240 mL water, 50 drops of orange flavoring (McCormick & Co, Inc, Hunt Valley, MD), and 2 g aspartame. Once again, the soy puddings were indistinguishable in color and taste, whereas the rice pudding was distinguishable from the soy products.

To minimize baseline breath-hydrogen excretion on the day of the study, the subjects ingested a rice and hamburger dinner the night before the challenge (7) and then fasted until the next morning. At ≈0600, the test meals were ingested as rapidly as possible (within 30 min). No food was allowed for the next 8 h, but water and black coffee were allowed ad libitum. After the 8-h test period, subjects were allowed to resume their regular diets. End-alveolar breath samples for measurements of hydrogen and methane concentrations were collected before ingestion of the test meals and hourly for the next 8 h. The severity of each abdominal symptom (fullness, nausea, bloating, abdominal pain, and subjective impression of rectal gas) was rated by the subject on a linear 6-cm scale (0 cm = none, >0–1.5 cm = trivial, 1.5–3 cm = mild, 3–4.5 cm = moderate, and 4.5–6 cm = severe) (6). Symptom severity was recorded over 2 time periods: from the time of test meal ingestion until 1200 and from 1200 until dinner. Each flatus passage from the time of test meal ingestion until 1800 was recorded and arbitrarily rated as "small" or "large."

Analysis of breath gases

Hydrogen and methane were quantified by gas chromatography and carbon dioxide was quantified by infrared spectroscopy as described previously (8). In rare cases, samples had a carbon dioxide concentration <3.75% and were thus considered to have excessive atmospheric contamination; such a value was assumed to be the mean of the concentration obtained 1 h before and 1 h after the contaminated sample.

TABLE 1
Composition of the conventional and low-oligosaccharide soy flours

Components	Conventional soy flour	Low-oligosaccharide soy flour
	%	
Fat	19.07	17.07
Protein	43.89	48.20
Crude fiber	0.19	0.19
Moisture	4.54	4.76
Ash	5.27	5.47
Nitrogen-free extract	27.04	24.31
Total dietary fiber	10.87	10.50
Stachyose	3.33	0.46
Raffinose	0.51	0.16
Galactinol	0.0	2.16
Sucrose	6.37	6.52

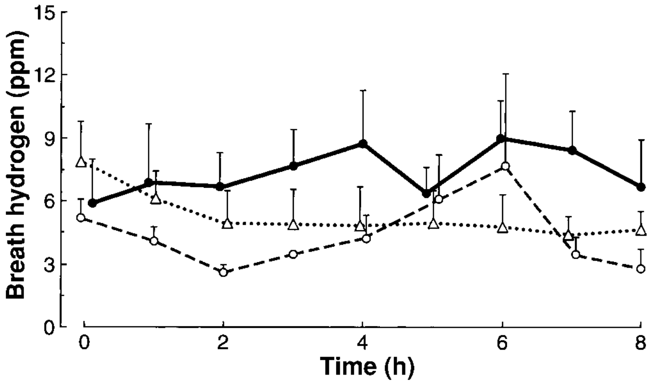

FIGURE 2. Mean (±SEM) hourly breath-hydrogen concentrations in 20 subjects after ingestion of 500 mL of the 3 test meals: 34 g conventional soy concentrate (●), 34 g low-oligosaccharide soy concentrate (O), and lactose-hydrolyzed cow milk (control, △). The sum of the hourly breath-hydrogen concentrations from 0 to 8 h was significantly greater ($P = 0.005$) after ingestion of conventional soy than after ingestion of low-oligosaccharide soy or lactose-hydrolyzed milk.

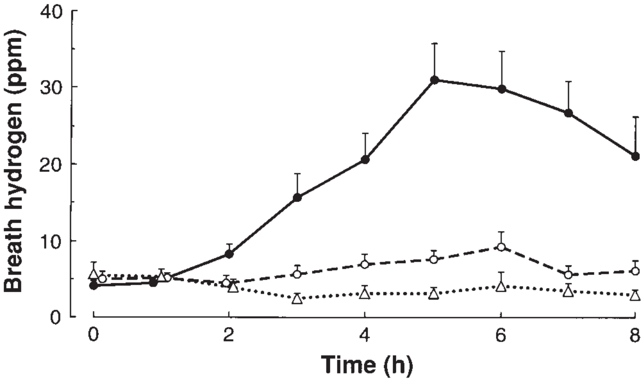

FIGURE 3. Mean (±SEM) hourly breath-hydrogen concentrations in 20 subjects after ingestion of puddings containing 80 g conventional soy concentrate (●) or 80 g low-oligosaccharide soy concentrate (O) or 80 g of a white rice cereal (control, △). The sum of the hourly breath-hydrogen concentrations from 0 to 8 h was significantly greater ($P < 0.001$) after ingestion of conventional soy than after ingestion of low-oligosaccharide soy or rice cereal.

Statistical analysis

Data were analyzed by repeated-measures analysis of variance (ANOVA) (9, 10). The differences between treatments were calculated by paired *t* test, and the *P* value was adjusted by Bonferroni correction. A binomial distribution was used to calculate the *P* value (two-tailed). The sum of breath-hydrogen concentrations and mean symptom scores for each treatment period were compared.

RESULTS

Study 1

Breath-hydrogen concentrations resulting from the ingestion of 34 g of the 2 soymilks and the lactose-hydrolyzed cow milk are shown in **Figure 2**. The sum of breath-hydrogen concentrations from 1 to 8 h after ingestion of lactose-hydrolyzed milk (39.5 ± 9.4 ppm) and low-oligosaccharide soymilk (34.3 ± 8.1 ppm) were not significantly different, whereas breath-hydrogen excretion after ingestion of conventional soymilk (60.4 ± 9.4 ppm) was significantly greater than that after the other 2 treatments (overall $P = 0.005$; $P = 0.006$ when compared with low-oligosaccharide soymilk; and $P = 0.033$ when compared with lactose-hydrolyzed milk). Neither the severity of symptoms nor flatus frequency differed significantly with either soy product from that observed with the control, lactose-hydrolyzed milk (data not shown).

Study 2

Breath-hydrogen concentrations after ingestion of puddings containing 80 g of the soy concentrates or rice are shown in **Figure 3**. Although there appeared to be a trend toward greater total breath-hydrogen concentrations after the low-oligosaccharide soy (50.8 ± 6.8 ppm) than after the rice control (28.5 ± 6.1 ppm), this difference was not significant by ANOVA ($P = 0.325$). Total breath-hydrogen concentrations after conventional soy (157.9 ± 19.4 ppm) were significantly greater ($P < 0.001$) than concentrations observed after each of the other 2 treatments. Ingestion of conventional soy resulted in a flatus frequency (7.5 ± 1.9 times/12 h) that was significantly greater ($P = 0.039$) than that with rice (3.2 ± 0.8), whereas the frequency observed after ingestion of the low-oligosaccharide soy (3.9 ± 0.8) was comparable with that after rice (**Figure 4**). No abdominal symptoms differed significantly with either soy product from those observed with the rice control (**Table 2**). The ingestion of the meals did not result in a significant alteration in breath-methane concentrations from the zero time values, which averaged 18 ± 2.5 ppm. The sums of breath-methane concentrations for the 8 h after ingestion of the 3 meals were not significantly different in either study (data not shown).

DISCUSSION

The soluble oligosaccharides raffinose and stachyose are present in high concentrations in soybeans and other legumes (2, 11). These sugars consist of sucrose linked via α-galactoside bonds to 1 or 2 galactose moieties to form raffinose and stachyose,

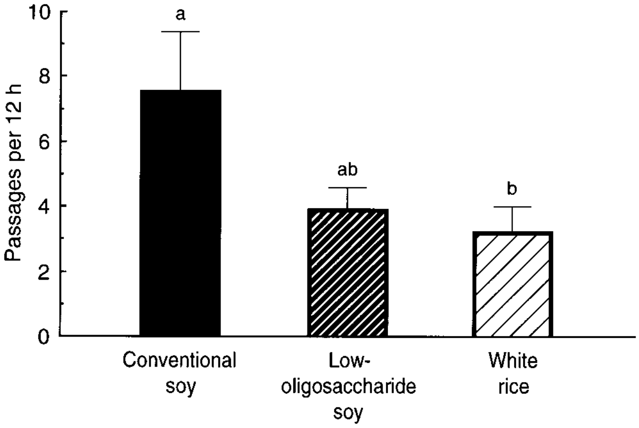

FIGURE 4. Mean (±SEM) frequency of flatus passages in 20 healthy subjects in the 12 h after ingestion of puddings containing 80 g conventional soy concentrate or 80 g low-oligosaccharide soy concentrate or 80 g of a white rice cereal. Different letters indicate significant differences between groups, $P < 0.05$.

TABLE 2
Severity of symptoms over 12 h after ingestion of puddings containing 80 g of the soy concentrates or rice (control)[1]

Gastrointestinal symptoms	Control	Low-oligosaccharide soy flour	Conventional soy flour
Daily severity			
Excessive gas	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
Bloating	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Abdominal pain	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
Nausea	0.1 ± 0.1	0.1 ± 0.05	0.1 ± 0.1
Feeling of fullness	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Daily frequency			
Bowel movement	1.2 ± 0.2	1.2 ± 0.1	1.3 ± 0.2
Diarrhea	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Small flatus	2.6 ± 0.6	3.4 ± 0.6	6.7 ± 1.8
Large flatus	0.7 ± 0.3	0.6 ± 0.2	0.8 ± 0.2
Total flatus	3.2 ± 0.8[a]	3.9 ± 0.7[a,b]	7.5 ± 1.9[b]

[1] $\bar{x}$. Symptoms were rated on a continuous 6-cm scale: 0 cm = none; >0–1.5 cm = trivial; >1.5–3 cm = mild, >3–4.5 cm = moderate; and >4.5–6 cm = severe. Each flatus passage was recorded as large or small, and bowel movements were assessed with regard to frequency, consistency, and straining. Means with different superscript letters are significantly different, $P \leq 0.05$ (repeated-measures ANOVA).

respectively. The small intestine of humans (and animals) does not synthesize the α-galactosidase required for the hydrolysis of these oligosaccharides to their constituent simple sugars. As a result, virtually 100% of ingested stachyose and raffinose reaches the colon. Colonic bacteria possess the enzyme systems necessary to ferment these sugars, and copious quantities of carbon dioxide and hydrogen may be released during the fermentation process. In addition to the well-known ability of soy and other legumes to cause gas in humans (1, 8, 12), soy products have been reported to induce digestive problems in animals (5). Soy is an inexpensive source of high-quality protein. In addition, recent studies have suggested that soy may have other beneficial features, including antineoplastic properties (13). Thus, a soy that lacks gas-producing factors could be useful for both humans and animals.

Recent studies have elucidated the metabolic pathway involved in raffinose and stachyose synthesis in soybeans (Figure 1). At the end of this pathway, galactinol (1-*O*-α-D-galactopyranosyl-D-myo-inositol) serves as a galactose donor for reactions in which sucrose is converted to raffinose and raffinose to stachyose (14). These reactions are catalyzed by galactinol–sucrose galactosyltransferase and galactinol–raffinose galactosyltransferase, respectively. Beans with a naturally low activity of these enzymes have been identified and compared with conventional soybeans; these low-oligosaccharide soybeans are low in raffinose and stachyose but rich in galactinol (Table 1). The low concentration of the oligosaccharides presumably should reduce the gas-producing propensity of these beans. However, the fate of galactinol in the intestinal tract of humans and animals has not been studied. It seems likely that the small-intestinal mucosa will be impermeable to a water-soluble molecule the size of intact galactinol, which consists of galactose bound to myo-inositol. However, in the absence of information concerning the digestibility of galactinol and the ability of the colonic microflora to ferment nonabsorbed galactinol, the gas-producing propensity of a soybean low in oligosaccharides but rich in galactinol could not be predicted with certainty.

The purpose of the present study was to compare, in healthy human volunteers, gas production after ingestion of soy flour made from conventional soybeans with that after ingestion of a new variety of soybean that is naturally low in raffinose and stachyose. To this end, in double-blind, randomized, crossover studies, breath-hydrogen excretion and gaseous symptoms were assessed after ingestion of the 2 types of soy and rice.

Malabsorbed fermentable material is converted to hydrogen by multiple species of colonic bacteria. This hydrogen can be absorbed and excreted in the breath, passed through the rectum, or utilized by other bacterial species for the reduction of carbon dioxide to methane, or of sulfate to sulfide (15). Most of the hydrogen released during the slow fermentation of insoluble substrates such as fiber and resistant starch is utilized by other bacteria (16). However, an appreciable fraction of the hydrogen liberated during rapid fermentation of soluble carbohydrates—such as lactose, raffinose, and stachyose—is absorbed and excreted in the breath. Breath-hydrogen measurements have been shown to provide a semiquantitative assessment of the quantity of soluble carbohydrate reaching the colon (17).

Ingestion of conventional soy in quantities of 34 g (Figure 2) and 80 g (Figure 3) was associated with significantly greater increases in breath-hydrogen concentrations than those observed after ingestion of low-oligosaccharide soy. On the basis of these breath-hydrogen concentrations, it can be concluded that low-oligosaccharide soy contains less nonabsorbable, rapidly fermented substrate than does conventional soy. Thus, the advantage of low-oligosaccharide soy is that it produces less gas than does conventional soy; however, because it contains less nonabsorbable carbohydrate than conventional soy, the putative benefit to the colonic mucosa (18) of organic acids derived from nonabsorbable carbohydrate is lacking.

White rice has been shown to be the one complex carbohydrate that is nearly completely absorbed and hence results in minimal breath-hydrogen excretion (7). Although there appeared to be a trend toward greater hydrogen excretion after 80 g low-oligosaccharide soy than after 80 g white rice (Figure 3), this difference was not significant when assessed by ANOVA. However, this difference was significant ($P = 0.026$) when assessed by paired *t* test. Thus, it seems likely that some component of the low-oligosaccharide soy, such as galactinol or fiber, provided substrate that supported a small amount of hydrogen production in the colon.

The widespread belief that legume ingestion causes excessive rectal gas is supported by a large body of anecdotal evidence and many objective measurements (1, 8, 12). In the present investigation, no significant increase in flatus frequency was observed after ingestion of 34 g conventional soy, whereas ingestion of 80 g conventional soy (3.1 g raffinose and stachyose) resulted in a significant increase in flatus frequency. The relatively minor increase in flatus frequency induced by conventional soy flour (about 4 passages/12 h) was not totally unexpected given the relatively small quantity of oligosaccharides (3.06 g) in the 80-g dose. Christl et al (19) showed that as the rate of gas production in the colon increases, a smaller fraction of the gas is absorbed and a greater fraction is excreted through the rectum. Thus, it is possible that a slightly greater dose of conventional soy flour might have resulted in a disproportionate increase in rectal gas output and a more readily recognizable increase in flatus frequency.

Techniques to reduce the raffinose and stachyose contents of soybean products have been the subject of extensive investigation. Cooking does not eliminate these heat-stable oligosaccharides; however, germination for 1–4 d results in the utilization of the bulk of these sugars (20). Incubation with various microorganisms or enzymes derived from microorganisms yields products with a markedly reduced oligosaccharide content (21, 22). Ultrafiltration of the aqueous fraction of soybeans has also been successful in removing oligosaccharides from soybeans (23). The present study showed that soy seeds naturally low in oligosaccharides produced little flatulence, significantly less than that associated with conventional soy. Thus, this new seed may represent a useful alternative to conventional soybeans.

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