Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 1331 – 1340
Metabolism
Clinical and Experimental
www.metabolismjournal.com

The effect on the blood lipid profile of soy foods combined with a prebiotic: a randomized controlled trial

Julia M.W. Wong[a,b], Cyril W.C. Kendall[a,b], Russell de Souza[a,b], Azadeh Emam[a,b], Augustine Marchie[a,b], Ed Vidgen[a,b], Candice Holmes[a,c], David J.A. Jenkins[a,b,d,e,*]

[a]*Clinical Nutrition and Risk Factor Modification Center, St. Michael's Hospital, Toronto, Ontario, Canada M5C 2T2*
[b]*Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 3E2*
[c]*Division of Family Medicine, Department of Medicine, St. Michael's Hospital, Toronto, Ontario, Canada M5C 2T2*
[d]*Division of Endocrinology and Metabolism, Department of Medicine, St. Michael's Hospital, Toronto, Ontario, Canada M5C 2T2*
[e]*Department of Medicine, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 3E2*

Received 6 July 2009; accepted 15 December 2009

Abstract

The value of soy protein as part of the cholesterol-lowering diet has been questioned by recent studies. The apparent lack of effect may relate to the absence of dietary factors that increase colonic fermentation and potentiate the cholesterol-lowering effect of soy. Therefore, unabsorbable carbohydrates (prebiotics) were added to the diet with the aim of increasing colonic fermentation and so potentially increasing the hypocholesterolemic effect of soy. Twenty-three hyperlipidemic adults (11 male, 12 female; 58 ± 7 years old; low-density lipoprotein cholesterol [LDL-C], 4.18 ± 0.58 mmol/L) completed three 4-week diet intervention phases—a low-fat dairy diet and 10 g/d prebiotic (oligofructose-enriched inulin, a fermentable carbohydrate), a soy food–containing diet (30 g/d soy protein, 61 mg/d isoflavones from soy foods) and 10 g/d placebo (maltodextrin), and a soy food–containing diet with 10 g/d prebiotic—in a randomized controlled crossover study. Intake of soy plus prebiotic resulted in greater reductions in LDL-C (−0.18 ± 0.07 mmol/L, $P = .042$) and in ratio of LDL-C to high-density lipoprotein cholesterol (−0.28 ± 0.11, $P = .041$) compared with prebiotic. In addition, high-density lipoprotein cholesterol was significantly increased on soy plus prebiotic compared with prebiotic (0.06 ± 0.02 mmol/L, $P = .029$). Differences in bifidobacteria, total anaerobes, aerobes, and breath hydrogen did not reach significance. Soy foods in conjunction with a prebiotic resulted in significant improvements in the lipid profile, not seen when either prebiotic or soy alone was taken. Coingestion of a prebiotic may potentiate the effectiveness of soy foods as part of the dietary strategy to lower serum cholesterol.

1. Introduction

Early studies emphasized the potential value of soy in the cholesterol-lowering diet [1,2], but the use of soy has now been questioned [3,4]. Most recently, the US Food and Drug Administration (FDA), which previously accepted a coronary heart disease (CHD) risk reduction health claim for soy foods [5] based on their cholesterol-lowering ability, is reassessing the health claim for soy. Because relatively few cholesterol-lowering dietary components are recognized, it is important to retain the few that currently exist if dietary strategies to lower cholesterol are to have any relevance. It has been suggested that one means of increasing the hypocholesterolemic effect of soy is through enhancing the effectiveness of the isoflavone component of soy [6,7].

Financial disclosure: Dr Jenkins reported serving on the Scientific Advisory Board of Unilever, the Sanitarium Company, and the California Strawberry Commission and receiving research grants from Loblaws, Unilever, Barilla, and the Almond Board of California. Drs Jenkins and Kendall have been on the speaker's panel for the Almond Board of California. Dr Kendall and Mr Vidgen have received partial salary funding from research grants provided by Unilever, Loblaws, and the Almond Board of California. Dr Jenkins has received honoraria for scientific advice from the Almond Board of California, Barilla, Unilever Canada, and The Solae Company. Dr Wong was the recipient of a Canadian Institutes of Health Research Doctoral Research Award.

Clinical Trial Registration: #NCT00516594.

* Corresponding author. Clinical Nutrition and Risk Factor Modification Center, St. Michael's Hospital, Toronto, Ontario, Canada M5C 2T2. Tel.: +1 416 978 4752; fax: +1 416 978 5310.

E-mail address: cyril.kendall@utoronto.ca (D.J.A. Jenkins).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.12.017

Colonic fermentation of soy isoflavones to produce cholesterol-lowering selective estrogen receptor modulators has been suggested as a mechanism for the cholesterol-lowering property of soy [8]. Therefore, the primary aim of this study was to determine the effect on serum lipids (low-density lipoprotein cholesterol [LDL-C] and the ratio of total cholesterol [TC] to high-density lipoprotein cholesterol [HDL-C]) of adding a nonabsorbable fermentable carbohydrate (prebiotic), in the form of a polyfructan, to a diet containing soy. The hypothesis to be tested was that a combination of soy and a prebiotic could be more effective in lowering serum lipid risk factors for cardiovascular disease than soy consumed alone. Secondary end points of interest included the effect of the soy and prebiotic combination on apolipoproteins, C-reactive protein, blood pressure, and body weight, and the fermentation-related markers and symptoms including breath gases, fecal microbiology, satiety, and gastrointestinal symptoms.

2. Methods

2.1. Participants

Participants were recruited from newspaper advertisements and enrolled between September 2003 and March 2004 (Fig. 1). Thirty-seven men and postmenopausal women with hyperlipidemia were randomized with a mean age of 59.6 ± 7.7 years (range, 44-83 years), body mass index of 26.3 ± 3.3 kg/m^2, and LDL-C of 4.19 ± 0.53 mmol/L (Table 1). Twenty-three participants completed all 3 phases (Fig. 1). No participant had a history of cardiovascular disease, untreated hypertension (blood pressure >140/90 mm Hg), diabetes, or renal or liver disease. No participants were taking medications known to influence serum lipids apart from 4 women who were on stable doses of a statin and were admitted to the trial in error and 1 man who was taking levothyroxine before and during the study. Data from the statin users did not differ from the group as a whole and are included in the final analysis. All participants were nonsmokers, and none had taken antibiotics in the last 3 months. Three participants were taking antihypertensive medications at a constant dose before and during the study, with 1 participant starting the medication during the second phase. Participants were asked to keep constant across treatments the intakes of prescription and nonprescription medications and supplements, and the level of physical activity.

2.2. Study protocol

The study consisted of three 4-week diet phases in a randomized controlled crossover design, with each phase separated by a minimum 2-week washout period.

Clinic visits were at weekly intervals during the 1-month treatment phases. At each visit, body weight and blood pressure were measured; and blood was taken every 2 weeks after an overnight fast (10-12 hours).

Seven-day weighed diet records and supplement checklists were completed and returned weekly during each 4-week diet phase. Symptom diaries (ie, flatus, bloating, and abdominal discomfort) were also completed daily during each 1-month phase using a 7-point semantic scale, where 0 was none, +3 was moderate, and +6 was severe. Participants rated their overall feeling of satiety on the diet at weekly intervals using a 9-point bipolar semantic scale, where −4 was very hungry, 0 was neutral, and +4 was uncomfortably full.

Forced end expiratory samples of alveolar air were collected hourly by the subjects over 12 hours at the end of each treatment period using a modified Haldane-Priestley tube for analysis of hydrogen and methane [9]. In addition, 3-day fecal collections were obtained at baseline and at the end of each treatment [10,11]. Breath gases and fecal microbiology were analyzed as indirect measures of colonic fermentation.

Participants were randomized based on sex and baseline LDL-C using SAS software (SAS Institute, Cary, NC) [12] by the statistician in a location removed from the clinic. Study dietitians were not blinded to the diet because they were responsible for packaging and providing the study foods to the participants and for reviewing their diet records. The laboratory staff responsible for analyses were blinded to the treatment and received samples labeled with participant codes and dates.

The study was approved by the Ethics Committees of the University of Toronto and St Michael's Hospital. Written informed consent was obtained from the participants. The study clinical trial registration number is NCT00516594, which contains 2 independent clinical trials (only study 2 is presented here).

2.3. Diets

Subjects were expected to be following a therapeutic low–saturated fat and cholesterol diet before starting the study [13] (Table 2). Two weeks before each dietary treatment and throughout the study period, subjects were given a list of foods containing oligofructose and inulin [14] and foods with FDA-approved health claims for CHD reduction including soy-containing foods, soluble fiber (oats, barley, and psyllium), plant sterols, and nuts, which they were to avoid [5,15-18]. Participants were also asked to avoid any herbal supplements that contained soy isoflavones and/or fructooligosaccharides.

The 3 phases consisted of (1) oligofructose-enriched inulin (10 g/d) and low-fat dairy and egg protein foods (prebiotic); (2) soy protein foods (30 g/d soy protein, total isoflavone 61 mg/d) with prebiotic (10g/d) (soy plus prebiotic); or (3) soy protein foods without prebiotic (soy), based on a 2000-kcal diet (Table 3). The dairy and egg protein foods included skim milk, fat-free cheese and yogurt, and egg substitute and liquid egg white. The soy protein foods included soy beverage, low-fat tofu, and a variety of

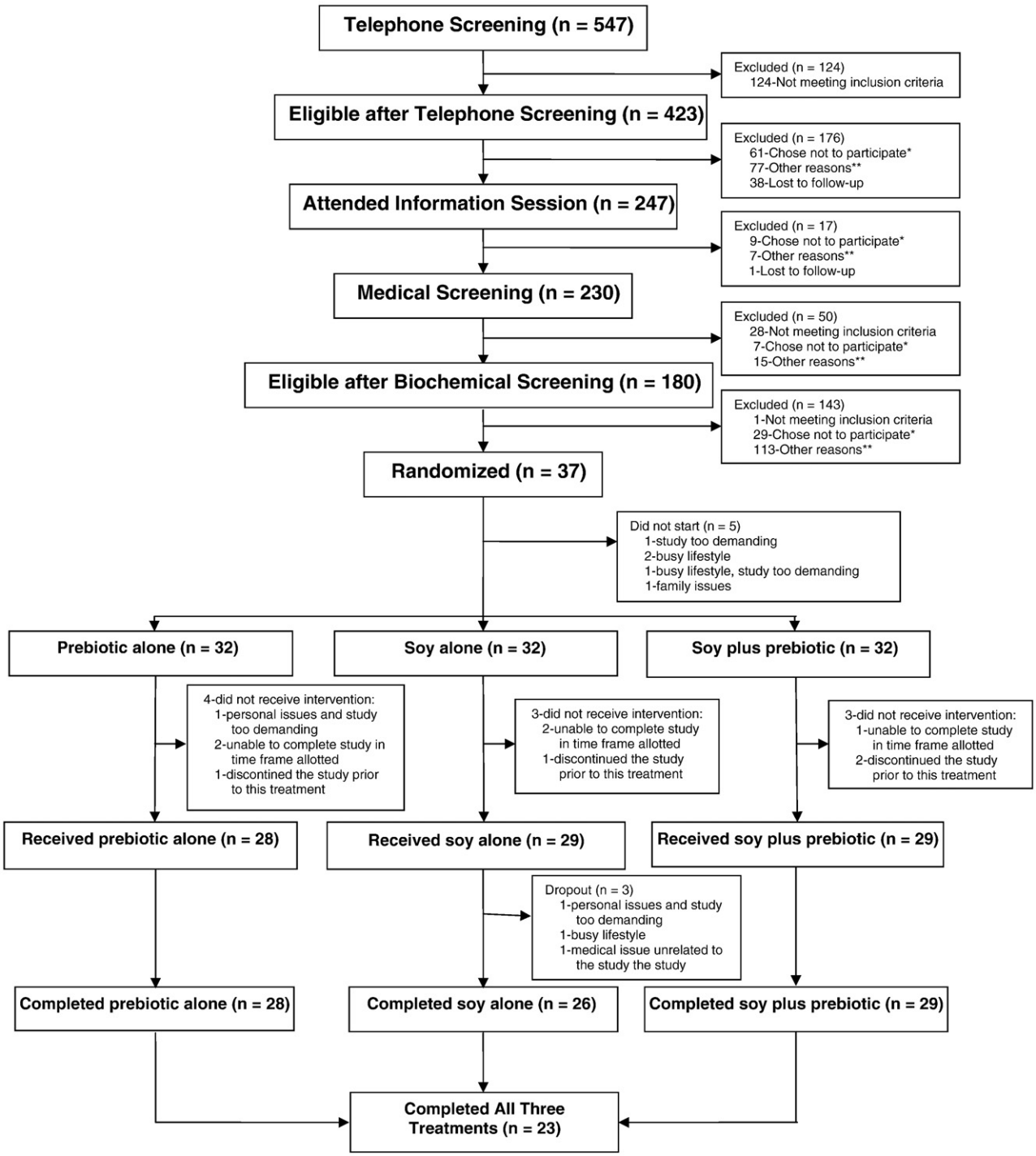

Fig. 1. Patient flow diagram. *Chose not to participate (106): not interested (56), busy lifestyle (26), study too demanding (19), no compensation (3), decided to start statin (1), currently on another diet (1). **Other reasons (212): unable to contact (70), joining Portfolio study† (48), joining FOS-fiber study† (36), unable to come to clinic (21), away during study period (11), medical issue unrelated to study (11), GP not consent (5), wants to lose weight (3), does not want to discontinue statin (2), personal issue (2), B12 deficiency (1), hepatitis B (1), cancer history, and personal issues (1). †Subject recruitment occurred concurrently with 2 other clinical trials. Those subjects who met the eligibility criteria for more than one study were place in their preferred study.

soy meat analogues such as deli slices, hot dogs, burgers, and breakfast patties. The soy and control supplements were matched for macronutrient composition except that soy protein replaced other proteins in the soy treatment arms. Fatty acid composition was balanced between the 2 diets with the use of butter and a mixture of sunflower and safflower oil. Dietary fiber was slightly higher and dietary cholesterol was slightly lower on the soy foods (based on a 2000-kcal diet: +3.9 g and −9.5 mg, respectively). The amount of soy protein and dairy foods was estimated based

Table 1
Subjects' baseline characteristics at randomization

	Completers (n = 23)	Noncompleters (n = 14)
Age (y)	58.1 ± 6.8	62.1 ± 8.8
Male/female	11/12	3/11
Body weight (kg)	74.2 ± 14.8	72.8 ± 7.6
Body mass index (kg/m^2)	25.9 ± 2.6	26.8 ± 4.3
Cholesterol (mmol/L)		
TC	5.96 ± 0.66	6.11 ± 0.75
LDL-C	4.18 ± 0.58	4.20 ± 0.45
HDL-C	1.12 ± 0.31[a]	1.39 ± 0.48
TG	1.44 ± 0.78	1.16 ± 0.32
Ratios		
TC/HDL-C	5.62 ± 1.21[a]	4.74 ± 1.15
LDL-C/HDL-C	3.96 ± 0.94[a]	3.30 ± 0.94
Blood pressure (mm Hg)		
Systolic	122.8 ± 10.9	118.8 ± 10.3
Diastolic	76.7 ± 7.6	75.0 ± 9.4
Medications		
Lipid lowering before and during the study	3	1
Blood pressure	2	1
Thyroid	1	0

Data expressed as mean ± SD unless otherwise noted. To convert TC, LDL-C, and HDL-C to milligrams per deciliter, divide by 0.0259; to convert TG to milligrams per deciliter, divide by 0.0113.

[a] Significantly different from noncompleters using 2-sample *t* test.

Table 3
Nutritional profiles of prescribed NCEP (control) and soy food (test) supplements (based on 2000-kcal diet)

	NCEP (control) supplements	Soy food (test) supplements
Energy (kcal/d)	416.1	415.7
Total protein (g/d)	38.3	38.1
Soy protein (g/d)	0.0	30.0
Soy isoflavones (mg/d)	0.0	60.5
Daidzein	0.0	28.4
Genistein	0.0	29.7
Glycitein	0.0	2.4
Available carbohydrate (g/d)	39.1	39.0
Total dietary fiber (g/1000 kcal)	0.0	2.0
Total fat (g/d)	11.8	12.1
SFA (g/d)	2.4	2.6
MUFA (g/d)	4.4	4.6
PUFA (g/d)	4.3	4.5
Dietary cholesterol (mg/1000 kcal)	5.4	0.6[a]

NCEP indicates National Cholesterol Education Program; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[a] The source of cholesterol was from butter, which was used in conjunction with sunflower and safflower oil to balance the fatty acids between treatments.

on the Lipid Research Clinic tables for energy requirements [19] for each participant to provide 30 g of soy or dairy protein per 2000-kcal diet. This level of soy protein was chosen to be in excess of the minimal level of soy protein (25 g/d) in the current FDA health claim for CHD risk reduction (Table 3) [5]. The amount of prebiotic consumed (10 g/d) remained constant for all participants; and the prebiotic used was Synergy1 (Orafti Group, Tienen, Belgium), a 50:50 mixture of inulin and oligofructose. Maltodextrin (10 g/d) was provided on the soy-alone phase as a placebo (Table 2). Participants were blinded to the prebiotic and maltodextrin during the study treatments.

During the study period, food supplements to be consumed by the participants were provided weekly at clinic visits. Participants were given a 7-day rotating menu of control or soy supplements (Table 4) on which they checked off the weight of each supplement as eaten and confirmed the

Table 2
Mean nutrient profiles during the study treatments (n = 23)*,†

	Baseline			Mean treatment		
	Prebiotic alone	Soy alone	Soy plus prebiotic	Prebiotic alone	Soy alone	Soy plus prebiotic
Calories (kcal)	1581.9 ± 99.9	1590.8 ± 105.0	1651.0 ± 122.1	1687.4 ± 103.1	1698.1 ± 109.5	1690.3 ± 110.1
% of total calories						
Protein	17.9 ± 1.0	18.9 ± 1.1	18.7 ± 0.9	21.4 ± 0.6	21.3 ± 0.6	20.7 ± 0.6
Vegetable protein	8.0 ± 0.3	8.1 ± 0.6	7.7 ± 0.4	7.1 ± 0.2[a]	15.8 ± 0.4[b]	15.8 ± 0.5[b]
Soy protein	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0[a]	7.3 ± 0.3[b]	7.3 ± 0.3[b]
Available carbohydrate	57.3 ± 1.8	56.9 ± 2.2	57.5 ± 2.2	55.8 ± 1.3	55.9 ± 1.2	56.3 ± 1.2
Dietary fiber (g/1000 kcal)	17.8 ± 1.0	19.6 ± 1.5	17.9 ± 0.9	16.5 ± 0.8[a]	18.1 ± 0.8[b]	18.1 ± 0.8[b]
Fat	22.9 ± 1.1	22.1 ± 1.4	22.7 ± 1.5	21.0 ± 1.0	21.4 ± 0.9	21.6 ± 1.0
Saturated	6.1 ± 0.5	5.9 ± 0.5	6.3 ± 0.6	4.9 ± 0.3[a]	5.5 ± 0.4[b]	5.7 ± 0.4[b]
Monounsaturated	8.9 ± 0.4	8.0 ± 0.6	8.6 ± 0.6	7.7 ± 0.4	7.8 ± 0.4	7.8 ± 0.4
Polyunsaturated	5.4 ± 0.4	5.6 ± 0.6	5.5 ± 0.5	6.1 ± 0.3	6.2 ± 0.3	6.2 ± 0.4
Dietary cholesterol (mg/1000 kcal)	82.8 ± 10.8	86.2 ± 11.0	87.4 ± 13.1	51.9 ± 5.0[a]	48.6 ± 5.8[ab]	42.9 ± 5.2[b]
Alcohol (% of total calories)	1.9 ± 0.7	2.1 ± 0.6	1.1 ± 0.4	1.7 ± 0.5	1.5 ± 0.5	1.4 ± 0.4
Soy protein (g/d)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0[a]	29.8 ± 1.5[b]	29.8 ± 1.6[b]
Prebiotic/maltodextrin (g/d)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.8 ± 0.1	9.9 ± 0.1	9.9 ± 0.1

* Unadjusted mean values.

† Superscripts with a different letter in a row denote a significant difference. Values with a superscript letter that is the same indicate no significant difference. Significance of differences was calculated using least square means with change from baseline to the mean of all weeks postbaseline as the response variable; the diet, sex, diet by sex, treatment sequence, and subject ID nested within sex-by-sequence interaction as main effects; and baseline as the covariate, with a Tukey-Kramer adjustment ($P < .05$).

Table 4
Example diets based on 2000 kcal

	Soy + prebiotic or maltodextrin		Dairy + prebiotic	
Breakfast	Bran flakes cereal	1 c	Bran flakes cereal	1 c
	Soy beverage	1 c	Milk–skim	3/4 c
	Whole wheat toast	2 slices	Whole wheat toast	2 slices
	With light margarine and	1 T	With light margarine and	1 T
	Double fruit jam	1 T	Double fruit jam	1 T
	Strawberries	1 c	Strawberries	1 c
	Tea/coffee/water		Tea/coffee/water	
	Prebiotic[a] or maltodextrin	5 g	Prebiotic[a]	5 g
Snack	Grapes	1/2 c	Grapes	1/2 c
Lunch	*Sandwich:*		*Sandwich:*	
	Soy deli slices	3 slices	Fat-free cheese slices	2 slices
	Whole wheat bread	2 slices	Whole wheat bread	2 slices
	Tossed salad	1.5 c	Tossed salad	1.5 c
	(mixed greens and lettuce, tomato, cucumber)		(mixed greens and lettuce, tomato, cucumber)	
	With vinaigrette		With vinaigrette	
	Olive oil	1.25 T	Olive oil	1.25 T
	Balsamic vinegar	1 T	Balsamic vinegar	1 T
	Pear	1	Pear	1
Dinner	*Stir fry:*		*Egg omelet:*	
	Extra-firm low-fat tofu	82 g (1/4 block)	Egg white and egg substitute with	150 g
	Broccoli, red peppers, and onions	1 c	Broccoli, red peppers, and onions	1c
	Butter or sunflower and safflower oil[b,c]	1/2 t	Sunflower and safflower oil[c]	1 t
	Brown rice	1.25 c	Brown rice	1.25 c
	Whole wheat toast	1 slice	Whole wheat toast	1 slice
	Cantaloupe	1 c	Cantaloupe	1 c
	Prebiotic[a] or maltodextrin	5 g	Prebiotic[a]	5 g
Snack	Whole wheat crackers	8 each	Whole wheat crackers	8 each
	Light margarine	1 T	Light margarine	1 T
	Soy beverage	1 c	Milk–skim	3/4 c

T indicates tablespoon; t, teaspoon; c, cup.
[a] Prebiotic used was Synergy1, a 50:50 mixture of inulin and oligofructose.
[b] On the soy plus prebiotic or maltodextrin treatments, butter was used 2 d/wk; and safflower and sunflower oil were used the remaining days of the week.
[c] Butter and/or vegetable oils were used to balance the fatty acid profile of the soy and dairy foods prescribed.

weight. The same menu was used for all participants; but when necessary, the menu was modified to suit individual preferences providing the goals for soy protein or for dairy and egg protein were met. All participants were instructed to weigh all other foods consumed during the study period with self-taring electronic foods scale provided (Salter Housewares, Kent, England). Compliance was assessed from the completed weekly checklists and from the return of uneaten supplement food items.

2.4. Analyses

Serum was analyzed according to the Lipid Research Clinic protocol [20] for TC, triglyceride (TG), and HDL-C after dextran sulfate–magnesium chloride precipitation in the J Alick Little Lipid Research Laboratory [21]. Low-density lipoprotein cholesterol was calculated by the method of Friedewald et al [22] in millimoles per liter (LDL-C = TC − [TG/2.2 + HDL-C]). C-reactive protein was analyzed by endpoint nephelometry (Behring BN-100, N high-sensitivity C-reactive protein reagent; Dade-Behring, Mississauga, Ontario, Canada).

Dietary isoflavone concentrations were measured as the 3 aglycones (genistein, daidzein, and glycitein) in study supplements. The samples were analyzed in duplicate using a modification of a previously described trimethylsilyl derivatization procedure for isoflavones [23].

Freeze-dried soy and control foods were analyzed using the methods of the Association of Official Analytical Chemists for fat, protein, and fiber with available carbohydrate by difference [24,25].

Bacterial populations were expressed as the $\log_{10}$ of colony-forming units per gram of fresh sample of total aerobes, total anaerobes, bacteroides, bifidobacteria, and fusobacteria [10,26].

End-expiratory samples were analyzed for breath hydrogen and methane using a Quintron gas chromatograph (Quintron Microanalyzer Model DP; Quintron, Milwaukee, WI) [9].

Diet histories were assessed for macronutrients, fatty acids, cholesterol, and fiber using a computer program based on US Department of Agriculture data [27,28].

2.5. Statistical analysis

Data analysis was conducted using SAS software Version 9.1 (SAS Institute, Cary, NC) [12]. Results are expressed as mean ± SE. Twenty-three subjects who

completed all 3 treatments were included in the statistical analyses. No significant treatment differences were observed between the sexes. Paired treatment comparisons were tested using least square means (LSM option/PROC MIXED) with mean change from baseline (ie, mean of values for all weeks postbaseline) as the response variable; the diet, sex, diet by sex, treatment sequence, and subject ID nested within sex-by-sequence interaction as main effects; and baseline as the covariate with a Tukey-Kramer adjustment for multiplicity of pairwise comparisons between all 3 treatments. Outcomes with only end values were analyzed using a similar model without baseline as a covariate.

No first-order carryover effects were seen in the data except in the comparison of prebiotic with soy alone for the apolipoprotein (apo) B to A-1 ratio ($P = .020$).

Breath hydrogen data were also analyzed using the CONTRAST statement in SAS [12] to combine the 2 prebiotic treatments in the comparison with soy alone because individually significantly higher or near significantly higher breath hydrogen levels were seen on the prebiotic treatments in individual comparisons with soy alone.

3. Results

Compliance for the protein (dairy or soy) was 99% on the prebiotic phase, 95% on soy, and 94% on the soy plus prebiotic phase. Mean compliance for the prebiotic or maltodextrin over the 4 weeks was 98% on the prebiotic, 99% on the soy plus prebiotic, and 99% on the soy plus maltodextrin (soy) phases (Table 2).

3.1. Lipids, apolipoproteins, and C-reactive protein

No between-group differences were seen in serum lipids at baseline. Adding a prebiotic to soy resulted in the largest reductions in serum lipids and their ratios (Table 5), but no significant differences were seen between the soy treatments with and without prebiotic. However, treatment reductions were observed on soy plus prebiotic compared with prebiotic alone for LDL-C: −0.18 ± 0.07 mmol/L ($P = .042$), TC/LDL-C: −0.30 ± 0.13 ($P = .065$), and LDL-C/HDL-C: −0.28 ± 0.11 ($P = .041$). Furthermore, HDL-C increased on the soy plus prebiotic compared with prebiotic (0.06 ± 0.02 mmol/L, $P = .029$). No significant differences were observed in TC and TG. On the other hand, both soy treatments improved apo B and the apolipoprotein ratio compared with prebiotic, but did not reach significance except for a greater reduction on the soy vs the prebiotic for change in apo B/apo A-1: −0.04 ± 0.02 ($P = .048$) (Fig. 2). No significant difference was observed in apo A-1 between the 3 treatments (Fig. 2). C-reactive protein was not altered among the 3 dietary treatments (Table 5).

The percentage changes reflected the absolute changes, with greater reductions in TC/HDL-C and LDL-C/HDL-C on the soy plus prebiotic compared with prebiotic alone

Table 5
Effect of study treatments on body weight, blood lipids, apolipoproteins, blood pressure, and C-reactive protein (n = 23)*,†

	Baseline			Mean treatment		
	Prebiotic alone	Soy alone	Soy plus prebiotic	Prebiotic alone	Soy alone	Soy plus prebiotic
Body weight (kg)	72.9 ± 3.1	73.1 ± 3.1	73.4 ± 3.1	72.6 ± 3.1	72.9 ± 3.1	72.9 ± 3.1
Body mass index (kg/m^2)	25.5 ± 0.5	25.6 ± 0.5	25.7 ± 0.5	25.4 ± 0.5^{ab}	25.5 ± 0.5^{a}	25.5 ± 0.5^{b}
Waist circumference (cm)	89.9 ± 2.3	89.2 ± 2.2	90.6 ± 2.3	89.4 ± 2.2	89.1 ± 2.2	89.5 ± 2.3
Satiety (−4 to 4)	0.4 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	1.0 ± 0.2
Cholesterol (mmol/L)						
TC	6.16 ± 0.20	6.17 ± 0.16	6.20 ± 0.18	6.12 ± 0.18	5.99 ± 0.17	5.97 ± 0.15
LDL-C	4.18 ± 0.16	4.16 ± 0.13	4.25 ± 0.14	4.21 ± 0.15^{a}	4.04 ± 0.13^{ab}	4.03 ± 0.12^{b}
HDL-C	1.23 ± 0.08	1.20 ± 0.06	1.19 ± 0.08	1.16 ± 0.07^{a}	1.17 ± 0.06^{ab}	1.20 ± 0.08^{b}
TG	1.66 ± 0.15	1.80 ± 0.17	1.68 ± 0.14	1.65 ± 0.10	1.73 ± 0.14	1.64 ± 0.14
Ratios						
TC/HDL-C	5.27 ± 0.22	5.40 ± 0.24	5.61 ± 0.30	5.52 ± 0.27	5.35 ± 0.22	5.35 ± 0.28
LDL-C/HDL-C	3.60 ± 0.19	3.66 ± 0.20	3.89 ± 0.25	3.84 ± 0.24^{a}	3.63 ± 0.18^{ab}	3.65 ± 0.23^{b}
Apolipoproteins (g/L)						
Apo A-1	1.57 ± 0.06	1.56 ± 0.06	1.55 ± 0.06	1.50 ± 0.05	1.55 ± 0.06	1.53 ± 0.06
Apo B	1.31 ± 0.04	1.33 ± 0.04	1.34 ± 0.05	1.33 ± 0.05	1.28 ± 0.04	1.28 ± 0.04
Apo B/apo A-1	0.86 ± 0.04	0.88 ± 0.05	0.89 ± 0.05	0.91 ± 0.05^{a}	0.85 ± 0.04^{b}	0.87 ± 0.05^{ab}
C-reactive protein (mg/L)	1.59 ± 0.35	1.81 ± 0.36	1.29 ± 0.20	1.98 ± 0.43	1.60 ± 0.27	1.41 ± 0.22
Blood pressure (mm Hg)						
Systolic	122.8 ± 2.9	122.1 ± 2.2	125.6 ± 3.3	120.9 ± 2.5	121.9 ± 2.2	122.0 ± 2.3
Diastolic	75.8 ± 1.8	76.1 ± 1.3	77.7 ± 1.9	74.8 ± 1.4	75.8 ± 1.5	75.8 ± 1.4

* Unadjusted mean values.

† Superscripts with a different letter in a row denote a significant difference. Values with a superscript letter that is the same indicate no significant difference. Significance of differences was calculated using least square means with change from baseline to the mean of all weeks postbaseline as the response variable; the diet, sex, diet by sex, treatment sequence, and subject ID nested within sex-by-sequence interaction as main effects; and baseline as the covariate, with a Tukey-Kramer adjustment ($P < .05$).

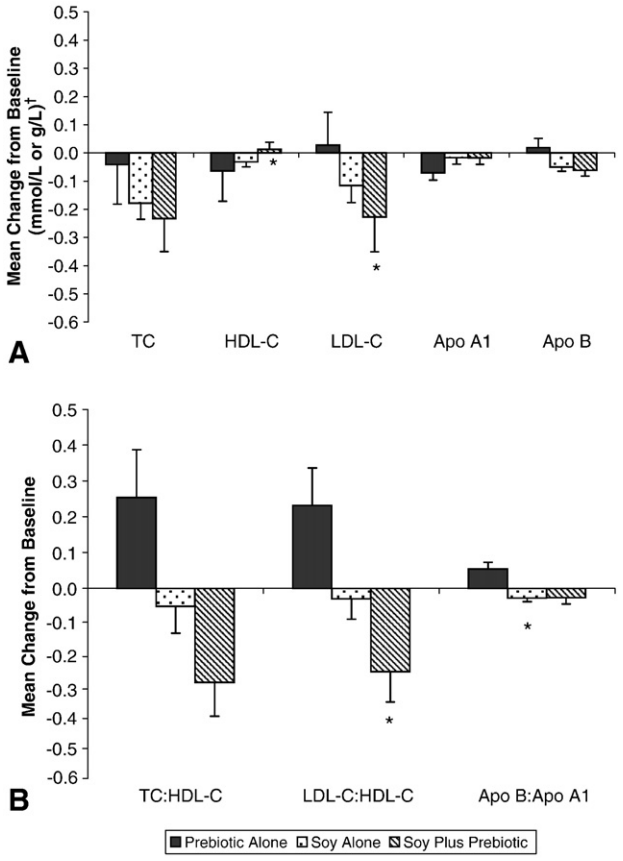

Fig. 2. Changes in lipids and apolipoproteins (A) and the associated ratios (B) (n = 23) (unadjusted mean changes). *Significantly different from prebiotic alone; change from baseline to the mean of all weeks postbaseline as the response variable; the diet, sex, diet by sex, treatment sequence; and subject ID nested within sex-by-sequence interaction as main effects; and baseline as the covariate, with a Tukey-Kramer adjustment ($P < .05$). †Units of millimoles per liter for TC, HDL-C, and LDL-C. Units of grams per liter for apo A-1 and apo B.

(−9.2% ± 2.7%, $P = .004$ and −12.2% ± 3.4%, $P = .003$, respectively). Again, the differences between the 2 soy treatments did not reach significance. The apo B/apo A-1 ratio was reduced similarly on the soy plus prebiotic and soy alone compared with prebiotic alone (−8.3 ± 2.8, $P = .015$ and −7.7 ± 2.8, $P = .027$, respectively).

3.2. Body weight, satiety, and gastrointestinal symptoms

No treatment differences were seen in body weight, satiety, bloating, or flatus. The mean change in rating of abdominal discomfort was significantly increased on the prebiotic compared with soy, but the absolute difference in rating was small (0.5 ± 0.1, $P = .008$). No other treatment differences were observed (Table 6).

3.3. Blood pressure

No significant changes were observed in either systolic or diastolic blood pressure among the 3 treatments (Table 5).

3.4. Breath gas data and fecal microbiology

No differences in mean hydrogen and methane production were observed among the 3 treatments (Fig. 3). However, the difference in breath hydrogen between prebiotic supplementation and soy alone approached significance when prebiotic alone and soy plus prebiotic data were combined ($P = .056$, CONTRAST statement in SAS). Furthermore, in the unadjusted model, the mean breath hydrogen concentration on prebiotic alone was significantly higher than on soy alone ($P = .043$); and the 2 prebiotic treatments were not different from each other ($P = .875$), although the differences between soy plus prebiotic and soy alone did not reach significance ($P = .065$). A greater increase in fusobacteria was observed on the prebiotic compared with soy (4.8 ± 1.7 $\log_{10}$ colony-forming units per gram of fresh sample, $P = .023$). No other treatment differences were seen in fecal microbiology.

4. Discussion

No differences were seen between the 2 soy treatments, but only the soy plus prebiotic showed significant lipid reductions by comparison to the prebiotic (control).

These data support the lipid-lowering basis for the current FDA health claim for soy foods. They demonstrate how a nonsignificant (~3%) LDL-C reduction seen when soy was consumed alone can be converted to a significant (~5%) LDL-C reduction when soy was taken with a prebiotic. The

Table 6
Gastrointestinal symptoms during the study treatments where the scale is 0 (none) to 6 (severe) (n = 23)*,†

	Baseline			Mean treatment		
	Prebiotic alone	Soy alone	Soy plus prebiotic	Prebiotic alone	Soy alone	Soy plus prebiotic
Scale from 0 (none) to 6 (severe)						
Bloating	0.4 ± 0.2	0.5 ± 0.3	0.6 ± 0.3	0.8 ± 0.2	0.6 ± 0.2	0.7 ± 0.2
Flatus	1.1 ± 0.3	1.4 ± 0.3	1.5 ± 0.3	1.7 ± 0.3	1.3 ± 0.2	1.8 ± 0.2
Abdominal Discomfort	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.2[a]	0.4 ± 0.2[b]	0.5 ± 0.1[ab]

* Unadjusted mean values.
† Superscripts with a different letter in a row denote a significant difference. Values with a superscript letter that is the same indicate no significant difference. Significance of differences was calculated using least square means with change from baseline to the mean of all weeks postbaseline as the response variable; the diet, sex, diet by sex, treatment sequence, and subject ID nested within sex-by-sequence interaction as main effects; and baseline as the covariate, with a Tukey-Kramer adjustment ($P < .05$).

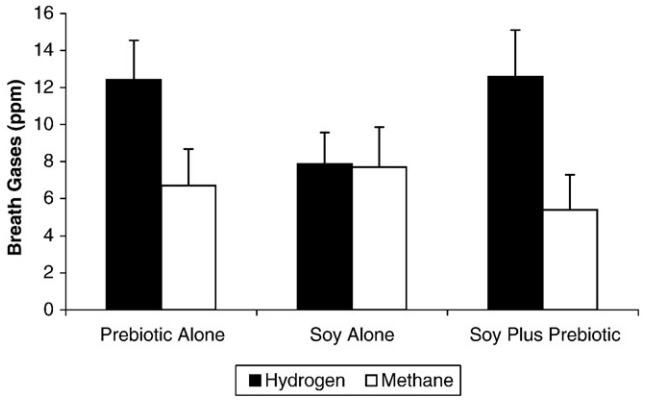

Fig. 3. Mean breath hydrogen and methane production (n = 22) (unadjusted mean values). No significant differences among treatments using least square means with end values as the response variable and the diet, sex, diet by sex, treatment sequence, and subject ID nested within sex-by-sequence interaction as main effects, with a Tukey-Kramer adjustment, $P > .05$.

nonsignificant ~3% LDL-C reduction is similar to the nonsignificant ~3% LDL-C reduction reported in the recent American Heart Association Scientific Advisory that contributed to the FDA reassessment of the value of soy as a cholesterol-lowering agent. The current study shows that although no significant difference existed between the 2 soy treatments, only when soy was taken with a prebiotic was there a significant reduction in LDL-C compared with prebiotic taken alone. Thus, the ability of soy to alter serum lipids may be influenced by other aspects of the diet that should be accounted for if effective use is to be made of diet in cholesterol reduction.

Possible cholesterol-lowering components found in soy include the soy peptides [29], saponins [30], and isoflavones [6]. To date, no link between altered colonic metabolism and enhanced cholesterol-lowering potential by soy peptides or saponins has been established. However, increased colonic metabolism has been associated with increased colonic soy isoflavone biotransformation. The resulting equol production, a metabolite of daidzein, has been suggested to be the most estrogenic isoflavone, with the ability to bind to both estrogen receptor–α and estrogen receptor–β (as selective estrogen receptor modulators) [7]. Selective estrogen receptor modulators, such as tamoxifen and raloxifene, have been shown to lower LDL-C, whereas toremifene has been shown to lower LDL-C and raise HDL-C [31].

In general, purified isoflavones isolated from soy protein have not resulted in lower cholesterol levels [32], with the exception of a high-isoflavone soy germ–enriched pasta that reduced LDL-C by 8.6% [33].

The ability of a prebiotic to enhance the cholesterol-lowering ability of soy is illustrated by a study in which resistant (nonabsorbable) starch, as the prebiotic, was given with soy [34,35]. The increase in colonic fermentation resulted in a significant reduction in TC after soy where no significant effect was seen with soy in the absence of resistant starch [34]. Nevertheless, no increase in isoflavone, specifically equol, was detected in the urine as a result of the increased colonic fermentation; and it is possible that increased colonic production of the short-chain fatty acid, propionate, which has been shown to result in lower TC and LDL-C [36], was responsible for the improved lipid profile seen when soy was taken with a prebiotic. The assessment of changes in both urinary equol production and serum propionate concentrations would be useful for future studies of soy taken with prebiotics because both equol and propionate have been associated with improved blood lipid profiles [33,36].

In the current study, changes in colonic fermentation, measured by breath gases and fecal microbiology, were not significantly altered, although numerically breath hydrogen concentrations were higher on the prebiotic treatments, as were bifidobacterial counts. Short-chain fatty acid production was not measured. For breath hydrogen, based on the observed treatment difference and standard deviation, more than 50 subjects would have been required to see a significant difference between the soy and prebiotic compared with soy alone, after adjustment for multiple comparisons. The inability to detect differences in fecal microbiology may be related to the collection method and storage before plating. However, this same method of processing did not prevent the confirmation of increased bifidobacteria with antibiotic use [37]. It is possible that changes in the colonic microflora may have occurred earlier in the supplementation period and reversion to the previous pattern of microbiota may have occurred by the end of 1 month. It has been suggested that the indigenous microflora remain rather constant over time, despite changes in dietary intake. In the current study, fecal samples were only collected at the end of the supplementation period; therefore, we cannot determine if there were any earlier changes.

These data have relevance for the design of cholesterol-lowering diets. There are implications for the heart health claim for soy that is now being reconsidered by the US FDA because of the relatively poor performance of soy in recent studies [4]. There are relatively few specific foods or food components that actively lower serum cholesterol; and for the most part, all of these have been singled out for heart health claim status by the FDA. The foods and food components are few and include soy, nuts, viscous fibers (psyllium, β-glucan in oats and barley), and plant sterols (in vegetable oils and leafy green vegetables and enriched in certain commercial margarines) [5,16-18,38]. Although individually these dietary components may only lower serum cholesterol by 5% to 10%, when incorporated into the same diet as a dietary portfolio and provided under metabolically controlled conditions, they have been shown to result in LDL-C reductions of up to 30%, similar to early statins [39]. We believe the present study therefore supports the value of soy as one of the few cholesterol-lowering foods, in the 5% reduction range, especially when given with fermentable substrates such as would be naturally

present in diets that also contained viscous fibers to lower serum cholesterol.

In conclusion, both soy treatments were effective in reducing the apolipoprotein ratio compared with prebiotic; however, in this relatively small study, a significant reduction in LDL-C and the lipid ratios was only observed with soy after coingestion of a fermentable substrate or prebiotic. The provision of fermentable substrates may be one means, possibly through colonic microbial biotransformation of lipid-lowering components including isoflavones or production of specific short-chain fatty acids, to increase the effectiveness of soy foods as part of a dietary strategy for cardiovascular disease risk reduction. The maintenance of soy's status as one of a few cholesterol-lowering foods recognized by the FDA, therefore, appears warranted.

Acknowledgment

We thank all the study participants for their attention to detail and enthusiasm. We also thank The Orafti Group for their donation of Synergy1 (prebiotic) and Loblaw Companies Limited (Toronto, Ontario, Canada), Unilever Research and Development, the Hain-Celestial Group (Melville, NY), Kraft Foods (Glenview, IL), and Burnbrae Farms Ltd (Naturegg Simply Egg Whites) (Upton, Quebec, Canada) for donation of foods used in the study. Our thanks go to Jan Van Loo and Anne Franck, The Orafti Group; Sherry Casey and Paul Uys, Loblaw Companies Limited; Gerry Amantea and Wendy Willis, the Hain-Celestial Group; and Margaret C. Martini, Kraft Foods, for their assistance on this project.

Sources of funding: This study was supported by the Heart and Stroke Foundation of Ontario and The Orafti Group.

References

[1] Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;333:276-82.

[2] Sirtori CR, Agradi E, Conti F, Mantero O, Gatti E. Soybean-protein diet in the treatment of type-II hyperlipoproteinaemia. Lancet 1977;1:275-7.

[3] Dewell A, Hollenbeck CB, Bruce B. The effects of soy-derived phytoestrogens on serum lipids and lipoproteins in moderately hypercholesterolemic postmenopausal women. J Clin Endocrinol Metab 2002;87:118-21.

[4] Sacks FM, Lichtenstein A, Van Horn L, Harris W, Kris-Etherton P, Winston M. Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for professionals from the Nutrition Committee. Circulation 2006;113:1034-44.

[5] US Food and Drug Administration. FDA final rule for food labeling: health claims: soy protein and coronary heart disease. 64 Fed Regist 1999:57699-733.

[6] Setchell KD, Cassidy A. Dietary isoflavones: biological effects and relevance to human health. J Nutr 1999;129:758S-67S.

[7] Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr 2002;132:3577-84.

[8] Setchell KD. Soy isoflavones—benefits and risks from nature's selective estrogen receptor modulators (SERMs). J Am Coll Nutr 2001 (5 Suppl):354S-62S [discussion 381S-383S].

[9] Metz G, Gassull MA, Leeds AR, Blendis LM, Jenkins DJ. A simple method of measuring breath hydrogen in carbohydrate malabsorption by end-expiratory sampling. Clin Sci Mol Med 1976;50:237-40.

[10] Rao AV, Shiwnarain N, Koo M, JDJ A. Effect of fiber-rich foods on the composition of intestinal microflora. Nutr Res 1994;14:523-35.

[11] Cummings JH, Wiggins HS, Jenkins DJ, et al. Influence of diets high and low in animal fat on bowel habit, gastrointestinal transit time, fecal microflora, bile acid, and fat excretion. J Clin Invest 1978;61:953-63.

[12] SAS Institute. SAS/STAT user's guide (ed 9.1) program. SAS Institute; 2003.

[13] Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-421.

[14] Roberfroid MB. Inulin-type fructans: functional food ingredients. Boca Raton (Fla): CRC Press; 2005.

[15] US Food and Drug Administration. Food labeling: health claims: soluble fiber from whole oats and risk of coronary heart disease. Washington, DC: US Food and Drug Administration; 2001. p. 15343-4. Docket 95P-0197.

[16] US Food and Drug Administration. Food labeling: health claims: soluble fiber from certain foods and coronary heart disease. Washington, DC: US Food and Drug Administration; 1998. Docket 96P-0338.

[17] US Food and Drug Administration. Food labeling: health claims: nuts and heart disease. Washington, DC: US Food and Drug Administration; 2003. Docket No. 02P-0505.

[18] US Food and Drug Administration. FDA authorizes new coronary heart disease health claim for plant sterol and plant stanol esters. Washington, DC: US Food and Drug Administration; 2000. Docket 001-1275, OOP-1276.

[19] Lipid Research Clinics, population studies data book II. The prevalence study: nutrient intake Washington, DC: NIH Publication No. 82-2014, U.P.H.S. Department of Health and Human Services, National Institutes of Health; 1982).

[20] Lipid Research Clinics. Manual of laboratory operations. Lipid and lipoprotein analysis (revised 1982). Washington, DC: US Government Printing Office, US Department of Health and Human Services; 1982.

[21] Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982;28:1379-88.

[22] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

[23] Thompson LU, Boucher BA, Liu Z, Cotterchio M, Kreiger N. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. Nutr Cancer 2006;54:184-201.

[24] Association of Official Analytical Chemists. AOAC official methods of analysis. Washington, DC: United States Department of Agriculture; 1980.

[25] Prosky L, Asp NG, Furda I, DeVries JW, Schweizer TF, Harland BF. Determination of total dietary fiber in foods and food products: collaborative study. J Assoc Off Anal Chem 1985;68:677-9.

[26] Onderdonk AB, Zamarchi GR, Walsh JA, Mellor RD, Munoz A, Kass EH. Methods for quantitative and qualitative evaluation of vaginal microflora during menstruation. Appl Environ Microbiol 1986;51: 333-9.

[27] Jenkins DJ, Kendall CW, Faulkner D, et al. A dietary portfolio approach to cholesterol reduction: combined effects of plant sterols, vegetable proteins, and viscous fibers in hypercholesterolemia. Metabolism 2002;51:1596-604.

[28] USDA nutrient database for standard reference, release 19. Nutrient Data Laboratory Home Page. U.S. Department of Agriculture, Agricultural Research Service; 2006.

[29] Lovati MR, Manzoni C, Gianazza E, et al. Soy protein peptides regulate cholesterol homeostasis in Hep G2 cells. J Nutr 2000;130: 2543-9.

[30] Sidhu GS, Oakenfull DG. A mechanism for the hypocholesterolaemic activity of saponins. Br J Nutr 1986;55:643-9.
[31] Riggs BL, Hartmann LC. Selective estrogen-receptor modulators—mechanisms of action and application to clinical practice. N Engl J Med 2003;348:618-29.
[32] Yeung J, Yu TF. Effects of isoflavones (soy phyto-estrogens) on serum lipids: a meta-analysis of randomized controlled trials. Nutr J 2003;2:15.
[33] Clerici C, Setchell KD, Battezzati PM, et al. Pasta naturally enriched with isoflavone aglycons from soy germ reduces serum lipids and improves markers of cardiovascular risk. J Nutr 2007;137:2270-8.
[34] Larkin TA, Astheimer LB, Price WE. Dietary combination of soy with a probiotic or prebiotic food significantly reduces total and LDL cholesterol in mildly hypercholesterolaemic subjects. Eur J Clin Nutr 2009;63:238-45.
[35] Larkin TA. Soy isoflavone bioavailability—effects of probiotic and prebiotic consumption and oil supplementation. University of Wollongong; 2005.
[36] Wolever TM, Spadafora P, Eshuis H. Interaction between colonic acetate and propionate in humans. Am J Clin Nutr 1991;53:681-7.
[37] Jenkins DJ, Kendall CW, Hamidi M, et al. Effect of antibiotics as cholesterol-lowering agents. Metabolism 2005;54:103-12.
[38] US Food and Drug Administration. Food labeling: health claims: soluble fiber from whole oats and risk of coronary heart disease. Washington, DC: US Food and Drug Administration; 2001. p. 15343-4. Docket 95P-0197.
[39] Jenkins DJ, Kendall CW, Marchie A, et al. Effects of a dietary portfolio of cholesterol-lowering foods vs lovastatin on serum lipids and C-reactive protein. JAMA 2003;290:502-10.