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Effect of a low glycemic index diet with soy protein and phytosterols on CVD risk factors in postmenopausal women

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Abstract

Objectives: Cardiovascular disease (CVD) is the leading cause of death in women. Hyperlipidemia is a major risk factor for CVD, but research suggests that metabolic syndrome and type 2 diabetes are also key factors in CVD in postmenopausal women. Most dietary programs, however, focus only on hyperlipidemia and not on insulin resistance associated with diabetes and metabolic syndrome. This 12-wk trial compared the effects of a dietary program combining a low glycemic index diet with a functional food delivering 30 g of soy protein and 4 g of phytosterols per day (LGID) with a standard dietary program (American Heart Association Step 1 diet; AHAD) in postmenopausal women.

Methods: Fifty-nine postmenopausal women (average age 54.6 y, range 44–65 y) with a body mass index of 27 to 39 kg/m^2 were randomly assigned to the LGID or the AHAD program for 12 wk. Total caloric intake and exercise were matched in each arm.

Results: Twenty-seven women completed the LGID program, and 26 completed the AHAD program. The participants on the LGID program showed statistically significant decreases in total cholesterol (15.8%, $P = 0.0036$ between-group comparison), low-density lipoprotein cholesterol (14.8%, $P = 0.004$ between-group comparison), and triacylglycerol (44.8%, $P = 0.006$ between-group comparison). In addition, significant improvements were observed in ratios of total to high-density lipoprotein cholesterol and of triacylglycerol to high-density lipoprotein cholesterol, blood pressure, and Framingham risk assessment for coronary heart disease compared with the AHAD program.

Conclusions: A significantly greater improvement was observed in CVD risk factors in postmenopausal women on the LGID program (incorporating 30 g of soy protein and 4 g of phytosterols per day) than with a standard therapy.

Keywords: Low-density lipoprotein cholesterol; Total cholesterol; High-density lipoprotein cholesterol; Triacylglycerol; Soy protein; Phytosterols; Cardiovascular disease; Cholesterol; Hyperlipidemia; Glycemic load; Glycemic index; Soy isoflavones

Introduction

Cardiovascular disease (CVD) is the leading cause of death in women, and postmenopausal women show the greatest susceptibility to CVD. A large body of evidence indicates that hyperlipidemia is a major clinical consideration in managing risk of CVD [1,2]. Recent data also indicate that factors other than increased total blood cholesterol (tChol) and low-density lipoprotein cholesterol (LDL-C) contribute to CVD. In particular, metabolic syndrome, which is characterized by a high level of triacylglycerols (TG), a low level of high-density lipoprotein cholesterol (HDL-C), hypertension, central obesity, and hyperinsulinemia, is associated with CVD [3]. Low HDL-C is also independently associated with increased risk of CVD, and cumulative data indicate that low HDL-C and an in-

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creased ratio of tChol to HDL-C are better predictors of CVD mortality in women than are increased levels of tChol and LDL-C [1,4,5].

Although diet and exercise are primary interventions for hyperlipidemia and are endorsed by organizations such as the National Cholesterol Education Program (NCEP) and the American Heart Association (AHA), the optimal dietary guidelines remain controversial [6,7]. A large body of evidence documents the relation among total fat, saturated fat, dietary cholesterol, and increased blood cholesterol. Data indicate that decreases in dietary total fat, saturated fat, and cholesterol results in decreased blood tChol and LDL-C by 9% to 13% in women with hyperlipidemia [4,5,8]. However, low-fat high-carbohydrate diets, such as the AHA Step 1 diet (AHAD), have also been shown to result in substantial decreases in HDL-C [4,8].

Unfortunately, diets that promote a low fat intake also promote increased carbohydrates and, even with a focus on complex carbohydrates, often result in an increased glycemic load (GL). Evidence is accumulating that an increased GL is contraindicated for cardiovascular health, especially for the large proportion of individuals with metabolic syndrome [9,10]. Some research suggests that low-fat high-carbohydrate diets also may not provide adequate satiety, especially in obese patients, to promote weight loss [11]. Therefore, identifying an approach that promotes healthy blood lipids, including TG and HDL-C, provides for a low GL, and generates enough satiety to prevent excess caloric intake is a critical clinical endpoint.

Specific dietary components have been shown to benefit individuals with hyperlipidemia. The efficacy of soy protein with isoflavones in lowering blood cholesterol in hyperlipidemic patients has been consistently reported. For example, a meta-analysis of 38 controlled human clinical trials indicated that significant decreases of 9% for tChol, 13% for LDL-C, and 11% TG can be obtained from an average daily intake of around 47 g of soy protein [12,13]. The U.S. Food and Drug Administration (FDA) has allowed the following food claim for soy protein: Diets low in saturated fat and cholesterol that include 25 g of soy protein a day may decrease the risk of heart disease [14]. Some data suggest that soy may have a preferential benefit in women as opposed to men [15,16]. Phytosterols have also been shown to have a statistically significant lipid-lowering effect. A review of 16 published human studies with a total of 590 subjects reported that 4 g of phytosterols per day showed an average 10% decrease in tChol and a 13% decrease in LDL-C [17]. The FDA has also authorized a food claim for phytosterols: "Food containing at least 0.65 g per serving of plant sterol esters, eaten twice a day with meals for a daily total intake of at least 1.3 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease" [18].

It is our hypothesis that addressing concerns of risk factors for hyperlipidemia and metabolic syndrome provides a comprehensive and beneficial approach to managing CVD risk in postmenopausal women. In this report, we describe a controlled, randomized, 12-wk study that compared a standard therapy for high cholesterol (AHAD) with a test program of a low glycemic index diet with 30 g of soy protein and 4 g of phytosterols per day (LGID) on CVD risk factors in postmenopausal women. The soy protein and phytosterols were provided as part of a powdered beverage that could be easily incorporated into the daily routine. Both programs also included weight management, by setting caloric intake goals to result in a 1- to 2-lb weight loss per week, and standard exercise guidelines. Satiety was measured, as were blood lipids, metabolic syndrome markers, and other relevant CVD risk markers.

Materials and methods

Subjects

The study was performed at the Functional Medicine Research Center (Gig Harbor, WA, USA) from July 2003 through January 2004. Menopausal women between 40 and 65 y of age with a blood LDL-C level of 3.36 to 5.17 mmol/L (130 to 200 mg/dL) and a body mass index of 27 to 39 kg/m^2 were recruited through newspaper, e-mail, and radio advertisements. Menopause was defined as 12 mo of spontaneous amenorrhea, as 6 mo of spontaneous amenorrhea with serum levels of follicle-stimulating hormone higher than 40 mIU/mL, or as surgical bilateral oophorectomy at least 6 wk previously (with or without hysterectomy).

Exclusion criteria included use of oral corticosteroids, oral estrogens, birth control medication, or creams or patches containing estrogen, progestin, or progesterone, supplements containing isoflavones, or cholesterol-lowering prescriptive or non-prescriptive medications (e.g., niacin, guggul lipids) in the preceding 4 wk; a history of significant liver or kidney disease (e.g., glomerulonephritis, recent or ongoing hepatitis, cirrhosis, dialysis treatment); a history of heart disease (heart attack, angina, cardiac surgery, arrhythmia, or congestive heart failure); uncontrolled hypertension (blood pressure $>$ 140/90 mmHg); diabetes mellitus; cancer or a history of cancer (with exception of skin cancer); autoimmune disease such as multiple sclerosis or rheumatoid arthritis; human immunodeficiency virus or acquired immunodeficiency syndrome; a history of serious mental illness or an episode of attempted suicide within the preceding 5 y; menstruation within the preceding 6 mo; allergy to at least one ingredient in the functional food; abnormal complete blood cell count, glucose, or kidney or liver function test.

Diet and functional food

The LGID, with 30 g of soy protein (containing 34 mg of isoflavones) and 4 g of phytosterols per day, was provided

Table 1
Nutrient profile of the soy protein and phytosterol beverage

Nutrient	Amount per serving (50 g)
Calories	160
Protein (g)	15
Carbohydrate (g)	24
Dietary fiber (g)	5
Sugars (g)	15
Fat (g)	2
Saturated fat (g)	0.5
Cholesterol (g)	0
Isoflavones† (mg)	17
Phytosterols‡ (g)	2
Sodium (mg)	170
Potassium (mg)	660
Vitamin A (IU)	1750
Vitamin C (mg)	60
Vitamin D (IU)	200
Vitamin E (IU)	11
Vitamin K (μg)	40
Vitamin B1 (μg)	750
Vitamin B2 (μg)	850
Vitamin B3 (mg)	10
Vitamin B6 (mg)	25
Vitamin B12 (μg)	30
Folate* (μg)	500
Biotin (μg)	150
Pantothenate (mg)	5
Calcium (mg)	600
Phosphorus (mg)	460
Magnesium (mg)	180
Iron (mg)	3
Zinc (mg)	9
Copper (mg)	1
Manganese (mg)	1
Selenium (μg)	35
Chromium (μg)	100
Molybdenum (μg)	75

* Provided as folic acid, L-5-methyltetrahydrofolate, and 5-formyl tetrahydrofolate

† Genistein, daidzein, and glycetein.

‡ Betasitosterol, campesterol, stigmasterol, and brassicasterol.

as part of a powdered beverage whose composition is presented in Table 1 (Metagenics, Inc, Gig Harbor, WA, USA). The LGID (including the soy protein and phytosterol beverage) was designed to provide a daily nutrient intake of 44% to 47% carbohydrates, 27% to 30% protein, 25% to 27% fat, and a total GL no higher than 65. The dietary guidelines indicated that legumes should be consumed at one serving (half cup) per day for the 1300-kcal diet (equivalent to 100 kcal from legume protein per day) and two servings of legumes per day for the 1600-kcal dietary program (equivalent to 200 kcal from legume protein per day). The dietary guidelines also suggested two to three servings (3 oz/serving) of another concentrated protein source, which could include poultry, fish, lean cuts of lamb, eggs, dairy, and soy products.

The AHAD was designed to provide less than 30% energy from fat, less than 10% energy from saturated fat, less than 300 mg of dietary cholesterol per day, 50% to 60% carbohydrate, and 20% to 30% protein. The dietary program guidelines included 6 oz of animal protein per day with the allowance of one cup of legumes to be substituted for 3 oz of animal protein per day if desired by the subject.

Dietary compliance was determined by analysis of GL from 3-d diet diaries collected at each returning visit. Diet diaries were analyzed using Genesis R&D 6.30 (ESHA Research, Salem, OR, USA), and GL was calculated as described previously [19]. Compliance for the LGID program was determined as consumption of the powdered beverage within 80% of target as determined by weight of returned canisters at each visit and a diet diary indicating a GL no higher than 65. Compliance with the AHAD program was determined as a diet dairy analysis indicating a GL of at least 70.

Study design

Potential subjects were assessed for inclusion at a screening visit (V0) that examined history, physical examination, and laboratory results (complete blood cell count, chemistry panel [Complete Medical Profile], and fasting blood lipids, i.e., tChol, LDL-C, HDL-C, and TG). If a subject met the criteria and agreed to participate, she returned for visit 1 (V1), when baseline laboratory and clinical assessments were obtained. Randomization was also performed at V1 by using a standard randomization chart (Excel, Microsoft, Redmond, WA, USA).

All subjects were counseled on their respective dietary programs, which were adjusted for a target caloric intake to achieve a weight loss of 1 to 2 lb/wk (based on booklets with dietary guidelines of 1300 to 1400 kcal or of 1600 to 1700 kcal and modified appropriately for each individual's caloric target). Target caloric intake was determined from the subject's basal metabolic rate assessed at V1, which was multiplied by an activity factor (1.375) and then decreased by 600 kcal to achieve weight loss. The basal metabolic rate was obtained by bioelectric impedance analysis measurement and calculation with the Katch-McArdle formula: *basal metabolic rate = 370 + (21.6 × lean body mass in kilograms)*. All subjects were also counseled on the importance of exercise and placed on AHA standard exercise recommendations of 150 min/wk of aerobic exercise.

Subjects returned for 2-wk (V2), 4-wk (V3), 8-wk (V4), and 12-wk (V5) visits. Throughout the study, all subjects were individually counseled by a clinician about compliance to the diet, appropriate foods, caloric intake, and physical activity. Anthropometric measurements and a brief physical examination were obtained at each visit. Blood lipids were assessed initially (V1) and at V4 and V5. Exercise compliance was also assessed at each return visit by questionnaire that rated different physical activity levels at each visit. Satiety was assessed using a 63-mm scale visual analog scale in which subjects were asked to assess feelings of hunger during the preceding 2 wk at three different times during the day; a higher score indicated more feelings of

hunger. Individual scores were averaged and then converted mathematically to a 10-cm scale score for overall satiety per subject. The Framingham score was calculated as described previously [20] by using age and relevant laboratory and questionnaire data per individual.

Laboratory assessments

All laboratory tests were performed by Laboratories Northwest (Tacoma, WA, USA). Each laboratory analysis was prequalified by using split-sample comparisons to assess variance. For trial analysis, lipids were obtained from prefrozen fasting blood samples (V1, V4, and V5) and assayed in batches. Lipids were assayed enzymatically and quantified on an Ortho Clinical Diagnostics Vitros 950IRC analyzer (Ortho-Clinical Diagnostics, Raritan, NJ, USA). LDL-C was determined directly for V0 samples and indirectly for the batch analysis using the Friedewald formula: LDL-C = tChol − HDL-C − TG/5 [21]. Hemoglobin A1c was quantified by ion exchange high pressure liquid chromatography on a Bio-Rad Variant II (Hercules, CA, USA). Glucose was assayed by a colorimetric glucose oxidase reaction and quantified on an Othro Clinical Diagnostics Vitros 950IRC analyzer. Insulin was determined using the DPC Coat-A-Count Insulin solid-phase radioimmunoassay (Diagnostics Products Corporation, Los Angeles, CA, USA). Homocysteine was assessed with a fluorescence polarization immunoassay (Abbott Axsym, Abbott Park, IL, USA). High-sensitivity C-reactive protein (hs-CRP) was determined by particle-enhanced immunonephrometry (Dade BNII, Deerfield, IL, USA).

Ethics

The study protocol was approved by the human subjects review committee of Bastyr University (Bothell, WA, USA). This study was conducted in accordance with good clinical practice International Conference on Harmonisation guidance and the ethical principles of the Declaration of Helsinki. Candidates who agreed to participate signed informed consent forms before the start of the trial. A copy of the informed consent was provided to each subject.

Statistics

Data were analyzed for within-arm comparisons and are reported as means ± standard errors or medians with 95% confidence intervals (95% CIs), which were calculated using Excel (Microsoft). Parametric data were compared using Student's two-tailed, paired *t* test with significance predetermined as $P < 0.05$. Cross-treatment comparisons by one-way analysis of variance and regression analyses were performed with JMP (SAS Institute, Cary, NC, USA). Nonnormally distributed data were log transformed before analysis.

Table 2
Initial characteristics of subjects*

	Arm 1†	Arm 2‡
Age (y)	55.6 ± 5.5	54.8 ± 5.9
Weight (kg)	84.4 ± 2.7	88.0 ± 2.3
BMI (kg/m^2)	32.5 ± 0.6	32.4 ± 0.7
Blood pressure (mm Hg)		
Systolic	126 ± 1.6	127 ± 1.8
Diastolic	84 ± 0.8	83 ± 0.9
LDL-C (mmol/L)§	4.24 ± 0.13	4.27 ± 0.10

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol

* Mean ± standard error of the mean. There were no significant differences between groups for any of the characteristics listed.

† Low glycemic index diet with a functional food delivering 30 g of soy protein and 4 g of phytosterols per day and a glycemic load no higher than 65.

‡ American Heart Association Step 1 diet (control).

§ To convert cholesterol to milligrams per deciler, multiply by 38.67.

Results

Subjects

Fifty-nine subjects qualified for selection to the trial; 30 were randomized to the LGID program (arm 1) and 29 to the AHAD program (arm 2). Both groups were matched in age, body mass index, weight, LDL-C, and blood pressure (Table 2). Of the 30 subjects assigned to the LGID, 27 completed the trial and compliance assessment indicated that 22 of these subjects (73%) had been compliant with the dietary protocol and the soy protein and phytosterol beverage (Fig. 1). Similarly, of the 29 subjects assigned to the AHAD, 26 subjects completed the trial and 20 of these subjects (69%) were compliant with the diet.

All general laboratory parameters remained constant and within the reference range for subjects (compliant and noncompliant) in both arms of the trial. Exercise was also similar between the compliant subjects on the LGID and the AHAD programs. For example, of the 22 compliant subjects on the LGID program, 16 reported performing aerobic exercise longer than 100 min/wk, 5 reported exercising 60 to 100 min/wk, and 1 reported exercising less than 60 min/wk. Of the 20 compliant subjects on the AHAD program, 15 reported performing aerobic exercise longer than 100 min/wk, 4 reported exercising 60 to 100 min/wk, and 1 reported exercising less than 60 min/wk.

Diet assessment

Analysis of diet diaries collected at each returning visit confirmed that the compliant subjects on the AHAD maintained intakes of less than 30% total energy from fat (27% energy), less than 10% of total energy from saturated fat (8% energy), and less than 300 mg/d of dietary cholesterol (180 mg/d; Table 3). This group also consumed a relatively high level of fiber (average 24.4 g/d) and a low intake of salt

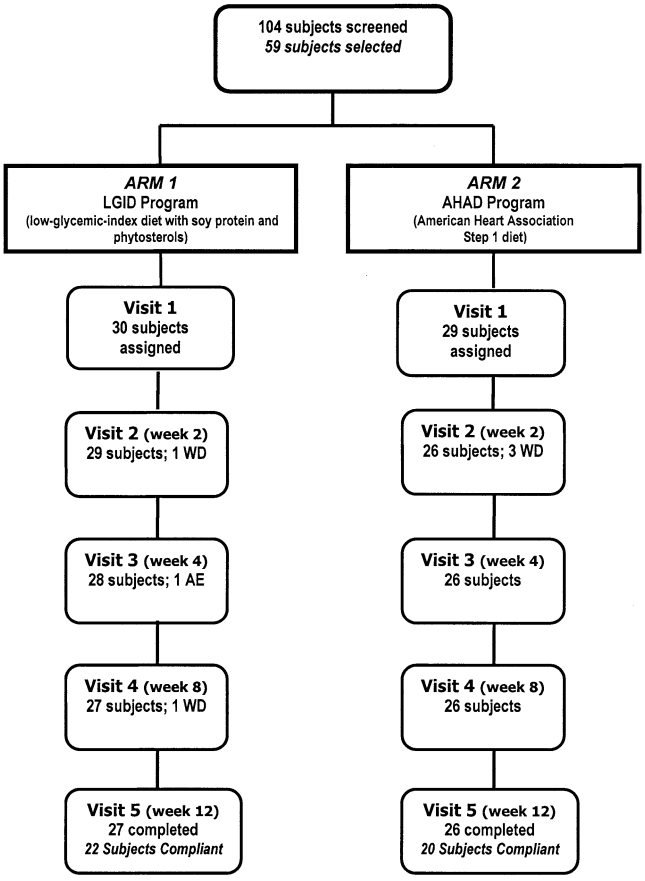

Fig. 1. Schematic of the trial. At visit 0, 104 subjects were screened and 59 were selected for the trial: 30 subjects were randomized to arm 1 (LGID program with a glycemic load lower than 65) and 29 subjects were randomized to arm 2 (AHAD control diet). Fifty-three subjects completed the trial: 27 in arm 1 and 26 in arm 2. Of the six subjects who did not complete the trial, one was WD because of starting hormone replacement therapy, four were WD for personal reasons or were lost to follow-up, and one had an AE that was determined to be unrelated to the trial. AE, adverse event; AHAD, American Heart Association Step 1 diet; LGID, low glycemic index diet with a functional food delivering 30 g of soy protein and 4 g of phytosterols per day; WD, withdrawn.

(2.0 g/d). Subjects on the LGID program were also compliant with the prescribed diet with respect to carbohydrate and GL. Fat intake was slightly higher than dietary guidelines provided, at 29% total energy (dietary guidelines allowed 25% to 27% kcal from fat). As expected, the LGID program resulted in a higher intake of protein and a lower intake of total carbohydrates than did the AHAD program. Fiber (37.3 g/d) and cholesterol (245 mg/d) intakes were also higher in the LGID group than in the AHAD group.

Caloric intake, satiety, and weight

The average preintervention prescribed caloric intake was similar for both groups (1555 kcal for LGID and 1571 kcal for AHAD), but actual caloric intake for the AHAD group was 91% of the prescribed level, whereas the LGID group consumed only 84% of prescribed calories. Both groups had begun the trial indicating that the satieties of their standard diets were similar (3.1 cm for LGID and 3.3 cm for AHAD), and even with the lower caloric intake with the LGID, both groups showed similar satieties during the trial (2.9 cm for LGID and 2.4 cm for AHAD; Table 3). Both groups also showed significant weight loss during the trial (Table 4); however, subjects on the LGID lost significantly more weight overall than did subjects on the AHAD ($P = 0.0031$ between-group comparison; Table 4).

Analysis of data for the non-compliant subjects also indicated decreases in weight of 4.6 ± 2.0% ($n = 5$) and 6.0 ± 1.7% ($n = 6$) for the LGID and AHAD groups, respectively. Those non-compliant with the AHAD showed diets with a GL lower than 70. Two of the five subjects non-compliant with the LGID program indicated diets with a GL higher than 65, whereas the other three non-compliant subjects did not consume at least 80% of the prescribed amount of the soy protein and phytosterol beverage.

Lipids

Both groups began the trial with substantially high levels of tChol, LDL-C, and TG (Table 4). After 12 wk on the program, the LGID group showed significant decreases in tChol (15.8%, $P = 0.00006$), LDL-C (14.8%, $P = 0.002$), and TG (44.8%, $P = 0.00006$), whereas the AHAD group showed no significant decrease in tChol or LDL-C. The AHAD group showed a substantial decrease in TG (23.5%); however, this change was not significant ($P = 0.06$). Be-

Table 3
Average daily nutrient intake

Nutrient	Arm 1*	Arm 2†	Difference‡
Total kilocalories	1287	1522	235
Total mean energy (% kcal)§			
Protein	29	20	−9
Carbohydrate	43	54	11
Fat	29	27	−2
SFA	7	8	1
MUFA	9	8	−1
PUFA	5	4	−1
Mean dietary cholesterol level (mg/1000 kcal)	190	118	−72
Mean dietary fiber (g/1000 kcal)	29	16	−13
Mean Sodium (g/1000 kcal)	1.4	1.35	−0.05
Mean fatty acids (g/1000 kcal)			
ω-3	0.72	0.40	−0.32
ω-6	4.43	3.0	−1.42
Satiety (10-cm scale)	2.9	2.4	−0.5

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

* Low glycemic index diet with a functional food delivering 30 g of soy protein and 4 g of phytosterols per day and a glycemic load no higher than 65.

† American Heart Association Step 1 diet (control).

‡ Difference in nutrient intake of arm 1 from arm 2.

§ Percentages may not equal 100 because of rounding.

Table 4
Cardiovascular disease risk markers for compliant subjects at baseline and weeks 8 and 12*

	Arm 1 (n = 22)†				Arm 2 (n = 20)‡				P‖
	Week 0	Week 8	Week 12	% Difference§	Week 0	Week 8	Week 12	% Difference§	
Body weight (kg)	84.5 ± 2.2	79.1 ± 2.0	77.7 ± 2.0##	−8.0	89.4 ± 2.5	86.8 ± 2.4	86.0 ± 2.4‖‖	−3.8	0.0031
Cholesterol#									
tChol (mmol/L)	7.10 ± 0.27	5.85 ± 0.23	5.98 ± 0.18§	−15.8	6.63 ± 0.25	6.32 ± 0.24	6.56 ± 0.27	−1.1	0.0036
LDL-C (mmol/L)	4.79 ± 0.20**	4.02 ± 0.16	4.08 ± 0.14§§	−14.8	4.43 ± 0.20**	4.36 ± 0.19**	4.58 ± 0.23	+3.4	0.0041
HDL-C (mmol/L)	1.25 ± 0.03	1.17 ± 0.03	1.32 ± 0.04††	+5.6	1.22 ± 0.07	1.16 ± 0.07	1.23 ± 1.18	+0.82	NS
TG (mmol/L)¶	2.39 ± 0.32	1.22 ± 0.11	1.32 ± 0.13##	−44.8	2.34 ± 0.41	1.92 ± 0.22	1.79 ± 0.21	−23.5	0.0061
Blood pressure (mmHg)									
Systolic	130 ± 1.8	123 ± 2.2	124 ± 2.8‡‡	−4.6	128 ± 2.7	127 ± 2.8	125 ± 2.1	−2.3	NS
Diastolic	84 ± 1.0	78 ± 1.4	77 ± 1.5§§	−8.3	83 ± 1.5	80 ± 1.8	78 ± 1.5‡‡	−6.0	NS

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NS, not significant; tChol, total cholesterol; TG, triacylglycerols
* Mean ± standard error.
† Low glycemic index diet with a functional food delivering 30 g of soy protein and 4 g of phytosterols per day and a glycemic load no higher than 65.
‡ American Heart Association Step 1 diet (control).
§ Difference calculated as: ([week 12 − week 0]/week 0) × 100.
‖ Repeated measures analysis of variance on log-transformed data.
To convert cholesterol milligrams per deciliter, multiply by 38.67; a cholesterol level of 120 mg/dL is equal to 3.10 mmol/L and a level of 260 mg/dL is equal to 6.7 mmol/L.
¶ To convert TG to milligrams per deciliter, multiply by 88.57; a TG level of 115 mg/dL is equal to 1.3 mmol/L and a level of 213 mg/dL is equal to 2.4 mmol/L.
** Data for week 0 for two subjects in arm 1 and those for weeks 0 and 8 for one subject in arm 2 were not available.
†† $P = 0.05$ within treatment change.
‡‡ $P < 0.02$ within treatment change.
§§ $P < 0.01$ within treatment change.
‖‖ $P < 0.001$ within treatment change.
$P < 0.0001$ within treatment change.

tween-group comparison indicated that these changes with the LGID were significantly greater than the AHAD changes for tChol ($P < 0.005$), LDL-C ($P < 0.005$), and TG ($P < 0.01$). Initial HDL-C values were similar for both groups, but the final HDL-C value was significantly increased with the LGID program (to 1.32 ± 0.13 mmol/L, $P = 0.05$) but not with the AHAD (1.23 ± 1.18 mmol/L, $P = 0.7$).

Multiple regression analyses showed no significant relations between change in weight and change in individual lipid levels (Table 5). Although not significant, stronger relations were seen between change in weight and change in tChol and TG for the LGID group and between weight change and HDL-C for the AHAD group. To more fully explore the potential role of weight loss on lipid changes, we also used the proposed equation of Poobalan et al. [22], which was derived from review of 13 independent long-term studies, to predict the lipid changes that might be expected from the observed weight loss. The slopes and intercepts derived from the published analysis were 0.032 and 0.071 mM/L for tChol, 0.02 and −0.001 mM/L for LDL-C, and 0.02 and 0.05 mM/L for TG, respectively. Table 6 lists the predicted changes in lipids from the observed weight loss using these relations. As shown, the change in tChol with the AHAD program was similar to the predicted level, whereas the change with the LGID program was nearly an order of magnitude greater than predicted.

Table 5
Correlation results for weight loss and changes in lipid values*

	LGID	AHAD‡
tChol	0.096 (22)	0.017 (20)
LDL-C	0.038 (20)	0.001 (19)
HDL-C	0.017 (22)	0.084 (20)
TG	0.087 (22)	0.031 (20)

AHAD, American Heart Association Step 1 diet (control); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LGID, low glycemic index diet; tChol, total cholesterol; TG, triacylglycerols
* Values are r^2 (number of subjects). No correlations were significant at $P < 0.05$.

CVD risk assessment

The tChol/HDL-C and TG/HDL-C ratios have been proposed as markers for CVD risk and the metabolic syndrome, respectively. Due to decreases in TG and tChol and the significant increase in HDL-C, it was not surprising that subjects on the LGID showed significant decreases in tChol/HDL-C (from 5.77 ± 0.25 to 4.59 ± 0.17, $P < 0.0001$) and TG/HDL-C (from 4.61 ± 0.70 to 2.38 ± 0.29, $P < 0.0001$). The tChol/HDL-C ratio did not significantly change for the

Table 6
Predicted changes in lipid parameters as a factor of weight change using the equations from Poobalan et al.[22] compared with actual changes observed in the trial

	LGID		AHAD	
	Predicted	Actual	Predicted	Actual
tChol (mM/L)	−0.15 ± 0.02	−1.12 ± 0.23	−0.04 ± 0.02	−0.07 ± 0.24
LDL-C (mM/L)	−0.14 ± 0.01	−0.65 ± 0.19	−0.07 ± 0.01	+0.14 ± 0.19
TG (mM/L)	−0.009 ± 0.01	−1.08 ± 0.22	−0.02 ± 0.01	−0.55 ± 0.28

AHAD, American Heart Association Step 1 diet (control); LDL-C, low-density lipoprotein cholesterol; LGID, low glycemic index diet; tChol, total cholesterol; TG, triacylglycerols

AHAD group (from 5.66 ± 0.31 to 5.47 ± 0.25, $P = 0.36$), and the change in the TG/HDL-C ratio approached, but did not reach, significance (from 5.11 ± 1.15 to 3.55 ± 0.47, $P = 0.06$).

The Framingham risk score for coronary heart disease was determined for subjects in each group as described previously [20]. Subjects in both groups had similar scores at initiation of the trial (group 1: median 10.0, 95% CI 8.8 to 11.2; group 2: median 10.0, 95% CI 8.6 to 11.5). After the intervention, however, subjects on the LGID program showed a much lower risk (median 6.0, 95% CI 4.4 to 7.6) compared with the AHAD group (median 9.0, 95% CI 7.9 to 10.1; Fig. 2).

Other metabolic risk markers

Fasting blood glucose levels were not substantially increased for either group before the trial and remained constant at week 12; however, fasting insulin decreased significantly with the LGID program compared with the AHAD (Table 7). Hemoglobin A1c, another marker of abnormal blood glucose, also decreased significantly with LGID. Homocysteine levels were seen to moderately decrease in the LGID group, and hs-CRP also showed a significant decrease with the LGID, from 4.99 mg/L (95% CI 2.59 to 7.39) to 3.31 mg/L (95% CI 1.75 to 4.37, $P = 0.055$). The hs-CRP value for the AHAD group remained relatively constant; however, the two groups did not begin the trial with comparable hs-CRP values.

Discussion

Although hyperlipidemia is a well-understood risk factor for CVD, it appears that for women, particularly women older than 65 y, a low HDL-C level (<1.29 mmol/L or 50 mg/dL) is a stronger predictor than a tChol level higher than 6.21 mmol/L (240 mg/dL) or an LDL-C level higher than 4.14 mmol/L (160 mg/dL) [23]. Further, data suggest that each 1% increase in HDL-C is associated with a 2% to 3% decrease in death or myocardial infarction [24,25]. In our study, the LGID program resulted in a 5.6% increase in HDL-C over 12 wk, which corresponds to a 11% to 17% decrease in risk by this relation. Not only did the LGID program result in an increase in HDL-C, it also resulted in significant decreases of 45% in absolute TG levels and 42% in the TG/HDL-C ratio. The TG/HDL-C ratio is an important, if underutilized, marker of the metabolic syndrome, which is also correlated with increased risk for CVD in women [26,27]. Although we did not initially assess the subjects for metabolic syndrome by the NCEP criteria, the decrease in the TG/HDL-C ratio suggests that CVD risk was lowered in women on the LGID program.

Epidemiologic evidence over the past 5 y indicates that foods high on the glycemic index are associated with lower HDL-C levels, higher TG levels, and an increased risk for CVD [28–32]. Data with low GL (low glycemic index) diets promoting weight loss have suggested a low GL diet alone can result in substantial decreases in TG but do not support substantial increases in HDL-C. Therefore, the significant effect on both lipid factors is likely due to more than just the dietary changes alone. The AHAD program did not

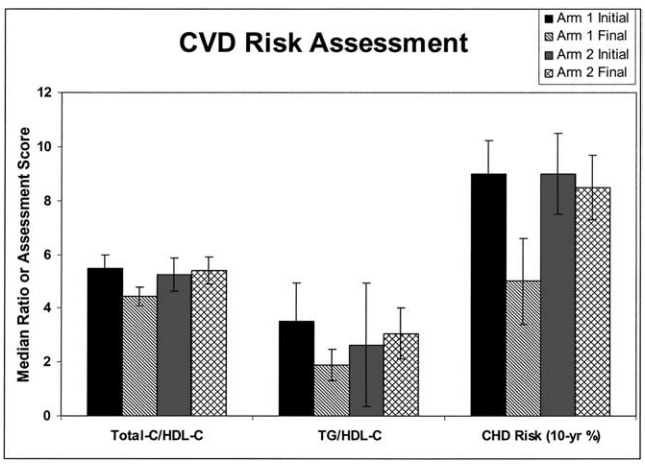

Fig. 2. Median Total-C/HDL-C and TG/HDL-C ratios and Framingham CHD risk assessment score with 95% confidence intervals are shown for the LGID with soy protein and phytosterols at baseline (closed bars) and after 12 wk on the program (slashed bars) and for the AHAD control diet at baseline (open bars) and after 12 wk (cross-hatched bars). CHD risk assessment was calculated using the Framingham cardiovascular risk equation as described. AHAD, American Heart Association Step 1 diet; CHD, coronary heart disease; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LGID, low glycemic index diet with a functional food delivering 30 g of soy protein and 4 g of phytosterols per day; TG, triacylglycerols; Total-C, total cholesterol.

Table 7
Observation data for blood glucose and insulin markers, homocysteine, and C-reactive protein for compliant subjects at baseline and week 12*

	Arm 1 (n = 22)†			Arm 2 (n = 20)‡		
	Week 0	Week 12	P	Week 0	Week 12	P
Fasting glucose (mmol/L)§	5.23 ± 0.16	5.03 ± 0.11	0.14	4.97 ± 0.12	5.06 ± 0.12	0.46
Fasting insulin (pmol/L)‖	59.48 ± 6.60	44.20 ± 5.67	0.0096	59.12 ± 6.67	53.10 ± 6.46	0.16
Hemoglobin A1c (proportion total hemoglobin)	0.0559 ± 0.009	0.0540 ± 0.008	0.006	0.0547 ± 0.010	0.0539 ± 0.011	0.19
Homocysteine (μmol/L)	8.02 ± 0.38	7.65 ± 0.31	0.09	8.51 ± 0.26	8.75 ± 0.29	0.17
C-reactive protein (mg/L)#	4.99 ± 1.20	3.31 ± 0.53	0.05	3.02 ± 0.58	2.80 ± 0.48	0.62

* Mean ± standard error.
† Low glycemic index diet with a functional food delivering 30 g of soy protein and 4 g of phytosterols per day and a glycemic load no higher than 65.
‡ American Heart Association Step 1 diet (control).
§ To convert to milligrams per deciliter, divide by 0.05551; the reference range is 3.9 to 6.1 mmol/L.
‖ To convert to micro international units per milliliter, divide by 7.175; the reference range is 21.53 to 215.25 pmol/L.
Assayed as high-sensitivity C-reactive protein.

improve HDL-C. Published studies have shown that a low-fat high-carbohydrate diet alone can lead to substantial decreases of approximately 4% to 8% in HDL-C in postmenopausal women [4,8]. Taken together with the decreased HDL-C, which is commonly observed with the AHAD diet, these data continue to suggest that the LGID program is more successful in decreasing laboratory values associated with CVD risk.

Although published data have indicated that the AHAD can promote a decrease in high blood lipids, we observed no significant change in tChol or LDL-C. We cannot explain the failure of this dietary approach to lower LDL-C in this study. It has been noted by several researchers that the magnitude of change in blood lipids is related to the initial values. Using the correlation presented by Nicklas et al. [4], we would predict the AHAD and weight loss to lead to an average decrease in LDL-C of 0.5 mmol/L for the AHAD group. Given this change, the LGID program still resulted in a 3.5% greater decrease in LDL-C than published values.

Regression analysis suggested only a small contribution of weight loss to lipid change; however, too few subjects may have been in this study to adequately assess for this relation. Our data are similar to those of others, in which a much stronger relation was noted between waist-to-hip ratio or waist circumference and change in blood lipids [33,34]. Poobalan et al. [22] explored the relation between change in weight and change in blood lipids seen in long-term studies and, using an optimal weighted least squares approach, developed a model to predict long-term blood lipid changes with weight change. Using this approach, the changes observed with the LGID program cannot be explained by weight loss alone. Moreover, short-term studies have suggested that a decrease in tChol of 10% may be expected for every 10 kg of weight loss, which is consistent with the observed changes seen with the AHAD (average of 11.72% decrease in tChol per 10 kg of weight loss). However, an average of 20.9% decrease in tChol was seen for every 10 kg of weight loss with the LGID program. These studies have indicated that the distribution of body fat is an important factor and a more relevant marker than body mass index or weight alone and implicates metabolic changes over mere total amount of fat as contributing to the health benefits of the dietary program.

Recent discussions on the most efficacious lifestyle interventions, such as the Therapeutic Lifestyle Change (TLC) approach, suggest the addition of specific foods such as phytosterols and soy protein in addition to dietary changes for successful non-pharmacologic intervention of hyperlipidemia [35]. Moreover, the differing mechanisms of action of phytosterols and soy proteins suggest they may be additive or even synergistic: phytosterols primarily decrease cholesterol absorption and soy protein decreases hepatic cholesterol synthesis and increases LDL receptor production. Cicero et al. [36] explored this concept by providing 8 g of soy protein and 2 g of phytosterols as a single dose for 40 d and observed a 15% decrease in blood LDL-C in subjects with hypercholesterolemia. In another combination study, soy protein (16.2 g/1000 cal), phytosterols (1.2 g/1000 cal), viscous fibers (8.3 g/1000 cal), and almonds (16.6 g/1000 cal) were compared with the NCEP Step 2 diet for effect on blood lipids in subjects with hyperlipidemia [37]. In this study, significant decreases of 26.6% tChol and 35.0% LDL-C were observed with the combination diet, whereas the NCEP Step 2 diet resulted in decreases of 9.9% and 12.1% of tChol and LDL-C, respectively. Soy protein and phytosterols were provided at therapeutic levels, but the diet was very low in saturated fat (4% to 5% intake), and the influence of different fibers and the presence of therapeutic levels of almonds is not known. We observed effects of 16% and 15% decreases in tChol and LDL-C, respectively. Because phytosterols and soy protein alone have been suggested to account for a 6% to 15% decrease in these lipids, we cannot assess whether these components provided an additive or synergistic effect. Neither substance has been shown to be effective for high TG; however, studies with soy protein have shown a moderate benefit on HDL-C (averaging ~2% to 3% increase). Given these observations, it is likely that in our study the soy protein and phytosterol beverage was largely responsible for the decreases in LDL-C and tChol and likely contributed to the increase in

HDL-C. In addition, the convenience of a beverage may increase compliance with such an approach. Therefore, a comprehensive diet and targeted phytonutrient intake with a supplement may be more beneficial for overall CVD risk than dietary changes alone.

Recently, the AHA Nutrition Committee has adopted the Step 2 diet, which is similar to the AHAD (Step 1 diet) in promoting decreased total fat (<30%), saturated fat (<7%), and dietary cholesterol (<200 mg/d) [6]. Because these diets result in an increased consumption of simple carbohydrates, the guidelines also include promotion of fiber (≥25 g/d). In addition, these recent guidelines promote a diet with energy restrictions to promote weight losses of 1 to 2 lb/wk. Although we used the AHAD for our primary dietary guidelines, analysis of actual diet diaries in the compliant subjects showed that these individuals met the criteria for the TLC/Step 2 diet, with the exception of only a slightly higher saturated fat intake. Therefore, it is likely that the changes we observed would be similar to using a Step 2 approach.

Although both groups received the same caloric guidelines, the LGID program resulted in a lower daily caloric intake with a similar level of satiety to the AHAD and to the predietary intervention experience. There is evidence that a low GL/glycemic index diet may result in increased satiety; therefore, the decreased caloric intake with similar satiety levels in the LGID compared with the AHAD may be explained by this mechanism. Many problems that occur in weight-loss programs arise from an inability of the dietary programs to produce satiety. We often hear from patients on high GL/glycemic index dietary programs about the difficulty in staying on such programs and the LGID may be an easier program to implement. For example, the LGID does not limit fat intake but eliminates full-fat dairy, red meat, and processed meats, which results in decreased intake of saturated fat. Moreover, although both programs promote fruits and vegetables, the beverage provided as part of the LGID provided a consistent intake of 10 g/d of fiber. These factors may have contributed to the increased satiety per caloric intake seen with the LGID. If satiety is an important parameter in maintaining a dietary program, the LGID may make it easier for women to engage in such a weight-loss program.

We calculated the Framingham score for both groups of women. Because the Framingham score takes into account blood pressure, blood sugar, hyperlipidemia, and other factors, it is a more comprehensive assessment of risk for coronary heart disease [20]. The Framingham score also indicated a greater decrease in relative risk for coronary heart disease with the LGID program. In the LGID program, 68% of subjects were initially at the moderate to high level of relative risk, and after the intervention this number decreased to 18% of subjects. In the AHAD, 45% of subjects had initial moderate to high risk, which was decreased to 35% of subjects after 12 wk.

In conclusion, the LGID program resulted in significant decreases in TG, LDL-C, and tChol, with a concurrent increase in HDL-C, even though the dietary cholesterol was higher with the LGID than with the AHAD. This observation further supports the observation that dietary cholesterol is not the primary factor in increased blood lipids. Moreover, based on established risk markers, the LGID program appeared to substantially decrease the risk of CVD in this group of postmenopausal women. Therefore, the LGID program may provide a viable option for postmenopausal women who have concerns of CVD, hyperlipidemia, and the metabolic syndrome.

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