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Phytosterol-deficient and high-phytosterol diets developed for controlled feeding studies

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Abstract

Phytosterols reduce cholesterol absorption and low-density lipoprotein (LDL) cholesterol concentrations, but the quantity and physiological significance of phytosterols in common diets are generally unknown because nutrient databases do not contain comprehensive phytosterol data. The primary aim of this study was to design prototype phytosterol-deficient and high-phytosterol diets for use in controlled feeding studies of the influence of phytosterols on health. A second aim was to quantify the phytosterol content of these prototype diets and three other diets consumed in the United States. This study was conducted from June, 2001 to September, 2008 and involved designing, preparing, and then analyzing five different diets: an experimental phytosterol-deficient ‘control’

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diet, a relatively high-phytosterol diet based on the Dietary Approaches to Stop Hypertension (DASH) diet, American Heart Association (AHA) diet, Atkins® lifetime maintenance plan, and a vegan diet. A single day of meals for each diet was homogenized and the resulting composites were analyzed for free, esterified, and glycosylated phytosterols by gas chromatography. Independent samples t tests were used to compare the diets' total phytosterol content. The total phytosterol content of the experimental phytosterol-deficient diet was 64 mg/2000 kcal, with progressively larger quantities in Atkins®, AHA, vegan, and the high-phytosterol DASH diet (163, 340, 445 and 500 mg/2000 kcal, respectively). Glycosylated phytosterols, which are often excluded from phytosterol analyses, comprised $15.9 \pm 5.9\%$ (mean $\pm$ SD) of total phytosterols. In summary, phytosterol-deficient and high-phytosterol diets that conform to recommended macronutrient guidelines and are palatable can now be used in controlled feeding studies.

Keywords

cholesterol; glycosylated phytosterol; DASH diet

INTRODUCTION

Phytosterols are plant sterols and stanols that reduce cholesterol absorption (1–4) and blood cholesterol concentrations (5,6). These benefits form the basis of the National Cholesterol Education Program recommendation to add 2 g/day of phytosterols to the diet to reduce low-density lipoprotein (LDL) cholesterol concentrations and coronary heart disease risk (7). The therapeutic potential of naturally-occurring dietary phytosterols was demonstrated by Jenkins et al. (8), who showed that an experimental plant-based diet that was naturally high in phytosterols dramatically reduced LDL-cholesterol concentrations within one week.

Although dietary phytosterols are recognized as a natural constituent of many foods (9–16), little is known regarding the phytosterol content of diets commonly consumed by Americans because nutrient databases do not contain comprehensive phytosterol data. The United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference (Release 21) (17) contains phytosterol values for only 524 of its 7,412 foods (7.1%), and many of these values are underestimates because only three major sterols (sitosterol, campesterol, stigmasterol) are typically included in the totals, despite the contribution of other sterols (eg, avenasterol). While sitosterol is the predominant phytosterol in most plant foods and campesterol is often second in abundance, plants always contain a mixture of phytosterols, and the contribution of other sterols can be significant, particularly in nuts and seeds (10).

Another concern with the relatively few phytosterol values that are available in the nutrient database is that the standard methodology for quantifying phytosterols assesses free and esterified phytosterols, but not glycosylated phytosterols that have an attached glucose which must be liberated before quantification (18). As shown in Table 1, phytosterols are present in the glycosylated form in some foods, while the free and esterified forms are ubiquitous in plants. The role of phytosterols in plant membranes is similar to the function of cholesterol in mammalian cell membranes, but the specific role of glycosylated phytosterols is unknown. Preliminary evidence indicates that glycosylated phytosterols are biologically active (19).

The potential health benefits of dietary phytosterols are significant, but their therapeutic role cannot be quantified accurately without comparing a high-phytosterol diet to a control diet that is deficient in phytosterols. A phytosterol-deficient control diet would enable a dose-response relationship to be determined for phytosterols at various intake levels or supplement doses. Because phytosterols are present naturally in many common foods, a phytosterol-deficient diet can only be achieved by specially formulating recipes and purifying vegetable oils to remove

the naturally-occurring phytosterols. A high-phytosterol diet can be achieved naturally with careful planning and sufficient knowledge of the phytosterol content of individual foods. Therefore, the primary aim of this study was to design a phytosterol-deficient diet and a high-phytosterol diet for use in controlled feeding studies of the influence of phytosterols on health. A second aim was to quantify the phytosterol content of these prototype diets and three other diets consumed in the United States.

METHODS

Study Design

The study was carried out in two phases between July, 2001 and September, 2008. The first phase involved designing two diets that could be used in controlled feeding studies: an experimental phytosterol-deficient diet to serve as a control diet, and a high-phytosterol diet to serve as an intervention diet to evaluate the effects of dietary phytosterols on health. As part of this phase, it was necessary to test a variety of individual foods and beverages for phytosterol content. The second phase involved preparation of a single-day of meals for five different diets, followed by analytical quantification of the phytosterols in each diet using gas chromatography. The following five diets were evaluated: 1) the experimental phytosterol-deficient control diet; 2) the high-phytosterol diet that was based upon the Dietary Approaches to Stop Hypertension (DASH) diet (20,21); 3) the American Heart Association (AHA) diet that was revised in 2000 (22); 4) the AtkinsR “Lifetime Maintenance” low-carbohydrate diet plan (23); and 5) a vegan diet that contained soymilk, soybean- and vegetable-based meat alternatives in place of dairy products, meat, poultry, seafood, and eggs. This study was approved by the Washington University Human Studies Committee.

Phytosterol-Deficient Diet

In order to design the experimental phytosterol-deficient diet, it was necessary to identify and minimize foods with high phytosterol concentrations. Phytosterol values of individual foods were derived from the USDA database (Release 16, 16-1, and 17) (24), published studies (12–14,16,25), and phytosterol analyses performed using gas chromatography/mass spectrometry in the Mass Spectrometry Resource at Washington University School of Medicine (26). Because wheat and whole rice are relatively rich sources of phytosterols, gluten-free recipes were used to make biscuits and muffins, but were adapted to contain rice flour that was made by milling white Minute® Rice three times with a milling attachment on a KitchenAid® mixer (St. Joseph, MI). To lower the phytosterol content even further, these recipes contained Crisco® shortening that was heated to 72°C until melted, and then purified to remove 73% of its phytosterols by a purification process described previously (3). In addition, the meal plan contained high-oleic safflower oil that was purified to remove 86% of its phytosterols (3). Based upon the previous finding that 150 mg of phytosterols inhibits cholesterol absorption by 12% (2), the goal in the current study was to create a palatable diet comprised of common foods, but which contained minimal phytosterols that would not influence cholesterol absorption.

High-Phytosterol DASH Diet

The high-phytosterol diet was modeled after the DASH diet (21), which is recommended in the 2005 *Dietary Guidelines for Americans* (27) due to its documented health benefits (28–30). This high-phytosterol DASH diet had a nutrient profile similar to the combination diet that was reportedly consumed by participants in the DASH trial (20) (ie, containing fruits, vegetables, fiber, and whole grains, with limited saturated fat, total fat, trans fatty acids, cholesterol, sodium and added sugars). The phytosterol content of this diet was enhanced by incorporating wheat germ (3), canola oil, and other high-phytosterol foods.

Preparation of Diet Composites

A one-day meal plan for each diet was prepared in the metabolic kitchen of the Washington University General Clinical Research Center. All foods and beverages were purchased in local grocery stores, and each item was weighed on a digital scale (Acculab model VI-4800, Edgewood, NY) to the nearest 0.1 g. The foods were prepared as specified in Table 2, and the contents of each meal plan were homogenized with water (70–120 ml) in a Waring® blender (model CB15, Waring Pro® Torrington, CT). Aliquots (25 g) of each diet composite were transferred to 50-ml conical plastic tubes (Fisherbrand, Fisher Scientific, Pittsburgh, PA) and stored at –70C. Two sets of aliquots were shipped (overnight) on dry ice to Virginia Polytechnic Institute and State University and stored at –70C until analysis.

Determination of Phytosterols

A 2–5 g aliquot of each diet composite was taken from the larger (25 g) aliquots and assayed in duplicate for the quantification of phytosterols at Virginia Polytechnic Institute and State University. Two analytical methods were used: 1) alkaline hydrolysis—the standard single hydrolysis method (31) that quantifies free and esterified phytosterols; and 2) acid hydrolysis followed by alkaline hydrolysis—the double hydrolysis method (32) that enables recovery of glycosidic phytosterols in addition to free and esterified phytosterols. Lipid extracts of the hydrolysates were derivatized to trimethylsilyl derivatives and analyzed by gas chromatography, as described previously (32).

The individual sterols quantified were sitosterol, campesterol, stigmasterol, brassicasterol, sitostanol, campestanol, and $\Delta 5$ -avenasterol. The total phytosterol content of each diet, including glycosides, was computed as the sum of the individual phytosterols determined by the double hydrolysis method. Because $\Delta 5$ -avenasterol is acid labile (33), total $\Delta 5$ -avenasterol in each diet was computed as the $\Delta 5$ -avenasterol determined by single hydrolysis plus the $\Delta 5$ -avenasterol glucoside measured by direct analysis (32). Phytosterol values were expressed as mg/100g and then converted to mg/2000 kcal for comparison between diets. The percentage standard deviation of replicate phytosterol analyses of a control diet composite analyzed with each set of samples was computed as an indicator of analytical quality control. The difference between total phytosterols determined using acid plus alkaline hydrolysis and phytosterols determined from alkaline hydrolysis alone provided an estimate of the total glycosylated sterol content.

Nutrient Composition of Diets

Nutrition Data System for Research (NDSR) software version 5.0 (2004, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN) was used to determine the total energy content and nutrient composition of each diet. Information for foods that were not in the NDS-R database was obtained by request from the Nutrition Coordinating Center, and these items were added to the database.

Statistical Analysis

The total phytosterol content of the phytosterol-deficient and high-phytosterol diets was compared to each of the other diets using independent samples t tests in SPSS (version 17.0, SPSS Inc, Chicago, IL). The tests were 2-tailed with equal variances not assumed. Significance was accepted at $P < 0.05$.

RESULTS

Diet Composition

Table 2 shows the individual menu items in each diet composite. By design, the macronutrient and micronutrient composition of the diets differed, as shown in Table 3. The phytosterol-deficient, high-phytosterol DASH, AHA, and vegan diets generally conformed to the 2005 *Dietary Guidelines for Americans* (27) with respect to total fat, saturated fat, cholesterol, and sodium, but the phytosterol-deficient diet was low in fiber. The vegan diet was lowest in cholesterol due to the exclusion of animal products; the Atkins® diet had the least carbohydrate and the most fat and cholesterol.

Total Phytosterol Content

The high-phytosterol DASH diet contained the highest concentration of phytosterols, with progressively smaller quantities in the vegan, AHA, Atkins®, and phytosterol-deficient diets (Table 3). The high-phytosterol DASH diet contained 1.5 times more phytosterols than the AHA diet, 3.1 times more than the Atkins® diet, and 7.8 times more than the phytosterol-deficient diet.

Contribution of Individual Phytosterols

Quantitatively, sitosterol was the most abundant phytosterol, comprising an average of 64% of total phytosterols in the five diets (range: 60% in the phytosterol-deficient diet to 69% in the DASH diet). Campesterol and stigmasterol comprised 16% and 10% of total phytosterols, respectively, while sitostanol, campestanol, and $\Delta 5$ -avenasterol collectively contributed 10%.

Contribution of Glycosylated Phytosterols

Glycosylated phytosterols comprised an average of $15.9 \pm 5.9\%$ of total phytosterols in the five diets, with the highest contribution in the high-phytosterol DASH (108 mg/2000 kcal, 21.8%) and AHA (69 mg/2000 kcal, 20.3%) diets. The absolute quantities of glycosylated phytosterols in the vegan, Atkins®, and phytosterol-deficient diets were 45, 14, and 12 mg/2000 kcal, respectively; the relative contributions were 10.0, 8.7, and 18.6%, respectively.

DISCUSSION

The major outcome of this study is the design of phytosterol-deficient and high-phytosterol diets for use in controlled diet studies. A phytosterol-deficient background diet is essential for evaluating the physiological significance of low-dose phytosterol supplements or of naturally-occurring phytosterols in common diets. Although it was not possible to create a phytosterol-free diet, the use of wheat-free muffin and biscuit recipes and purified vegetable oils enabled a significantly lower phytosterol content relative to the other diets while maintaining palatability, making this an ideal control diet for phytosterol studies. The high-phytosterol DASH diet can serve as a prototype intervention for studies evaluating the role of naturally-occurring dietary phytosterols on health. Another novel outcome of this study is the analytical quantification of naturally occurring dietary phytosterols, including glycosylated phytosterols, in American Heart Association, Atkins®, and vegan diets.

The high-phytosterol DASH diet in the present study contained more than double the quantity of phytosterols than has been reported previously for the DASH diet (34,35). Most (34) calculated the phytosterol content of the DASH diet to be 94 mg/2000 kcal using the USDA nutrient database (Release 16) (24), whereas analytical quantification by Phillips et al. (35) revealed a phytosterol content of 214 mg/2000 kcal in a seven-day diet composite from the DASH trial. The low calculated value (34) is attributable to the paucity of phytosterol values in the nutrient database, and to the phytosterol underestimates from the standard analytical

methodology that was employed. As shown by results of the present study (Table 3) and trends reported for individual foods (15,32), glycosylated phytosterols contribute meaningfully to total phytosterols in mixed diets, necessitating the use of the double hydrolysis methodology rather than the standard single hydrolysis method that does not capture this form of phytosterols. Foods that contain a relatively high proportion of glycosylated phytosterols include nuts, seeds, legumes, fruit, vegetables, and whole grains (32). Based upon recent evidence that sterol glycosides cause a 38% reduction in cholesterol absorption in humans (19), which is comparable to the effect demonstrated for sitostanol (36), soy stanols (37), corn oil (2) and wheat germ (3), the quantification of glycosylated phytosterols is important.

Development of a high phytosterol diet that conforms to the DASH guidelines was feasible by incorporating foods that are naturally rich in phytosterols. Foods containing the highest concentration of naturally occurring phytosterols include oils (wheat germ oil, rice bran oil, corn oil, canola oil), seeds (sesame seed, sunflower seed), nuts (pistachio, pine nut, almond), whole grains such as wheat germ, bran (rice bran, corn bran, wheat bran), flour (soy flour, buckwheat flour, rye flour, whole wheat flour), and soybeans (9,10,12). Fruits and vegetables, while relatively low in phytosterols by weight (11,14), enhanced the phytosterol content of the high-phytosterol DASH diet due to their relative abundance.

The AHA and Atkins® diets in the current study are representative of phytosterol intakes in the general American population, ranging from 163 to 340 mg/2000 kcal, or 204 to 425 mg/d for adults consuming 2500 kcal/d. In comparison, a 1984 study revealed phytosterol intakes as low as 78 mg/d in the general U.S. population, and 344 mg/d among Seventh-day Adventists who were lacto-ovo vegetarians (38). However, these may be underestimates due to the older analytic method used. Reported phytosterol intakes in Finland, the Netherlands, Spain, Japan, and Mexico fall within a similar range of 237 to 400 mg/d (i.e., 270 to 335 mg/2000 kcal) (39–43). The highest dietary phytosterol content was reported for an experimental Myocene diet that was designed to mimic that of the earliest humans and contained 774 mg/2000 kcal due to a very high volume of vegetables, fruits, and nuts (8,44). Collectively, these results suggest that dietary phytosterol intake comparable to the 500 mg/2000 kcal observed in the high-phytosterol DASH diet of the present study is relatively uncommon in the absence of supplementation, but can be attained with careful planning and knowledge of the phytosterol content of individual foods. This phytosterol level is approximately 25%–30% of the 2 g/d therapeutic phytosterol dose recommended by the National Cholesterol Education Program (7).

The primary limitation of the present study is that each diet composite was comprised of meals from a single day rather than multiple days. Because foods with similar macronutrient profiles can differ markedly with respect to phytosterol content, the selection of different foods as part of a five-day menu cycle may shift the total phytosterol level higher or lower than the quantities achieved in this study.

CONCLUSIONS

This is the first study to introduce a phytosterol-deficient diet and a high-phytosterol DASH diet, which will enable controlled diet studies to be conducted on the influence of dietary phytosterols on cholesterol metabolism and health. Results of the analytical quantification of phytosterols in these and three other diets illustrate the important contribution of plant foods to phytosterol intake. In particular, minimally processed whole grains, non-hydrogenated vegetable oils, seeds, nuts, fruits, and vegetables are the richest sources of naturally-occurring phytosterols, and are foods recommended in the DASH diet and the Dietary Guidelines for Americans. Building upon evidence that phytosterols improve LDL-cholesterol concentration,

dietitians will be able to use the results of the current study to modify an individual's diet to enhance its phytosterol content.

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TABLE 1

Forms of phytosterols and examples of food sources.

Phytosterol Form	Food Sources
Free Phytosterol	wheat germ oil, corn oil, vegetable oils, all plants
Phytosterol Ester	wheat germ oil, corn oil, vegetable oils, whole wheat, all plants, Benecol® spreads (stanol esters), Promise activ® spreads (sterol esters)
Phytosterol Glycoside	unrefined plant-derived lecithin, nuts, seeds, legumes, wheat germ, whole grains, bran, fruit, vegetables

TABLE 2
Menu items with gram weights and estimated phytosterol content^a for 5 single-day diet plans at the 2,000 kcal/day level.

Phytosterol-Deficient ^b		High-Phytosterol DASH c		American Heart Association		Atkins® Lifetime Maintenance		Vegan						
Wt ^d (g)	PS ^e (mg)	Wt (g)	PS (mg)	Wt (g)	PS (mg)	Wt (g)	PS (mg)	Wt (g)	PS (mg)					
BREAKFAST														
261	1	Ardmore Farms® grape juice, ready-to-drink	247	22	Ardmore Farms® orange juice, ready-to-drink	250	23	Tropicana pure premium orange juice	243	32	coffee, caffeinated	233	f	organic peach smoothie
44	11	Kellogg's corn flakes				30	20	Kellogg's bran flakes						
256	0	Pevely skim milk	139	22	banana, edible portion	240	0	Pevely 1% milk	123	0	Schnucks scrambled eggs, no fat added	177	f	Health Valley Organic Fiber 7 Flakes
5	0	sugar	30	12	Kellogg's Special K	47	25	Wonder wheat bread, toasted						Silk® soymilk, vanilla
			13	53	Kretschmer original cereal toasted wheat germ				30	f	Kraft cheddar cheese, regular fat			
			243	0	Pevely skim milk		10	Fleischmann's Original Soft Spread	15	1	Land O Lakes® butter			
			50	32	Wonder 100% whole wheat bread, toasted									
			5	8	Fleischmann's Original Soft Spread									
			18	2	Smucker's jelly									
LUNCH														
250	0	water	250	0	water	240	0	Pevely 1% milk	243	0	diet Pepsi	177	f	Silk® soymilk, vanilla
94	0	chicken breast, skinless, baked	41	21	Beefsteak rye bread	47	25	Wonder wheat bread	152	0	ground chuck, 81% lean, cooked on stovetop	87	14	banana, edible portion
			59	3	sliced ham, baked, no visible fat	10	10	Kraft Miracle Whip				127	33	blueberries, from frozen
														burritos:

Phytosterol-Deficient ^b			High-Phytosterol DASH ^c			American Heart Association			Atkins® Lifetime Maintenance			Vegan		
Wt ^d (g)	PS ^e (mg)		Wt (g)	PS (mg)		Wt (g)	PS (mg)		Wt (g)	PS (mg)		Wt (g)	PS (mg)	
10	3	Hain expeller pressed high-oleic safflower oil, purified ^g				60	0	roast beef, top round, baked, no fat added						
			28	f	Kraft American cheese				61	f	Kraft cheddar cheese, regular fat	63	f	Azteca flour tortillas
63	3	sauce: Campbell's® Healthy Request® Cream of Chicken soup (45g), water (18g)	5	f	yellow mustard salad:	10	1	iceberg lettuce leaf				85	85	black beans, canned
						120	6	tomato slice, raw			salad:	80	23	brown rice, boiled
			50	5	iceberg lettuce			salad:	86	7	iceberg lettuce	27	f	Morningstar Farms® veggie sausage patties, thawed
			20	1	tomato, chopped	50	5	iceberg lettuce	44	3	green pepper, raw, no seeds			
21	f	Kraft parmesan cheese	20	3	carrot, chopped	120	6	tomato, raw						
			20	10	black olives	60	5	cucumber, raw, with skin	26	2	onion, raw, sliced	28	6	red pepper, raw, no seeds
157	2	Minute® rice, boiled, cooked weight	5	35	canola oil				46	2	tomato, raw, chopped			
			10	0	Heinz® cider vinegar	60	11	mushrooms, raw	30	2	Kraft Ranch salad	24	f	Newman's Own® salsa
26	2	green pepper, raw, no seeds	139	18	Red Delicious apple, with skin	30	36	Kraft Zesty Italian salad dressing	30	60	almonds, raw dressing	103	13	Red Delicious apple, with skin
42	1	buttermilk biscuit, low-phytosterol recipe ^h				21	f	graham crackers				18	f	dried dates
						120	24	red grapes, seedless				18	35	almonds, raw
103	1	JELL-O gelatin dessert, regular												
DINNER														
250	0	water	243	0	Pevely skim milk	120	f	Chardonnay wine	243	f	iced tea, instant, unsweetened	177	f	Celestial Seasonings Lemon Zinger® herbal salad: tea
94	f	top round beef roast, oven roasted	119	f	Atlantic salmon, baked in foil with lemon juice and Italian herbs, raw weight	60	0	chicken breast, baked, skinless						

Phytosterol-Deficient ^b		High-Phytosterol DASH ^c				American Heart Association				Atkins® Lifetime Maintenance				Vegan			
Wt ^d (g)	PS ^e (mg)	Wt (g)	PS (mg)	Wt (g)	PS (mg)	Wt (g)	PS (mg)	Wt (g)	PS (mg)	Wt (g)	PS (mg)	Wt (g)	PS (mg)	Wt (g)	PS (mg)	Wt (g)	PS (mg)
209	8	baked potato, no skin				120	5	baked potato, no skin						41	4	green leaf lettuce	
26	0	Land O Lakes® sour cream, fat-free				5	8	Fleischmann's Original Soft Spread						17	3	spinach, raw	
3	0	chives, raw, chopped												32	13	broccoli, raw	
			56	23	egg noodles, boiled, dry weight												
						60	5	onion, cooked, raw weight						44	7	carrots, steamed cooked in microwave, raw weight	
		<u>salad:</u>	182	71	frozen broccoli, heated in microwave									26	4	celery, raw	
52	5	iceberg lettuce				60	10	carrot sticks, steamed						22	2	green onions, raw	
21	1	tomato, raw	89	36	frozen cauliflower, heated in microwave	80	194	frozen green peas, heated in microwave		26	16	Brownberry Carb Counting 100% whole wheat bread		22	2	cucumber, with skin, raw	
7	f	real bacon bits															
21	6	Hain expeller pressed high-oleic safflower oil, purified ^g	5	8	Fleischmann's Original Soft Spread	40	9	white dinner roll						50	2	tomato, raw	
			396	71	pink grapefruit, fresh	5	8	Fleischmann's Original Soft Spread		15	1	Land O Lakes® butter		32	24	Hass avocado, raw	
16	0	Heinz® cider vinegar	28	38	Planters dry roasted peanuts, unsalted	120	51	Del Monte © canned peaches, juice-packed		81	8	strawberries, fresh, edible portion		63	46	Newman's Own® olive oil & vinegar dressing	
58	6	dinner muffin, low-phytosterol recipe ^h												10	f	brewer's yeast	
						80	f	orange sherbet						21	55	pumpkin seeds, raw	
237	1	Dannon® Light & Fit® fruit yogurt, non-fat												52	f	Soy Dream® non-dairy frozen dessert, French vanilla	
Sum	51		494				503				163				399		

^aPhytosterol content for each food item was estimated from similar foods in the United States Department of Agriculture National Nutrient Database for Standard Reference (17), from published studies (12–14,16,25), and from phytosterol analyses performed in the Washington University School of Medicine Mass Spectrometry Resource (unpublished data).

^b The phytosterol-deficient diet was designed to serve as a control diet in studies assessing the influence of phytosterols on health.

^c DASH=Dietary Approaches to Stop Hypertension diet (21). This diet was modified to enhance its phytosterol content.

^d Wt=weight of each food item included in the diet composite for each meal plan at the 2,000 kcal level.

^e PS=phytosterol content of each item, expressed in mg per quantity of food included in the diet composite.

^f Items for which phytosterol values were not available.

^g This safflower oil was purified to remove most of the naturally-occurring phytosterols.

^h This recipe was specially formulated to minimize the phytosterol content. Ingredients included rice flour milled from Minute® rice, shortening that was purified to remove most of the naturally-occurring phytosterols, potato starch, and xanthan gum

TABLE 3
Phytosterol content^a and nutrient composition^b of 5 single-day diet plans at the 2,000 kcal/day level.

Nutrient		Diet Plans				
		Phytosterol-Deficient (Control) ^c	High-Phytosterol DASH ^d	American Heart Association	Atkins® Lifetime Maintenance	Vegan
Total Phytosterols	mg	64 ^e	500 ^f	340	163	445
Glycosylated Phytosterols	% of total	19	22	20	9	10
Carbohydrate	% of kcal	52	54	57	8	52
Protein	% of kcal	22	20	17	23	13
Fat	% of kcal	27	26	26	68	35
Carbohydrate	g	259	278	284	42	274
Protein	g	110	105	85	118	68
Fat	g	59	60	56	153	83
Saturated Fatty Acid	g	13	14	14	59	12
Monounsaturated Fatty Acid	g	34	24	19	63	39
Polyunsaturated Fatty Acid	g	9	16	19	19	26
Cholesterol	mg	198	186	131	839	8
Fiber	g	8	29	28	12	41
Calcium	mg	1194	1084	947	849	1072
Potassium	mg	3230	4496	4332	2220	3641
Sodium	mg	2102	2708	2338	1504	1993

^aTotal phytosterols were quantified analytically in diet composites that were subjected to acid plus alkaline hydrolysis followed by gas chromatography. Glycosylated phytosterols were derived as the difference between total phytosterols determined using acid plus alkaline hydrolysis and phytosterols determined from alkaline hydrolysis alone.

^bAll nutrient values other than phytosterols were derived from Nutrition Data System for Research software version 5.0 (2004, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).

^cThe phytosterol-deficient diet was designed to serve as a control diet in studies assessing the influence of phytosterols on health.

^dDASH=Dietary Approaches to Stop Hypertension diet (21). This diet was modified to enhance its phytosterol content.

^eP<0.01 when comparing the phytosterol content of this diet to each of the other diets.

^fP<0.003 when comparing the phytosterol content of this diet to the phytosterol-deficient, American Heart Association, and Atkins® diets.