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A Combination Therapy Including Psyllium and Plant Sterols Lowers LDL Cholesterol by Modifying Lipoprotein Metabolism in Hypercholesterolemic Individuals[1]

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Abstract

We conducted a randomized, double blind, crossover, placebo-controlled study to determine the effects of a combination therapy including plant sterols (PS) and psyllium (PSY), provided via cookies, on plasma lipids and on the size and subfraction distribution of VLDL, LDL, and HDL. Thirty-three healthy free-living individuals (11 males and 22 females), aged 35–65 y, with a BMI between 25 and 35 kg/m^2 and initial plasma LDL cholesterol (LDL-C) concentrations between 2.6 and 4.1 mmol/L (100 and 160 mg/dL), were randomly assigned to receive treatment cookies (7.68 g/d PSY and 2.6 g/d PS) or placebo cookies (0 g PSY+PS) for 4 wk. After a 3-wk washout period, subjects received the other cookies for an additional 4 wk. Plasma total cholesterol concentrations were significantly reduced for all subjects, from 5.65 ± 0.72 mmol/L after the placebo period to 5.28 ± 0.76 mmol/L after the PSY+PS cookie period ($P < 0.01$). These reductions were primarily in LDL-C, which decreased from 3.48 ± 0.70 to 3.14 ± 0.78 mmol/L after PSY+PS cookie consumption ($P < 0.01$). Intake of the PSY+PS cookie decreased the number of intermediate density lipoprotein (IDL), LDL, and HDL particles ($P < 0.05$) and plasma apo B concentrations ($P < 0.01$). The decreases in LDL and HDL particles were in the small subfractions. Because smaller LDL particles are associated with an increased risk of heart disease and because smaller HDL particles are indicative of diminished reverse cholesterol transport, we conclude that the combination therapy resulted in a less atherogenic lipoprotein profile. In addition, the evaluation of lipoprotein subfractions resulting from the action of the fiber and plant sterols in the intestinal lumen provides an insight on the secondary mechanisms of plasma LDL-C lowering. J. Nutr. 136: 2492–2497, 2006.

Introduction

Coronary heart disease (CHD)[6] is associated with high morbidity and mortality rates (1). Hypercholesterolemia is a major risk factor for CHD and atherosclerosis and high serum lipid concentrations are linked with the development of CHD (2,3). The National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III recognizes that elevated LDL cholesterol (LDL-C) is a major cause of CHD and has highlighted LDL-lowering as a primary therapy to reduce the risk for CHD. The new guidelines released by NCEP emphasize dietary and lifestyle changes as a primary therapy to maintain the desired plasma LDL-C concentrations (4).

Dietary therapy has been shown to be effective in reducing the cardiovascular risk and mortality associated with high cholesterol (5,6). Soluble fiber and plant sterols (PS) have therefore been recommended by the NCEP-ATP III (4) and the American Heart Association (7) as options in the dietary strategy to reduce blood cholesterol concentrations. The gel forming properties of dietary soluble fiber are responsible for its physiologic responses (8,9) such as improved glucose homeostasis and lipid and lipoprotein profiles (10). PS, on the other hand, closely resembles the structure of cholesterol and competes with cholesterol during micelle formation. This action of PS interferes with the intestinal absorption of cholesterol (11).

The consumption of 2–10 g/d soluble fiber was reported to cause significant decreases in total cholesterol, with an effect independent of study design, treatment length, and background dietary fat content (12). A significant reduction of serum total and LDL-C concentrations was reported in men and women with primary hypercholesterolemia after consumption of 5.1 g

[1] This study was supported by RD Foods, Kenilworth, NJ.

[6] Abbreviations used: APO, apolipoprotein; ATP, adult treatment panel; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; FA, fatty acids; IDL, intermediate density lipoprotein; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; NCEP, National cholesterol education program; PS: plant sterol; PSY, psyllium; TC, total cholesterol; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

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of psyllium (PSY) twice daily (13). Miettinen et al. (11) reported that 1.8 or 2.6 g/d of sitostanol, supplied via margarine to subjects with mild hypercholesterolemia, significantly reduced serum cholesterol (14% reduction in the treatment group compared with only 1% reduction in the control group). Katan et al. (14), in a metaanalysis of 41 trials, reported that intake of 2 g/d of stanols independent of background diet resulted in a 10% reduction of LDL-C.

Both PS and soluble fiber can be preferable for the long-term management of hypercholesterolemia (15,16). In combination, these functional components are more effective than either component alone. A dietary portfolio including soluble fiber, PS, soybean protein and nuts with a very low saturated fat has been as effective as the cholesterol lowering drug lovastatin in hypercholesterolemic subjects (6,17,18). Moreover, sex and hormonal status have influenced lipid lowering response of dietary soluble fiber in both human and animal models (9,19). The combined effect of soluble fiber and PS to evaluate clinical markers of CHD in free living and mildly hypercholesterolemic subjects has not been tested. We have previously demonstrated in humans that PSY intake affects the intravascular processing of lipoproteins by reducing cholesteryl ester transfer protein (CETP) activity, via changes in the composition of VLDL (19). These alterations in lipoprotein metabolism partially explain the hypolipidemic effect of PSY. However, to our knowledge, there are no reports evaluating changes in lipoprotein morphology and subclass distribution resulting from the primary action of PSY and PS in the intestinal lumen.

This study was conducted with the following 2 main objectives: *1*) to determine the combined effects of PS and PSY on lowering LDL-C, and *2*) to evaluate whether this dietary intervention modified lipoprotein size and subfraction distribution, which could further explain the hypocholesterolemic actions of these compounds. Our hypothesis was that the combined nutritional therapy would lower plasma LDL-C in hypercholesterolemic individuals and that lipoprotein metabolism would be modified.

Materials and Methods

Materials. Placebo and test cookies were obtained from RD Foods. Enzymatic cholesterol and triglyceride kits were obtained from Roche diagnostics. EDTA, aprotinin, sodium azide, and phenyl methyl sulfonyl fluoride were obtained from Sigma Chemical. Glucose kits were obtained from Wako Chemicals. An insulin ELISA (DSL-10–1600) kit was obtained from Diagnostics Systems Laboratories. The ELISA for plasma insulin determination was obtained from Diagnostic Systems Laboratories.

Subjects. Thirty three healthy adults, 11 men, 8 premenopausal women, and 14 postmenopausal women, aged 35–65 y with initial plasma LDL-C between 2.6 and 4.1 mmol/L (100 and 160 mg/dL) and a BMI between 25 and 35 kg/m^2 were recruited. All participants completed the study. The exclusion criteria were diabetes, cardiovascular disease or lipid-lowering drug treatment, and fiber or sterol supplementation. All subjects gave a written informed consent to participate, and the study protocol was approved by the Committee on the use of Human Subjects in Research of the University of Connecticut.

Study design. This was a randomized, double blind, crossover, placebo-controlled study that was designed to determine the effects of PS and PSY, provided via cookies, on plasma lipids and on the size and subfraction distribution of VLDL, LDL, and HDL. Participants were randomly assigned to the test cookie (10 g of PSY yielding 7.68 g/d of soluble fiber and 2.6 g/d PS) group or placebo cookie (0 g PSY + 0 g PS) group, in a crossover design. Participants were asked to consume 2 cookies (labeled A or B)/d, for a period of 30 d. The design included a 21-d washout period. During the first treatment period, 17 subjects were eating the test cookies and 16 subjects consumed the PSY+PS cookie. Subjects were asked to maintain their habitual diet and level of exercise. Dietary intake during both periods was assessed by the completion of 5-d weighted dietary records that included 2 weekend days. Participants' weight, height, and blood pressure were recorded at the beginning and end of each treatment period.

Dietary supplement. Macronutrient, fiber, and plant sterol contents of the cookies are listed in **Table 1**. A regimen of 2 cookies/d was packed in individually labeled bags and provided to the subjects on a weekly basis. Participants returned empty bags or bags containing the uneaten portion of the cookies, and the weight was recorded to calculate the amount of cookies consumed per individual during each dietary period. We developed a questionnaire to evaluate whether subjects had any difficulty adhering to the supplementation protocol. Subjects were asked about any gastrointestinal disturbances or physiological changes. At the end of each treatment period, participants completed the questionnaire, reporting any discomfort or side effects.

Dietary analysis. The nutrient intake and nutrient composition of the cookies was calculated using the Nutrition Data System for Research (NDS-R) software version 5.0, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 28. If an analytic value is not available for a nutrient in a food, NDS calculates the value based on the nutrient content of other nutrients in the same food or on a product ingredient list, or it estimates the value based on the nutrient content of similar foods. Nutrient intake was calculated as a mean of the 5-d dietary records, including 2 test cookies/d for both dietary periods. Energy derived from total fat, saturated fat, monounsaturated fat, polyunsaturated fat, carbohydrates, and protein were also calculated.

Data collection. At the end of each treatment period, 2 blood samples drawn on different days to control for day-to-day variability were collected. Plasma was separated by centrifugation at 2000 × *g* for 20 min, and aprotinin (0.5 mL/100 mL), sodium azide (0.1 mL/100 mL), and phenyl methyl sulfonyl fluoride (0.1 mL/100 mL) were added for preservation. Plasma was stored in individual aliquots at –80°C for later analysis.

Plasma lipids. Our laboratory has been participating in the Centers for Disease Control–National Heart, Lung, and Blood Institute (CDC-NHLBI) Lipid Standardization Program since 1989 for quality control and standardization for plasma total cholesterol and TG assays. CV were 1.53–2.02 for total cholesterol, 1.91–2.62 for HDL-C, and 1.7–3.78 for TG during the time of this study. Plasma TC and TG concentrations were determined by enzymatic methods (20,21). HDL-C concentration in the supernatant was measured by an enzymatic method after selective precipitation of apo B-containing lipoproteins, by magnesium chloride and dextran sulfate (22). LDL-C was calculated as described by Friedewald et al. (23). Apo B was measured using an immunoturbidimetric method with turbidity determined at 340 nm (24).

VLDL, LDL, and HDL size and subfraction distribution. H-NMR analysis was performed on 400 MHz NMR analyzer (Bruker BioSpin) as

TABLE 1 Nutrient composition of placebo and test cookies

	Placebo cookie	Test cookie
Total energy, *kJ/cookie*	652	644
Total fat, *g/cookie*	7.6	6
Total saturated fat, *g/cookie*	2.2	1.9
Total monounsaturated fat, *g/cookie*	3.4	2.6
Total polyunsaturated fat, *g/cookie*	1.6	1.2
Total carbohydrate, *g/cookie*	20	23
Protein, *g/cookie*	1.88	1.96
Total fiber, *g/cookie*	1.42	4.67
Soluble fiber, *g/cookie*	0.19	3.8
Plant sterols, *g/cookie*	0	1.3
Cholesterol, *mg*	13.1	10.3

previously described (25). Briefly, lipoprotein subclasses of different sizes produce a distinct lipid methyl signal, whose amplitude is directly proportional to lipoprotein particle concentration. NMR simultaneously quantifies >30 lipoprotein subclasses that are empirically grouped into 9 smaller subclasses based on particle diameters: large VLDL (>60 nm), medium VLDL (27–35 nm), small VLDL (23–27 nm), large LDL (21.2–23 nm), medium LDL (19.8–21.2), small LDL (18–19.8 nm), large HDL (8.8–13 nm), medium HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm). Weighted mean lipoprotein particle sizes in diameters were calculated based on the diameter of each lipoprotein subclass multiplied by its respective relative concentration.

Plasma glucose and insulin. Plasma glucose was determined enzymatically (25). Insulin concentrations were determined in duplicate using an ELISA. The intra-assay CV was 1.6%.

Statistical analysis. A 1-way ANOVA was used to test for baseline differences among the subjects in body weight, plasma lipids, and systolic and diastolic blood pressures. A 2-way repeated measures ANOVA was performed to test the significant effects of PSY+PS intake (test vs. placebo), group effects (men, premenopausal women, and postmenopausal women) or their interaction on plasma lipids, VLDL, LDL, or HDL size and subfraction distribution, insulin, glucose, apolipoprotein B, and nutrient intake. Tukey's post-hoc test was used to find differences among means. A paired *t* test was used to compare placebo vs. test periods. Data are presented as means ± SD. Differences of $P < 0.05$ were considered significant.

Results

Baseline measurements. Participants were grouped depending on sex and hormonal status (**Table 2**). Baseline characteristics, including BMI, systolic and diastolic blood pressures, plasma LDL, HDL, and total cholesterol, and plasma TG did not differ among the subjects except for HDL-C, which was higher in postmenopausal women than in men. Plasma HDL-C in premenopausal women did not differ from either men or postmenopausal women.

Dietary intake and compliance. There was high dietary compliance during both treatment periods. Subjects collected their respective cookies every week and returned the package with or without uneaten portions of the cookies. Total energy intake and energy distribution in carbohydrate, fat, and protein did not differ between treatment and placebo periods (**Table 3**). Total and soluble fiber intakes were higher during the PSY+PS period ($P < 0.0001$). The Nutrition Data System does not provide analysis for plant sterols in food. However, the additional 2.6 g/d of plant sterols that the subjects consumed during the treatment period indicates that consumption of plant sterols would have been greater during the placebo period.

TABLE 2 Baseline plasma lipids, BMI, and systolic and diastolic blood pressures of participating subjects[1]

	Men	PreMW[2]	PostMW
n	11	8	14
BMI, *kg/m²*	31.4 ± 5.0	33.7 ± 3.9	30.0 ± 4.4
Diastolic blood pressure, *mm Hg*	79.6 ± 8.0	85.0 ± 4.0	81.4 ± 3.0
Systolic blood pressure, *mm Hg*	124.0 ± 10.5	132.4 ± 9.7	123.3 ± 10.9
Total cholesterol,[3] *mg/dL*	203.2 ± 23.2	216.8 ± 18.9	214.9 ± 44.8
LDL cholesterol,[3] *mg/dL*	131.0 ± 20.6	129.8 ± 28.1	125.8 ± 39.7
HDL cholesterol,[3] *mg/dL*	51.8 ± 28.9[a]	60.4 ± 9.9[ab]	65.2 ± 17.5[b]
Triglycerides,[4] *mg/dL*	105.4 ± 51.0	143.3 ± 65.4	116.9 ± 46.8

[1] Data are presented as means ± SD. Means in a row without a common letter differ, $P < 0.05$.
[2] PreMW, premenopausal women; PostMW, postmenopausal women.
[3] To convert to mmol/L, divide by 38.67.
[4] To convert to mmol/L, divide by 88.54.

Symptoms. Symptoms reported in the questionnaire did not differ between the placebo and test periods (data not shown).

Body weight and plasma lipids. Body weights did not change during either period (**Table 4**). PSY+PS lowered plasma total and LDL-C ($P < 0.01$) in all subjects compared with the placebo. In contrast, there were no changes in HDL-C or TG. There was no significant influence of sex and hormonal status or their interactions on plasma lipid concentrations (data not shown).

Plasma apoprotein B and lipoprotein size and subfraction distribution. The mean size of VLDL, LDL, and HDL particles did not change during the test or placebo periods (data not shown). However, dietary treatment significantly affected the total number of lipoprotein particles (**Table 5**). The combined effects of PSY+PS reduced the number of intermediate density lipoprotein (IDL), LDL, and HDL particles ($P < 0.05$) in all subjects. In contrast, the number of VLDL particles was not affected by the treatment. In agreement with these observations, there was a significant reduction of plasma apo B concentrations due to PSY+PS (**Table 6**), which was linked with a reduction in the medium-small LDL subfractions ($P < 0.05$). Similarly, the reductions in HDL numbers were associated with lower concentrations of the small HDL (**Table 7**). There was a significant correlation between changes in LDL particles and IDL particles from the placebo to the PSY+PS periods (**Fig. 1**), suggesting that the lower concentrations of medium LDL particles during the treatment period were due to a reduction in the conversion of IDL to LDL.

Glucose and insulin. Plasma glucose concentration was lower during the treatment period for all subjects (5.0 ± 0.5 mmol/L) than during the placebo period (5.3 ± 0.5 mmol/L) ($P < 0.05$). Plasma insulin concentrations were not influenced by diet or gender/ hormonal status (data not shown).

Discussion

In this study, we sought to assess the efficacy of a combination of functional components provided via a novel delivery vehicle to reduce LDL-C, rather than aiming to evaluate the separate contribution of individual components. This was a weight maintenance study in which test cookies were consumed without changing the background diet and physical activity.

PSY and PS significantly decreased the plasma TC and LDL-C concentrations by ~7 and 10%, respectively. According to the literature, when these dietary components are consumed together, each is suggested to individually contribute to a 4- to 7% LDL-C lowering (6). Our results suggest that there might be an additive effect of psyllium and plant sterols, because the decrease in LDL-C was 10%. Dietary intervention in adults <65 y aiming to reduce LDL-C concentrations by 10% would decrease the number of people who qualify for drug therapy by as much as 7% (26). This study produced modest but sustained reductions in LDL-C.

The treatment cookies improved the plasma glucose concentration. Associations between soluble fiber and glucose and insulin responses have been reported (10,27). Riguad et al. (28)

TABLE 3 Total energy intake, distribution of energy intake, and intakes of cholesterol, fiber, and plant sterols by men and pre- and postmenopausal women during the test and placebo periods[1,2]

	Men, $n = 11$		PreMW, $n = 8$		PostMW, $n = 14$	
	Placebo	Test	Placebo	Test	Placebo	Test
Total energy, *kJ/d*	9601 ± 2454	9025 ± 2029	8277 ± 2203	8394 ± 1613	9390 ± 2272	8794 ± 2080
Fat, *% energy*	39 ± 8.3	38.4 ± 9.1	35.3 ± 5.4	36.4 ± 6.4	41.1 ± 6.4	39.2 ± 8.7
SFA, *% energy*	13 ± 4.4	12.7 ± 5	11.9 ± 2.6	11.7 ± 3.7	13 ± 3.2	13.3 ± 2.4
MUFA, *% energy*	14.4 ± 3.8	14.1 ± 4	12.5 ± 2.6	12.5 ± 3.5	16.1 ± 3	15.2 ± 4.6
PUFA, *% energy*	7.4 ± 1.2	7.4 ± 1.3	7.9 ± 4.2	8.6 ± 5.5	7.3 ± 2.2	8.4 ± 6.1
Carbohydrate, *% energy*	44.5 ± 8.3	46 ± 9.2	49.4 ± 6.1	49.5 ± 8	41.1 ± 8.5	44.6 ± 9.2
Protein, *% energy*	16.5 ± 3.4	16.5 ± 2.7	13.6 ± 1.8	15.1 ± 2.3	16.2 ± 3.3	17 ± 2.1
Cholesterol, *mg/d*	373 ± 97.6	407.9 ± 162.3	238.9 ± 138.5	210.5 ± 144	372.4 ± 124	345.4 ± 156
Total fiber,*g/d*	22.5 ± 9	28 ± 7.2*	14.6 ± 5.3	22 ± 6.2*	19.6 ± 6.5	26.6 ± 5.1*
Soluble fiber, *g/d*	5 ± 2	11.6 ± 1.8	4.4 ± 2.4	10.7 ± 1	3.8 ± 1.7	11.2 ± 1.2
Plant sterols,[2] *g*	–	2.6	–	2.6	–	2.6

[1] Data are presented as means ± SD. *Different from placebo period, $P < 0.05$.
[2] 2.6 g/d PS was provided via test cookies. The evaluation of plant sterols was not included in the NSD program.

found that consumption of 7.4 g PSY/d significantly reduced postprandial increases in serum glucose, TG, and insulin concentrations. PSY also been improves both glycemic and lipid profiles in diabetic individuals (29,30).

Sex and hormonal status influence the lipoprotein changes, as evidenced by the fact that men are at higher risk of CVD than women at younger ages, whereas postmenopausal women are at a higher risk than their younger counterparts (31,32). Vega-Lopez et al. (19) reported that sex and hormonal status influence the PSY-induced responses to plasma lipids by increasing plasma TG concentrations in postmenopausal women, whereas there is a decrease in plasma TG in men, and no change in premenopausal women. We did not find any influence of sex and hormonal status on PSY and PS effects on plasma lipids. One possible explanation could be that combination of PSY and PS may have outweighed the effects of PSY alone as influenced by sex and hormonal status.

PSY, PS, and LDL metabolism. Normal blood lipid profiles are not always connected with a lower risk for CVD. Lipoprotein subclasses may be other risk factors that cause CHD in individuals with normal lipid profiles (33). Studies have shown that small, dense LDL particles are associated with the etiology of atherosclerosis and larger, less dense LDL particles are less atherogenic (34,35). Elevated apo B as an indicator of elevated LDL particle number could also be involved in CVD risk (34).

There are limited reports on soluble fiber supplementation in diet and LDL phenotype. Behall et al. (36) reported that the addition of barley to a healthy diet revealed favorable changes in plasma lipids in both men and women. The result showed large LDL fractions and increased mean LDL particles in postmenopausal women. Lambarche et al. (37) reported that dietary portfolio of plant sterols, vegetable protein, viscous fiber, and almonds had a favorable effect on reducing cholesterol concentrations from all LDL subfractions and reduced serum concentration of all LDL subfractions, including small dense LDL. Oat fiber supplementation produced lower concentrations of small, dense LDL particles than placebo wheat cereal (38). Despite the lack of effect of phytosterols on LDL size phenotypes (39,40), dietary manipulation has been shown to modify LDL electrophorotic characteristics by shifting the LDL particles toward a less atherogenic pattern A (41). Pedersen et al. (42) showed that rapeseed and sunflower oil containing sterol had favorable effects on LDL size and subfractions compared with olive oil. Varady et al. (43) reported that PS significantly decreased the cholesterol content in small, medium, and large LDL particles; however, the changes were not linked with an increase in LDL particle size. In our study, PSY+PS supplementation resulted in a decrease in LDL-C that was associated with a decrease in the number of LDL particles, but not in the amount of cholesterol per particle. Further, the reduction in LDL occurred in the small subfractions. The decrease in the number of LDL particles was in conjunction with decreases in apo B concentration and with a decrease in IDL particles, suggesting less conversion of IDL to LDL during the test period. Gyalling and Miettinen (44) reported the similar results, that supplementation of sitostanol lowered LDL apo B production rate that was associated with decrease in LDL-C concentration in plasma.

TABLE 4 Body weight and plasma lipid concentrations of all subjects at the end of the placebo and test periods[1]

Variable	Placebo	Test
Body weight, *kg*	84.4 ± 13.9	84.0 ± 13.9
Total cholesterol,[2] *mg/dL*	217.4 ± 27.8	203.2 ± 29.6*
LDL cholesterol,[2] *mg/dL*	132.7 ± 27.1	119.8 ± 30.1*
HDL cholesterol,[2] *mg/dL*	59.9 ± 16.5	59.6 ± 14.9
Triglycerides,[3] *mg/dL*	128.0 ± 56.0	121.4 ± 54.4

[1] Data are presented as means ± SD as determined by paired *t* test, $n = 33$.
*Different from placebo period, $P < 0.01$.
[2] To convert to mmol/L, divide by 38.67.
[3] To convert to mmol/L, divide by 88.54.

TABLE 5 Plasma total VLDL, IDL, LDL, and HDL particle concentrations of all subjects after the test and placebo periods[1]

Variable	Placebo	Test
VLDL, *nmol/L*	84.7 ± 40.0	79.8 ± 37.7
IDL, *nmol/L*	66.1 ± 55.4	48.6 ± 53.1*
LDL, *nmol/L*	1390.4 ± 333.7	1300.2 ± 376.1*
HDL, *μmol/L*	34.3 ± 6.1	33.4 ± 5.0*

[1] Data are presented as means ± SD as determined by paired *t* test, $n = 33$.
*Different from the placebo period, $P < 0.05$.

TABLE 6 Plasma apo B concentration and numbers of large, small, medium small, and very small LDL particles of all subjects after the test and placebo periods[1]

Variable	Placebo	Test
Plasma apo B, *mg/L*	91.3 ± 16.6	81.3 ± 16.6*
Large LDL, *nmol/L*	446.0 ± 224.3	425.1 ± 197.0
Small LDL, *nmol/L*	881.0 ± 454.0	826.6 ± 479.2
Medium small LDL, *nmol/L*	183.8 ± 85.7	164.9 ± 85.0*
Very small LDL, *nmol/L*	696.9 ± 359.9	661.7 ± 385.3

[1] Data are presented as means ± SD as determined by paired *t* test, $n = 33$.
*Different from placebo period, $P < 0.05$.

A previous study in our laboratory showed that PSY supplementation induced formation of phospholipid (PL) enriched LDL particles. This compositional modification of LDL was connected with reduced CETP activity (45). The decrease in LDL-C and the number of smaller LDL subfractions seen in our study may be due to compositional modification of PSY+PS-induced LDL particles and associated intravascular processing. It is possible that PSY+PS could decrease plasma LDL-C by an increase in receptor mediated endocytosis of LDL. PSY interrupts bile acids absorption and PS inhibits cholesterol absorption. The action of both reduces the bile acid pool in the hepatocyte, leading to increase in bile acid synthesis to compensate for the loss of bile acids through the actions of these components. To supply more free cholesterol as a substrate for the bile acid synthesis pathway, the hepatocytes express more LDL receptors, through which LDL is removed from the circulation, resulting in a decrease in LDL-C concentration. The increased expression of mRNA for LDL receptors, in hepatocytes as well as in mononuclear cells, by PS+PSY have been well documented (9,46,47). The correlation between decreases in LDL and IDL during the PSY+PS supplementation suggests that an up-regulation of the receptor may have contributed to the decreased conversion of IDL to LDL, as we previously observed in guinea pig studies (8,9).

PSY, PS, and HDL metabolism. Like LDL, HDL particles are heterogeneous, and HDL subclasses play interrelated metabolic functions. Changes in HDL subclasses distribution might be linked with atherosclerosis. Studies have suggested that an increased CETP activity and a diminished LCAT activity are associated with variation of HDL subclasses distribution. CETP plays a role in exchanging neutral lipids, cholesteryl ester, and TG between triglyceride rich lipoproteins and HDL particles. Increased activity of CETP leads to TG-rich HDL particles, which by the action of hepatic lipase are modified into smaller HDL particles. Smaller HDL particles can create an environment of reverse cholesterol transport (48). In this study, although plasma HDL-C concentrations were not affected by the treatment, HDL subfraction distribution was changed, with a

TABLE 7 Numbers of large, medium, and small HDL particles of all subjects after the test and placebo periods[1]

Variable	Placebo	Test
Large HDL, *μmol/L*	7.6 ± 4.7	7.1 ± 4.4
Medium HDL, *μmol/L*	4.4 ± 3.6	4.8 ± 5.2
Small HDL, *μmol/L*	22.4 ± 4.5	20.1 ± 5.3*

[1] Data are presented as means ± SD as determined by paired *t* test, $n = 33$.
*Different from placebo period, $P < 0.01$.

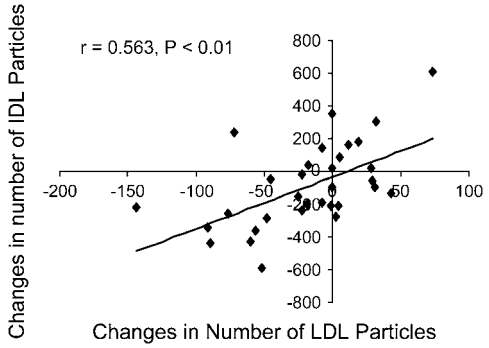

Figure 1 Correlation between the changes in the numbers of IDL and LDL particles from the placebo to the PSY+PS period for men and pre- and postmenopausal women ($n = 33$).

reduction in the small HDL particle concentration. This has been linked with diminished reverse cholesterol transport. Vega-Lopez et al. (19) reported that PSY supplementation significantly decreased CETP activity, whereas LCAT activity was not affected. We, therefore, contend that the decrease in smaller HDL subfractions in our study may be associated with a decrease in CETP activity during the treatment period.

In summary, this study presents novel information regarding the effects of a PSY+PS nutrition therapy on plasma lipoprotein subfraction distribution. We provide evidence that dietary interventions affect plasma lipoprotein metabolism in the intravascular compartment, resulting in the formation of lipoprotein subclasses with distinct associations with CHD risk. The reductions in LDL-C induced by PSY+PS treatment were expected, and these results by themselves are beneficial for CHD risk. However, the more detailed evaluation of LDL and HDL subfraction distribution provides more precise information regarding the formation of less atherogenic LDL and HDL subclasses, important results which should be considered when this combined therapy is recommended.

Literature Cited

1. Heart Disease and Stroke Statistics — 2005 Update. American Heart Association:2–3.
2. Stamler J, Wentforth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary hearth disease continuous or graded? Findings in 356,222 primary screen of the multiple risk factor intervention trial (MR-FIT). JAMA. 1986;256: 2823–8.
3. Sarman B. New results in the management of hypercholesterolemia. Orv Hetil. 2005;146:1999–2004.
4. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the 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). JAMA. 2001;285:2486–97.
5. Brown L, Rosner B, Willen WW, Sacks FM. Cholesterol-lowering effects of dietary fiber: a meta analysis. Am J Clin Nutr. 1999;69:30–42.
6. Jenkins D, Marchie CKA, Faulkner D, Wong J, Souza R, Emam A, Parker T, Edward Vidgen, Elke A Trautwein, et al. Direct comparison of a dietary portfolio of cholesterol-lowering foods with a statin in hypercholesterolemic participants. Am J Clin Nutr. 2005;81:380–7.
7. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW, Jr., Kris-Etherton P, Goldberg IJ, et al. AHA dietary guidelines revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation. 2000;102:2284–99.

8. Fernandez ML. Soluble fiber and nondigestible carbohydrate effects on plasma lipids and cardiovascular risk. Curr Opin Lipidol. 2001;12: 35–40.
9. Roy S, Vega-Lopez S, Fernandez ML. Gender and hormonal status affect the hypolipidemic mechanisms of dietary soluble fiber in guinea pigs. J Nutr. 2000;130:600–7.
10. Moreno LA, Tresaco B, Bueno G, Fleta J, Rodriguez G, Garagorri JM, Bueno M. Psyllium fibre and the metabolic control of obese children and adolescents. J Physiol Biochem. 2003;59:235–42.
11. Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med. 1995;333: 1308–12.
12. Brown L, Rosner B, Willett WW, Sacks FM. Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr. 1999;69:30–42.
13. A Anderson JWDavidson MH, Blonde L, Brown WV, Howard WJ, Ginsberg H, Allgood LD, Weingand KW. Long-term cholesterol-lowering effects of psyllium as an adjunct to diet therapy in the treatment of hypercholesterolemia. Am J Clin Nutr. 2000;71:1433–8.
14. Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R. Stresa Workshop Participants. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc. 2003;78:965–78.
15. O'Neill FH, Sanders TA, Thompson GR. Comparison of efficacy of plant stanol ester and sterol ester: short-term and longer-term studies. Am J Cardiol. 2005;96:29D–36D.
16. Jensen CD, Haskell W, Whittam JH. Long-term effects of water-soluble dietary fiber in the management of hypercholesterolemia in healthy men and women. Am J Cardiol. 1997;79:34–7.
17. Jenkins DJ, Kendall CW, Marchie A, Faulkner DA, Wong JM, de Souza R, Emam A, Parker TL, Vidgen E, 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.
18. Jenkins DJ, Kendall CW, Faulkner D, Vidgen E, Trautwein EA, Parker TL, Marchie A, Koumbridis G, Lapsley KG, et al. A dietary portfolio approach to cholesterol reduction: Combined effects of plant sterols, vegetable proteins, and viscous fibers in hypercholesterolemia. Metabolism. 2002;51:1596–1604.
19. Vega-Lopez S, Vidal-Quintanar RL, Fernandez ML. Sex and hormonal status influence plasma lipid responses to psyllium. Am J Clin Nutr. 2001;74:435–41.
20. Allain CC, Poon LC, Chan CS, Richard W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;20:470–475.
21. Carr TP, Anderssen CJ, Rudel LL. Enzymatic determination of triglycerides, free cholesterol and cholesterol in tissue lipid extracts. Clin Biochem. 1993;26:39–42.
22. Warnick GR, Benderson J, Albert JJ. Dextran sulfate-Mg2+ precipitation procedure for quantification of high-density lipoprotein cholesteol. Clin Chem. 1982;28:1379–88.
23. Friedewald WT, Levy RI, Fredirckson D. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;8:499–502.
24. Rifai N, King ME. Immunoturbidimetric assays of apolipoproteins A, AI, AII, and B in serum. Clin Chem. 1986;32:957–61.
25. Reljic R, Ries M, Anic N, Ries B. New Chromogen for assay of glucose in serum. Clin Chem. 1992;38:522–5.
26. Sempos CT, Cleeman JI, Carroll MD, Johnson CL, Bachorik PS, Gordon DJ, Burt VL, Briefel RR, Brown CD, Lippel K. Prevalence of high blood cholesterol among US adults. An update based on guidelines from the second report of the National Cholesterol Education Program Adult Treatment Panel. JAMA. 1993;269:3009–14.
27. Lovejoy J, DiGirolamo M. Habitual dietary intake and insulin sensitivity in lean and obese adults. Am J Clin Nutr. 1992;55:1174–9.
28. Rigaud D, Paycha F, Meulemans A, Merrouche M, Mignon M. Effect of psyllium on gastric emptying, hunger feeling and food intake in normal volunteers: a double blind study. Eur J Clin Nutr. 1998;52:239–45.
29. Song YJ, Sawamura M, Ikeda K, Igawa S, Yamori Y. Soluble dietary fibre improves insulin sensitivity by increasing muscle GLUT-4 content in stroke-prone spontaneously hypertensive rats. Clin Exp Pharmacol Physiol. 2000;27:41–5.
30. Rodriguez-Moran M, Guerrero-Romero F, Lazcano-Burciaga G. Lipid- and glucose-lowering efficacy of Plantago Psyllium in type II diabetes. J Diabetes Complications. 1998;12:273–8.
31. Schaefer EJ, Lamon-Fava S, Cohn SD, Schaefer MM, Ordovas JM, Castelli WP, Wilson PW. Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. J Lipid Res. 1994;35:779–92.
32. Do KA, Green A, Guthrie JR, Dudley EC, Burger HG, Dennerstein L. Longitudinal study of risk factors for coronary heart disease across the menopausal transition. Am J Epidemiol. 2000;151:584–93.
33. Mack W, Krauss R, Hodis H. Lipoprotein subclasses in the monitored atherosclerosis regression study (MARS). Arterioscler Thromb Vasc Biol. 1996;16:697–704.
34. Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres JP. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Circulation. 1997; 95:69–75.
35. Gardner C, Fortman S, Krauss R. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA. 1996;276:875–81.
36. Behall KM, Cholfield DJ, Hallfrisch J. Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. Am J Clin Nutr. 2004;80:1185–93.
37. Lamarche B, Desroches S, Jenkins DJ, Kendall CW, Marchie A, Faulkner D, Vidgen E, Lapsley KG, Trautwein EA, et al. Combined effects of a dietary portfolio of plant sterols, vegetable protein, viscous fibre and almonds on LDL particle size. Br J Nutr. 2004;92:657–63.
38. Davy BM, Davy KP, Ho RC, Beske SD, Davrath LR, Melby CL. High-fiber oat cereal compared with wheat cereal consumption favorably alters LDL-cholesterol subclass and particle numbers in middle-aged and older men. Am J Clin Nutr. 2002;76:351–8.
39. Charest A, Desroches S, Vanstone CA, Jones PJH, Lamarche B. Unesterified plant sterols and stanols do not effect LDL electrophoretic characteristics in hypercholesterolemic subjects. J Nutr. 2004;134: 592–5.
40. Charest A, Vanstone C, St-Onge MP, Parson W, Jones PJ, Lamarche B. Phytosterols in nonfat and low-fat beverages have no impact on the LDL size phenotype. Eur J Clin Nutr. 2005;59:801–4.
41. Desroches S, Mauger JF, Ausman LM, Lichtenstein AH, Lamarche B. Soy protein favorably affects LDL size independently of isoflavones in hypercholesterolemic men and women. J Nutr. 2004;134:574–9.
42. Pedersen A, Baumstark MW, Marckmann P, Gylling H, Sandstrom B. An olive oil-rich diet results in higher concentrations of LDL cholesterol and a higher number of LDL subfraction particles than rapeseed oil and sunflower oil diets. J Lipid Res. 2000;41:1901–11.
43. Varady KA, St-Pierre AC, Lamarche B, Jones PJ. Effect of plant sterols and endurance training on LDL particle size and distribution in previously sedentary hypercholesterolemic adults. Eur J Clin Nutr. 2005;59:518–25.
44. Gylling H, Miettinen TA. Effects of inhibiting cholesterol absorption and synthesis on cholesterol and lipoprotein metabolism in hypercholesterolemic non-insulin-dependent diabetic men. J Lipid Res. 1996;37: 1776–85.
45. Vega-Lopez S, Conde-Knape K, Vidal-Quintanar RL, Shachter NS, Fernandez ML. Sex and hormonal status influence the effects of psyllium on lipoprotein remodeling and composition. Metabolism. 2002;51:500–7.
46. Vega-Lopez S, Freake HC, Fernandez ML. Sex and hormonal status modulate the effects of psyllium on plasma lipids and monocyte gene expression in humans. J Nutr. 2003;133:67–70.
47. Plat J, Mensink RP. Effects of plant stanol esters on LDL receptor protein expression and on LDL receptor and HMG-CoA reductase mRNA expression in mononuclear blood cells of healthy men and women. FASEB J. 2001;16:258–60.
48. Xu Y, Fu M. Alterations of HDL subclasses in hyperlipidemia. Clin Chim Acta. 2003;332:95–102.