Sex and Hormonal Status Influence the Effects of Psyllium on Lipoprotein Remodeling and Composition

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We evaluated the influence of sex and hormonal status on the effect of psyllium (PSY) supplementation on parameters of plasma lipoprotein metabolism. Twenty-four men, 23 premenopausal women, and 21 postmenopausal women (PMW) were randomly assigned to a fiber supplement (15 g PSY/d) or a control, provided via cookies, in a crossover design. Plasma lipids, insulin, apoprotein (apo) B, apo CI, apo CIII, and apo E concentrations and the composition and size of low-density lipoprotein (LDL) and very–low-density lipoprotein (VLDL) particles were measured at the end of each 30-day treatment period. Compared with control, PSY intake decreased plasma LDL cholesterol by an average of 8% ($P < .0001$) in men and pre- and PMW. There was a fiber-sex/hormonal status interaction on plasma triglycerides (TG) in the response to the intervention. Men had a 17% decrease in TG, while PMW had a 16% increase with PSY ($P < .01$). Plasma levels of apo C III, apo E, and insulin followed the same pattern as plasma TG with PSY consumption and decreased by an average of 12% in men ($P < .05$), but increased by 10% in PMW ($P < .05$). These reductions in apoproteins suggest an increased peripheral removal of TG in men, perhaps due to decreased insulin resistance, while in PMW, the increases in apoproteins may be related to an enhanced VLDL production. The lack of effect of PSY on VLDL metabolism in premenopausal women could be associated with the protective effect of estrogen. No prominent changes in VLDL and LDL composition were observed with PSY intake other than an increase in LDL phospholipid ($P < .05$). In addition, compared with men and PMW, the amount of TG per VLDL particle was less, and VLDL diameter was smaller in premenopausal women ($P < .05$). These results indicate an important role of sex and hormonal status in determining the effects of PSY on lipoprotein metabolism.

PSYLLIUM (PSY) HUSKS, obtained from the seeds of *Plantago ovata*, are a commonly available source of dietary soluble fiber with significant hypocholesterolemic effects[1-4] that may contribute to coronary heart disease risk reduction. Several human studies have shown that PSY is effective in reducing low-density lipoprotein-cholesterol (LDL-C) without decreasing high-density lipoprotein-cholesterol (HDL-C).[1,2,5]

Jenkins et al[6] reported a greater decrease in plasma total cholesterol in men fed a high-fiber diet for 4 months when compared with postmenopausal women (PMW), suggesting that sex and hormonal status modify plasma lipid responses to fiber. In addition Dallongeville et al[7] reported that both plasma LDL-C and triglycerides (TG) increase after menopause, which suggests that sex and hormonal status can also affect plasma TG.

TG metabolism can be affected by the apoproteins (apo) composition of very–low-density lipoprotein (VLDL). Accumulation of apo E in VLDL has been associated with decreased lipolysis of VLDL in human apo E3 transgenic mice[8] and human apo E3 transgenic rabbits.[9] In addition, Huang et al[9] reported that the overexpression of human apo E3 in transgenic rabbits stimulated VLDL production. Both reports suggested that apo E displaces apo CII, an activator of lipoprotein lipase (LPL),[10] from VLDL. Apo CIII also contributes to increases in plasma TG due to its function as a LPL inhibitor.[11] Moreover, Breyer et al[11] reported that the addition of apo CIII to human plasma resulted in particles almost completely depleted of apo E, an important ligand for VLDL remnant removal.[12]

The secondary mechanisms by which dietary soluble fiber affects the intravascular processing of lipoproteins, in particular VLDL, in humans have not been well elucidated. In guinea pigs, lipoprotein modifications induced by PSY include the production of larger nascent VLDL particles with a lower proportion of cholesteryl ester (CE) due to a reduction in cholesteryl ester transfer protein (CETP) and lecithin:cholesterol acyl transferase (LCAT) activities.[13] LDL particles have also been reported to have less CE and more TG after PSY consumption.[14] In addition, a favorable decrease in LDL susceptibility to oxidation in guinea pigs fed either pectin or PSY has been reported.[15]

Previous results from our study[16] indicated that PSY consumption significantly decreased plasma total and LDL-C in men and premenopausal women and PMW. However, TG responses to PSY were not uniform and varied based on sex and hormonal status. PSY intake resulted in significant decreases in plasma TG in men, but significant increases in plasma TG in PMW with no changes seen in premenopausal women, suggesting that sex and hormonal status might affect the responsiveness to PSY. Therefore, our objectives were to study the mechanisms by which PSY may exert a hypertriglyceridemic effect in PMW and to further investigate the hypocholesterolemic mechanisms of this source of soluble fiber by measuring its effects on lipoprotein remodeling and composition.

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MATERIALS AND METHODS

Materials

Powdered PSY husks were purchased from Frutarom Meer Corporation (North Bergen, NJ). Enzymatic cholesterol and TG kits were from Boehringer-Mannheim (Indianapolis, IN). Free cholesterol and phospholipid enzymatic kits, and apo CIII and apo E kits were from Wako Pure Chemical (Osaka, Japan). EDTA, aprotinin, sodium azide, phenylmethylsulfonyl fluoride (PMSF) and the kit for apo B were obtained from Sigma Chemical (St Louis, MO). Malonaldehyde bis (diethyl acetal) was obtained from Aldrich (Arlington Heights, IL). Human insulin specific radioimmunoassay (RIA) kit was from Linco Research (St Charles, MO).

Subjects

We recruited 68 healthy adults: 24 men, 23 premenopausal women, and 21 PMW. All recruited participants completed the study. The exclusion criteria were diabetes, cardiovascular disease, or lipid-lowering drug treatment. Participants were randomly assigned to a fiber supplement (15 g PSY/d) or a control (0 g PSY/d), both provided as cookies, in a crossover design. Participants were asked to consume 100 g of cookies/day for a period of 30 days, followed by a 21-day washout period. Subjects were instructed to follow the National Cholesterol Education Program step I diet (less than 30% of total energy from fat, less than 10% of energy from saturated fat, and less than 300 mg of cholesterol/d) during each treatment period. Dietary compliance during both periods was assessed by the completion of 7-day dietary records that included 2 weekend days. Subjects were also asked to return the uneaten portion of cookies to evaluate compliance. Written informed consent was obtained from each subject, and the study protocol was approved by the Committee on the use of Human Subjects in Research of the University of Connecticut. Composition of the dietary supplement, characteristics of participants at baseline, compliance, and diet evaluation during treatment periods have been reported in detail elsewhere.[16]

Plasma Lipids

Two blood samples, drawn on different days, were collected in EDTA-containing tubes at the end of each treatment period. Plasma was separated by centrifugation at 2,400 rpm for 20 minutes, and aprotinin (0.5 mL/100 mL), sodium azide (0.1 mL/100 mL), and PMSF (0.1 mL/100 mL) were added as preservatives. Plasma total cholesterol, LDL-C, HDL-C, and TG concentrations were determined as previously reported.[16]

Plasma Apoproteins

Plasma apo E[17] and apo CIII[18] were measured from 150 μL of sample using WAKO kits in a Hitachi Autonalyzer 740. Plasma apo B concentration was determined using an immunoturbidimetric method[19]; turbidity was measured in a microplate spectrophotomer at 340 nm. Plasma apo CI was measured by enzyme-linked immunosorbent assay (ELISA).[20]

Plasma Insulin

Insulin was measured in plasma using a RIA kit that uses the double-antibody/polyethylen glycol (PEG) technique.[21] Briefly, 100 μL of plasma was incubated with ^{125}I-labeled human insulin and guinea pig antihuman insulin antiserum. After an overnight incubation, a precipitating reagent containing goat antiguinea pig IgG was added, and samples were mixed and incubated for 20 minutes. Samples were then centrifuged at 2,500 $\times$ g for 20 minutes, the liquid was decanted, and tubes containing the pellet were counted for 1 minute using a Cobra II-Auto Gamma Counting System (Packard Instruments, Meriden, CT).

Lipoprotein Isolation

VLDL and LDL were isolated from the plasma of a representative subsample of each group (13 men, 13 premenopausal women, and 14 PMW) by sequential ultracentrifugation in an LE-80K ultracentrifuge (Beckman Instruments, Palo Alto, CA) at 200,000 $\times$ g and 15°C using a Ti-65 vertical rotor, as previously described.[22] Separation was based on $d < 1.006$ mg/mL for VLDL and d = 1.019 to 1.063 mg/mL for LDL. Isolated lipoprotein samples were dialyzed overnight (0.01% Na_2 EDTA, 0.9% NaCl pH 7.2 to 7.4) at 4°C.

Lipoprotein Characterization

VLDL and LDL composition were calculated after the determination of the concentration of their main components: free cholesterol (FC), CE, TG, phospholipids (PL), and protein. Total and FC, TG, and PL concentrations were measured using enzymatic methods.[23,24] Esterified cholesterol was calculated by subtracting FC from total cholesterol. Protein concentration was measured by a modified Lowry procedure.[25] The number of component molecules of LDL and VLDL were calculated assuming one apo B molecule (molecular weight, 550 kd)/particle. The apo B concentration of VLDL was adjusted after subtracting the non-apo B protein concentration, measured after a selective precipitation of apo B with isopropanol.[26] The molecular weights of TG, FC, CE, and PL used were 885.4, 386.6, 664, and 734, respectively.[27] VLDL and LDL diameters were calculated as described by Van Heek and Zilversmit.[28]

LDL Susceptibility to Oxidation

The apo B-containing lipoprotein fraction, consisting of VLDL, intermediate-density lipoprotein (IDL), and LDL, was isolated from plasma by ultracentrifugation in an LE-80K ultracentrifuge (Beckman Instruments) at 200,000 $\times$ g and 15°C using a Ti-65 vertical rotor.[22] Separation was based on $d = 1.063$ mg/mL. Samples were dialyzed overnight in an EDTA-free phosphate-buffered saline (PBS; 10 nmol/L NaH_2PO_4, 0.15 mol/L NaCl, pH 7.4), at 4°C. In vitro LDL susceptibility to oxidation was determined by the measurement of the formation of thiobarbituric acid reactive substances (TBARS), after copper-mediated oxidation of the apo B-containing lipoprotein fraction, as previously reported.[15] The lipid peroxide content was expressed as malondialdehyde equivalents.[29]

Plasma α-Tocopherol Concentration

The α-tocopherol concentrations were determined following the procedure described by Barua et al.[30] A total of 10 μg/mL α-tocopherol acetate was added as an internal standard. Samples were analyzed by high-performance liquid chromatography (HPLC) using a Rainin Microsorb 3 μm C18, 15-cm column (Varian Instruments), with 100% methanol as mobile phase. Tocopherol was detected at 290 nm. Concentrations were corrected for recovery using the internal standard.

Statistical Analysis

Repeated measures analysis of variance was performed to test the significant fiber effects (fiber *v* control), group effects (men, premenopausal, and PMW) or their interaction on plasma lipids, lipoprotein composition, plasma apoproteins, LDL susceptibility to oxidation, and α-tocopherol concentrations. Tukey's post hoc test was used to find differences among means. Data are presented as mean $\pm$ SD for the number of subjects in each group. Analyses were conducted at the 0.05 alpha level.

RESULTS

Plasma Lipids

There were no significant differences in total or LDL-C concentrations or body mass index (BMI) at baseline among

Table 1. Plasma Apoproteins B, CI, CIII, and E of Men, Women, and PMW During Control and Fiber Periods

	Plasma Apoproteins (mg/dL)			
	Apo B	Apo C I	Apo C III	Apo E
Control				
Men	115.7 ± 27.2	8.47 ± 2.45	15.45 ± 5.03[b]	4.51 ± 1.42[a]
Women	97.37 ± 27.7	6.65 ± 1.84	12.32 ± 4.88[d]	3.72 ± 1.05[c]
PMW	104.5 ± 22.8	7.14 ± 2.09	15.13 ± 4.61[b]	4.16 ± 1.09[b]
Fiber				
Men	106.4 ± 23.4	8.02 ± 2.51	13.70 ± 3.76[c]	3.94 ± 1.00[b]
Women	94.2 ± 29.2	6.71 ± 1.93	12.03 ± 4.84[d]	3.60 ± 0.94[c]
PMW	103.9 ± 28.8	7.78 ± 2.94	16.90 ± 5.56[a]	4.54 ± 1.32[a]
Repeated measures ANOVA				
Fiber effect	NS	NS	NS	NS
Group effect	NS	$P < .05$	$P < .02$	$P = .06$
Interaction	NS	NS	$P < .02$	$P < .02$

NOTE. Data are presented as mean ± SD. N = 24, 23, and 21 for men, premenopausal, and PMW, respectively. Superscripts indicate interactive effect. Values in the same column with different superscripts are statistically different.

Abbreviations: PMW, postmenopausal women; ANOVA, analysis of variance; NS, not significant.

groups, as previously reported.[16] Men had 23% lower HDL-C than PMW and 69% higher plasma TG than premenopausal women ($P < .01$).

PSY consumption produced a significant decrease in total and LDL-C concentrations without affecting HDL-C concentrations (data not shown, $P < .0001$), as reported elsewhere.[16] There was an interactive effect between fiber and sex/hormonal status for TG ($P < .01$). Men experienced a 17% reduction in TG with fiber (1.90 ± 1.38 mmol/L and 1.57 ± 0.81 mmol/L for the control and fiber period, respectively), while PMW had a 16% increase in TG with fiber intake (1.43 ± 0.64 mmol/L and 1.65 ± 0.85 mmol/L for the control and fiber period, respectivley). Plasma TG were not affected by fiber in premenopausal women (1.02 ± 0.58 mmol/L and 1.11 ± 0.62 mmol/L for the control and fiber period, respectively).

Plasma Apo

As shown in Table 1, concentrations of plasma apo B, apo CI, apo CIII, and apo E were not modified by PSY intake. However, plasma apo CI was 23% higher in men than in premenopausal women ($P < .05$). Plasma apo CIII was 24% lower in premenopausal women compared with PMW ($P < .02$). Similarly to what was observed for plasma TG, there was a significant interaction between fiber intake and sex/hormonal status for plasma apo CIII ($P < .02$) and apo E ($P < .02$). Men had an 11% and 13% decrease, while PMW had a 12% and 9% increase in plasma apo CIII and plasma apo E, respectively, with fiber intake. Changes in plasma apo B concentration with PSY intake were significantly and positively correlated with changes in plasma apo CIII, apo E, and TG (Table 2; $P < .001$ for all correlations).

Significant correlations were also observed between changes in plasma apo and plasma lipids between both the PSY and the control period, particularly among the components of VLDL (Table 2). For example, changes in plasma apo CI concentrations between control and PSY periods were positively correlated with changes in plasma apo CIII ($P < .001$), apo E and TG ($P < .01$), while changes in plasma apo CIII were positively correlated with changes in plasma apo E and TG ($P < .001$). In addition, changes in plasma apo E with PSY intake were also positively correlated with changes in plasma TG ($P < .001$).

Plasma Insulin

As shown in Fig 1, plasma insulin followed the same pattern as plasma TG, apo CIII, and apo E. Fiber intake and sex/hormonal status had an interactive effect on plasma insulin. Men experienced a decrease in this parameter with PSY consumption, while PMW had an increase ($P < .05$). Plasma insulin did not change in premenopausal women with fiber intake. In addition, premenopausal women were the group with the lowest concentration of insulin ($P < .01$). Differences in plasma insulin between control and fiber periods were positively correlated with differences in plasma apo CI, apo CIII, and apo E (Table 2, $P < .05$).

Lipoprotein Composition

Composition and size of VLDL during both treatment periods are shown in Table 3. There were no significant differences in the number of molecules of CE, FC, PL, and TG found in VLDL or the percentage of apo B in VLDL or VLDL diameter with fiber intake. Premenopausal women had the lowest number of TG molecules ($P < .05$) and the highest percentage of apo B in VLDL ($P < .05$). Premenopausal women had the smallest VLDL particles ($P = .05$). Changes in VLDL-TG with fiber intake were negatively correlated with changes in VLDL

Table 2. Significant Correlations Between Changes in Plasma Apoproteins and Changes in Plasma Lipids With PSY Intake

	ΔApoB	ΔApoCI	ΔApoCIII	ΔApoE
ΔApo CIII	0.494*	0.402*		
ΔApo E	0.530*	0.353†	0.843*	
ΔTG	0.621*	0.339†	0.564*	0.586*
ΔPlasma insulin	—	0.295‡	0.262‡	0.280‡

*$P < .001$.
†$P < .01$.
‡$P < .05$.

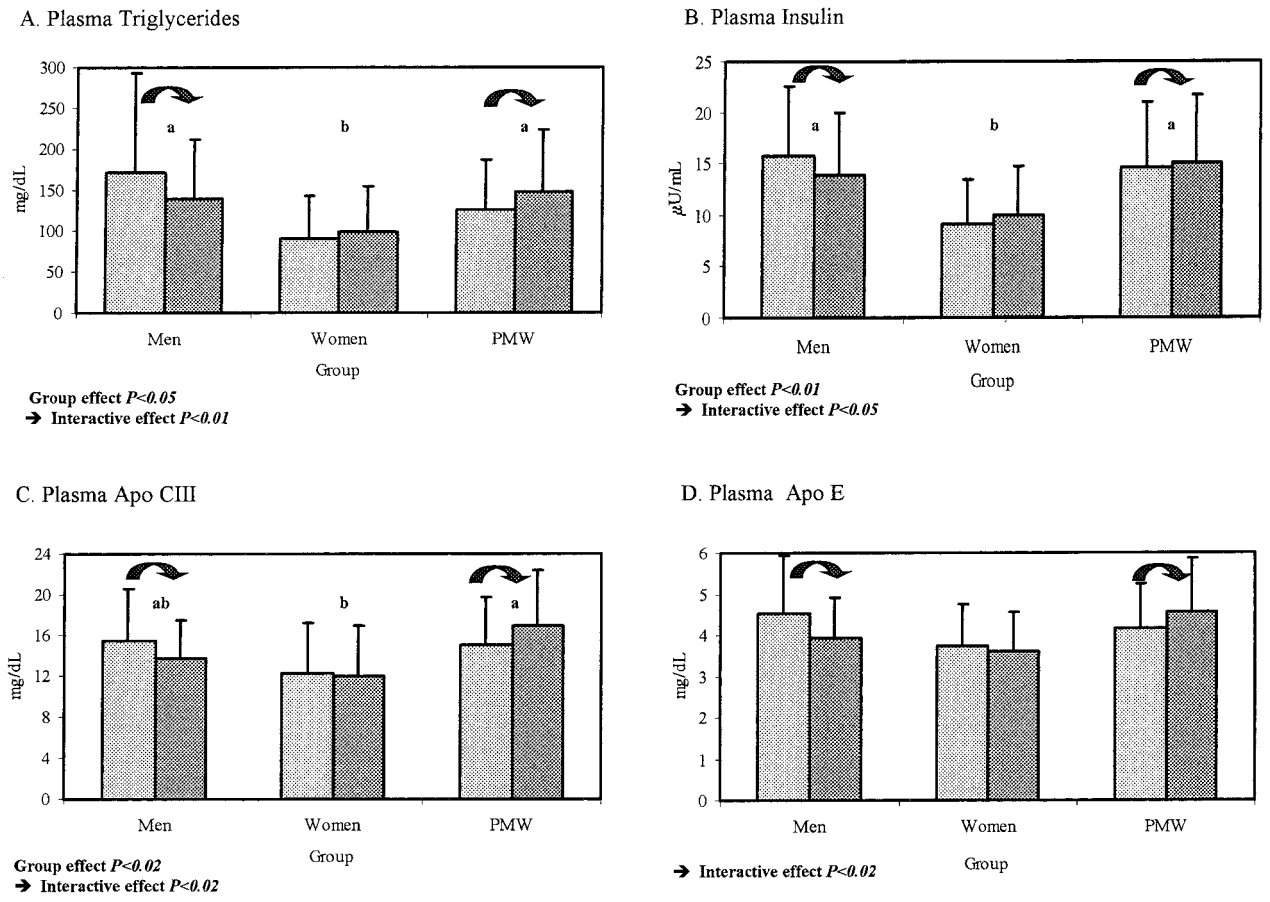

Fig 1. Plasma TG (A), insulin (B), apo CIII (C), and apo E (D) of men, women, and PMW after control (light bars) or PSY (dark bars) intake. Arrows indicate interactive effects between PSY intake and sex/hormonal status. Letters indicate sex/hormonal status effect. Groups of bars with different letters are statistically different.

apo B content (r = -.729, $P < .001$) and positively correlated with changes in VLDL diameter (r = .488, $P < .001$), which shows the contribution of TG to VLDL particle size.

The composition and size of LDL particles for both treatment periods are shown in Table 4. There were no significant differences in LDL diameter or number of CE, FC, PL, or TG

Table 3. Number of CE, FC, PL, and TG in VLDL, Apo B Content, and Particle Size for Men, Women, and PMW During Control and Fiber Periods

	VLDL No. of Molecules				Apo B	Diameter
	CE	FC	PL	TG	(%)	(Å)
Control						
Men	1,285 ± 396	872 ± 371	1,204 ± 371	3,125 ± 1,504	11.1 ± 3.9	335 ± 61
Women	960 ± 491	829 ± 564	1,050 ± 296	2,142 ± 1,090	14.3 ± 4.6	268 ± 80
PMW	1,369 ± 804	1,044 ± 787	1,557 ± 1,226	3,502 ± 2,174	10.5 ± 3.7	322 ± 72
Fiber						
Men	1,447 ± 333	922 ± 307	1,343 ± 299	3,324 ± 1,417	10.1 ± 3.4	336 ± 71
Women	1,343 ± 541	563 ± 292	1,124 ± 254	2,027 ± 631	13.1 ± 2.9	293 ± 74
PMW	1,208 ± 322	899 ± 284	1,239 ± 324	2,945 ± 1,021	11.1 ± 3.4	318 ± 59
Repeated measures ANOVA						
Fiber effect	NS	NS	NS	NS	NS	NS
Group effect	NS	NS	NS	$P < .05$	$P < .05$	$P = .05$
Interaction	NS	NS	NS	NS	NS	NS

NOTE. Data are presented as mean ± SD. N = 13, 13, and 14, for men, premenopausal, and PMW, respectively.

Abbreviations: CE, cholesteryl ester; FC, free cholesterol; PL, phospholipids; TG, triglycerides; VLDL, very–low-density lipoprotein; PMW, postmenopausal women; ANOVA, analysis of variance; NS, not significant.

Table 4. Number of CE, FC, PL, and TG in LDL and Particle Size for Men, Women, and PMW During Control and Fiber Periods

	LDL No. of Molecules				Diameter (Å)
	CE	FC	PL	TG	
Control					
Men	1,635 ± 261	675 ± 310[b]	745 ± 87	222 ± 91	190 ± 28
Women	1,815 ± 345	657 ± 218[b]	787 ± 134	195 ± 67	196 ± 27
PMW	1,729 ± 228	783 ± 346[a]	832 ± 96	215 ± 66	185 ± 30
Fiber					
Men	1,645 ± 218	673 ± 287[b]	780 ± 85	225 ± 91	188 ± 29
Women	1,826 ± 238	698 ± 219[b]	824 ± 129	206 ± 70	193 ± 22
PMW	1,663 ± 265	706 ± 328[b]	840 ± 89	232 ± 89	185 ± 32
Repeated measures ANOVA					
Fiber effect	NS	NS	$P < .01$	NS	NS
Group effect	NS	NS	NS	NS	NS
Interaction	NS	$P < .01$	NS	NS	NS

NOTE. Data are presented as mean ± SD. N = 13, 13, and 14 for men, premenopausal, and PMW, respectively. Superscripts indicate interactive effect. Values in the same column with different superscripts are statistically different.

molecules in LDL among groups. However, there was an increase in LDL PL in men, premenopausal women, and PMW with PSY intake ($P < .01$), which was negatively correlated with plasma cholesterol concentrations ($r = -.365$, $P < .05$). In addition, PMW had the highest FC content in LDL during the control period ($P < .01$). Changes in LDL diameter between treatment periods were directly correlated with differences in LDL-CE ($r = .729$, $P < .001$), which points out the contribution of this neutral component to the size of the particle.

Susceptibility of LDL to Oxidation

As indicated in Table 5, neither sex/hormonal status nor PSY affected susceptibility of apo B-containing lipoproteins to oxidation. However, susceptibility to oxidation tended to be lower during the PSY period ($P = .068$).

To account for factors that could modify changes in LDL susceptibility to oxidation, α-tocopherol concentrations in plasma were measured, and vitamin E intake was calculated from the 7-day dietary records. PMW had the highest vitamin E intake ($P < .05$). However, there were no differences in plasma α-tocopherol concentration in either period among the groups. There was a positive correlation between plasma α-tocopherol concentrations and dietary vitamin E for all subjects ($r = .48$, $P < .0001$). However, there was no correlation between plasma α-tocopherol levels and susceptibility of apo B-containing lipoproteins to oxidation (data not shown). In contrast, the changes in CE present in LDL between treatment periods was positively correlated with changes in LDL susceptibility to oxidation and negatively correlated with changes in plasma vitamin E ($r = .380$, $P < .05$).

DISCUSSION

In our previous report,[16] we documented that the effects of diet supplementation with PSY on plasma lipids are dependent on sex and hormonal status. Our results were consistent with the widely reported hypocholesterolemic responses to PSY.[2,3,5,6] However, while men experienced a decrease in plasma TG, PMW had an increase in TG with PSY intake. In this study, we examined some of the factors associated with sex and hormonal status that may have contributed to the different effects of PSY on plasma TG. We further studied the hypocholesterolemic mechanisms of dietary soluble fiber by examining lipoprotein remodeling and composition after PSY intake.

Table 5. Non-HDL Oxidation, Plasma Vitamin E, and Dietary Vitamin E of Men, Women, and PMW During Control and Fiber Periods

Control	Non-HDL Oxidation nmol TBARS/mg Protein	Plasma Vitamin E μg/mL	Dietary Vitamin E mg/d
Men	28.9 ± 8.8	13.6 ± 5.4	67 ± 131
Women	29.6 ± 7.5	12.3 ± 6.7	22 ± 34
PMW	31.2 ± 8.4	16.6 ± 6.9	107 ± 127
Fiber			
Men	27.6 ± 8.9	12.5 ± 5.1	53 ± 102
Women	28.2 ± 6.2	12.6 ± 6.7	22 ± 28
PMW	28.1 ± 8.6	16.0 ± 7.5	105 ± 122
Repeated measures ANOVA			
Fiber effect	$P = .068$	NS	NS
Group effect	NS	NS	$P < .05$
Interaction	NS	NS	NS

NOTE. Data are presented as mean ± SD. N = 24, 23, and 21 for men, premenopausal, and PMW, respectively.
Abbreviation: TBARS, thiobarbituric acid reactive substances.

Sex/Hormonal Status-Mediated TG Response to Dietary Soluble Fiber

Plasma TG responses to PSY intake vary widely according to numerous reports of dietary interventions that used PSY as a source of soluble fiber. In a meta-analysis of 11 trials evaluating the hypocholesterolemic effects of dietary soluble fiber, the average net change in plasma TG was 0.003 mmol · L^{-1} · g PSY^{-1}, with a range from −0.007 to 0.013 mmol · L^{-1} · g PSY^{-1}.[1] Several parallel trials in which PSY or placebo supplementation was given[4,31,32] failed to find significant changes from baseline in plasma TG with PSY treatment. However, these studies pooled data from men and women, therefore sex and hormonal status were not considered as variables when conducting the statistical analysis.

In humans, plasma apo CIII concentrations correlate with TG levels[33] and are known to be increased in hypertriglyceridemic individuals.[18] Apo CIII is known to inhibit TG hydrolysis by LPL,[34] decreasing the removal of TG from VLDL or chylomicrons. Ebara et al[35] reported a severe hypertriglyceridemia associated with an increase in TG-rich chylomicrons and VLDL in human apo CIII transgenic/apo E knockout mice, demonstrating that the reduction in VLDL-TG clearance due to LPL inhibition by apo CIII was independent of the displacement of apo E.

Apo E is an important apoprotein involved in VLDL removal through the apo B/E receptor.[12] Moreover, mice overexpressing apo E3 produced VLDL particles enriched with apo E3 and depleted in apo CII[8], which contributed to the decrease in LPL activity. Zsigmond et al[36] reported that apo E is not required for LPL activity; thus the decrease in TG removal may be more related to the decrease in apo CII observed when apo E is increased.[8] However, apo E has also been related to hypertriglyceridemia by actions to stimulate VLDL production and to directly inhibit VLDL-TG lipolysis by plasma lipases.[8]

Along with the effect of PSY on plasma TG, we found an interaction between PSY supplementation and sex/hormonal status on plasma apo CIII and apo E concentrations. While a reduction in these parameters was observed in men, PMW experienced an increase in plasma TG, apo CIII, and apo E with PSY intake; this is reflected by the positive correlations observed between changes in plasma TG and changes in plasma apoproteins B, CI, CIII, and E between control and fiber periods. These correlations are in agreement with the reported increases in these apoproteins in familial hypertriglyceridemic individuals compared with normolipidemic patients.[37]

Hyperinsulinemia represents a risk factor for cardiovascular disease especially for men.[38] The increased risk for cardiovascular disease may be a result of insulin resistance-related dyslipidemia, mainly characterized by elevated plasma TG, low HDL-C, and the presence of small, dense LDL particles.[39] Hypertriglyceridemia associated with insulin resistance is a product of increased VLDL secretion.[40] It has been suggested that this augmented VLDL production is caused by increased stability of newly synthesized apo B and enhanced expression of microsomal triglyceride transfer protein, resulting in facilitated assembly and secretion of VLDL.[41] In addition, there is an increase in flux of free fatty acids to the liver with insulin resistance, which further contributes to the increased VLDL synthesis.[39]

In our study, plasma insulin was decreased in men and increased in PMW after fiber intake. It is possible that men had a hypotriglyceridemic response due to the beneficial effect of PSY on insulin resistance that contributed to decreased VLDL production. Women, in contrast, are less insulin resistant than men due to the protective effect of estrogen.[42]

In the specific case of PMW, PSY may have a similar action to that reported for bile acid sequestrants, such as cholestyramine. Patients treated with cholestyramine have significant decreases in LDL-C, but they also experience increases in plasma TG,[43,44] possibly due to increased VLDL secretion. In addition, the presence of apo CIII and apo E in VLDL could also contribute to the increases in plasma TG observed in PMW by the mentioned potential inhibition of LPL.[8,11,35] Furthermore, changes in plasma insulin between treatment periods were positively correlated with changes in apoproteins CI, CIII, and E. Similarly, apoproteins B, CIII, and E are increased in patients with type 2 diabetes.[37]

Lipoprotein Remodeling and Composition

In addition to plasma lipids, it is important to identify lipoprotein composition to have a better understanding of the mechanisms responsible for the beneficial effects of dietary interventions. Results from the Cholesterol and Recurrent Events (CARE) Trial[33] identified cholesterol, TG, apo B, apo CIII, and apo E content of VLDL as independent predictors for coronary events.

Premenopausal women had the lowest number of TG molecules in VLDL and, therefore, had the largest percentage of apo B in VLDL particles. They were also the group with smallest VLDL particles. These results are in agreement with the findings that premenopausal women have the lowest plasma TG, apo CI, apo CIII, and apo E concentrations, which suggests they also had the least inhibition of LPL.

PSY intake produced an increase in LDL-PL. These results reflect changes in the surface components of LDL, which may be related to increased LDL catabolism with fiber intake.[45] Changes in surface components of the lipoproteins, especially of PL content, may affect the affinity of CETP for the different lipoproteins, decreasing the ability of this transfer protein for neutral lipid transfer.[46] The lower CETP activity we found in our earlier report[16] and the higher concentrations of PL in LDL reported here may very well be correlated and partially explain the lower concentrations of LDL-C with PSY treatment.

Normal LDL particles per se do not induce atherosclerosis. It is only when LDL particles are oxidatively modified that they bind scavenger receptors in the monocytes or macrophages, initiating the process of foam cell formation.[47] Therefore, oxidized LDL plays an important role in the atherosclerotic plaque formation process. Some animal studies have suggested an increased resistance of LDL oxidation with PSY intake.[15,48] In this study, the reduced susceptibility of LDL to oxidation after PSY consumption was only borderline significant. It is possible that a more sensitive method, such as the measurement of conjugated diene formation, might have provided more accurate information about the effects of PSY on LDL oxida-

tion. However, changes in LDL susceptibility to oxidation were positively correlated with changes in LDL-CE following fiber intake, which suggests a possible decrease in oxidative modifications in LDL among those people who had a reduction in LDL-CE with PSY.

Several studies have documented the protective effect of α-tocopherol supplementation against LDL susceptibility to oxidation.[49-51] This effect is further corroborated with the negative correlation found in this study between changes in plasma vitamin E and changes in LDL susceptibility to oxidation between the control and fiber periods. In addition, Todd et al[52] reported a negative association between antioxidant intake and risk of coronary heart disease, which supports the protective effect of vitamin E. Despite the reduction in LDL, the major carrier of vitamin E in plasma, there were no differences in plasma vitamin E between treatment periods. Kersting et al[53] reported similar effects on LDL-C and vitamin E after a cholestyramine treatment and suggested an improvement in antioxidant status as indicated by the reduction in the vitamin E/LDL-C ratio.

In conclusion, we have shown the important influence of sex and hormonal status on the effects of PSY on lipoprotein metabolism. It was clear from this study that premenopausal women had the best lipid and apoprotein profile among the 3 groups evaluated, and that these characteristics were not altered by PSY intake. The beneficial plasma LDL-C lowering of PSY was also present in premenopausal women, although no other changes were observed in VLDL metabolism. An interesting finding of this study is that male participants had additional benefits from PSY intake, mainly a decrease in plasma TG. This finding could be, in part, related to a beneficial effect on insulin resistance that contributes to decreased VLDL production with a concomitant reduction in apoproteins associated with elevated levels of plasma TG. In contrast, PSY intake was associated with higher plasma TG in PMW. Further studies using other sources of soluble fiber are still needed to evaluate whether hormonal status and sex have similar effects as PSY on TG metabolism.

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