Sex and hormonal status influence plasma lipid responses to psyllium1–3

Sonia Vega-López, Reyna Luz Vidal-Quintanar, and Maria Luz Fernandez

ABSTRACT
Background: The primary mechanisms by which soluble fiber lowers plasma cholesterol are well known. However, specific effects of fiber on lipoprotein metabolism and how sex and hormonal status influence these effects are not well defined.

Objective: The effects of a psyllium supplement in men, premenopausal women, and postmenopausal women were examined to determine the mechanisms by which psyllium lowers plasma LDL-cholesterol concentrations and affects lipoprotein remodeling in the intravascular compartment.

Design: We designed a crossover trial in which 24 men, 23 premenopausal women, and 21 postmenopausal women were randomly assigned for 30 d to a fiber supplement (15 g psyllium/d) or a control. Plasma lipids and cholesteryl ester transfer protein and lecithin-cholesterol acyltransferase (phosphatidylcholine-sterol O-acyltransferase) activities were measured after each treatment.

Results: When compared with the control, psyllium intake lowered plasma LDL-cholesterol concentrations by 7–9% (P < 0.0001) without affecting plasma HDL-cholesterol concentrations. An interactive effect between fiber and sex and hormonal status was observed for plasma triacylglycerol. Psyllium supplementation significantly lowered plasma triacylglycerol concentrations in men by 17% and raised triacylglycerol concentrations in postmenopausal women by 16% (P < 0.01). The dietary treatment did not significantly affect plasma triacylglycerol in premenopausal women. Lecithin-cholesterol acyltransferase was unaffected by psyllium intake whereas cholesteryl ester transfer protein activity was 18% lower after psyllium supplementation than after the control treatment (P < 0.0001).

Conclusions: This trial showed that the psyllium-induced responses to plasma lipids were associated with sex and hormonal status and that psyllium, through its action in the intestinal lumen, indirectly affected the intravascular processing of lipoproteins.

KEY WORDS Cholesteryl ester transfer protein, lecithin-cholesterol acyltransferase, dietary soluble fiber, sex, hormonal status, lipoproteins, plasma cholesterol, plasma triacylglycerol, psyllium, men, premenopausal women, postmenopausal women

INTRODUCTION
Until a few decades ago, male sex was considered a risk factor for the development of cardiovascular disease (CVD). However, although men are at higher risk of CVD than are women at younger ages, women's risk of CVD becomes greater as they reach and go beyond menopause (1–4). Estrogen may play a protective role against coronary risk factors (5, 6). When administered to postmenopausal women, estrogen raises HDL-cholesterol concentrations and reduces LDL-cholesterol concentrations, which results in a less atherogenic lipoprotein profile (7).

Increased consumption of dietary fiber is considered an appropriate diet therapy for reducing plasma cholesterol concentrations in mildly hypercholesterolemic patients (8–11). Dietary soluble fibers delay intestinal absorption of cholesterol and bile acids and boost bile acid excretion, which contributes to the hypocholesterolemic effect (12, 13). Psyllium husks are one of the sources of dietary soluble fiber that have consistently shown hypocholesterolemic effects (10, 14–17). Several studies in both normal and hypercholesterolemic individuals showed that psyllium husks effectively reduce LDL-cholesterol concentrations without adversely affecting HDL-cholesterol concentrations (10, 13, 15, 16, 18, 19). Because clinical studies have focused mainly on measuring plasma lipid changes induced by psyllium, little information is available on the secondary mechanisms by which psyllium, through its action in the intestinal lumen, reduces plasma LDL-cholesterol concentrations in humans.

Studies in animals have provided important information about potential mechanisms involved in the hypocholesterolemic response to dietary soluble fiber. Trautwein et al (12) reported that the hypocholesterolemic effect of psyllium was induced by more fecal bile acid excretion, leading to more bile acid synthesis in hamsters. In studies in guinea pigs, it was documented that, compared with controls, psyllium induced a reduction in apolipoprotein (apo) B secretion as well as a faster fractional catabolic rate of LDL (20). It was also shown in studies in guinea pigs that psyllium affects the intravascular processing of lipoproteins by...
reducing plasma cholesteryl ester transfer protein (CETP) activity (21). Roy et al (22) reported the effects of sex and hormonal status on the hypocholesterolemic mechanisms of psyllium in guinea pigs. Ovariectomized guinea pigs (a model for menopause) had higher plasma cholesterol, apo B, and triacylglycerol concentrations after psyllium treatment than did male or nonovariectomized guinea pigs.

The present study was conducted to evaluate whether sex and hormonal status influence psyllium effects on plasma lipids and lipoprotein remodeling in the intravascular compartment. We hypothesized that sex would influence psyllium’s effects on lipoprotein metabolism and that psyllium intake would have a less beneficial effect on plasma lipid concentrations in postmenopausal women because of their lack of estrogen.

SUBJECTS AND METHODS

Materials

Powdered psyllium husks were purchased from Frutarom Meer Corporation (North Bergen, NJ). Enzymatic cholesterol and triacylglycerol kits were obtained from Boehringer-Mannheim (Indianapolis). The free cholesterol enzymatic kit was obtained from Wako Pure Chemical (Osaka, Japan). EDTA, aprotinin, sodium azide, and phenyl methyl sulfonyl fluoride were obtained from Sigma Chemical (St Louis).

Subjects

Sixty-eight healthy adults (24 men, 23 premenopausal women, and 21 postmenopausal women) were recruited. All recruited participants completed the study. The exclusion criteria were diabetes, CVD, or a lipid-lowering drug treatment. A dietary supplement (cookies) was provided to participants, who were randomly assigned to the fiber supplement (15 g psyllium/d) or the control (0 g psyllium/d) in a crossover design. Participants were asked to consume 100 g cookies/d for 30 d. The macronutrient and fiber content of the cookies except for the psyllium, which was replaced by wheat flour in the control cookies. The cookies were weighed and packaged in individually labeled bags that contained the daily dose (5 cookies/d). Participants returned empty bags or bags containing the uneaten portion of the cookies, and the weight of the bags was recorded to calculate the amount of cookies consumed per individual during each dietary period. At the end of each treatment period, participants completed a questionnaire in which they reported any discomfort and side effects (more defecation, bloating, flatulence, or fullness or a greater need for liquid intake) caused by the supplements. Side effects were self-reported by using the following scale: 1 = none, 2 = weak, 3 = moderate, and 4 = strong.

Plasma lipids

At the end of each treatment period, 2 blood samples that were drawn on different days to control for day-to-day variability were collected. Plasma was separated by centrifugation at 1000 × g for 20 min at 4°C, and aprotinin (0.5%), sodium azide (0.1%), and EDTA, aprotinin, sodium azide, and phenyl methyl sulfonyl fluoride were obtained from Sigma Chemical (St Louis).
phenyl methyl sulfonyl fluoride (0.1%) were added for preservation purposes.

Plasma total cholesterol and triacylglycerol concentrations were measured with enzymatic methods (23, 24). HDL-cholesterol concentrations were measured in the supernatant fluid after selective precipitation of apo B–containing lipoproteins (25). LDL-cholesterol concentrations were calculated as described by Friedewald et al (26). Our laboratory has been participating in the Centers for Disease Control–National Heart, Lung, and Blood Institute Lipid Standardization Program since 1989 for quality control and standardization for plasma total cholesterol and triacylglycerol assays (CV: 1.57–2.01% for total cholesterol; 1.28–1.86% for HDL cholesterol; and 1.86–3.24% for triacylglycerol).

Activity of plasma lecithin-cholesterol acyltransferase

Physiologic plasma lecithin-cholesterol acyltransferase (LCAT; phosphatidylcholine–sterol o-acyltransferase) activity was determined by measuring the reduction in the mass of endogenous free cholesterol of plasma samples after a 6-h incubation (21). Freshly isolated plasma samples were either incubated at 37°C for 6 h or kept at −70°C (time = 0). After incubation, all samples were stored at −70°C until analyzed. Free cholesterol concentrations were measured by an enzymatic method. LCAT activity was calculated as the reduction in plasma endogenous free cholesterol after the 6-h incubation and was expressed as the molar esterification rate (µmol decrease in unesterified cholesterol/L plasma·h⁻¹).

Activity of plasma cholesterol ester transfer protein

CETP activity was calculated as the reduction in cholesteryl ester of HDL samples after a 6-h incubation (27). Freshly isolated plasma samples were either incubated at 37°C for 6 h or MgCl₂-dextran sulfate was added to precipitate apo B–containing lipoproteins (25) and the samples were stored at −70°C (time = 0). After 6 h, apo B–containing lipoproteins were also precipitated from the incubated samples, and these were stored at −70°C until analyzed. Free and total cholesteryl concentrations were determined by using enzymatic methods. Cholesteryl ester was calculated as the difference between total and free cholesterol in the samples. CETP activity was calculated as the reduction in cholesteryl ester in HDL in samples incubated for 6 h compared with the nonincubated samples. Activity was expressed as µmol decrease in cholesteryl ester·L plasma⁻¹·h⁻¹.

Food record analysis

Nutrient intake was calculated by using the NUTRITION DATA SYSTEM FOR RESEARCH (NDS-R) software version 4.01, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis [Food and Nutrient Database 29] (28). If an analytic value is not available for a nutrient in a food, the Nutrition Coordinating Center calculates the value on the basis of the nutrient content of similar foods. Nutrient intake was calculated as an average of the 7-d dietary records for both dietary periods. Nutrients evaluated included total energy, total fiber, soluble fiber, total vitamin E, and dietary cholesterol. Energy derived from total fat, saturated fat, monounsaturated fat, polyunsaturated fat, carbohydrates, protein, and alcohol was also calculated.

Statistical analysis

A one-way analysis of variance was used to test for baseline differences among means in men, premenopausal women, and postmenopausal women. A repeated-measures analysis of variance was performed to test the significant effects of fiber (fiber period compared with control period), group (men, women, and postmenopausal women), and their interaction on plasma lipid, CETP and LCAT activities, and nutrient intake. Post hoc multiple comparisons were performed with Tukey’s test to find differences among means. Data are presented as means ± SDs for the number of subjects in each group. Analyses were conducted at the α level of 0.05. Statistical analyses were conducted by using SPSS version 10.0 for WINDOWS (SPSS Inc, Chicago).

RESULTS

Baseline measurements

Both systolic and diastolic blood pressures were higher in men than in premenopausal women or postmenopausal women as shown in Table 1. There were no significant differences in baseline total or LDL-cholesterol concentrations among the groups. However, the men had 23% lower HDL-cholesterol concentrations than did the postmenopausal women and 69% higher plasma triacylglycerol concentrations than did the premenopausal women.

Diet and dietary supplement consumption

The percentage of cookies returned was higher during the fiber period for the 3 groups as shown in Table 3. Nevertheless, according to the information provided by the 7-d dietary records, total and soluble fiber intakes were significantly higher during the fiber period. There were no significant group differences in total or soluble fiber consumption, even when the data were corrected for energy intake (data not shown).

Energy intake was 5.9%, 3.6%, and 4.3% higher during the control period than during the fiber period for the men, premenopausal women, and postmenopausal women, respectively. The energy intake of the women (both pre- and postmenopausal) was on average 17% lower than the men’s. In addition, the percentage of energy provided by carbohydrates was lower during the control period. The percentage of energy provided by polyunsaturated fat was affected by sex and hormonal status: postmenopausal women had the highest intake and premenopausal women had the lowest. Dietary cholesterol intake was 17% and 15% higher during the control period than during the fiber period for men and postmenopausal women, respectively.
Also, women consumed 35% less dietary cholesterol than did men, indicating a significant group effect.

As reported by participants, fiber intake resulted in more defecation, bloating, and fullness and a greater need for liquid intake as shown in Table 4. When compared with the men, premenopausal women experienced more bloating and a greater need for liquid intake during the fiber period.

**Plasma lipids**

Consumption of the psyllium supplement resulted in a significant reduction in both total and LDL-cholesterol concentrations (Table 5). Plasma total cholesterol concentrations were 7%, 5%, and 4% lower for men, premenopausal women, and postmenopausal women, respectively, after fiber intake than after the control period. The decrease in LDL-cholesterol concentrations with psyllium intake was 7% for men and 9% for premenopausal and postmenopausal women. HDL-cholesterol concentrations were not significantly affected by the fiber intake. Similar to what was seen at baseline, the men had the lowest HDL-cholesterol concentrations. The concentrations of plasma VLDL-cholesterol concentrations and triacylglycerol were lowest in the premenopausal women in both periods. In addition, there was a significant interactive effect between fiber and sex and hormonal status for both VLDL-cholesterol and triacylglycerol concentrations. In men, VLDL-cholesterol and triacylglycerol concentrations were lowered by 18% and 17% after fiber intake. By contrast, postmenopausal women’s VLDL-cholesterol concentrations were 17% higher and their triacylglycerol concentrations were 16% higher after fiber intake.

Although there were no significant differences in the hypocholesterolemic response to psyllium among groups, a significant interactive effect between fiber and sex and hormonal status was seen for plasma triacylglycerol concentrations (Table 5). Plasma triacylglycerol concentrations were significantly lower after psyllium intake in men whereas they did not differ significantly by dietary period in premenopausal women. By contrast, postmenopausal women had significantly higher plasma triacylglycerol concentrations after the fiber period than after the control period.

**Activity of plasma of cholesteryl ester transfer protein and lecithin-cholesterol acyltransferase**

Effects of diet and sex and hormonal status on CETP and LCAT activities are shown in Table 6. Psyllium intake resulted in 24%, 18%, and 12% lower CETP activity in men, premenopausal women, and postmenopausal women, respectively. By contrast, and in agreement with the lack of effect on plasma HDL-cholesterol concentrations, psyllium intake did not significantly affect LCAT activity.

**Table 4**

<table>
<thead>
<tr>
<th>Side effects</th>
<th>Control period</th>
<th>Fiber period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Premenopausal women</td>
</tr>
<tr>
<td>Defecation$^{2,4}$</td>
<td>1.38 ± 0.71</td>
<td>1.43 ± 0.73</td>
</tr>
<tr>
<td>Bloating$^{2,4}$</td>
<td>1.21 ± 0.66</td>
<td>1.91 ± 1.12</td>
</tr>
<tr>
<td>Flatulence$^3$</td>
<td>1.33 ± 0.64</td>
<td>1.74 ± 0.81</td>
</tr>
<tr>
<td>Fullness$^3$</td>
<td>1.79 ± 1.02</td>
<td>2.26 ± 1.13</td>
</tr>
<tr>
<td>Need for liquid intake$^2$</td>
<td>1.54 ± 0.83</td>
<td>2.04 ± 1.15</td>
</tr>
</tbody>
</table>

$^1\bar{x} \pm SD; n = 24, 23, and 21 for men, premenopausal women, and postmenopausal women, respectively. Side effects were self-reported by using the following scale: 1 = none, 2 = weak, 3 = moderate, and 4 = strong. There were no significant fiber \times group interactions.

$^2,3$ Significant effect of fiber (repeated-measures ANOVA): $^2 P < 0.0001$, $^3 P < 0.005$.

$^4$ Significant effect of group, $P < 0.05$ (repeated-measures ANOVA).
group that showed a significant reduction in VLDL-cholesterol concentrations after psyllium supplementation. Jenkins et al (33) found a greater reduction in plasma total cholesterol and apo B concentrations in men who were fed a high-fiber diet for 4 mo than in postmenopausal women fed the same diet. However, Jenkins et al’s data cannot be compared with the present study because the differences in plasma triacylglycerol concentrations between men and postmenopausal women after fiber treatment were not reported (33).

An elevated plasma triacylglycerol concentration is an important risk factor for CVD, especially in postmenopausal women (34, 35). Results from the Framingham Offspring Study (4) documented that although plasma triacylglycerol concentrations are lower in women than in men, triacylglycerol concentrations become higher with age for both sexes and can be significantly high for postmenopausal women. In agreement with these findings, results from our study showed that plasma triacylglycerol concentrations were lower in premenopausal women than in men or postmenopausal women, which indicates a potential beneficial effect of estrogen in reducing plasma triacylglycerol concentrations. Estrogen has been associated with a retardation of the progress of atherosclerosis (36, 37), which reduces the risk of CVD. Estrogen’s major effect is a less atherogenic lipoprotein profile with lower plasma LDL-cholesterol concentrations and possibly higher HDL-cholesterol concentrations (7, 38).

Several studies in animals reported a reduction in plasma triacylglycerol concentrations with psyllium intake (12, 22, 28). In our study, psyllium intake reduced plasma triacylglycerol concentrations only in men whereas concentrations in postmenopausal women were higher. van Beek et al (39) suggested that in addition
to a loss of estrogen postmenopausal women have a reduced ability to regulate plasma triacylglycerol, which might partially explain the hypertriglyceridemic response to psyllium in this subject group.

VLDL is the major carrier of triacylglycerol during fasting periods. Thus, any dietary modifications that affect VLDL metabolism will alter plasma triacylglycerol concentrations. The higher plasma triacylglycerol concentrations in postmenopausal women after psyllium supplementation could be caused by more VLDL synthesis by the liver, reduced VLDL removal, or delayed conversion into intermediate-density lipoprotein and LDL due to lower lipoprotein lipase activity. Psyllium could have an action similar to one that has been reported for bile acid–binding resins such as cholestyramine (40). After drug treatment, patients had lower plasma cholesterol concentrations accompanied by higher plasma triacylglycerol concentrations that were associated with more VLDL synthesis (40). In addition, lipoprotein lipase activity was lower after the loss of estrogen (41), and the delayed uptake of triacylglycerol by extra-hepatic tissues could explain accumulation of plasma triacylglycerol concentrations. By contrast, more lipoprotein lipase activity or reduced VLDL synthesis could explain the lower plasma triacylglycerol concentrations observed in men after psyllium supplementation. What is clear in this study is that the triglyceridemic response to psyllium varied among men, premenopausal women, and postmenopausal women. This may indicate important alterations in VLDL metabolism associated with sex and hormonal status.

Lipoprotein remodeling

A reduction in CETP activity was observed in this study after psyllium supplementation suggesting that psyllium indirectly affected the intravascular processing of lipoproteins by reducing the transfer of cholesterol ester from HDL to VLDL. By contrast, LCAT activity remained unchanged with psyllium intake, which is in agreement with the lack of effect of psyllium on plasma HDL-cholesterol concentrations.

Although there is controversy about the pro- or anti-atherogenic role of CETP, studies in humans suggest that if CETP deficiency is associated with lower HDL-cholesterol concentrations, CETP’s role appears to be proatherogenic (42). In the present study, psyllium lowered plasma LDL-cholesterol concentrations and CETP activity without adversely affecting HDL-cholesterol concentrations. Thus, a beneficial effect of psyllium in reducing proatherogenic lipoproteins can be postulated.

The results in the present study in humans agree with reported data from studies in guinea pigs (21) and hamsters (43) in which a lower CETP activity was observed after soluble fiber intake. In guinea pigs, the lower CETP activity induced by psyllium is partially responsible for a smaller cholesteryl ester–depleted LDL associated with faster fractional catabolic rates (44, 45) and with a large and nascent VLDL that is triacylglycerol enriched and has less affinity for CETP (21). This large triacylglycerol-enriched VLDL is preferentially removed from the circulation by hepatic receptors and is not easily converted into LDL in the delipidation cascade (21). Psyllium intake also slows apo B secretion rate and raises LDL turnover by up-regulation of LDL receptors (44, 45).

These compositional changes in the secreted VLDL as well as the faster removal of LDL from plasma and the slower conversion of VLDL to LDL are an indirect result of depletion of hepatic cholesterol pools, more precisely of microsomal free cholesterol (20) induced by psyllium. Psyllium is primarily active in the intestinal lumen, where it interrupts the enterohepatic circulation of bile acids, which in turn results in mobilization of hepatic cholesterol to bile acid synthesis, resulting in a significant reduction in hepatic cholesterol. In guinea pigs, the combination of these secondary mechanisms that take place in the liver and in plasma result in lower plasma LDL-cholesterol concentrations.

The ability of psyllium to elevate bile acid secretion without affecting cholesterol absorption has been reported in studies in humans (32) and guinea pigs (45). The higher plasma triacylglycerol concentrations after psyllium supplementation in ovariec-tomized guinea pigs (22) is similar to the higher plasma lipid concentrations observed in the present study. Reductions in CETP activity after psyllium supplementation in the present study are similar to the reported data from studies in guinea pigs (21) and hamsters (42). It is possible that the observed metabolic alterations in hepatic cholesterol and lipoprotein metabolism influenced by psyllium intake in guinea pigs are also present in humans. The multiple secondary effects of psyllium, which affect hepatic cholesterol homeostasis, lipoprotein synthesis, remodeling, and catabolism, suggest that the hypolipidemic action of psyllium is not simplistic and warrants further investigation in the clinical setting.

With this study, we showed that sex and hormonal status influence the effects of psyllium on plasma lipids. Men had more favorable alterations in plasma lipids—reductions in both total cholesterol and triacylglycerol concentrations—than did premenopausal and postmenopausal women. Although plasma LDL-cholesterol concentrations were reduced by psyllium intake in postmenopausal women, the beneficial effects of this fiber supplement were somewhat diminished by the higher plasma triacylglycerol concentrations. In addition, our data suggest that the hypocholesterolemic mechanisms of psyllium are partially related to reduced CETP activity. Changes in the intravascular processing of lipoproteins are associated with modifications in LDL composition, size, and potential for atherogeneity. How sex and hormonal status may influence these variables needs to be further explored.

We thank Juan Flores Alvarez for his enthusiastic and professional participation in this study.

REFERENCES


