Psyllium reduces plasma LDL in guinea pigs by altering hepatic cholesterol homeostasis

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Abstract Male Hartley guinea pigs were fed semipurified diets containing various levels of psyllium and cholesterol to determine mechanisms by which psyllium lowers plasma low density lipoprotein (LDL) concentrations. Four diets were tested: control diets with 12.5% (w/w) cellulose, and psyllium diets in which cellulose was partially replaced with 7.5% (w/w) psyllium. Two levels of dietary cholesterol were used, either low (LC, 0.04%, w/w) or high (HC, 0.25%, w/w). Plasma LDL was reduced by 30 and 54% with psyllium intake in the LC and HC groups, respectively ($P < 0.001$), while plasma very low density lipoprotein (VLDL) was lowered only in the HC group ($P < 0.001$). Psyllium intake modified LDL composition and size compared to LDL from control animals with a lower proportion of cholesteryl ester and higher proportion of triacylglycerol, lower molecular weight, smaller diameter, and higher peak density ($P < 0.001$). Plasma VLDL from animals fed the psyllium-HC diet compared to the control-HC contained lower relative proportions of free and esterified cholesterol and a higher proportion of triacylglycerol, compositional characteristics similar to VLDL from animals fed LC diets. Hepatic free and esterified cholesterol concentrations were significantly reduced by psyllium an average of 25 and 55%, respectively, while hepatic HMG-CoA reductase activity was increased in both psyllium groups compared to the respective controls ($P < 0.001$). In addition, psyllium intake reduced hepatic acyl-CoA:cholesterol acyltransferase (ACAT) activity in both the LC and HC groups ($P < 0.001$) and increased hepatic membrane apoB/E receptor number (B_{max}) by 17 and 52% for animals fed LC and HC diets, respectively ($P < 0.005$). Significant psyllium-induced increases in cholesterol 7α-hydroxylase of 4- and 1.6-fold were also observed in animals fed the LC and HC diets respectively ($P < 0.001$). These results indicate that psyllium generates a negative cholesterol balance across the liver which results in induction of cholesterol 7α-hydroxylase and HMG-CoA reductase and suppression of ACAT activities, up-regulation of apoB/E receptors, and secretion of smaller VLDL particles, metabolic alterations that contribute to a lowering of plasma LDL cholesterol levels.—**Fernandez, M. L., L. R. Ruiz, A. K. Conde, D-M. Sun, S. K. Erickson, and D. J. McNamara.** Psyllium reduces plasma LDL in guinea pigs by altering hepatic cholesterol homeostasis. *J. Lipid Res.* 1995. **36:** 1128–1138.

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Ischemic heart disease mortality has been negatively correlated with dietary fiber intake in population studies (1). This protection against coronary heart disease is related to the intake of soluble fibers such as pectins, gums, and mucilages which reduce serum lipids, lower blood pressure, and improve glucose tolerance (2).

Psyllium, a hydrophilic gel-forming polymer of arabinoxylan from the seeds of the plant *Plantago ovata,* has been successfully used as a hypocholesterolemic agent in clinical trials with normal and hypercholesterolemic individuals (3–7). The possible mechanisms by which psyllium decreases plasma LDL cholesterol concentrations have been investigated in humans (7, 8) and animal models (9–12). Miettinen and Tarpila (7) reported that in humans *Plantago ovata* lowered plasma LDL through increased bile acid elimination. Similarly, Everson et al. (8) concluded that the primary mechanism by which psyllium lowers plasma cholesterol is through stimulation of bile acid synthesis. Turley, Daggy, and Dietschy (9) reported that the plasma LDL lowering in hamsters fed a psyllium-based diet was due to an increase in the rate of catabolism of cholesterol to bile acids (9). Horton, Cuthbert, and Spady (10) reported that increases in cholesterol 7α-hydroxylase activity in the hamster may be partially responsible for the hypocholesterolemic effect of dietary psyllium. These investigators also observed an increase in LDL turnover with psyllium intake, possibly the result of up-regulation of LDL receptors in response to the depletion of hepatic cholesterol pools (10). In contrast, McCall et al. (12) did not observe any changes in LDL apoB turnover in the African green monkey with psyllium intake

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; ACAT, acyl-CoA:cholesterol acyltransferase; LC, low cholesterol; HC, high cholesterol.

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and concluded that the hypocholesterolemic effect of psyllium was related to a decrease in LDL synthesis (12). In addition, these investigators found no changes in cholesterol absorption or in the activity of hepatic HMG-CoA reductase activity with psyllium intake (11).

We have previously demonstrated that dietary pectin, another soluble fiber, in the presence of high dietary cholesterol (0.25%, w/w) alters hepatic cholesterol homeostasis in the guinea pig by increasing the activity of HMG-CoA reductase, decreasing acyl-CoA:cholesterol acyltransferase (ACAT) and up-regulating hepatic membrane apoB/E receptor number, responses that result in a significant hypocholesterolemia specific for LDL (13). Modest effects on these variables were observed when pectin was fed to guinea pigs with low cholesterol (0.04%, w/w) intake (13). In contrast, dietary guar gum had significant effects on hepatic enzyme activities and plasma LDL concentrations when given with low cholesterol (14) while a more modest response was observed when guar gum was given in combination with high dietary cholesterol (14). These studies suggest that the primary mechanisms that induce plasma cholesterol lowering vary among different sources of soluble fibers (13, 14).

Based on the significant hypocholesterolemia observed in clinical studies (1–5), and on the discrepancy in the proposed mechanisms for lowering plasma LDL in hamster and African green monkeys (8–12), the effects of psyllium intake in the guinea pig were tested. The purpose of this study was to examine the metabolic responses to psyllium intake with previously defined responses to pectin and guar gum (13, 14) on cholesterol and lipoprotein metabolism to characterize the unique hypocholesterolemic properties of each type of soluble fiber, and mechanisms by which plasma LDL levels are decreased. The use of the guinea pig model for these studies is based on its unique plasma lipoprotein profile, with high LDL relative to HDL, and that guinea pigs, similar to humans, respond to dietary fat and cholesterol challenges by alterations specific to the LDL fraction (15, 16).

MATERIALS AND METHODS

Materials

Reagents were obtained from the following sources: DL-hydroxy-[3-^{14}C]methylglutaryl coenzyme A (1.81 GBq/mmol), DL-[5-^{3}H]mevalonic acid (370 GBq/mmol), cholesteryl [1,2,6,7-^{3}H]oleate (370 GBq/mmol), [^{14}C]cholesterol, aquasol, and liquifluor were purchased from New England Nuclear (Boston, MA); [1-^{14}C]oleoyl coenzyme A (1.8 GBq/mmol) and DL-3-hydroxy-3-methylglutaryl coenzyme A from Amersham; cholesteryl oleate, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and NADP from Sigma (St. Louis, MO). Enzymatic cholesterol kits, cholesterol oxidase, cholesterol esterase, and hydroperoxidase were purchased from Boehringer Mannheim (Indianapolis, IN). Powdered psyllium husks, ffl40 purified 95% and containing less than 3% fat and 1% protein, were obtained from Meer Corporation (North Bergen, NJ).

Diets

Diets were prepared and pelleted by Research Diets, Inc. (New Brunswick, NJ). The four diets had the same composition except for the fiber source and cholesterol content as indicated in **Table 1.** The fiber source was either 12.5% (w/w) cellulose (control diets) or 7.5% (w/w) psyllium plus 5% (w/w) cellulose (psyllium diets) (Table 1). Diets contained 15% (w/w) palm oil (C16:0 43.3%, C18:0 4.1%, C18:1 39.8%, C18:2 9.7%) and fat represented 35% of the energy content. Cholesterol content was either 0.04% (w/w) low cholesterol (LC) or 0.25% (w/w) high

TABLE 1. Composition of semipurified diets

Component	Diet Composition[a]				
	CNT-Low	PSY-Low	CNT-High	PSY-High	
	Weight (%)				Energy (%)
Soy protein	22.4	22.4	22.4	22.4	23.0
Palm oil	15.1	15.1	15.1	15.1	35.1
Sucrose/corn starch[b]	39.6	39.6	39.6	39.6	41.9
Cellulose	12.5	5.0	12.5	5.0	
Psyllium	0	7.5	0	7.5	
Mineral mix[c]	8.2	8.2	8.2	8.2	
Vitamin mix[c]	1.1	1.1	1.1	1.1	
Cholesterol	0.04	0.04	0.25	0.25	

[a]CNT-Low: control (0% psyllium), low cholesterol; PSY-Low: psyllium, low cholesterol; CNT-High: control (0% psyllium), high cholesterol; PSY-High: psyllium, high cholesterol.
[b]Sucrose–starch ratio 1.43.
[c]Mineral and vitamin mix are adjusted to meet NRC requirements for guinea pigs (8, 9).

cholesterol (HC) diets. These dietary cholesterol concentrations were chosen to define the effects of psyllium intake when the amount of absorbed dietary cholesterol is equivalent to 0.25 (0.04%) and 1.5 (0.25%) times the daily endogenous cholesterol synthesis rate in guinea pigs (15).

Animals

Guinea pigs weighing 250–300 g (six per group) were randomly assigned to one of four dietary groups for 4 weeks. They were housed in a light cycle room (light 7 AM to 7 PM) and had access to diets and water ad libitum. Animals were killed by heart puncture after halothane anesthesia, and plasma and liver were harvested for analysis of lipoproteins and isolation of hepatic membranes and microsomes. All animal experiments were conducted in accordance with U.S. Public Health Service/U.S. Department of Agriculture guidelines, and experimental protocols were approved by the University of Arizona Institutional Animal Care and Use Committee.

Plasma and liver lipids

Total plasma and lipoprotein cholesterol concentrations were determined by enzymatic analysis (17). Very low density lipoprotein (VLDL) + intermediate density lipoprotein (IDL), LDL, and HDL were separated by sequential ultracentrifugation in an L8-M ultracentrifuge (Beckman Instruments, Palo Alto, CA) at 125,000 *g* at 15°C for 19 h in a Ti-50 rotor. Separation was based on the following density fractionations: d < 1.019 g/ml for VLDL and IDL; d 1.019–1.09 g/ml for LDL, and d 1.09–1.24 g/ml for HDL. Hepatic concentrations of total and free cholesterol were measured according to the method of Sale et al. (18) and cholesteryl ester concentrations were calculated as the difference between free and total cholesterol.

VLDL and LDL characterization

VLDL and LDL composition were calculated by determining free and esterified cholesterol (19), protein (20), triacylglycerol, and phospholipids as previously described (13, 14). VLDL apoB was selectively precipitated with isopropanol (21). The number of constituent molecules of VLDL and LDL was calculated on the basis of one apoB per LDL (purity of LDL verified by SDS-PAGE) with a molecular mass of 412,000 kD (22) and apoB represented approximately 60% of total VLDL protein. The molecular weights were: 885.4, 386.6, 646, and 734 for triacylglycerol, free and esterified cholesterol, and phospholipids as previously reported (23). The ratio of LDL core to surface components was determined by dividing the relative percentages of cholesteryl ester plus triacylglycerol by the relative percentage of protein, free cholesterol plus phospholipids. LDL diameters were calculated according to the model of Van Heek and Zilversmit (24).

LDL binding assays

Pooled LDL samples from each dietary group were iodinated by the method of Goldstein, Basu, and Brown (25) for measurement of LDL binding to guinea pig hepatic membranes of the homologous diet. Hepatic membranes from animals fed the various diets were isolated as previously described (15) and incubated with varying concentrations of ^{125}I-labeled LDL for 2 h at 37°C. After incubation, membranes were pelleted and washed by ultracentrifugation and counted in a gamma counter. To determine apoB/E (LDL) receptor number (B_{max}) and affinity (K_d), hepatic membranes were incubated with ^{125}I-labeled LDL over a range of 10–80 μg of LDL protein/ml in the presence or absence of 1 mg/ml of unlabeled human LDL. Human LDL has been shown in previous studies to be an effective competitor for guinea pig LDL binding to hepatic apoB/E receptors at 37°C (13). Values for B_{max} and K_d were calculated from Woolf plots by plotting free LDL (μg/ml) versus free/bound LDL [(μg/ml)/(μg/mg membrane protein)] (26).

Hepatic HMG-CoA reductase assay

Hepatic microsomes for measurement of HMG-CoA reductase, cholesterol 7α-hydroxylase, and ACAT activities were isolated as previously described (16). Microsomal HMG-CoA reductase (EC 1.1.1.34) activity was measured by incubation of 200 μg microsomal protein with 7.5 nmol (0.33 GBq/nmol) [3-^{14}C]HMG-CoA, 4.5 μmol glucose-6-phosphate, 3.6 μmol of EDTA, 0.45 μmol of NADP, and 0.3 I.U. of glucose-6-phosphate dehydrogenase in a final volume of 0.20 ml for 15 min at 37°C, using [^{3}H]mevalonic acid as an internal recovery standard (0.024 GBq per assay) (27). HCl was added (25 μl) to stop the reaction and samples were further incubated for 30 min. After incubation, microsomal protein was precipitated by microfuging for 1 min. An aliquot of the supernatant was applied on TLC silica gel plates (Alltech, Deerfield, IL) and developed in acetone–benzene 1:1 (v/v). The area containing mevalolactone (R_f 0.6 to 0.9) was removed and mixed with 5 mL of Aquasol and counted for radioactivity in a scintillation counter. HMG-CoA reductase activity is expressed as pmol of [^{14}C]mevalonate synthesized per min per mg microsomal protein. Recoveries of the internal standard were between 60 to 80%.

Hepatic ACAT assay

Hepatic ACAT activity (EC 2.2.26) of guinea pig hepatic microsomes was determined by preincubating microsomal protein (0.7 to 1.0 mg per assay) with 84 mg/ml albumin as previously described (28) and ACAT buffer (50 mmol/l KH_2PO_4, 1 mol/l sucrose, 50 mmol/l KCl, 30 mmol/l EDTA, and 50 mmol/l NaF) in a final volume of 0.18 ml. After 5 min at 37°C, 20 μl (500 μmol/l)

of [1-^{14}C]oleoyl-coenzyme A (0.15 GBq/pmol) was added and the reaction proceeded for 15 min at 37°C. The reaction was stopped by addition of 2.5 ml of chloroform–methanol 2:1. The [^{3}H]cholesteryl oleate (0.045 GBq per assay) recovery standard was added with an additional 2.5 ml chloroform and 1 ml water. Tubes were vortexed and left to stand overnight. The aqueous phase was removed and after evaporation of the organic phase to dryness, samples were resuspended in 150 μl chloroform containing 30 μg of unlabeled cholesteryl oleate. Samples were applied to 20 × 20 cm glass silica gel TLC plates (Alltech, Deerfield, IL) that were developed in hexane–diethyl ether 9:1 (v/v). Cholesteryl esters were visualized with iodine vapors, scraped from the TLC plates, and counted in a scintillation counter after addition of 5 ml Liquifluor. ACAT activity is expressed as pmol of [^{14}C]cholesteryl oleate synthesized per min per mg microsomal protein. Recoveries of the internal standard were between 75 and 90%.

Hepatic cholesterol 7α-hydroxylase assay

Cholesterol 7α-hydroxylase (EC 1.14.13.7) activity was assayed by the method of Shefer and Mosbach (29) as modified by Jelinek et al. (30) using [^{14}C]cholesterol as substrate, except that cholesterol was delivered as cholesterol:phosphotidylcholine liposomes (1:8 by weight) prepared by sonication and an NADPH-regenerating system (glucose-6-phosphate dehydrogenase, NADP, and glucose-6-phosphate) was included in the assay as a source of NADPH. After addition of glucose-6-phosphate dehydrogenase (0.3 I.U), samples were incubated for an additional 30 min. The reaction was stopped by addition of 5 ml chloroform–methanol 3:1 and 1 ml acidified water (5% sulfuric acid). Tubes were mixed, the top layer was discarded, and samples were dried under nitrogen. Samples and 7α- and 7β-hydroxycholesterol standards each were dissolved in 100 μl of chloroform, applied to silica gel TLC plates, and developed with ethyl acetate–toluene 3:2. The plate was placed on XAR-5 film with intensifying screen overnight and placed in iodine vapors to mark the 7α- and 7β-hydroxycholesterol standards. Using the film as a guide, the location of the [^{14}C]7α-hydroxycholesterol spots was determined, scraped from the plate, and counted in a liquid scintillation counter.

Statistical analysis

Student's *t*-test was used to identify differences in the measured variables within the same LC or HC groups. Two way ANOVA (GBSTAT, Silver Spring, MD) was used to test significant effects in plasma lipids, VLDL and LDL composition, hepatic cholesterol concentrations, apoB/E receptor number (B_{max}) and affinity (K_d), and activities of the hepatic enzymes HMG-CoA reductase, ACAT, and cholesterol 7α-hydroxylase mediated by psyllium and dietary cholesterol.

RESULTS

Psyllium effects on plasma lipids and lipoproteins

No significant differences in either daily or total weight gain per group were observed for animals fed the test diets indicating comparable consumption of diets (**Table 2**). Both dietary psyllium and cholesterol affected plasma total cholesterol concentrations (Table 2) and plasma VLDL and LDL cholesterol concentrations (**Table 3**) with no effects on plasma triacylglycerol levels (Table 2). Psyllium reduced plasma cholesterol by 30 and 54% in animals fed the LC and HC diets, respectively ($P < 0.0001$). The hypocholesterolemic effect of psyllium was specific to LDL for guinea pigs from the LC group where LDL cholesterol was 40% lower than in animals fed the control diet (Table 3). For animals fed the HC diets, VLDL cholesterol was 80% and LDL cholesterol 50% lower than in the control groups ($P < 0.0001$).

TABLE 2. Weight gain and plasma lipids of guinea pigs fed semi-purified diets containing 0 or 7.5% (w/w) psyllium with low (0.04% w/w) or high (0.25% w/w) dietary cholesterol

Diet	Weight Gain		Plasma Lipids (mg/dl)	
	g/ 5 wks	g/day	Cholesterol	Triacylglycerol
0.04% Cholesterol				
0% Psyllium	249 ± 28^{a}	7.1 ± 1.0^{a}	71 ± 1^{b,c}	86 ± 24^{a}
7.5% Psyllium	209 ± 51^{a}	5.8 ± 2.1^{a}	51 ± 9^{c}	80 ± 23^{a}
0.25% Cholesterol				
0% Psyllium	248 ± 37^{a}	7.3 ± 1.4^{a}	198 ± 31^{a}	85 ± 16^{a}
7.5% Psyllium	256 ± 51^{a}	6.9 ± 1.0^{a}	92 ± 21^{b}	65 ± 22^{a}
P value determined by two-way ANOVA				
Psyllium effect	NS	NS	< 0.0001	NS
Cholesterol effect	NS	NS	< 0.0001	NS
Interaction	NS	NS	0.002	NS

Data are presented as mean ± SD for n = 6 per dietary group. Values in the same column not sharing the same superscript are significantly different; NS, not significant.

TABLE 3. Lipoprotein cholesterol concentrations of guinea pigs fed semipurified diets containing 0 or 7.5% (w/w) psyllium with low (0.04%) or high (0.25%) dietary cholesterol

Diet	Lipoprotein Cholesterol (mg/dl)		
	VLDL	LDL	HDL
0.04% Cholesterol			
0% Psyllium	2.2 ± 0.9^{c}	57.5 ± 13.6^{b,c}	11.5 ± 2.1^{a}
7.5% Psyllium	2.1 ± 1.0^{c}	35.2 ± 7.2^{c}	14.3 ± 1.6^{a}
0.25% Cholesterol			
0% Psyllium	33.4 ± 1.3^{a}	149.6 ± 38.1^{a}	14.6 ± 4.3^{a}
7.5% Psyllium	6.6 ± 2.9^{b}	76.2 ± 19.5^{b}	10.6 ± 3.8^{a}
P value determined by two-way ANOVA			
Psyllium effect	0.0002	< 0.0001	NS
Cholesterol effect	< 0.0001	< 0.0001	NS
Interaction	0.0002	0.011	NS

Data are presented as mean ± SD for n = 6 animals per dietary group. Values in the same column not sharing the same superscript are significantly different; NS, not significant.

Dietary cholesterol (HC diets) increased plasma LDL 2.8-fold in the HC control group and psyllium intake lowered plasma LDL cholesterol concentrations to values similar to those found in guinea pigs fed the control LC diet (Table 3). Although plasma VLDL cholesterol was decreased with psyllium intake in animals fed the HC diets, values were still higher than in animals fed LC diets. Plasma HDL cholesterol concentrations were not affected by the level of dietary psyllium or cholesterol (Table 3).

Psyllium effects on VLDL and LDL composition and size

VLDL free cholesterol, cholesteryl ester, triacylglycerol, and protein content were affected by both psyllium and cholesterol intake. The cholesterol effects were associated with increases in the relative proportion of free and esterified cholesterol and a corresponding reduction in triacylglycerol (**Table 4**). In addition, the relative protein content was reduced with the high cholesterol intake, suggesting a reduction in the percent of surface components of the VLDL particle since phospholipid concentrations were not affected. No significant differences were observed in VLDL compositions of guinea pigs fed the 0 or 7.5% psyllium-LC diets (Table 4) in agreement with the lack of effect on plasma VLDL cholesterol levels (Table 3). In contrast, psyllium intake significantly affected the composition of VLDL from guinea pigs fed the HC diet. VLDL free cholesterol and cholesteryl ester were lower and the protein percentage was higher in the VLDL from guinea pigs fed the psyllium-HC diet compared to the control-HC group (Table 4). The compositional differences induced by both cholesterol and psyllium on VLDL are shown in **Fig. 1** where the number of molecules of triacylglycerol, phospholipid, free cholesterol, and cholesteryl ester was calculated for VLDL particles from guinea pigs in the four dietary groups. The largest particle containing more cholesteryl ester and free cholesterol was VLDL from guinea pigs fed the control-HC diet, while VLDL from the control-LC and both psyllium diets presented similar values for the number of molecules of

TABLE 4. Composition of VLDL from guinea pigs fed semipurified diets containing 0 or 7.5% psyllium with low (0.04%) or high (0.25%) dietary cholesterol

Diet	VLDL Composition (%)					
	CE	FC	TAG	PL	Protein	ApoB
0.04% Cholesterol						
0% Psyllium	0.9 ± 0.4^{b}	5.5 ± 0.8^{b}	67.0 ± 5.0^{a}	12.0 ± 2.9^{a}	15.1 ± 3.9^{a}	8.7 ± 2.6^{b}
7.5% Psyllium	1.3 ± 1.4^{b}	5.3 ± 0.6^{b}	65.5 ± 7.3^{a}	15.0 ± 1.9^{a}	14.9 ± 2.2^{a}	9.8 ± 2.0^{b,c}
0.25% Cholesterol						
0% Psyllium	12.9 ± 3.5^{a}	9.0 ± 1.6^{a}	49.8 ± 7.3^{b}	14.4 ± 1.0^{a}	11.6 ± 1.5^{b}	6.9 ± 0.9^{c}
7.5% Psyllium	5.4 ± 3.4^{b}	6.3 ± 1.8^{b}	57.4 ± 7.9^{b}	14.1 ± 2.1^{a}	16.6 ± 2.6^{a}	12.0 ± 2.9^{a}
P value determined by two-way ANOVA						
Psyllium effect	0.0001	0.004	NS	NS	0.043	0.0033
Cholesterol effect	0.004	0.014	0.0003	NS	NS	NS
Interaction	0.001	0.02	NS	NS	0.03	0.047

Data are presented as mean ± SD for n = 6 animals. Values in the same column not sharing the same superscript are significantly different; NS, not significant. Abbreviations: CE, cholesteryl ester; FC, free cholesterol; TAG, triacylglycerol and PL, phospholipids.

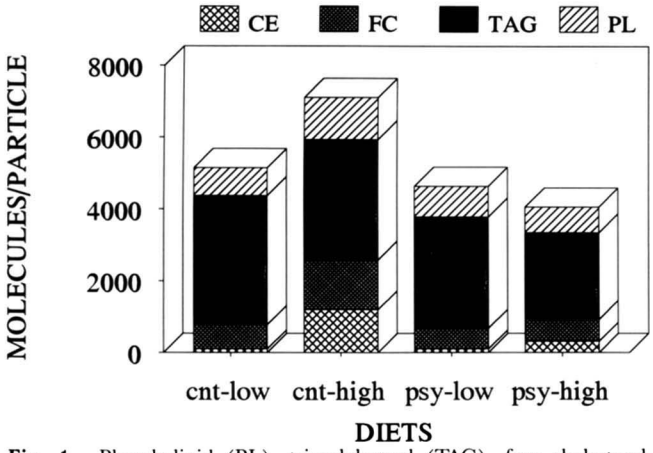

Fig. 1. Phospholipid (PL), triacylglycerol (TAG), free cholesterol (FC), and cholesteryl ester (CE) molecules per VLDL particle from guinea pigs fed control (CNT) or psyllium (PSY) diets with low (0.04%, w/w) or high (0.25% w/w) dietary cholesterol. The number of FC, CE, and PL molecules was significantly higher in animals fed the CNT-high diet ($P < 0.001$). CE molecules were higher in the PSY-high group compared to both low cholesterol groups ($P < 0.001$). Control diets are equivalent to 0% psyllium.

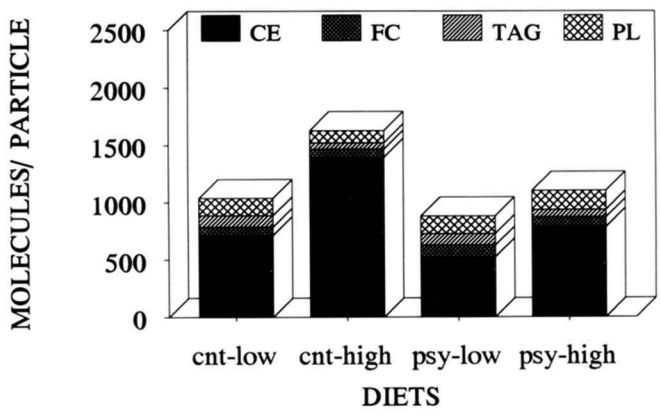

Fig. 2. Cholesteryl ester (CE), free cholesterol (FC), triacylglycerol (TAG), and phospholipids (PL) molecules per LDL particle from guinea pigs fed control (CNT) or psyllium (PSY) diets with low (0.04%, w/w) or high (0.25%, w/w) dietary cholesterol. LDL from psyllium-fed guinea pigs had a lower number of CE molecules and higher number of TAG molecules in both the low and high cholesterol groups ($P < 0.001$). The highest number of CE and lowest of TAG molecules was from animals fed the control-high cholesterol diet. Control diets are equivalent to 0% psyllium.

each VLDL component and therefore similar size (Fig. 1).

LDL size and composition were significantly affected by psyllium and cholesterol intake (**Table 5**). Intake of psyllium resulted in smaller LDL particles in both the LC and HC groups as demonstrated by the smaller diameters, lower molecular weights (M.W.), decreased core to surface ratios, and higher peak densities (Table 5). Although cholesterol intake significantly increased LDL particle size ($P < 0.0001$), psyllium in the diet reduced the size of LDL to that of guinea pigs fed the control-LC diet (Table 5). **Figure 2** illustrates the size differences of the LDL particles by presenting the number of molecules of each LDL component based on one apoB per LDL. SDS-PAGE electrophoresis documented the presence of only apoB in the isolated LDL (data not shown). The largest LDL particles were from animals fed the control-HC diet while the smallest was from animals fed the psyllium-LC diet (Fig. 2), clearly documenting that dietary cholesterol and psyllium affect LDL size and composition.

Psyllium effects on hepatic cholesterol

Similar to effects on plasma lipoprotein cholesterol levels, hepatic cholesterol concentrations were significantly altered by psyllium and cholesterol in the diet. Reductions of 25 and 42% of hepatic total cholesterol were observed in guinea pigs fed the psyllium-LC and psyllium-HC diets, respectively ($P < 0.005$) (**Table 6**). The effect of psyllium was on both the free and esterified cholesterol pools with an average reduction of 25 and 55% in the hepatic free and esterified cholesterol concentrations,

TABLE 5. Size characteristics of LDL from guinea pigs fed semipurified diets containing 0 or 7.5% (w/w) psyllium with low (0.04% w/w) or high (0.25% w/w) dietary cholesterol

Diet	LDL Characteristics			
	Diameter (Å)	M.W. ($\times 10^6$)	Core/Surface	Peak Density (g/ml)
0.04% Cholesterol				
0% Psyllium	214 ± 10^b	1.10 ± 0.04^b	1.00 ± 0.03^b	1.045 ± 0.002^b
7.5% Psyllium	169 ± 30^c	0.76 ± 0.14^c	0.82 ± 0.15^c	1.052 ± 0.004^c
0.25% Cholesterol				
0% Psyllium	378 ± 30^a	1.48 ± 0.07^a	1.79 ± 0.33^a	1.037 ± 0.003^a
7.5% Psyllium	224 ± 17^b	0.97 ± 0.08^b	1.23 ± 0.15^b	$1.050 \pm 0.002^{b,c}$
P value determined by two-way ANOVA				
Psyllium effect	< 0.0001	< 0.0001	< 0.0001	0.0011
Cholesterol effect	< 0.0001	< 0.0001	< 0.0001	0.029
Interaction	0.0001	0.0059	0.019	NS

Data are presented as mean ± SD for n ' 6 animals. Values in the same column not sharing the same superscript are significantly different; NS, not significant. Core to surface was determined by dividing the relative percentages of cholesteryl ester plus triacylglycerol by the relative percentage sum of protein, phospholipids, and free cholesterol.

respectively, compared to animals fed the control diets (Table 6). Animals fed the psyllium-HC diets had similar hepatic free cholesterol and slightly higher esterified cholesterol concentrations compared to animals fed the control-LC diet, thus documenting a notable effect of psyllium on hepatic cholesterol pools in animals fed the HC diet. This hepatic cholesterol lowering induced by psyllium had significant effects on hepatic enzyme activities and apoB/E receptor number as discussed below.

Psyllium effects on hepatic apoB/E receptor number

The number of hepatic apoB/E receptors was higher in animals fed both the LC and HC psyllium diets compared to the respective control groups (**Table 7**). Psyllium increased LDL receptor number (B_{max}) by 1.2- and 1.5-fold in animals fed LC and HC diets, respectively. Cholesterol intake decreased receptor number by 40% in animals fed the control-HC diet and dietary psyllium up-regulated hepatic apoB/E receptors to values found in animals fed the control-LC diet (Table 7). The dissociation constant, K_d, was not affected by either dietary treatment (Table 7). Plasma LDL cholesterol concentrations and hepatic apoB/E receptor B_{max} values exhibited a significant negative correlation ($r = -0.91$, $P < 0.001$) (**Fig. 3**).

Psyllium effects on hepatic enzymes

HMG-CoA reductase activity was suppressed with cholesterol intake (**Table 8**) and psyllium up-regulated enzyme activity in the HC group to values close to those found in animals fed the control-LC diet. A significant increase in reductase activity was also found in animals fed the psyllium-LC diet consistent with the observed reduction in hepatic cholesterol content associated with psyllium intake (Table 8). A negative correlation was observed between hepatic free cholesterol and HMG-CoA reductase activity ($r = -0.725$, $P < 0.01$) (**Fig. 4**, upper panel). Hepatic ACAT activity was higher in both control groups compared to animals fed the psyllium diets and the activity was correlated with the concentration of hepatic free cholesterol in animals from all dietary groups ($r = 0.82$, $P < 0.01$) (Fig. 4, lower panel). The highest ACAT activity was observed in animals fed the control-HC diet and the lowest in animals fed the psyllium-LC diet (Table 8). Cholesterol 7α-hydroxylase was not up-regulated by dietary cholesterol (Table 8); however, psyllium intake in animals fed the LC diet resulted in a 4-fold increase in enzyme activity while animals fed the HC diet had only a 1.6-fold increase in the activity of hepatic microsomal cholesterol 7α-hydroxylase (Table 8).

TABLE 6. Hepatic cholesterol concentrations of guinea pigs fed semipurified diets containing 0 or 7.5% psyllium with low (0.04%, w/w) or high (0.25% w/w) dietary cholesterol

Diet	Hepatic Cholesterol (mg/g)		
	Total	Free	Esterified
0.04% Cholesterol			
0% Psyllium	3.4 ± 0.2^{b}	3.1 ± 0.3^{b}	0.27 ± 0.10^{c}
7.5% Psyllium	2.6 ± 0.2^{c}	2.5 ± 0.2^{c}	0.13 ± 0.04^{d}
0.25% Cholesterol			
0% Psyllium	7.0 ± 2.1^{a}	4.7 ± 1.4^{a}	2.36 ± 1.13^{a}
7.5% Psyllium	4.1 ± 0.8^{b}	3.3 ± 0.3^{b}	0.93 ± 0.50^{b}
P value determined by two-way ANOVA			
Psyllium effect	0.0012	0.0032	0.0058
Cholesterol effect	0.0001	0.0016	0.0001
Interaction	0.038	NS	0.018

Data are presented as mean ± SD for n = 6 animals. Values in the same column not sharing the same superscript are significantly different; NS, not significant.

TABLE 7. LDL binding constants of hepatic membranes of guinea pigs fed semipurified diets containing 0 or 7.5% (w/w) psyllium with low (0.04% w/w) or high (0.25% w/w) dietary cholesterol

Diet	LDL Binding Constants	
	B_{max} (μg/mg)	K_d (μg/ml)
0.04% Cholesterol		
0% Psyllium	4.06 ± 0.03^{a,b}	26 ± 9
7.5% Psyllium	4.78 ± 0.39^{a}	34 ± 11
0.25% Cholesterol		
0% Psyllium	2.43 ± 0.30^{c}	33 ± 7
7.5% Psyllium	3.67 ± 0.81^{b}	39 ± 10
P value determined by two-way ANOVA		
Psyllium effect	0.002	NS
Cholesterol effect	0.0003	NS
Interaction	NS	NS

Data are presented as mean ± SD for n = 6 animals. Values in the same column not sharing the same superscript are significantly different; NS, not significant.

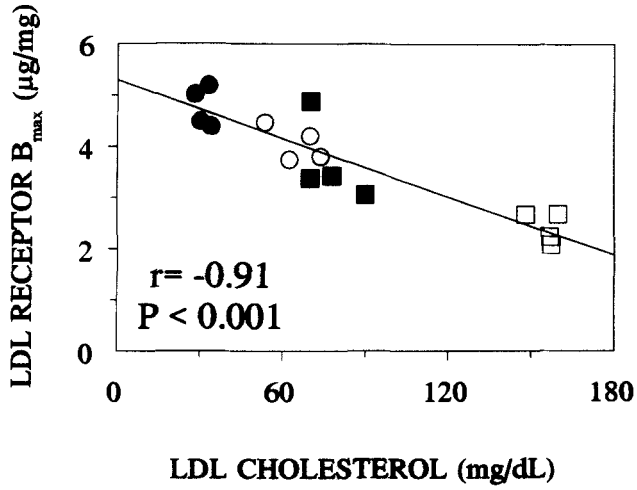

Fig. 3. Correlation between plasma LDL cholesterol concentrations and hepatic LDL receptor number (B_{max}) of guinea pigs fed control (CNT) low cholesterol (○), psyllium (PSY) low cholesterol (●), CNT, high cholesterol (□), and PSY high cholesterol (■) diets. Control diets are equivalent to 0% psyllium.

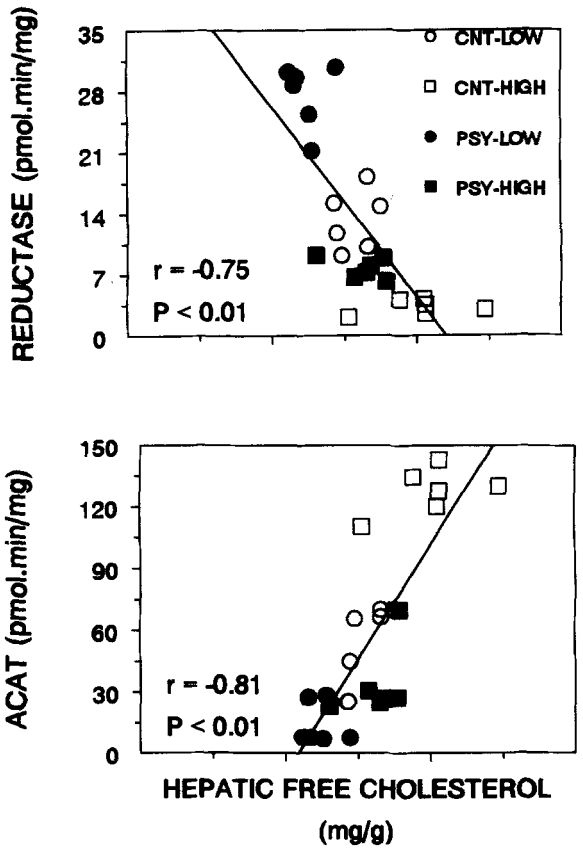

Fig. 4. Correlation between hepatic cholesterol concentrations and hepatic HMG-CoA reductase (upper panel) and ACAT (lower panel) activities of animals fed control (CNT), low cholesterol (LC) (○), psyllium (PSY) low cholesterol (●), control, high cholesterol (□), or PSY high cholesterol (■) diets. Control diets are equivalent to 0% psyllium.

DISCUSSION

Dietary soluble fiber has been shown to decrease plasma cholesterol concentrations in numerous clinical trials and animal studies (1-16). The primary mechanisms responsible for the hypocholesterolemic effects of soluble fiber have been a topic of investigation and debate over the last decade (2). In many cases the physical properties of the constitutive polymers of the different types of fiber are not consistent with their putative action in the small intestine. For example, pectin is generally of low viscosity in solution (31) and psyllium does not bind bile acids in vitro (5); however, the observed responses in some studies, including the present investigation, suggest that pectin to some extent decreases cholesterol absorption (13, 31-33) and that psyllium affects the rate-limiting enzyme of bile acid synthesis (10) indicating a direct effect of psyllium on the enterohepatic circulation of bile acids.

What is clear in this study, and studies in the hamster (9, 10), is that psyllium is an effective hypocholesterolemic agent when fed with either low or high levels of dietary cholesterol. The liver is the major organ involved in whole body sterol balance and regulation of plasma LDL cholesterol levels (34) and a major response to psyllium intake is a negative sterol balance in the liver that triggers a series of responses in hepatic enzymes and the LDL receptor culminating in significant reductions of plasma LDL. The homeostasis of cholesterol in the liver is maintained by a net balance between cholesterol delivered to the liver from dietary sources and lipoprotein uptake by hepatic receptors and rates of biliary cholesterol secretion, catabolism of cholesterol to bile acids, and cholesterol efflux in lipoproteins. In addition, the liver regulates plasma LDL cholesterol through its function as the sole site of VLDL synthesis and its major role in LDL catabolism through the apoB/E receptor (35). The primary mechanisms by which psyllium induces a negative hepatic sterol balance could be related to a decrease in cholesterol absorption (36), although this mechanism appears significant only in combination with a bile acid sequestrant such as cholestyramine (36), interruption of bile acid enterohepatic circulation (8), decreased micelle formation due to its viscosity (5), or a combination of

TABLE 8. Activity of hepatic enzymes of guinea pigs fed semipurified diets containing 0 or 7.5% (w/w) psyllium with low (0.04 w/w) or high (0.25% w/w) dietary cholesterol

Diet	Hepatic Enzymes (pmol/min-mg)		
	HMG-CoA Reductase	ACAT	7α-Hydroxylase
0.04% Cholesterol			
0% Psyllium	13 ± 3^{b}	57 ± 18^{b}	0.28 ± 0.20^{b,c}
7.5% Psyllium	28 ± 4^{a}	14 ± 10^{d}	1.15 ± 0.62^{a}
0.25% Cholesterol			
0% Psyllium	3 ± 1^{d}	128 ± 11^{a}	0.25 ± 0.08^{c}
7.5% Psyllium	8 ± 1^{c}	37 ± 18^{c}	0.42 ± 0.20^{c}
P value determined by two-way ANOVA			
Psyllium effect	< 0.0001	< 0.0001	< 0.001
Cholesterol effect	< 0.0001	< 0.0001	< 0.001
Interaction	0.0003	0.0007	0.025

Data are presented as mean ± SD for n = 6 animals. Values in the same column not sharing the same superscript are significantly different.

mechanisms that effectively reduce hepatic cholesterol pools and consequently plasma LDL levels.

From these studies it can be argued that the guinea pig is an appropriate model to investigate mechanisms of plasma hypocholesterolemia induced by psyllium or other fiber sources because, similar to humans, guinea pigs have higher levels of LDL than HDL and the plasma cholesterol lowering induced by fiber with physiological levels of dietary cholesterol is specific to the atherogenic LDL mimicking the human situation (6–8). In contrast to humans, other animals models, such as hamsters or rats, when fed psyllium or other sources of dietary fiber respond by lowering LDL modestly while HDL is lowered by a greater order of magnitude (6–8, 37).

Psyllium effects with low dietary cholesterol (LC diets)

Dietary psyllium lowered hepatic cholesterol pools and plasma LDL concentrations in animals fed the LC diet and the three regulatory enzymes involved in hepatic cholesterol homeostasis were significantly altered with psyllium intake. The significant up-regulation of cholesterol 7α-hydroxylase could be the key step that triggers the responses of the other two enzymes. Horton, Cuthbert, and Spady (10) have reported that increases in cholesterol 7α-hydroxylase activity, and parallel increases in 7α-hydroxylase mRNA levels, could account in part for the hypocholesterolemic effects of psyllium in the hamster. The decrease in hepatic cholesterol induced by psyllium intake may be due to mobilization of hepatic cholesterol for bile acid synthesis as suggested by the increase in cholesterol 7α-hydroxylase activity.

The regulation of cholesterol 7α-hydroxylase by dietary cholesterol appears to vary according to the animal model. The rat exhibits up-regulation of enzyme activity when challenged with dietary cholesterol (38) because rats use this route of cholesterol elimination (synthesis of bile acids) efficiently accounting for why this animal model is very unresponsive to dietary cholesterol. In contrast, studies with African green monkeys have shown that mRNA abundance and activity of hepatic cholesterol 7α-hydroxylase were down-regulated with an atherogenic diet (containing 0.33% cholesterol) (39) which suggests an attempt by the liver to decrease intestinal cholesterol absorption by reducing bile acid production. In the guinea pig model, similar to what was observed in the hamster (10), dietary cholesterol did not increase hepatic microsomal cholesterol 7α-hydroxylase activity. Horton et al. (10) did observe a higher activity of cholesterol 7α-hydroxylase in hamsters fed a high cholesterol-psyllium diet than observed in the present study in the guinea pig.

Increases in HMG-CoA reductase activity with psyllium intake paralleled those of cholesterol 7α-hydroxylase consistent with the theses that the reduced hepatic free cholesterol concentrations in animals fed the psyllium diets results from an increased production of bile acids due to the increase in cholesterol 7α-hydroxylase. This metabolic condition stimulates cholesterol production and up-regulates HMG-CoA reductase. Simultaneously, ACAT activity is down-regulated because the availability of free cholesterol for esterification is substantially reduced. The interplay of these three regulatory enzymes mediated by dietary psyllium results in an up-regulation of hepatic apoB/E receptors and plasma LDL concentrations are reduced, presumably due to an increase in LDL catabolism (13). In animals fed the LC diets, no significant modifications were observed in plasma VLDL concentrations or composition suggesting that psyllium in combination with a low cholesterol diet does not affect VLDL metabolism to a significant extent. Further studies measuring VLDL apoB turnover rates would be necessary to clarify specific effects on VLDL metabolism.

Psyllium effects with high dietary cholesterol (HC diets)

Psyllium intake induced a more pronounced response in plasma and hepatic cholesterol concentrations in guinea pigs fed the HC diet. In addition, not only were plasma LDL concentrations and physicochemical properties altered, but plasma VLDL levels and composition were clearly modified, suggesting that the hypocholesterolemic action of dietary psyllium occurs at multiple sites in the regulation of lipoprotein synthesis and intravascular processing, effects that were not detectable in animals fed psyllium with the LC diet. The composition of VLDL from the HC groups suggests that more cholesterol is packaged into VLDL in response to the higher concentrations of cholesterol in the liver which result in larger VLDL particles containing more cholesterol and less triacylglycerol than VLDL from animals fed the LC diet. Nascent VLDL from guinea pigs fed low cholesterol diets does not have a high concentration of cholesteryl ester (40, 41); the cholesteryl ester is likely acquired by cholesteryl ester transfer protein (CETP) activity. However, nascent VLDL from cholesterol-fed guinea pigs has been shown to have a relatively high proportion of esterified cholesterol derived from hepatic cholesterol pools (38), although it is possible that up-regulation of CETP activity by dietary cholesterol is also a contributing factor (42). At present the fate of these large VLDL particles, and their contribution to the increased plasma LDL pool, is unknown; however, the significantly higher concentrations of LDL in control-HC animals suggest that dietary cholesterol not only decreased the number of hepatic apoB/E receptors but possibly increased the production of VLDL and its rate of conversion to LDL. It is possible that the psyllium-induced hepatic hypocholesterolemia in animals fed the HC diet resulted not only in up-regulation of apoB/E receptors but also in decreased production of LDL from the smaller VLDL particles. In addition, the activities of hepatic enzymes regulating

cholesterol homeostasis were also significantly modified by psyllium intake. Although the up-regulation of cholesterol 7α-hydroxylase was not of the same magnitude as observed in animals fed the LC diet, enzyme activity was significantly increased. In addition, HMG-CoA reductase activity was increased in response to hepatic cholesterol depletion with psyllium intake and ACAT activity was substantially decreased as the hepatic free cholesterol pool was reduced.

From these studies we conclude that psyllium, similar to reports from clinical studies (2, 8), is an effective hypocholesterolemic agent in guinea pigs and, while some of the mechanisms responsible for plasma LDL lowering were investigated in this work and have been previously addressed by other investigators (8–10), our results suggest that there are multiple potential metabolic sites that are affected by psyllium intake including synthesis, intravascular processing, and catabolism of plasma VLDL.

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