

Distinct mechanisms of plasma LDL lowering by dietary fiber in the guinea pig: specific effects of pectin, guar gum, and psyllium

Maria Luz Fernandez

Lipid Metabolism Laboratory, Department of Nutritional Science and Interdisciplinary Nutritional Sciences Program, University of Arizona, Tucson, AZ 85721

Abstract Pectin (PE), guar gum (GG), and psyllium (PSY) lower plasma low density lipoprotein (LDL) cholesterol concentrations in guinea pigs with different orders of magnitude by inducing defined alterations in hepatic cholesterol homeostasis (Fernandez et al. 1994. *Am. J. Clin. Nutr.* **59**: 869–879; **61**: 127–134 and 1995. *J. Lipid Res.* **36**: 1128–1138). To further explore specific mechanisms responsible for the differences in plasma and hepatic cholesterol lowering, the effects of these fibers were evaluated on cholesterol absorption, hepatic cholesterol 7 α -hydroxylase activity, the rate-limiting enzyme of bile acid synthesis, and in vivo LDL transport to target specific primary and secondary mechanisms accounting for the observed responses. Fibers were fed with physiological (0.04%), low cholesterol (LC), or pharmacological high cholesterol (HC) (0.25%) levels to assess whether cholesterol intake influences plasma LDL lowering mechanisms. Intake of PE, GG, or PSY with LC or HC diets lowered plasma and hepatic cholesterol concentrations ($P < 0.001$). PE and PSY up-regulated 7 α -hydroxylase activity 3-fold with LC and PE by 5-fold with HC diets. In contrast, GG intake had no effect on 7 α -hydroxylase activity. Cholesterol absorption was reduced 30% by PE intake while no differences were found between control and PSY groups. GG reduced cholesterol absorption only with HC diets. Intake of PE, GG, or PSY with HC diets resulted in faster plasma LDL fractional catabolic rates (FCR) ($P < 0.01$) with no effect on LDL apoB flux rates (FR) or pool size, suggesting that fiber reduced LDL cholesterol concentration without decreasing the number of LDL particles. In addition to reducing LDL apoB FR, PE and PSY increased LDL FCR with HC diets while GG effects were limited to lowering LDL apoB FR. ■ These results indicate that the distinctive reductions in hepatic cholesterol induced by PE, GG, and PSY associated with plasma cholesterol lowering result from different mechanisms specific to each fiber and that the levels of dietary cholesterol contribute to the different metabolic responses.—**Fernandez, M. L.** Distinct mechanisms of plasma LDL lowering by dietary fiber in the guinea pig: specific effects of pectin, guar gum, and psyllium. *J. Lipid Res.* 1995. **36**: 2394–2404.

Supplementary key words LDL transport • cholesterol 7 α -hydroxylase • cholesterol absorption

Epidemiological studies have shown that dietary fiber is protective against coronary heart disease risk (1). Numerous clinical and animal studies have shown that intake of soluble non-starch polysaccharides lowers plasma LDL cholesterol concentrations, therefore reducing the associated risk to cardiovascular disease (2–11). Although some of the mechanisms responsible for the observed hypocholesterolemia have been reported for some animal models (5–8), controversy exists regarding specific actions of fiber in the small intestine that result in lower plasma LDL cholesterol concentrations. Abbey, Triantafyllidis, and Topping (7) have found that pectin lowers plasma LDL cholesterol in the rat only in combination with high cholesterol diets, while guar gum effects were not different from the control group fed methylcellulose. Decreases in apoB production rates with no effects on plasma LDL turnover were reported as a plasma LDL lowering mechanism by psyllium in the African green monkey (8). In addition, these authors did not find increases in hepatic HMG-CoA reductase activity by psyllium intake (8). In contrast, hamsters have been shown to increase LDL receptor expression by PSY intake with high cholesterol diets (5) and increases in cholesterol synthesis in the liver and extrahepatic tissues have been reported (6).

Many mechanisms have been suggested to explain dietary fiber-mediated effects on the resulting hypo-

Abbreviations: PE, pectin; GG, guar gum; PSY, psyllium; LDL, low density lipoprotein; LC, low cholesterol; HC, high cholesterol; FCR, fractional catabolic rate; FR, flux rate; RID, radial immunodiffusion.

cholesterolemia including decreases in cholesterol absorption (12), binding to bile acids (4), effects on hormones and other parameters (13), and production of volatile fatty acids (14, 15), although controversy exists regarding this last mechanism (16). Based on the effects of soluble fiber on cholesterol and lipoprotein metabolism in guinea pigs (9–11) and other studies (5–7) of the proposed mechanisms, decreases in cholesterol absorption and interruption of bile acid enterohepatic circulation are the most likely candidates to explain plasma LDL lowering. In these studies (5–10), soluble fiber has been shown to up-regulate hepatic HMG-CoA reductase activity (9–11) and increase cholesterol synthesis in hepatic and extrahepatic tissues (6), mechanisms not compatible with increased production of volatile fatty acids (16) which would result in decreased synthesis of cholesterol not increases in both liver and extra-hepatic tissues (6, 9–11).

Previous studies in guinea pigs have shown that the hypocholesterolemic effects of dietary fiber vary in the order of magnitude depending on the ability of the tested fiber to deplete hepatic cholesterol pools. This plasma cholesterol lowering has been associated to the type of fiber and the amount of dietary cholesterol present whether in physiological or pharmacological concentrations (9–11).

These studies propose that the plasma LDL lowering is directly related to the depletion of hepatic cholesterol by dietary fiber, that the primary and secondary mechanisms accounting for the lowering of hepatic cholesterol concentrations vary depending on the type of fiber, and also that the amount of dietary cholesterol directly

affects the metabolic responses. To test this hypothesis, guinea pigs were fed with one insoluble fiber (cellulose) (control diet) and three soluble fibers, pectin (PE), guar gum (GG), or psyllium (PSY), with two different levels of dietary cholesterol equivalent to an absorbed amount of 0.25- and 1.5-times the endogenous cholesterol synthesis in guinea pigs (17) to evaluate effects of these fibers on cholesterol absorption, hepatic cholesterol 7α -hydroxylase activity, and LDL transport as possible mechanisms associated with plasma LDL lowering.

Guinea pigs were chosen in these and previous investigations based on similarities to humans in their observed response to dietary fiber (9–11, 18), their lipoprotein profile (9–11), the distribution of hepatic cholesterol pools (19), absence of forestomach which complicates studies in other animal models (20), and gender similarities in response to dietary fiber (21, 22).

EXPERIMENTAL PROCEDURES

Materials

Reagents were obtained from the following sources: [1,2, 3 H(N)]cholesterol (1650.2 GBq/mmol), [4- 14 C]cholesterol (1.9GBq/mmol), Aquasol and Liquifluor were purchased from New England Nuclear (Boston, MA); cholesteryl oleate, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and NADP were from Sigma (St. Louis, MO); radioimmunoassay kits were from Bio-Rad (Hercules, CA); enzymatic cholesterol kits, cholesterol oxidase, cholesterol esterase, and hydroperoxidase were purchased from Boehringer Mannheim (Indian-

TABLE 1. Composition of test diets

Component	Diet Composition				Energy
	Control ^a	Pectin	Guar Gum	Psyllium	
	wt %				%
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
Mineral mix ^c	8.2	8.2	8.2	8.2	-
Vitamin mix ^c	1.1	1.1	1.1	1.1	-
Cholesterol ^d	0.04	0.04	0.04	0.04	-
Cellulose	12.5	0	0	5.0	-
Pectin	0	12.5	0	0	-
Guar gum	0	0	12.5	0	-
Psyllium	0	0	0	7.5	-

^aControl (12.5% cellulose).

^bSucrose-starch ratio 1.43.

^cMineral and vitamin mix adjusted to meet NRC requirements for guinea pigs (14).

^dHigh cholesterol diets contain 0.25% cholesterol.

apolis, IN). High methoxylated pectin made from lime peels and containing 6.7% methoxyl groups and 74% galacturonic acid was obtained from Grinsted Products Inc. (Industrial Airport, KA); guar gum type MMM/12 containing 84–89% fiber, 10% protein, and 1.5% ash was provided by Meer Corporation (North Bergen, NJ); powdered psyllium husks # 40 -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 eight 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), 12.5% pectin (PE), 12.5% guar gum (GG), or 7.5% (w/w) psyllium plus 5% (w/w) cellulose (PSY) (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. The amount of cholesterol was either 0.04% (w/w), low cholesterol (LC) or 0.25% (w/w), high cholesterol (HC) diets. These dietary cholesterol concentrations were chosen to define the effects of fiber intake when the amount of absorbed dietary cholesterol is equivalent to 0.25 (0.04%) or to 1.5 (0.25%) times the

TABLE 2. Plasma cholesterol and apoB concentrations of guinea pigs fed control, pectin, guar gum, and psyllium diets with low cholesterol (0.04%, w/w) or high cholesterol (0.25%, w/w)

Diets	Plasma Cholesterol	ApoB
	<i>mg/dl</i>	
Low cholesterol		
Control	68 ± 12 ^a	44.9 ± 13.3
Pectin	56 ± 17 ^b	46.2 ± 9.8
Guar gum	53 ± 15 ^b	29.8 ± 7.8
Psyllium	46 ± 10 ^b	45.1 ± 12.2
High cholesterol		
Control	167 ± 38 ^a	134.9 ± 39.0 ^a
Pectin	85 ± 29 ^b	83.6 ± 34.2 ^b
Guar gum	104 ± 38 ^b	95.8 ± 22.6 ^b
Psyllium	75 ± 25 ^b	70.1 ± 5.4 ^b
Two-way ANOVA		
Fiber effect	<i>P</i> < 0.0001	<i>P</i> = 0.03
Cholesterol effect	<i>P</i> < 0.0001	<i>P</i> < 0.0001
Interaction	N.S.	N.S.

Values are presented as mean ± SD for *n* = 21 animals per dietary group for plasma cholesterol and *n* = 6–8 for apoB concentrations. Values in the same column within the low or high cholesterol group with different superscripts are significantly different as determined by one-way ANOVA and Newman-Keuls post hoc test (*P* < 0.001).

daily endogenous cholesterol synthesis rate in guinea pigs (17).

Animals

Male guinea pigs weighing 250–300 g were randomly assigned to one of eight 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. No differences in weight gain/day were observed for animals fed the different diets in agreement with previous reports (9–11). Animals used for the in vitro experiments were killed by heart puncture after halothane anesthesia and plasma and livers were harvested. Animals used for the in vivo studies were killed by an excess of halothane vapors. 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 LDL isolation and labeling

Plasma cholesterol concentrations were determined by enzymatic analysis (23). Pooled LDL from each dietary group were separated by sequential ultracentrifugation in a L8-M ultracentrifuge (Beckman Instruments, Palo Alto, CA) at 125,000 *g* at 15°C for 19 h in a Ti-50 rotor at a density range of 1.02 to 1.09 g/ml and dialyzed against 0.09% NaCl and 0.01% EDTA for 24 h. Purity of the LDL preparations was checked by SDS-PAGE and only apoB was detected (data not shown).

Human LDL was isolated by sequential ultracentrifugation between densities 1.019 to 1.063 g/ml and washed at 1.063 g/ml. Methylated human LDL was prepared as described by Weisgraber, Innerarity, and Mahley (24) and the specificity of the methylation procedure was confirmed as previously described (25). Iodination of guinea pig LDL (pooled LDL from three animals fed the homologous diet) and human methylated LDL was performed according to the method of Goldstein, Basu, and Brown (26). The same batch of guinea pig LDL was used for all animals fed the same diet and the radiolabeled LDL were used within 2–3 days of preparation to minimize potential changes during oxidation (27).

Determination of apoB concentrations

Polyclonal antibodies against apoB-100 were prepared by injecting guinea pig purified LDL (checked by SDS-PAGE) into a sheep in one dose (300 µg/ml) followed by two booster doses (200 µg/ml) every 10 days. Antibodies were purified by use of antigen affinity column and apoB antibodies were eluted by modification of pH (28). The specificity of the polyclonal antibodies was tested by use of RID kits. ApoB concentrations in

TABLE 3. Hepatic cholesterol concentrations and cholesterol 7 α -hydroxylase activity of guinea pigs fed control, pectin, guar gum, and psyllium diets with low cholesterol (0.04%, w/w) or high cholesterol (0.25%, w/w)

Diets	Hepatic Cholesterol		Cholesterol 7 α -Hydroxylase
	Free	Esterified	
	mg/g		pmol/min-mg
Low cholesterol			
Control	3.10 \pm 0.31 ^a	0.31 \pm 0.02 ^a	1.19 \pm 0.17 ^b
Pectin	2.52 \pm 0.22 ^b	0.21 \pm 0.07 ^b	3.27 \pm 0.66 ^a
Guar gum	2.43 \pm 0.10 ^b	0.26 \pm 0.01 ^b	1.12 \pm 0.60 ^b
Psyllium	2.51 \pm 0.11 ^b	0.13 \pm 0.04 ^b	3.06 \pm 0.70 ^a
High cholesterol			
Control	5.42 \pm 1.12 ^a	2.81 \pm 0.50 ^a	1.54 \pm 0.22 ^c
Pectin	2.22 \pm 0.23 ^c	0.15 \pm 0.07 ^c	5.07 \pm 2.30 ^a
Guar gum	4.03 \pm 0.79 ^b	2.10 \pm 1.54 ^{a,b}	1.30 \pm 0.08 ^c
Psyllium	3.32 \pm 0.31 ^b	0.93 \pm 0.51 ^b	2.51 \pm 0.62 ^b
Two-way ANOVA			
Fiber effect	<i>P</i> < 0.001	<i>P</i> = 0.01	<i>P</i> = 0.05
Cholesterol effect	<i>P</i> < 0.001	<i>P</i> < 0.001	N.S.
Interaction	N.S.	<i>P</i> = 0.02	N.S.

Values are presented as mean \pm SD for n = 6 animals per dietary group. Values in the same column within the low or high cholesterol groups with different superscripts are significantly different as determined by one-way ANOVA and Newman-Keules post hoc test (*P* < 0.001).

plasma and LDL samples were measured by silver-enhanced radioimmunoassay (SERID) in which the antigen was allowed to diffuse radially from wells punched into gel media containing the antibody (29). RID plates were incubated at 37°C for 24–72 h. Nonspecific proteins were removed by applying pressure for 1 h and the gels were dyed with Coomassie blue. Diameters of the immunoprecipitate were read using an RID reader. Linear regression equations were generated for the standard calibrator curve to calculate sample concentrations. The standards were prepared by isolating guinea pig LDL at a more restricted density (d = 1.023–1.075 g/ml) and further purification by use of agarose column chromatography. The purity of LDL was checked by SDS-PAGE. Protein was determined by a modification of the Lowry method (30) and standards ranging in concentration from 0 to 650 μ g/ml were prepared.

Hepatic cholesterol concentrations and cholesterol 7 α -hydroxylase assay

Hepatic concentrations of total and free cholesterol were determined according to Carr, Andresen, and Rudel (31) and esterified cholesterol was calculated as the difference between free and total cholesterol. Cholesterol 7 α -hydroxylase (EC 1.14.13.7) activity was as-

sayed essentially as described by Jelinek et al. (32) using [¹⁴C]cholesterol as substrate except that cholesterol was delivered as cholesterol-phosphatidylcholine liposomes (1:8 by weight) prepared by sonication; 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 of chloroform-methanol 3:1 and 1 ml of acidified water (5% sulfuric acid). Tubes were mixed, the top layer was discarded, and samples were dried under nitrogen. Samples and 7 α - and 7 β -hydroxycholesterol standards each were dissolved in 100 μ l 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 7 α and 7 β standards. Using the film as a guide, the locations of the [¹⁴C]7 α -hydroxycholesterol spots were determined, scraped from the plate, and counted in a liquid scintillation counter.

Metabolic studies

Plasma LDL turnover kinetics were determined as previously described (25). FCR values were calculated

TABLE 4. Cholesterol absorption of guinea pigs fed control, pectin, guar gum, and psyllium diets with low cholesterol (0.04%, w/w) or high cholesterol (0.25%, w/w)

Diets	Cholesterol Absorption			
	Control	Pectin	Guar Gum	Psyllium
			%	
Low cholesterol	60.8 ± 19.5 ^a	37.1 ± 5.3 ^b	64.2 ± 12.4 ^c	64.6 ± 10.6 ^c
High cholesterol	64.1 ± 10.3 ^a	47.3 ± 12.7 ^b	44.5 ± 14.4 ^b	61.8 ± 15.8 ^c
Two-way Anova				
Fiber effect			N.S.	
Cholesterol effect			N.S.	
Interaction			N.S.	

Values are presented as mean ± SD for n = 6 animals per dietary group, except for pectin where n = 5. Values in the same row with different superscripts are significantly different as determined by ANOVA and Newman-Keules post hoc test ($P < 0.001$).

using a two pool model as described by Mathews (33). For all studies, guinea pig LDL kinetics were measured over a period of 28 h by sampling plasma LDL radioactivity at 5 min (zero time), and at 0.5, 1, 2.5, 5, 10, 20, 24, and 28 h after injection of the radiolabeled LDL. Samples were obtained by taking 300- μ l blood samples via an indwelling catheter of silastic tubing, I.D. 0.64 mm (Dow Corning, Corning NY) inserted into the femoral artery. ¹²⁵I-labeled LDL (70 μ g protein) from guinea pigs fed the homologous diet and ¹³¹I-labeled methylated human LDL (40 μ g protein) were simultaneously injected into guinea pigs to determine total and receptor-independent FCR, respectively (25). Receptor-mediated clearance was calculated by subtracting non-receptor LDL FCR from total FCR. LDL apoB pool size was calculated by multiplying the mg/ml obtained by radioimmunoassay \times plasma volume (ml/kg) = mg/kg with the assumption of 45 ml plasma/kg in guinea pigs, and LDL apoB flux rates were calculated by multiplying the pool size \times FCR.

Cholesterol absorption

Cholesterol absorption was determined by the method of Zilversmit (34) which has been validated in guinea pigs (35). Food was withdrawn from animals 12 h before they were given 370 GBq of [1,2³H(N)]cholesterol orally by adding 10 μ l of the radiolabeled isotope to one pellet of the corresponding diet. The [³H]cholesterol was promptly adsorbed by the pellet and guinea pigs consumed it within 2 min, followed by appropriate amounts of water and food. Once the pellet was consumed, animals were injected with 5.63 GBq of [4-¹⁴C]cholesterol through an indwelling catheter inserted in the femoral artery. The [¹⁴C]cholesterol was mixed

with guinea pig plasma, incubated for 15 min at 37°C, and vortexed for 10 min prior to injection. Blood samples (300 μ l) were taken every 12 h up to 72 h to ensure that the plasma isotope ratio had reached equilibrium (35). Plasma was separated from red blood cells, mixed with Aquasol, and counted. Cholesterol absorption was calculated from the ratio of ¹⁴C (injected dose) to ³H (oral dose) multiplied by the ratio of ³H to ¹⁴C in plasma at the time of equilibrium.

Statistical analysis

One-way ANOVA and the Newman-Keules post hoc test (GBSTAT, Silver Spring, MD) were used to determine differences between dietary treatments in plasma and hepatic cholesterol concentrations, cholesterol absorption, activity of cholesterol 7 α -hydroxylase, and LDL kinetics variables within the low and high cholesterol groups. Two-way ANOVA was used to determine fiber effects, cholesterol effects, and interactions. Values were considered significant with $P < 0.05$. Significant correlations were calculated by use of linear regression.

RESULTS

Plasma cholesterol concentrations were significantly decreased by dietary fiber in both the LC and the HC groups (Table 2). PE, GG, and PSY lowered plasma cholesterol by 18, 21, and 32% in the LC group and 50, 38, and 55% in the HC groups, respectively. Two-way ANOVA indicated a fiber effect in reducing plasma cholesterol concentrations and a cholesterol effect in increasing plasma cholesterol levels with higher levels of dietary cholesterol (Table 2). No effect of fiber on

TABLE 5. LDL kinetic parameters of guinea pigs fed control, pectin, guar gum, and psyllium diets with low cholesterol (0.04%, w/w) or high cholesterol (0.25%, w/w)

Diets	ApoB Pool Size mg/kg	FCR pools/h	ApoB Production tes mg/kg-h
Low cholesterol			
Control	19.2 ± 5.8	0.073 ± 0.005 ^b	1.42 ± 0.36
Pectin	19.7 ± 4.2	0.093 ± 0.0056 ^a	1.70 ± 0.53
Guar gum	12.7 ± 3.4	0.113 ± 0.020 ^a	1.58 ± 0.30
Psyllium	19.0 ± 5.2	0.103 ± 0.015 ^a	1.95 ± 0.62
High cholesterol			
Control	51.8 ± 13.8 ^a	0.057 ± 0.009 ^b	2.81 ± 0.50 ^a
Pectin	32.3 ± 13.6 ^b	0.078 ± 0.019 ^a	2.17 ± 0.49 ^b
Guar gum	37.3 ± 8.7 ^b	0.059 ± 0.016 ^a	2.10 ± 0.39 ^b
Psyllium	26.5 ± 2.0 ^b	0.080 ± 0.010 ^a	2.08 ± 0.40 ^b
Two-way ANOVA			
Fiber effect	<i>P</i> = 0.033	<i>P</i> < 0.0001	<i>P</i> = 0.003
Cholesterol effect	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> = 0.004
Interaction	N.S.	N.S.	<i>P</i> = 0.01

Values are presented as mean ± SD for n = 5–8 animals per dietary group. Values in the same column within the low or high cholesterol groups with different superscripts are significantly different as determined by one-way ANOVA and the Newman-Keules post hoc test (*P* < 0.001).

plasma apoB levels was observed for animals fed the physiological levels of dietary cholesterol (LC diets) while PE, GG, and PSY significantly reduced plasma apoB concentrations in the HC group, indicating that fiber reduced not only the amount of total cholesterol but, in addition, the number of lipoprotein particles (Table 2). A fiber effect in reducing plasma apoB concentrations was detected by two-way ANOVA while increasing the amount of dietary cholesterol resulted in increases in plasma apoB levels, suggesting a higher number of apoB-containing lipoproteins.

Hepatic cholesterol concentrations in both the free and esterified pools were reduced by dietary soluble fiber (Table 3). For animals fed LC diets, free cholesterol was reduced an average of 20% by intake of the three fiber sources while PSY had the most pronounced effect in lowering hepatic esterified cholesterol, followed by PE and GG (Table 3).

The lowering of hepatic cholesterol in animals fed HC diets differed in order of magnitude depending on the fiber source. PE had the most pronounced cholesterol-lowering effect as it reduced by 60 and 95% the free and esterified hepatic cholesterol pools, respectively; PSY had an intermediate effect while GG effects were moderate (Table 3). These differences in hepatic cholesterol-lowering result from different mechanisms as will be demonstrated in subsequent experiments. Hepatic cho-

lesterol 7 α -hydroxylase was up-regulated 3-fold by PE and PSY in animals fed LC diets. PE up-regulated the enzyme activity by 5-fold in animals fed HC diets while the effects of PSY on cholesterol 7 α -hydroxylase were more modest. In contrast to PE and PSY, GG intake did not affect hepatic cholesterol 7 α -hydroxylase activity in either the LC or HC groups (Table 3).

To determine whether the reduction in hepatic cholesterol concentrations was due to decreased delivery of cholesterol to the liver by chylomicron remnants, the effects of fiber on cholesterol absorption were measured. PE reduced cholesterol absorption by 40 and 26% in animals fed both the LC and HC diets, respectively (Table 4). GG decreased cholesterol absorption by 28% only in animals fed HC diets. PSY did not affect cholesterol absorption compared to animals fed control diets in the presence of either low or high concentrations of dietary cholesterol (Table 4).

LDL metabolic parameters were distinctively affected by dietary fiber type. LDL fractional catabolic rates (FCR) were increased by PE, GG, and PSY in animals fed LC diets while there was no effect on LDL apoB flux rates or LDL apoB pool size (Table 5). As dietary soluble fiber reduced plasma cholesterol concentrations and guinea pigs carry the majority of cholesterol in LDL, these data indicate that the number of LDL particles was not affected by fiber but the size of the LDL was,

resulting in smaller particles containing less cholesterol per particle as was observed previously (9–11).

In animals fed the HC diets, the mechanisms of plasma LDL lowering were different and dependent on the type of fiber. Dietary PE and PSY increased LDL FCR while GG did not affect plasma LDL turnover. However, a reduction of LDL apoB flux rates was observed in animals fed GG, PE, and PSY (Table 5). Two-way ANOVA indicated a fiber effect in increasing FCR and decreasing apoB flux rates although this last parameter was mostly affected by results from the HC group (Table 5). Dietary cholesterol significantly increased LDL apoB pool size, decreased LDL FCR, and increased LDL apoB flux rates as indicated by two-way ANOVA.

There was a significant positive correlation between plasma cholesterol concentrations and LDL FCR for animals from all dietary groups (Fig. 1) indicating that plasma LDL turnover rates are strong determinants of plasma cholesterol concentrations. The slower FCR observed in animals fed HC diets compared to the LC group are illustrated in Fig. 2 for animals from the PSY group. Similar curves were obtained for the PE, GG, and control groups (data not shown). Receptor-mediated LDL FCR was faster in animals fed PE, GG, and PSY with LC and HC diets than in control animals (Fig. 3). In addition, animals fed LC diets exhibited faster non-receptor-mediated clearance than animals fed HC diets which made a significant contribution to total LDL FCR (Fig. 3).

There was a significant positive correlation between plasma cholesterol levels and hepatic cholesterol concentrations in all dietary groups (Fig. 4) associated with the lowering of hepatic cholesterol pools resulting from dietary fiber effects on primary and secondary mecha-

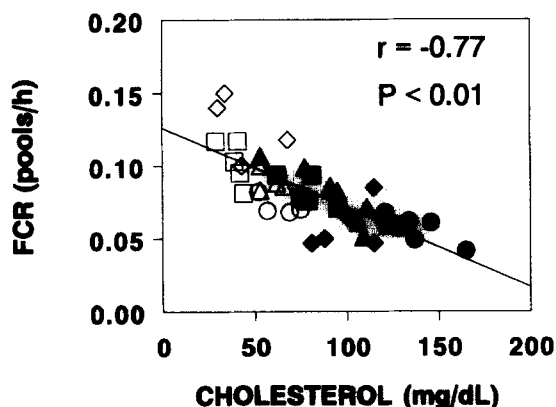


Fig. 1. Correlation between plasma cholesterol levels and total fractional catabolic rates (FCR) ($r = -0.77$, $P < 0.01$) of guinea pigs fed control (○, ●), pectin (△, ▲), guar gum (◇, ◆), or psyllium (□, ■). Open symbols represent low cholesterol (0.04%, w/w) and dark symbols high cholesterol (0.25%, w/w) groups.

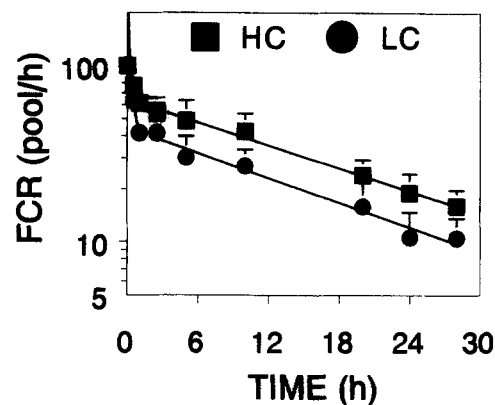


Fig. 2. Plasma decay curves of LDL isolated from animals fed psyllium with low (●) or high cholesterol (■) diets and injected into guinea pigs fed the homologous diet. Identical amounts of LDL protein were injected (70 μ g) and plasma decay curves were analyzed as described in Experimental Procedures. Each curve represents the mean \pm SD of 6 animals.

nisms, which in turn influenced plasma LDL cholesterol concentrations.

DISCUSSION

Soluble fiber and depletion of hepatic cholesterol pools: primary mechanisms

Numerous mechanisms have been proposed to explain the plasma cholesterol-lowering effects of non-starch polysaccharides. Although it is generally agreed that soluble fiber lowers plasma LDL cholesterol in clinical studies (2–4), the hypocholesterolemic responses vary depending on the physicochemical properties of the fiber tested (36), the type of diet that will affect fiber interaction with other nutrients in the small intestine (37), whether individuals are normal or hypercholesterolemic (38), and, recently, gender has been found to be an important variable to consider (21, 22).

Previous studies in the guinea pig have shown that PE, GG, and PSY decrease hepatic cholesterol concentrations, resulting in multiple effects on hepatic cholesterol homeostasis including increases in hepatic LDL receptor number, up-regulation of HMG-CoA reductase activity, and down-regulation of acyl CoA:acyltransferase (ACAT) (9–11). As the extent of hepatic cholesterol-lowering was dependent on the fiber type, it was postulated that this depletion in hepatic cholesterol concentrations resulted from different primary mechanisms specific to each fiber tested and closely related to the amount of dietary cholesterol.

The action of soluble fiber in the small intestine will play a major role in regulating whole body cholesterol

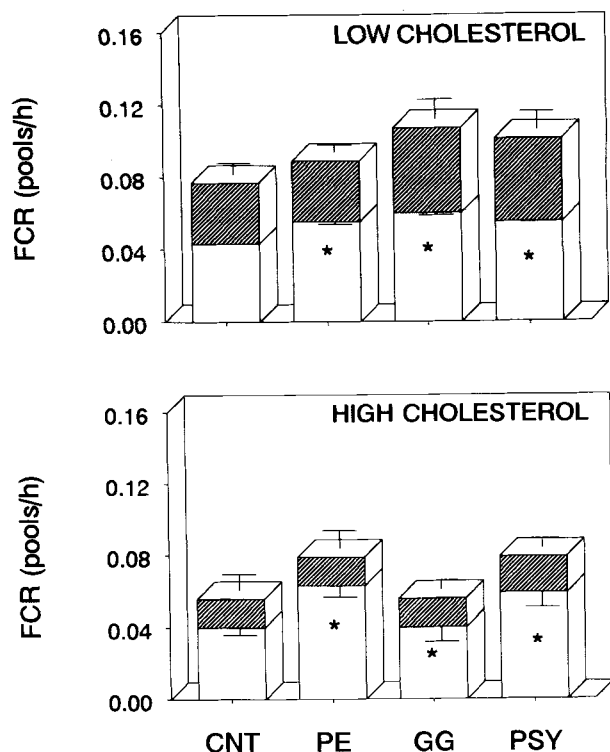


Fig. 3. Receptor-dependent (open bar) and receptor-independent (hatched bar) LDL turnover rates of guinea pigs fed control (CNT), pectin (PE), guar gum (GG), and psyllium (PSY) diets with low cholesterol (0.04%) (upper panel) and high cholesterol (0.25%) (lower panel). Each bar represents the mean \pm SD of 5–8 animals; *represents significantly different from the control diet. Two-way ANOVA indicated that receptor-independent FCR was significantly higher in animals fed the low cholesterol diets ($P < 0.05$).

balance as this organ is responsible for the absorption of dietary and biliary cholesterol and for the reabsorption of bile acids. Further, as the absorption of cholesterol through the small intestine is a linear function of its concentration in the lumen (39), it is expected that animals fed HC diets will have increased delivery of cholesterol to the liver through chylomicron remnants as is the case in the control guinea pigs. Therefore, it is not surprising that the effects of fiber in the gastrointestinal tract will be influenced by the amount of dietary cholesterol.

The present studies demonstrated that the significant depletion of hepatic cholesterol pools induced by non-starch polysaccharides was due to decreases in cholesterol absorption and alterations in the enterohepatic circulation of bile acids as manifested by the up-regulation of hepatic cholesterol 7α -hydroxylase, the regulatory enzyme of bile acid synthesis. The fiber sources evaluated in these studies had different actions possibly associated with their chemical structures and their physicochemical properties. Similar to the present results, PE although of low viscosity has been found to reduce

cholesterol absorption in animal and human studies (40, 41). This capacity of pectin to reduce cholesterol absorption and its possible interruption of the enterohepatic circulation of bile acids, as suggested in the present investigation by the up-regulation of cholesterol 7α -hydroxylase, resulted in a significant reduction of hepatic free and esterified cholesterol pools especially in guinea pigs fed the PE-HC diet compared to animals fed HC with GG or PSY. PSY effects in lowering hepatic cholesterol were mostly due to interruption of enterohepatic circulation of bile acids as suggested by the observed increases in 7α -hydroxylase activity as no effects on cholesterol absorption were observed in agreement with clinical and animal studies (2, 42). The effects of GG in the small intestine are harder to interpret although a decrease in cholesterol absorption was observed in animals fed GG with high cholesterol diets. However, no effects of GG in cholesterol 7α -hydroxylase activity were detected although studies in the rat (14) and in humans (4) do suggest that GG increases bile acid excretion. The primary mechanisms involved in reducing hepatic cholesterol concentrations are strongly correlated with the resulting cholesterol-lowering effects and it would appear from these results that bile acid excretion has a more potent effect in determining hepatic cholesterol concentrations in animals fed HC diets, as the lowest concentrations of hepatic cholesterol were observed in the PE group that had the highest enzyme activity while GG had the highest hepatic cholesterol concentrations and cholesterol 7α -hydroxylase activity was not different from the control. For animals fed GG-LC, the primary mechanisms of hepatic cholesterol-lowering were not evident in the present study as no effects of GG on cholesterol absorption or increased activity of cholesterol 7α -hydroxylase were detected. It is possible that a combination of multiple mechanisms including the ones evaluated in this experiment could have taken place but were not identified under the present experimental conditions.

Fiber effects on hepatic cholesterol metabolism and plasma cholesterol levels

It is well known that the liver plays a major role in the levels of circulating LDL through the regulation of VLDL synthesis and LDL catabolism by the apoB/E receptor (43). The decreases in hepatic cholesterol concentrations, specifically in the free pool, induced by PE, GG, and PSY in animals fed the LC diets resulted in an up-regulation of LDL receptors as indicated by the faster plasma total and receptor-mediated LDL turnover; these observations are in agreement with previous reports of increased LDL binding to hepatic membranes (9–12). Animals fed the LC diets independent of the non-starch polysaccharide source had a higher contribu-

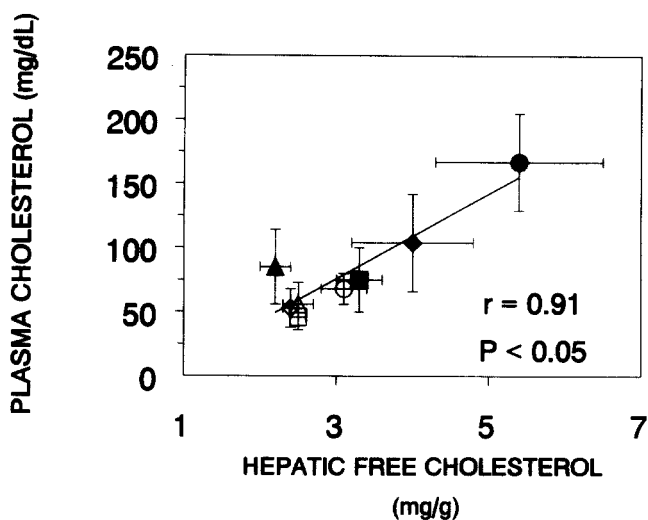


Fig. 4. Correlation between plasma cholesterol levels ($n = 21$) and hepatic free cholesterol concentrations ($n = 6$) of guinea pigs fed control (○, ●), pectin (△, ▲), guar gum (◇, ◆), or psyllium (□, ■). Open symbols represent low cholesterol (0.04%, w/w) and dark symbols high cholesterol (0.25%, w/w) groups.

tion of the non-receptor-mediated pathway than animals fed the HC diets, suggesting that dietary cholesterol possibly affected the packing of the membrane lipid bilayer decreasing non-receptor pathways via endocytosis (44). As no effects were observed in LDL apoB pool size, the results suggest the presence of smaller LDL particles containing less cholesterol. Previous studies in guinea pigs have shown that smaller LDL particles induced by polyunsaturated fat intake have faster plasma LDL FCR (45, 46) suggesting that the cholesteryl ester-depleted LDLs generated by fiber might have contributed to the faster plasma LDL turnover.

In animals fed the HC diets, PE and PSY not only increased LDL FCR, but decreases in LDL apoB flux rates were observed by intake of the three fiber sources, which suggests either decreased synthesis of VLDL or a decreased conversion of VLDL to LDL. Similar to these results, Turley and Dietschy (47) reported that the hypocholesterolemic effect of PSY in hamsters were related to decreases in LDL production and increases in LDL receptor-mediated activity although they found that the first mechanism was more important. Nascent VLDL from cholesterol-fed guinea pigs has been shown to have a high concentration of cholesteryl ester derived from hepatic pools (48). In addition, previous studies in guinea pigs have shown that animals fed high cholesterol diets exhibit a circulating VLDL that contains more cholesteryl ester and free cholesterol than VLDL isolated from animals fed LC (11). Further, PSY intake reversed the composition of this cholesteryl ester-enriched VLDL making it similar to VLDL from the LC group (11). As PSY also reduced the concentration of

plasma LDL cholesterol, one can speculate that the cholesteryl ester-depleted VLDL induced by PSY intake is not readily converted to LDL through the delipidation cascade, and the decreases in LDL apoB flux rates in animals fed PSY, PE, and GG with HC observed in the present study reinforce this hypothesis. However, as high cholesterol diets have been shown to up-regulate cholesteryl ester transfer protein (CETP) activity (49), and lipoprotein lipase (LPL) has a major role in the conversion of VLDL to LDL (50), a contribution of soluble fiber in decreasing CETP activity in the intravascular compartment or in affecting LPL activity cannot be overruled. As LPL and apoB/E receptor are regulated by insulin/glucagon ratios (51, 52), it could be that dietary fiber also contributes to plasma LDL lowering by affecting hormone metabolism as has been postulated (13), a possibility that should be explored further.

Another point to consider is that although a strong correlation was found between hepatic cholesterol concentrations and plasma cholesterol levels, individual fiber sources did not have similar plasma and hepatic cholesterol-lowering effects, suggesting that fiber may also influence transport proteins and enzymes in the intravascular compartment that contribute to plasma lipoprotein levels. In addition, results from the GG-LC group suggest that there must be other primary mechanisms affecting hepatic cholesterol concentrations as animals fed GG with LC diets exhibited similar cholesterol-lowering effects to PE or PSY and no changes in cholesterol absorption or inductions of 7α -hydroxylase activity were detected.

These studies have demonstrated that the source of dietary fiber and the amount of dietary cholesterol significantly determine the primary mechanisms that take place in the small intestine accounting for the depletion of hepatic cholesterol pools and that the amount of circulating LDL will be influenced to a great extent by the content of hepatic cholesterol with a possible contribution of metabolic alterations occurring in the intravascular compartment. These studies also suggest that in addition to decreases in cholesterol absorption and interruption of enterohepatic circulation of bile acids, there might be other primary mechanisms affecting the lowering of hepatic cholesterol and that fiber effects on plasma LDL concentrations are not limited to increases in LDL receptor catabolism but that other sites including VLDL synthesis, conversion of VLDL to LDL, and processing of VLDL in the intravascular compartment are potential sites of action. ■

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