Gender differences in response to dietary soluble fiber in guinea pigs: effects of pectin, guar gum, and psyllium

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Abstract Dietary soluble fiber significantly lowers plasma low density lipoprotein (LDL) cholesterol concentrations in humans and animals. In male guinea pigs, alterations in hepatic cholesterol homeostasis induced by dietary fiber in part account for the decrease in plasma LDL levels (Fernandez et al. 1994. *Am. J. Clin. Nutr.* **59:** 869–878; 1995. **61:** 127–134, and *J. Lipid Res.* 1995. **36:** 1128–1138). To test whether dietary fiber elicited similar hypocholesterolemic responses in both genders, female guinea pigs were fed diets containing 12.5% pectin (PE), 12.5% guar gum (GG), 7.5% psyllium (PSY), or 12.5% cellulose (control diet). In addition, physiological (0.04%) (LC) or pharmacological (0.25%) (HC) amounts of cholesterol were tested with the fibers to determine whether dietary cholesterol altered the plasma cholesterol response. Significant reductions in plasma cholesterol were observed in females fed LC diets with PE, GG, or PSY ($P < 0.01$) while the responses to fiber with high cholesterol intake were more moderate. Hepatic cholesterol concentrations were reduced in the LC group ($P < 0.001$) with increased HMG-CoA reductase and cholesterol 7α-hydroxylase and decreased acyl CoA:cholesterol acyltransferase (ACAT) activities accompanied by a reduction in hepatic cholesterol pools induced by fiber intake. In addition, plasma LDL lowering in animals fed the LC diets was associated with increases in hepatic LDL receptor B_{max} values. Effects of fiber on hepatic cholesterol in animals fed HC diets were moderate and hepatic enzymes were not altered to the same extent as in the LC groups. For the LC groups there was no gender effect on the magnitude of plasma LDL lowering, depletion of hepatic cholesterol, or alterations in hepatic cholesterol metabolism, although hepatic HMG-CoA reductase and ACAT activities were lower in females compared to males ($P < 0.01$). In contrast, females fed the control HC diet had higher plasma LDL levels than males and dietary fiber did not reduce hepatic cholesterol concentrations nor alter hepatic enzyme activities as effectively as in males. These studies demonstrate that female, compared to male, guinea pigs are more responsive to a dietary cholesterol challenge and, that with this pharmacological perturbation, fiber effects are moderate compared to males. In contrast, with low cholesterol intakes, the cholesterol lowering effects of fiber are similar in both genders.–**Fernandez, M. L., M. Vergara-Jimenez, A. L. Romero, S. K. Erickson, and D. J. McNamara.** Gender differences in response to dietary soluble fiber in guinea pigs: effects of pectin, guar gum, and psyllium. *J. Lipid Res.* 1995. **36:** 2191–2202.

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Elevated plasma LDL concentrations are a known risk factor for cardiovascular disease (1). Soluble fiber intake decreases risk by reducing plasma LDL cholesterol levels as shown in human (2, 3) and animal (4–9) studies. Many studies have investigated the mechanisms accounting for fiber-mediated plasma cholesterol lowering and suggest that soluble fiber's ability to bind bile acids (10, 11), its capacity to decrease bile acid reabsorption by the small intestine (12), the production of volatile fatty acids in the cecum (13), and decreases in dietary fat or cholesterol absorption (14) are involved in the hypocholesterolemic response. Few studies have addressed possible gender differences in the qualitative and quantitative plasma cholesterol responses to intake of various types of soluble fiber (15).

Previous studies in male guinea pigs have shown that the level of dietary cholesterol given with different types of soluble fiber elicits different hypocholesterolemic responses (7–9), possibly related to the specific action

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; PE, pectin; GG, guar gum; PSY, psyllium; LC, low cholesterol; HC, high cholesterol; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; TAG, triacylglycerol; ACAT, acyl-CoA:cholesterol acyltransferase.

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of each fiber type in the small intestine. Pectin, for example, has a significant plasma cholesterol lowering effect with a high cholesterol diet and a more moderate response with low cholesterol intake (7). In contrast, guar gum intake lowers plasma cholesterol more effectively in combination with low cholesterol rather than high intakes of cholesterol (8). Interestingly, psyllium had similar hypocholesterolemic responses in the guinea pig with either low or high cholesterol diets (9). These different responses suggest that the primary mechanisms responsible for decreasing plasma cholesterol concentrations are specific for each type of dietary fiber and in part dependent on the amount of dietary cholesterol.

Gender is a strong predictor of coronary heart disease susceptibility and reports from the Registrar's mortality data indicate that men are more likely to develop coronary heart disease than premenopausal women (16). Many carefully controlled studies have shown gender differences in lipoprotein responsiveness to changes in dietary patterns (17–19). For example, Mensink and Katan (17) observed that plasma triacylglycerol and HDL cholesterol responses to dietary fat saturation are gender specific, with females being less responsive than their male counterparts. In men eating a Western diet, high plasma triacylglycerol levels were not associated with cardiovascular disease risk while for women high triglycerides, and the associated low plasma HDL cholesterol concentrations, were highly related (18). When males and females were fed diets with low versus high polyunsaturated to saturated fat ratios, multivariate analysis of variance indicated that plasma triacylglycerol and VLDL and HDL cholesterol concentrations were significant gender-specific lipid variables (19). These gender-specific responses of lipoprotein metabolism to dietary interventions emphasize the importance of analyzing the effects of dietary factors in both male and female populations.

The present studies were undertaken to determine whether female guinea pigs have different responses to soluble fiber compared with male guinea pigs and whether the mechanisms of plasma LDL lowering in females differ when soluble fiber is consumed with low or high cholesterol intake. Guinea pigs were chosen for this and previous studies (7–9) because their plasma cholesterol is mainly transported in LDL and because hypocholesterolemic responses to fiber are mostly associated with decreases in LDL similar to the human situation. In addition, the distribution of hepatic cholesterol, with larger concentrations of the free compared to the esterified pool (20) and hepatic activities of HMG-CoA reductase (21), ACAT (22), and cholesterol 7α-hydroxylase (23) are similar to humans. Thus, this species has the necessary metabolic characteristics to be a suitable model for studying the regulatory responses of the liver to dietary interventions that affect plasma lipoprotein levels.

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), [14C]cholesterol, aquasol, and liquifluor were purchased from New England Nuclear (Boston, MA); oleoyl-[1-14C]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). 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); and 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 (22.4% soy protein, 15.1% palm oil, 39.6% carbohydrate, 8.2% mineral mix, and 1.1% vitamin mix) except for the fiber source and cholesterol content. 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). The fatty acid composition of palm oil was: 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). These dietary cholesterol levels 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 daily endogenous cholesterol synthesis rate in guinea pigs (24).

Animals

Male and female Hartley guinea pigs (Sasco Sprague-Dawley, Omaha, NE) weighing 250–300 g (six per

group) 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. 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 (25). Very low density lipoprotein (VLDL) + intermediate density lipoprotein (IDL), LDL, and HDL were separated by sequential ultracentrifugation 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 + IDL; d 1.019–1.09 g/ml for LDL, and d 1.09–1.24 g/ml for HDL. VLDL and LDL compositions were calculated by determining free and esterified cholesterol (26), protein (27), triacylglycerol and phospholipids (7–9). Hepatic concentrations of total and free cholesterol were measured according to the method of Carr, Andresen, and Rudel (27), and cholesteryl ester concentrations were calculated as the difference between free and total cholesterol.

LDL binding assays

Pooled LDL samples from each dietary group were iodinated by the method of Goldstein, Basu, and Brown (28) for measurement of LDL binding to hepatic membranes from female guinea pigs fed the homologous diet. Hepatic membranes were isolated as previously described (7) and incubated with varying concentrations of 125I-labeled LDL over a range of 10 to 80 μg of LDL protein per ml for 2 h at 37°C. After incubation, membranes were pelleted and washed by ultracentrifugation and counted in a gamma counter. 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)] (29).

Hepatic HMG-CoA reductase and ACAT assays

Hepatic microsomes for measurement of HMG-CoA reductase, cholesterol 7α-hydroxylase, and ACAT activities were isolated as previously described (7–9). Microsomal HMG-CoA reductase (EC 1.1.1.34) activity was measured by incubation of 200 μg microsomal protein with [3-14C]HMG-CoA as previously described (30). Hepatic ACAT activity (EC 2.3.2.26) was determined by preincubating microsomal protein (0.7–1.0 mg per assay) with 84 mg/mL albumin (31) in a final volume of 0.18 mL for 5 min at 37°C; then 20 μl (500 μmol/L) of oleoyl-[1-14C]coenzyme A (0.15 GBq/pmol) was added and assayed as previously described (7–9).

Hepatic cholesterol 7α-hydroxylase assay

Cholesterol 7α-hydroxylase (EC 1.14.13.7) activity was assayed by the method of Shefer, Hauser, and Mosbach (32) as modified by Jelinek et al. (33) using cholesterol:phosphatidylcholine liposomes (1:8 by weight) prepared by sonication and an NADPH-regenerating system (glucose-6-phosphate dehydrogenase, NADP, and glucose 6-phosphate) as previously described (9).

Statistical analysis

One-way ANOVA was used to identify differences in the measured variables within the LC and HC groups and the Newman-Keules post-hoc test was used to identify significant differences due to PE, GG, and PSY intake ($P < 0.05$). Two-way ANOVA (GBSTAT, Silver Spring, MD) was used to test significant effects on plasma lipids, VLDL and LDL composition, hepatic cholesterol, apoB/E receptor B_{max}, and affinity K_d, and activities of hepatic HMG-CoA reductase, ACAT, and cholesterol 7α-hydroxylase mediated by fiber and cholesterol in female guinea pigs. One-way ANOVA was used to compare PE, GG, and PSY effects within the male or female groups. Linear and power relationships were used to identify significant correlations between measurements ($P < 0.05$).

RESULTS

No significant differences were found in rates of body weight gain or final body weights in female animals fed high cholesterol (HC) versus low cholesterol (LC) diets nor between control versus fiber groups. Although female and male guinea pigs started the test diets at similar body weights, final weights were higher for males ($P < 0.001$) at the end of the 4-week experimental period (data not shown).

Fiber effects in female guinea pigs fed low and high cholesterol diets

Fiber and cholesterol effects on plasma lipid levels and lipoprotein composition in female guinea pigs. Plasma total cholesterol levels were significantly reduced by dietary fiber in female guinea pigs fed LC diets ($P < 0.01$) and dietary cholesterol significantly increased plasma cholesterol levels in the four dietary treatment groups (**Table 1**). No differences in plasma cholesterol, as determined by one-way ANOVA, were observed between the

TABLE 1. Effects of dietary fiber on plasma total and lipoprotein cholesterol and triacylglycerol levels of female guinea pigs

Diet	Plasma Cholesterol				Triacylglycerol
	Total	VLDL	LDL	HDL	
	mg/dL				mg/dL
Low cholesterol					
Control	77 ± 19[a]	2 ± 1	70 ± 21[a]	5 ± 2	91 ± 27
Pectin	54 ± 9[b]	2 ± 1	45 ± 10[b]	7 ± 2	104 ± 30
Guar gum	65 ± 10[b]	3 ± 3	56 ± 8[b]	6 ± 1	109 ± 33
Psyllium	63 ± 9[b]	2 ± 1	54 ± 9[b]	7 ± 1	86 ± 34
High cholesterol					
Control	270 ± 112	12 ± 6	246 ± 106	9 ± 1	102 ± 15
Pectin	188 ± 83	6 ± 3	171 ± 78	9 ± 2	76 ± 44
Guar gum	220 ± 93	10 ± 2	203 ± 89	6 ± 3	93 ± 27
Psyllium	171 ± 61	8 ± 5	157 ± 56	6 ± 2	93 ± 26
Cholesterol effect[c]	$P < 0.0001$	$P < 0.001$	$P < 0.0001$	NS	NS
Fiber effect[d]	$P = 0.033$	NS	$P = 0.035$	NS	NS
Interaction[e]	NS	NS	NS	NS	NS

Values are presented as mean ± SD for n = 6 animals per dietary group. Values in the same column with different superscripts are significantly different as determined by one-way ANOVA and the Newman-Keules post-hoc test ($P < 0.05$).
[c]Differences due to dietary cholesterol as determined by two-way ANOVA.
[d]Differences due to dietary fiber; NS, not significant.
[e]Interaction between fiber and cholesterol.

control and fiber groups for animals fed HC diets in part due to the rather large coefficients of variation among groups; however, two-way ANOVA indicated a significant plasma cholesterol lowering effect of fiber ($P = 0.033$). Plasma triacylglycerol (TAG) and HDL cholesterol levels were not affected by either dietary fiber or dietary cholesterol (Table 1), while plasma VLDL and LDL cholesterol levels were increased with cholesterol intake and LDL levels paralleled trends in total cholesterol, as expected for an animal with the majority of its plasma cholesterol in LDL (Table 1). Dietary fiber ($P < 0.035$) decreased plasma LDL cholesterol in animals fed the LC and HC diets and there were no significant differences in the cholesterol-lowering response among fiber groups (Table 1).

The major dietary effects on VLDL composition were due to changes in cholesterol intake regardless of fiber source. VLDL from animals fed LC diets had higher percentages of protein and TAG and lower free cholesterol and cholesteryl ester than VLDL from animals fed HC diets (**Table 2**). While these results document induction of a cholesterol-enriched VLDL with a high cholesterol intake, the effects of dietary fiber on VLDL composition were limited to changes in cholesteryl ester content which was significantly reduced with fiber intake (Table 2). Dietary fiber also apparently increased the surface components of VLDL as indicated by the relative increase in the percentage protein. The data

TABLE 2. Effects of dietary fiber on composition of VLDL from female guinea pigs

Diet	VLDL Composition				
	PRO	PL	TAG	FC	CE
			%		
Low cholesterol					
Control	14.6 ± 3.8	9.3 ± 1.8	69.8 ± 4.3	3.8 ± 1.0	3.1 ± 0.8[a]
Pectin	16.2 ± 5.7	10.6 ± 1.8	68.0 ± 4.5	4.0 ± 0.8	1.3 ± 1.0[b]
Guar gum	14.1 ± 2.6	10.2 ± 1.9	69.0 ± 2.5	5.2 ± 0.9	0.8 ± 0.5[b]
Psyllium	12.2 ± 2.2	9.2 ± 1.8	71.2 ± 3.2	6.1 ± 3.8	1.1 ± 0.9[b]
High cholesterol					
Control	9.8 ± 3.2	11.6 ± 3.3	61.7 ± 10.8	9.9 ± 1.8	11.8 ± 3.0
Pectin	11.7 ± 3.4	9.4 ± 3.3	62.8 ± 12.8	6.9 ± 4.3	9.1 ± 5.2
Guar gum	15.0 ± 4.9	12.1 ± 2.6	53.3 ± 14.1	9.7 ± 3.8	9.6 ± 3.2
Psyllium	12.2 ± 2.5	9.6 ± 3.4	62.8 ± 5.6	6.0 ± 2.8	9.5 ± 2.3
Cholesterol effect[c]	$P = 0.005$	NS	$P = 0.004$	$P < 0.0001$	$P < 0.0001$
Fiber effect[d]	$P = 0.07$	NS	NS	NS	NS
Interaction[e]	$P = 0.04$	NS	NS	NS	NS

Values represent mean ± SD for n = 6 animals per dietary group. Values in the same column with different superscripts are significantly different as determined by one-way ANOVA and the Newman-Keules post-hoc test ($P < 0.02$).
[c]Differences due to dietary cholesterol as determined by two-way ANOVA.
[d]Differences due to dietary fiber; NS, not significant.
[e]Interaction between fiber and cholesterol.

TABLE 3. Effects of dietary fiber on cholesteryl ester (CE), free cholesterol (FC), triacylglycerol (TAG), and phospholipid (PL) to protein ratios of LDL from female guinea pigs

Diet	LDL Composition			
	CE/Protein	FC/Protein	TAG/Protein	PL/Protein
	%			
Low cholesterol				
Control	3.3 ± 0.7[a]	0.30 ± 0.09	0.49 ± 0.19[b]	0.81 ± 0.31
Pectin	2.5 ± 0.2[b]	0.30 ± 0.03	0.66 ± 0.19[a]	0.86 ± 0.19
Guar gum	2.2 ± 0.8[b]	0.22 ± 0.06	0.41 ± 0.13[b]	0.59 ± 0.18
Psyllium	1.9 ± 1.0[b]	0.21 ± 0.08	0.40 ± 0.09[b]	0.57 ± 0.21
High cholesterol				
Control	4.1 ± 1.1	0.50 ± 0.16	0.21 ± 0.04[b]	0.88 ± 0.29
Pectin	3.0 ± 0.8	0.48 ± 0.11	0.18 ± 0.03[b]	0.80 ± 0.24
Guar gum	3.5 ± 0.9	0.46 ± 0.16	0.15 ± 0.04[b]	0.53 ± 0.17
Psyllium	3.2 ± 0.5	0.59 ± 0.11	0.29 ± 0.05[a]	0.74 ± 0.36
Cholesterol effect[c]	$P = 0.0021$	$P < 0.0001$	$P < 0.0001$	NS
Fiber effect[d]	$P = 0.0008$	NS	NS	NS
Interaction[e]	NS	NS	NS	NS

Values are presented as mean ± SD for n = 6 animals per dietary group. Values in the same column with different superscripts are significantly different as determined by one-way ANOVA and the Newman-Keules post-hoc test ($P < 0.02$).

[c]Differences due to dietary cholesterol as determined by two-way ANOVA.

[d]Differences due to dietary fiber; NS, not significant.

[e]Interaction between fiber and cholesterol.

indicate an interactive effect between dietary cholesterol and dietary fiber in determining the relative protein content of plasma VLDL (Table 2).

As LDL contains a single apoB, the ratios of the lipid components relative to protein were calculated to detect dietary effects on LDL composition. Dietary cholesterol increased the LDL cholesteryl ester ($P = 0.002$) and free cholesterol ($P < 0.0001$) to protein ratios and lowered the TAG/protein ratio ($P < 0.0001$). Intake of dietary fibers resulted in an LDL particle containing less cholesteryl ester compared to LDL derived from animals fed the control diet ($P < 0.001$). The effects of fiber on LDL TAG content were specific; pectin (PE) intake increased the amount of TAG in LDL from animals fed the LC diets while psyllium (PSY) intake increased TAG in LDL isolated from the HC group (**Table 3**).

TABLE 4 Effects of dietary fiber on hepatic total, free, and esterified cholesterol of female guinea pigs

Diet	Hepatic Cholesterol Concentrations		
	Total	Free	Esterified
	mg/g		
Low cholesterol			
Control	2.65 ± 0.27[a]	2.36 ± 0.19[a]	0.29 ± 0.12[a]
Pectin	1.97 ± 0.26[b]	1.80 ± 0.24[b]	0.17 ± 0.06[b]
Guar gum	2.02 ± 0.47[b]	1.88 ± 0.46[b]	0.15 ± 0.05[b]
Psyllium	1.39 ± 0.18[c]	1.23 ± 0.16[b]	0.16 ± 0.08[b]
High cholesterol			
Control	7.50 ± 3.40	4.71 ± 2.52	2.79 ± 1.50
Pectin	5.40 ± 3.10	3.20 ± 1.30	2.20 ± 1.80
Guar gum	5.20 ± 2.63	3.63 ± 1.52	1.70 ± 1.29
Psyllium	4.13 ± 3.30	2.78 ± 1.39	1.24 ± 1.60
Cholesterol effect[d]	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Fiber effect[e]	$P = 0.018$	$P = 0.009$	NS
Interaction[f]	NS	NS	NS

Values are presented as mean ± SD for n = 6 animals per dietary group. Values in the same column with different superscripts are significantly different as determined by one-way ANOVA and the Newman-Keules post-hoc test ($P < 0.01$).

[d]Differences due to dietary cholesterol as determined by two-way ANOVA.

[e]Differences due to dietary fiber; NS, not significant.

[f]Interaction between fiber and cholesterol.

Fiber and cholesterol effects on hepatic cholesterol concentrations, enzyme activities, and apoB/E receptors of female guinea pigs. Significant reductions in hepatic total, free and esterified cholesterol occurred with fiber intake in animals fed the LC diets (**Table 4**). PSY intake had the most pronounced effect and hepatic total and free cholesterol concentrations were the lowest in this group of animals ($P < 0.001$). PE, GG, and PSY intake in the LC group had similar lowering effects on hepatic esterified cholesterol concentrations compared to animals fed the control diet (Table 4). Cholesterol intake increased hepatic free and esterified cholesterol concentrations ($P < 0.0001$) and there was a dietary fiber-mediated lowering of hepatic free cholesterol levels ($P = 0.009$) (Table 4).

The most pronounced effects of fiber intake on hepatic enzyme activities were observed in animals fed the LC diets (**Table 5**). However, different responses were observed depending on the type of dietary fiber. PE and PSY intakes had similar effects resulting in up-regulation of hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase and decreased hepatic ACAT compared to animals fed the control diet. In contrast, ACAT and cholesterol 7α-hydroxylase activities were not different between animals fed the guar gum (GG) and the control

TABLE 5. Effects of dietary fiber on activities of hepatic HMG-CoA reductase, ACAT, and cholesterol 7α-hydroxylase of female guinea pigs

Diet	Hepatic Enzyme Activity		
	HMG-CoA Reductase	ACAT	7α-Hydroxylase
		pmol/min · mg	
Low cholesterol			
Control	4.1 ± 1.9[b]	11.5 ± 3.5[a]	1.72 ± 0.46[b]
Pectin	8.7 ± 2.7[a]	6.1 ± 0.9[b]	2.39 ± 0.32[a]
Guar gum	7.8 ± 1.5[a]	8.3 ± 3.0[a,b]	1.54 ± 0.50[b]
Psyllium	8.6 ± 4.0[a]	5.6 ± 1.2[b]	2.74 ± 0.65[a]
High cholesterol			
Control	1.27 ± 0.18	70 ± 30	0.82 ± 0.42
Pectin	1.45 ± 0.38	61 ± 31	1.12 ± 0.42
Guar gum	1.70 ± 0.57	72 ± 25	0.90 ± 0.32
Psyllium	1.82 ± 0.26	45 ± 30	1.51 ± 0.76
Cholesterol effect[c]	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Fiber effect[d]	$P = 0.009$	NS	$P = 0.05$
Interaction[e]	$P = 0.005$	NS	NS

Values are presented as mean ± SD for n = 6 animals per dietary group. Values in the same column with different superscripts are significantly different as determined by one-way ANOVA and the Newman-Keules post-hoc test ($P < 0.01$).

[c]Differences due to dietary cholesterol as determined by two-way ANOVA.

[d]Differences due to dietary fiber; NS, not significant.

[e]Interaction between fiber and cholesterol.

diet while GG intake increased HMG-CoA reductase activity (Table 5). While differences due to dietary fiber were not detected by one-way ANOVA in animals fed the HC diets, two-way ANOVA indicated significant fiber effects on the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase (Table 5). Similar to the other measured parameters, cholesterol intake significantly affected enzyme activities by decreasing HMG-CoA reductase and cholesterol 7α-hydroxylase and increasing ACAT ($P < 0.0001$). There was a significant negative relationship ($r = -0.73$, $P < 0.01$) between hepatic cholesterol concentrations and HMG-CoA reductase activities while there was a positive linear correlation ($r = 0.907$, $P < 0.001$) between hepatic cholesterol concentrations and ACAT activities (data not shown) in female guinea pigs. Similar correlations for male guinea pigs have been previously reported (7–9).

Hepatic membrane apoB/E receptor B_{max} was increased by all the dietary fibers in both the LC and HC groups (**Table 6**). Both dietary cholesterol and fiber affected LDL receptor number, cholesterol decreasing B_{max} and fiber increasing receptor number compared to the control group (Table 6). The receptor ligand affinity constant (K_d) was not modified by dietary fiber or dietary cholesterol (Table 6). There was a significant negative correlation ($r = -0.68$, $P < 0.01$) between hepatic cholesterol concentrations and LDL receptor B_{max} (data not shown).

Comparisons of gender and fiber effects on cholesterol and lipoprotein metabolism

A comparison of parameters of cholesterol metabolism between female and male guinea pigs fed the LC and HC control diets is presented in **Table 7**. Hepatic HMG-CoA reductase and ACAT activities were lower in females in both the LC and HC diet groups while plasma LDL levels were higher in females fed the high cholesterol diets (Table 7).

As male and female guinea pigs have different baseline values on the LC and HC control diets, comparisons of the responses to the dietary fiber are presented as percent of control values. Plasma LDL cholesterol levels

TABLE 6. Effects of dietary fiber on hepatic LDL receptor number (B_{max}) and affinity (K_D) of female guinea pigs

Diet	LDL Binding Parameters	
	B_{max}	K_d
	μg/mg	*μg/ml*
Low cholesterol		
Control	2.08 ± 0.39[b]	27 ± 15
Pectin	3.25 ± 0.35[a]	48 ± 24
Guar gum	3.12 ± 0.44[a]	35 ± 10
Psyllium	2.92 ± 0.26[a]	27 ± 6
High cholesterol		
Control	1.29 ± 0.39[b]	21 ± 14
Pectin	1.83 ± 0.32[a]	42 ± 9
Guar gum	1.84 ± 0.49[a]	48 ± 24
Psyllium	2.63 ± 0.33[a]	44 ± 12
Cholesterol effect[c]	$P < 0.0001$	NS
Fiber effect[d]	$P < 0.0001$	NS
Interaction[e]	NS	NS

Values are presented as mean ± SD for n = 6 animals per dietary group. Values in the same column with different superscripts are significantly different as determined by one-way ANOVA and the Newman-Keules post-hoc test ($P < 0.02$).

[c]Differences due to dietary cholesterol as determined by two-way ANOVA.

[d]Differences due to dietary fiber; NS, not significant.

[e]Interaction between fiber and cholesterol.

TABLE 7. Comparisons of plasma and hepatic cholesterol, enzyme activities, and LDL receptor B_{max} of male and female guinea pigs fed control (12.5% cellulose) diets with low (0.04%, w/w) or high (0.25%, w/w) cholesterol

Parameter	Low Cholesterol Diets		High Cholesterol Diets	
	Male	Female	Male	Female
Plasma cholesterol (mg/dL)	79 ± 16	77 ± 19	200 ± 45[b]	270 ± 112[a]
Hepatic cholesterol (mg/g)	3.0 ± 0.5	2.7 ± 0.3	7.8 ± 2.2	7.5 ± 3.4
HMG-CoA reductase (pmol/min · mg)	14.8 ± 9.2[a]	4.3 ± 1.9[b]	2.6 ± 1.0[a]	1.3 ± 0.2[b]
ACAT (pmol/min · mg)	18.9 ± 4.1[a]	10.7 ± 3.7[b]	130 ± 61[a]	73 ± 28[b]
7α-Hydroxylase (pmol/min · mg)	1.7 ± 1.2	1.7 ± 0.5	1.7 ± 0.5	1.5 ± 0.5
LDL receptor B_{max} (μg/mg)	1.76 ± 0.10	2.10 ± 0.48	1.37 ± 0.14	1.29 ± 0.23

Values represent mean ± SD for n = 18 male and n = 6 female guinea pigs except for the LDL receptor where n = 12 for males and n = 4 for females. Values in the same row within the low or high cholesterol groups are significantly different as determined by Student's *t*-test.

were significantly reduced with intake of PE, GG, or PSY in both the low and high cholesterol groups (**Fig. 1, upper and lower panels**). However, in males fed the high cholesterol diets, the magnitude of the response was more pronounced than in females and the responses to dietary fiber differed in males with PE and PSY having a more hypocholesterolemic effect than GG (Fig. 1, lower panel).

Hepatic cholesterol concentrations were reduced by dietary GG and PSY in all animals fed the LC diets and by PE intake in females. However, female guinea pigs uniformly had greater reductions in hepatic cholesterol by dietary fiber (50 to 75%) compared to males (30 to 46%) (**Fig. 2, upper panel**). PE intake reduced hepatic cholesterol concentrations by 75% in male guinea pigs fed the HC diets followed by 60% reduction with PSY and 40% with GG intake. With female guinea pigs, PSY reduced hepatic cholesterol concentrations by 46% and

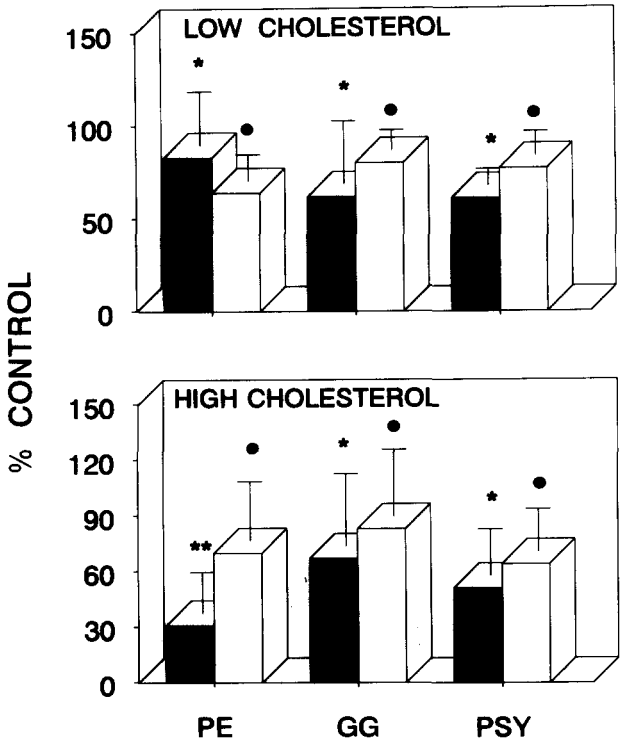

Fig. 1. Comparison of the dietary fiber effects on plasma LDL cholesterol concentrations in male and female guinea pigs. Bars represent the mean ± SD of % control for males (dark bars) (n = 18) and females (open bars) (n = 6) fed, dietary fiber (six male or female animals per group): pectin (PE), guar gum (GG), or psyllium (PSY) diets with low cholesterol (upper panel) or high cholesterol (lower panel). *Represents significantly different from control values in males and ● different from control values in females as determined by one-way ANOVA ($P < 0.01$).

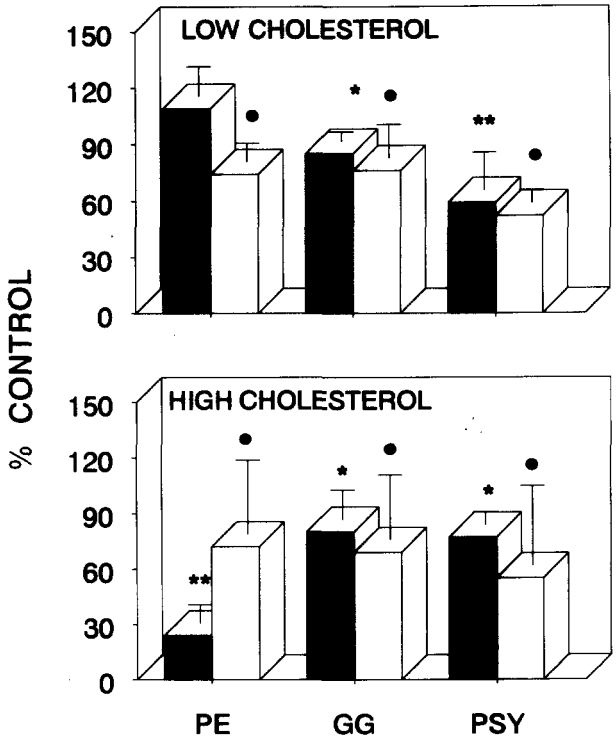

Fig. 2. Comparisons of dietary fiber effects on hepatic cholesterol concentrations of male and female guinea pigs. Bars represent the mean ± SD of % control for males (dark bars) (n = 18) and females (open bars) (n = 6) fed dietary fiber (six male or female animals per group): pectin (PE), guar gum (GG), or psyllium (PSY) diets with low cholesterol (upper panel) or high cholesterol (lower panel). *Represents significantly different from control values in males and ● significantly different from control values in females as determined by one-way ANOVA ($P < 0.01$).

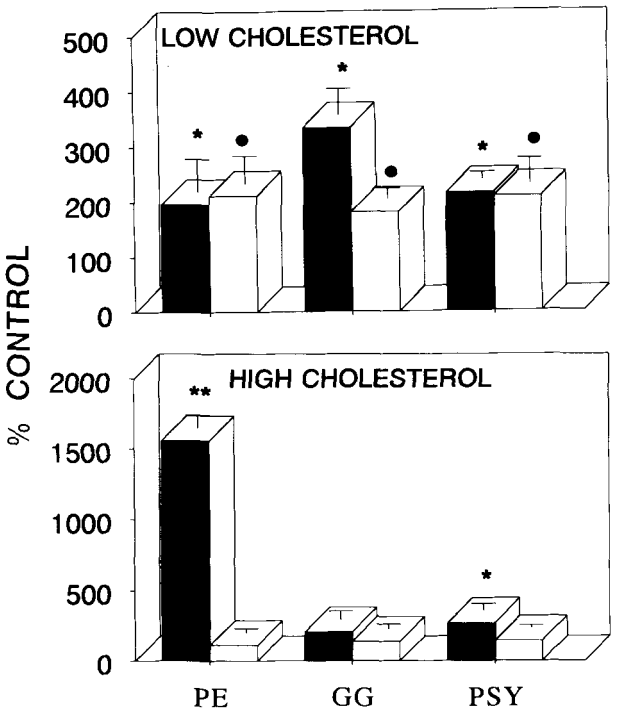

Fig. 3. Comparisons of dietary fiber effects on hepatic HMG-CoA reductase activity of male and female guinea pigs. Bars represent the mean ± SD of % control for males (dark bars) (n = 18) and females (open bars) (n = 6) fed dietary fiber (six male or female animals per group: pectin (PE), guar gum (GG), or psyllium (PSY) diets with low cholesterol (upper panel) or high cholesterol (lower panel). Hepatic HMG-CoA reductase activity was significantly higher in males for both the low and high cholesterol groups ($P < 0.001$). *Represents significantly different from control values in males and ● significantly different from control values in females as determined by one-way ANOVA ($P < 0.001$).

PE and GG resulted in reductions of 30% (Fig. 2, lower panel).

Intake of PE, GG, and PSY up-regulated reductase activity by the same order of magnitude in males and females (approximately 100%) (**Fig. 3, upper panel**). For animals fed the HC diets, PE and PSY had significant effects in increasing reductase activity in male animals (Fig. 3, lower panel) which appear related to decreases in hepatic cholesterol pools with these two fibers (Fig. 2, lower panel). The increases in reductase activity with fiber in females fed the HC diet were more modest ranging from 30 to 40% increase (Fig. 3, lower panel).

Different sources of fiber also affected hepatic ACAT and cholesterol 7α-hydroxylase activities in specific ways. While only PE and PSY decreased ACAT activity in females fed the LC diet, GG and PSY lowered ACAT activity in males (**Fig. 4, upper panel**). ACAT activities were not significantly modified by fiber in females fed HC diets while they were reduced in males with PE having the most pronounced effect (Fig. 4, lower panel).

PE and PSY increased cholesterol 7α-hydroxylase activity in both male and female guinea pigs fed LC and HC diets while no increases in enzyme activity in either sex was observed with GG intake (**Fig. 5, upper and lower panels**). In animals fed the HC diets, males fed the PE diet up-regulated cholesterol 7α-hydroxylase activity increasing it 4-fold compared to control values (Fig. 2, lower panel). No effects of dietary fiber on cholesterol 7α-hydroxylase activity were observed in female guinea pigs fed HC diets or in males fed GG or PSY.

Hepatic LDL receptor B_{max} values were significantly increased by intake of PE, GG, and PSY in both male and female guinea pigs fed LC and HC diets ($P < 0.0001$) (**Fig. 6, upper and lower panels**). There was a more pronounced fiber effect in female animals where PE and PSY intake resulted in significant increases of LDL receptor number relative to values observed in males (Fig. 6, upper panel). Although PE, GG, and PSY increased B_{max} values in both male and female guinea pigs fed the HC diets, PE had a greater effect in males (Fig. 6, lower panel) probably associated with the substantial

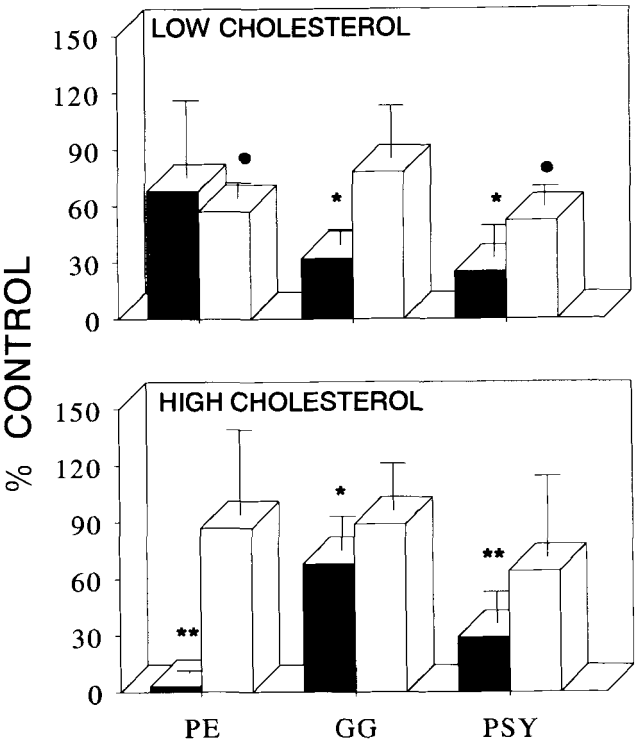

Fig. 4. Comparisons of dietary fiber effects on hepatic ACAT activity of male and female guinea pigs. Bars represent the mean ± SD of % control males (dark bars) (n = 18) and females (open bars) (n = 6) fed dietary fiber (six male or female animals per group): pectin (PE), guar gum (GG), or psyllium (PSY) diets with low cholesterol (upper panel) or high cholesterol (lower panel). Hepatic HMG-CoA reductase activity was significantly higher in males for both the low and high cholesterol groups ($P < 0.001$). *Represents significantly different from control values in males and ● significantly different from control values in females as determined by one-way ANOVA ($P < 0.001$).

reduction in hepatic cholesterol in these animals (Fig. 2, lower panel).

DISCUSSION

Dietary fiber effects on female guinea pigs fed low cholesterol and high cholesterol diets

The hypocholesterolemic effects of dietary fiber, and the mechanisms involved, vary depending on the type of fiber fed, the species under investigation, and the level of dietary cholesterol (34–38). Rats, for example, are known to be relatively resistant to dietary cholesterol effects on plasma and hepatic cholesterol concentrations (34, 35) while other animal models such as hamsters and guinea pigs are more susceptible to the effects of high dietary cholesterol and respond by increasing plasma cholesterol levels and suppressing hepatic LDL receptors (36, 37). This variability among species appears related to the efficiency of cholesterol absorption, the ability to suppress endogenous cholesterol synthesis, and rates of cholesterol catabolism to bile acids (38).

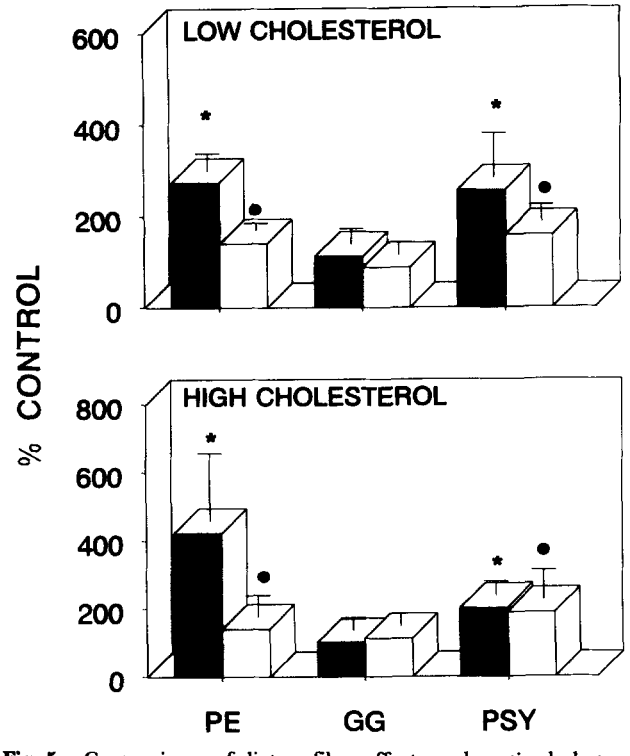

Fig. 5. Comparisons of dietary fiber effects on hepatic cholesterol 7α-hydroxylase activity of male and female guinea pigs. Bars represent the mean ± SD of % control males (dark bars) (n = 18) and females (open bars) (n = 6) fed dietary fiber (six animals per group): pectin (PE), guar gum (GG), or psyllium (PSY) diets with low cholesterol (upper panel) or high cholesterol (lower panel). *Represents significantly different from control values in males and ● significantly different from control values in females as determined by one-way ANOVA ($P < 0.001$).

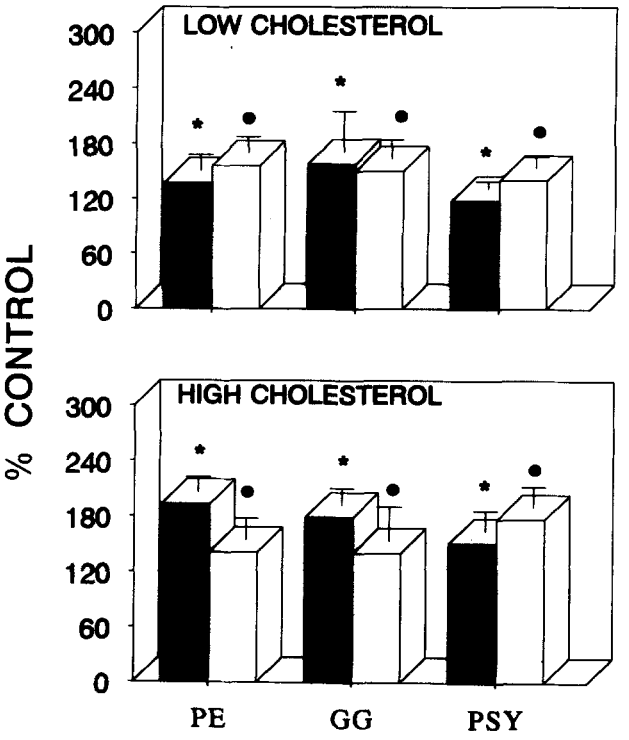

Fig. 6. Comparisons of dietary fiber effects on hepatic LDL receptor (B_{max}) of male and female guinea pigs. Bars represent the mean ± SD of % control for males (dark bars) (n = 16) and females (open bars) (n = 4) fed dietary fiber (four male or female animals per group) pectin (PE), guar gum (GG), or psyllium (PSY) diets with low cholesterol (upper panel) or high cholesterol (lower panel). *Represents significantly different from control values in males and ● significantly different from control values in females as determined by one-way ANOVA ($P < 0.01$).

Rats, for example, easily accommodate excess dietary cholesterol by increasing bile acid synthesis thereby maintaining steady state hepatic cholesterol concentrations (39). Another species difference is indicated by which plasma lipoproteins are affected by fiber intake; in hamsters and rats dietary fiber lowers LDL modestly while HDL is lowered to a great extent. In contrast, guinea pigs, similar to humans, carry the majority of plasma cholesterol in LDL, and dietary challenges aimed at altering plasma cholesterol levels are reflected by changes in LDL. When investigating the mechanisms of plasma LDL lowering by dietary fiber, these species differences in the responses to dietary cholesterol and fiber by different plasma lipoproteins complicate interpretation and integration of the available data.

In the present studies in guinea pigs we have shown that not only the amount of dietary cholesterol modifies the response to soluble fiber intake, but gender also significantly alters the hypocholesterolemic responses to dietary fiber. We previously demonstrated in male guinea pigs that dietary fiber types differ in the magnitude of the plasma cholesterol lowering response that

was associated with the ability of the soluble fiber to reduce hepatic cholesterol pools (7–9). This dietary fiber-mediated reduction in hepatic cholesterol, whether attained by decreasing cholesterol absorption or interruption of the enterohepatic circulation of bile acids, triggers a series of regulatory responses that culminate in the lowering of plasma LDL cholesterol. In female guinea pigs, alterations in hepatic enzymes and LDL receptor number were correlated with changes in hepatic cholesterol concentrations induced by fiber intake. In contrast, studies have shown that in the rat, alterations in the regulatory enzymes of cholesterol synthesis and catabolism occur in response to fiber intake in the absence of significant changes in hepatic cholesterol concentrations (13).

The present studies also demonstrate that female guinea pigs are hyper-responders to high dietary cholesterol and, while dietary fiber lowered plasma and hepatic cholesterol concentrations, the responses in females were moderate and more scattered than in males (7–9). We have previously observed that responses to dietary cholesterol vary among male guinea pigs and that there are both hypo- and hyper-responders (40). The present study suggests that the number of hyper-responders is increased in females. The decreases in hepatic cholesterol induced by dietary soluble fiber were also moderate due to substantial intragroup variation. Similarly, the effects of dietary fiber on hepatic enzymes were modest and correlated with the variations in hepatic cholesterol concentrations among female animals fed HC diets.

Although PE, GG, and PSY decreased plasma cholesterol in females fed the LC diets, the mechanisms appear to differ as these three types of fiber had different effects on hepatic cholesterol homeostasis. The reduction of hepatic cholesterol with fiber intake was more pronounced with PSY than with PE or GG and the effect was primarily on the free cholesterol pool. Interestingly, GG intake did not alter the activities of hepatic ACAT and cholesterol 7α-hydroxylase yet the concentration of hepatic cholesterol was similar to that in animals fed the PE and PSY diets. These differences in the changes of hepatic enzyme activities suggest that the primary mechanisms responsible for the reduction in hepatic cholesterol levels differ among PE, GG, and PSY, responses possibly related to their chemical structures, physical properties, and specific mode of action in the small intestine (41). For example, PE, although of low viscosity, decreases cholesterol absorption in rats (42, 43) and in humans (44) and, due to its gel-forming capacity, is an effective inhibitor of bile acid reabsorption in the ileum (13). These studies demonstrate that interruption of the enterohepatic circulation of bile acids, as suggested by the observed increases of cholesterol 7α-hydroxylase activity and possibly a decrease in cholesterol absorption, as observed by other investigators (42, 43), and in male guinea pigs (M. L. Fernandez, unpublished observations) account for the reduction in hepatic cholesterol concentration in animals fed the PE diet.

PSY intake has been shown to increase bile acid excretion (10, 45) with associated increases in the expression of hepatic cholesterol 7α-hydroxylase mRNA and enzyme activity in hamsters (4). In the present study, we observed an increase in cholesterol 7α-hydroxylase activity suggesting that psyllium binds to bile acids and increases hepatic bile acid synthesis resulting in the reduction in hepatic cholesterol pools. PSY could also induce a negative sterol balance by decreasing cholesterol absorption, yet decreases in cholesterol absorption with PSY intake were not observed in humans (45) or African green monkeys (46) and this mechanism was significant only with a bile acid sequestrant such as cholestyramine in the hamster (10). GG effects are less clear in that while reductions in hepatic cholesterol concentrations were observed, no increase in cholesterol 7α-hydroxylase activity was evident suggesting little effect of GG on bile acid excretion. These results are in contrast to what has been reported for rats where 40–50% increases in fecal bile acids were observed with guar gum intake although hepatic cholesterol pools were not altered (13). Miettinen and Tarpila (3) also reported increases in fecal bile acid output in humans consuming GG compared to a low fiber intake and Ikeda, Tomari, and Sugano (14) observed that GG decreased cholesterol absorption in rats. At this point, although it is not clear which mechanisms contribute to the observed reduction in hepatic cholesterol in female guinea pigs fed GG, the increase in hepatic LDL receptor number, decrease in plasma LDL levels, and lowering of hepatic cholesterol concentrations induced by GG combine to result in a significant plasma cholesterol lowering effect.

Gender differences in cholesterol and lipoprotein metabolism

Gender is a strong predictor of plasma lipoprotein patterns and susceptibility to dietary interventions (15–18). A main goal in these studies was to define differences in response between male and female guinea pigs to a high cholesterol challenge diet and to determine whether gender plays a role in determining the mechanisms by which dietary soluble fiber lowers plasma LDL. We demonstrated that female guinea pigs are more susceptible to a dietary cholesterol challenge than males and that the intervention, in this case intake of soluble fiber, was not as effective in decreasing plasma cholesterol levels as it was in males (7–9). Similar to our

observations, Jenkins et al. (15) reported a more significant effect of dietary fiber in lowering plasma total and LDL cholesterol levels in men compared to women. The reductions in plasma LDL cholesterol were 9.2% for mean versus 2.2% for women (15) which indicates a substantial gender difference in the responsiveness to dietary fiber of humans similar to what we have observed in the guinea pig.

The liver plays a central role in whole body cholesterol homeostasis as the site for cholesterol catabolism and excretion through bile acid and neutral sterol elimination and as the regulator of plasma cholesterol concentrations through synthesis of VLDL and catabolism of LDL via the apo B/E receptor (38). The more elevated plasma LDL levels observed in female guinea pigs fed the control HC diet could be due to a gender-associated effect related to reduced ability to maintain hepatic cholesterol homeostasis with high dietary cholesterol. Metabolic studies of the effects of dietary cholesterol on plasma cholesterol levels have shown that the major mechanisms associated with individual responses are decreases in the fractional absorption of dietary cholesterol, suppression of endogenous cholesterol synthesis (47), and, in the case of rats, an increase in bile acid synthesis and excretion (39). No gender effects on cholesterol 7α-hydroxylase activity were observed in response to the high cholesterol intake as this enzyme was not up-regulated with dietary cholesterol in male or female guinea pigs, consistent with the fact that guinea pigs do not utilize the conversion to bile acids to eliminate excess dietary cholesterol as efficiently as rats. However, hepatic HMG-CoA reductase activity was not suppressed in females to the same extent as males with high cholesterol intake (69 vs. 84%) suggesting that the more elevated plasma LDL levels in females relates to a less sensitive feedback control mechanism.

By these studies we have demonstrated that intake of PE, PSY, and GG effectively lowers plasma LDL cholesterol concentrations in female guinea pigs fed physiological levels of dietary cholesterol, and that gender plays an important role in the metabolic responses to both the challenge and intervention diets. The gender-associated responses to dietary fiber and the fact that males had a greater lowering of plasma LDL levels comparable to clinical observations support the appropriateness of the guinea pig model for these studies. Guinea pigs not only respond to the dietary factors by modification in plasma LDL levels and metabolism, but also gender differences in response to dietary fiber mimic the human situation thereby facilitating analysis of specific mechanisms of action of specific types of dietary soluble fiber.

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