PECTIN ISOLATED FROM PRICKLY PEAR (OPUNTIA SPP) MODIFIES LOW DENSITY LIPOPROTEIN METABOLISM IN CHOLESTEROL-FED GUINEA PIGS.

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INTRODUCTION

Epidemiologic studies have associated consumption of high fiber diets with a decreased risk for cardiovascular disease (1,2). Dietary fiber has been reported to reduce plasma cholesterol levels by binding to bile acids and increasing bile acid fecal excretion (3). In studies of dietary fiber, intake of pectin, guar gum, oat bran and other soluble fibers has consistently resulted in a decrease in plasma total and low density lipoprotein (LDL) cholesterol levels in humans (4) and animals models (5,6). In contrast, intake of insoluble fibers such as cellulose and wheat bran has been reported to have either a hypercholesterolemic effect (7) or no effect on plasma cholesterol levels (8).

The present study was undertaken to evaluate the effects of pectin isolated from prickly pear (Opuntia spp) on several parameters of cholesterol and lipoprotein metabolism in guinea pigs. Because this pectin has been used successfully to treat diabetic patients at concentrations as low as 60 mg/ dL (Augusto Trejo, unpublished data), the effects of this soluble fiber on plasma lipid levels, hepatic cholesterol content, hepatic HMG-CoA reductase and LDL binding to guinea pig hepatic membranes were investigated.

The advantages of using guinea pigs as the experimental animals model to measure the effects of prickly pear pectin on cholesterol and lipoprotein metabolism have been previously addressed: the similarity of the plasma lipoprotein profile to man (high LDL, low HDL), and similar responses to dietary fat and dietary cholesterol (9,10,11) constitute a unique animal model to study diet effects on LDL metabolism.

MATERIALS AND METHODS

Diets: Guinea pig nonpurified diet was obtained from Teklad (Madison, WI). The diet is reported to have 17.9% protein (mainly soybean meal), 1.9% vegetable fat, 49.3% carbohydrates and 14% fiber (mainly alfalfa); minerals and vitamins, including vitamin C, are added according to guinea pig requirements. Two diets were formulated from a single batch of the nonpurified diet by adding 0.25% (w/w) recrystallized cholesterol (HC diet) or 0.25% recrystallized cholesterol + 1% (w/w) prickly pear pectin (HC-P diet). The diets were repelleted. Pectin was isolated from Opuntia spp grown in Jiquilpan Michoacan in Mexico.

Animals: Male Hartley guinea pigs weighing between 250 to 300 g were randomly assigned to either the HC or the HC-P diet. After 25 days of dietary treatment, the

animals were anesthetized with halothane vapors and exsanguinated by cardiac puncture. Plasma was separated from blood cells for analysis of plasma lipids and isolation of plasma LDL. Livers were removed for preparation of hepatic membranes and hepatic microsomes and for determination of hepatic cholesterol content.

Plasma and Liver Lipids: Total plasma cholesterol and triglyceride levels were determined by enzymatic analysis (12). Very low density lipoprotein (VLDL), LDL and HDL were separated by sequential ultracentrifugation at 125,000 x g at 15°C for 19 h in a Ti-50 rotor. Separation was based on density fractions: d < 1.019 g/mL for VLDL; d 1.019 - 1.09 for LDL and 1.09 -1.21 for HDL (9,10,11). Hepatic concentrations of total and free cholesterol were determined according to Sale et al. (13).

LDL Isolation and Characterization: Plasma LDL was isolated by adjusting plasma density to 1.25 g/mL with solid KBr and by centrifuging for 36 h at 125,000 x g at 15°C in a Beckman Ti-50 rotor. The isolated lipoproteins were adjusted to a density of 1.3 g/mL with KBr and a 10-mL volume was overlayered with 30 mL of 0.9% NaCl solution in a Quickseal ultracentrifugation tube. Centrifugation was performed in a VC-53 vertical rotor for 3 h at 100,000 x g at 10°C to generate a density gradient fractionation of the lipoproteins. The lipoprotein profile was determined by measuring cholesterol in the isolated fractions, and density values were determined by measurement of the refractive index. The fractions corresponding to LDL were pooled from the d 1.02 - 1.09 g/mL portion of the gradient (9,10,11). To confirm the purity of LDL in the pooled fractions, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (4.5 to 20% linear gradient) were run for 45 min at constant voltage, 18-20 mA at room temperature and stained with Coomassie blue.

Microsome Isolation and Hepatic HMG-CoA reductase assay : Guinea pigs were killed at the nadir of the diurnal rhythm, livers were removed and hepatic microsomes were isolated as previously described (14). Microsomal HMG-CoA reductase (1.1.1.34) activity was measured by the radioisotopic method of Shapiro et al. (15).

Hepatic Membrane Isolation: Livers were homogenized with two 10s pulses of a Polytron at a setting of 10 in 10 mL of buffer A (150 mmol NaCl, 1 mmol $CaCl_2$, 10 mmol tris-HCL at pH 7.5). Hepatic membranes were isolated as previously described (11,13). For determination of LDL binding, frozen membranes were thawed and resuspended in buffer B (100 mmol NaCl, 0.5 mmol $CaCl_2$, 50 mmol Tris-HCl, 20 mg/mL bovine serum albumin at pH 7.5) and flushed 10 times through a 22-gauge needle. Membrane protein was determined according to Markwell (16).

Equilibrium Parameters: To determine hepatic membrane receptor affinity for LDL (K_d) and total apo B/E receptor number (B*max*), guinea pig LDL was radiolabeled according to Goldstein et al. (17) and incubated at 37°C with isolated membranes in the presence of 1 mg/mL of unlabeled human LDL over a range of 5 to 30 μg of LDL protein/mL. Scatchard analysis were used to determine (μg LDL protein/mL) and B_{max} (ng LDL bound/mg membrane protein).

Statistical Analysis: One-way analysis of variance was used to assess differences in the equilibrium parameters of K_d and B_{max}, in cholesterol content of livers and in plasma total, VLDL, LDL and HDL cholesterol levels. The least significant different test was used to evaluate differences between means (18).

RESULTS

Pectin Effects on Plasma and Hepatic Lipid Levels: Guinea pigs fed the HC-P diet exhibited a significant 26% reduction in plasma total cholesterol levels as compared to levels in animals fed the HC diet (**Table 1**) . A significant 33% decrease in plasma LDL and HDL cholesterol levels was observed in HC-P fed guinea pigs. VLDL cholesterol concentrations were elevated in guinea pigs fed the HC-P diet, although not significantly.

TABLE 1. Plasma Lipids in guinea pigs fed nonpurified diet + 0.25% cholesterol (HC diet) or nonpurified diet + 0.25% cholesterol + 1% pectin (HC-P diet)[1]

	Diet	
Plasma Lipids	HC	HC-P
	mg/dL	
Total cholesterol	89 ± 24[a]	66 ± 29[b]
VLDL cholesterol	5 ± 2	10 ± 12
LDL cholesterol	59 ± 24[a]	39 ± 15[b]
HDL cholesterol	22 ± 7[a]	15 ± 5[b]

[1] Data are presented as means ± SD. Values in the same row with different superscripts are significantly different ($P < 0.02$) as assessed by ANOVA and the least significance difference test.

Fractionation of plasma lipoproteins by density gradient vertical rotor ultracentrifugation indicated the presence of LDL subfractions in both groups (**Fig. 1**). In the HC- diet animals, the LDL peak density was 1.04 g/mL. Adding pectin to the diet increased the LDL peak density to 1.055 g/mL.

Hepatic concentrations of total, free and esterified cholesterol were significantly reduced in guinea pigs fed the HC-P diet ($P < 0.002$) (**Table 2**). However, hepatic HMG-CoA reductase activity was not affected by adding pectin to the HC-diet.

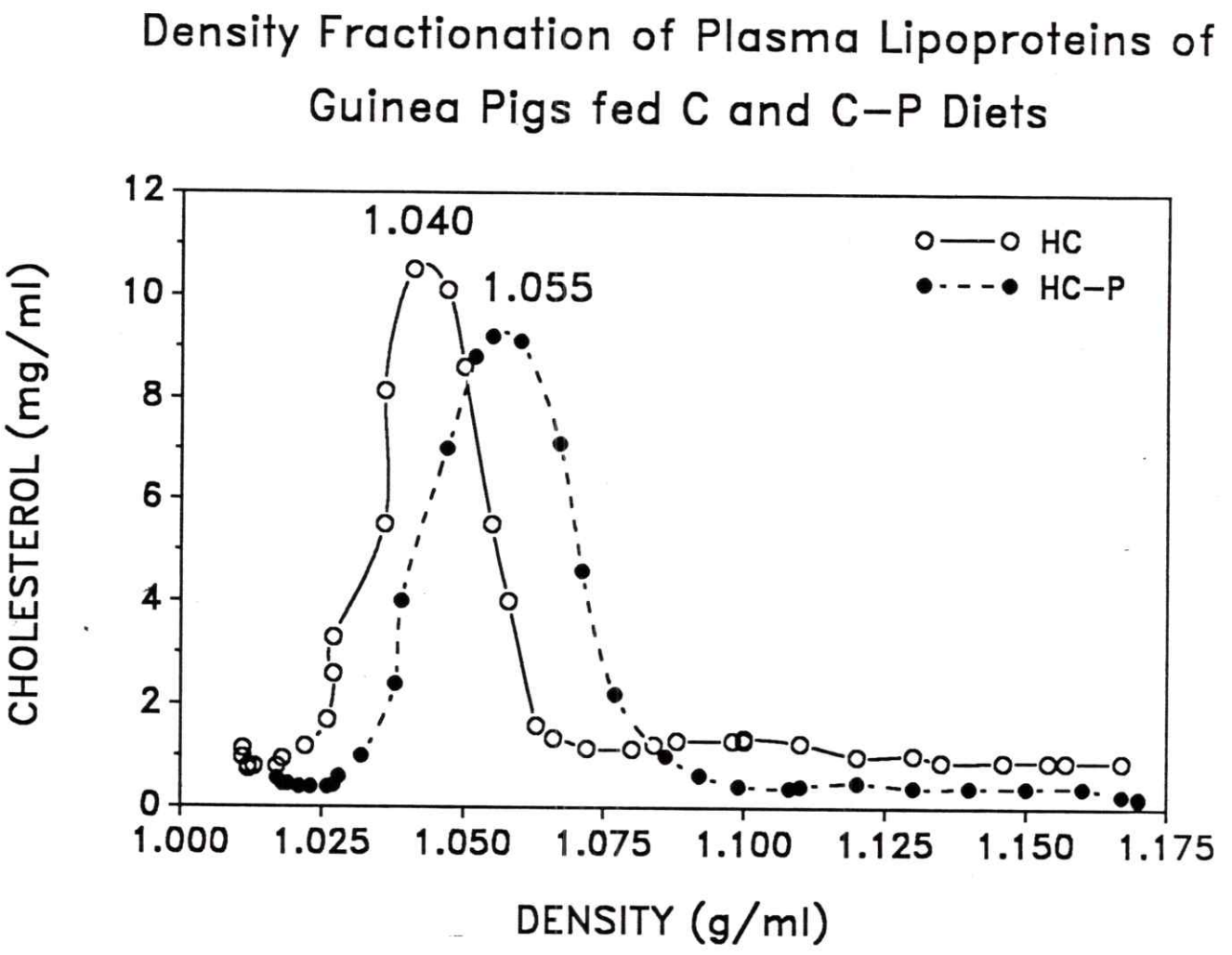

Figure 1. Typical density fractionation profiles of plasma lipoproteins of guinea pigs fed either the HC (O) or the HC-P (O) diets.

Table 2. Hepatic HMG-CoA reductase levels and cholesterol content in guinea pigs fed nonpurified diet + 0.25% cholesterol (HC diet) or nonpurified diet + 0.25% cholesterol + 1% pectin (HC-P diet)[1]

Diet	Cholesterol		HMG-CoA Reductase
	Free	Esterified	
	mg/g		pmol/(min.mg protein)
HC	4.1 ± 1.3[a]	2.0 ± 0.9[a]	2.03 ± 0.32
HC-P	2.3 ± 0.6[b]	0.3 ± 0.3[b]	1.99 ± 0.31

[1] Data are presented as means ± SD. Values in the same column with different superscripts are significantly different ($P < 0.002$) as assessed by ANOVA and the least significant difference test.

Pectin Effects on LDL Binding and in equilibrium parameters: Receptor-mediated binding of guinea pig ^{125}I-LDL to guinea pig hepatic membranes was significantly increased in animals fed the HC-P diet. A significant negative correlation ($r = -0.597$, $P < 0.005$) was found between plasma LDL cholesterol levels and receptor-mediated LDL binding to guinea pig hepatic membranes for animals fed the HC and HC-P diets (**Figure 2**).

Concentration dependent binding curves for the binding of LDL to guinea pig hepatic membranes from both group of animals are shown in Figure 3. Both membrane preparations exhibited saturation at 20 μg/mL. Scatchard plots are shown in the inset of Figure 3. The equilibrium parameters of K_d and B_{max} were analyzed in four different membrane preparations of both dietary groups. The affinity constant (K_d) was not affected by diet, but the number of receptors (B_{max}) was significantly increased 1.5-fold for hepatic membranes from guinea pigs fed the HC-P diet ($P < 0.001$) (**Table 3**).

TABLE 3. Equilibrium Constants for LDL Binding to Guinea Pig Hepatic Membranes from Animals fed non purified diet + 0.25% cholesterol (HC diet) or nonpurified diet + 0.25% cholesterol + 1% pectin (HC-P diet)[1]

Diet	K_d	B_{max}
	μg/mL	ng/mg protein
HC	19.8 ± 7.3	1628 ± 316[a]
HC-P	17.5 ± 4.2	2385 ± 342[b]

[1] Data are presented as means ± SD. Values with different superscripts in the same column are significantly different ($P < 0.002$) as assessed by ANOVA and the least significant difference test.

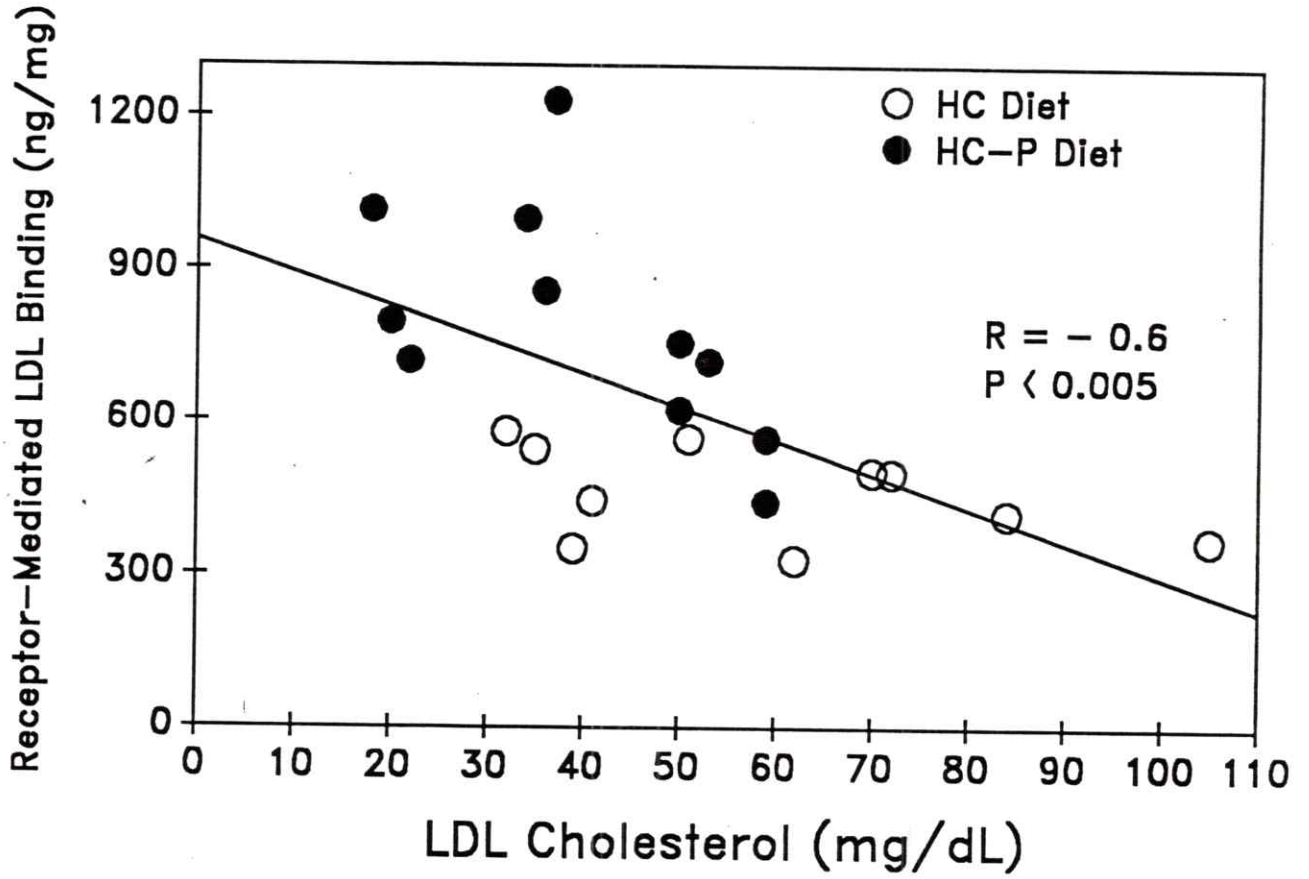

Figure 2. Regression analysis of relationship between plasma LDL cholesterol levels and receptor-mediated LDL binding in animals fed HC (O) or HC-P (O) diets.

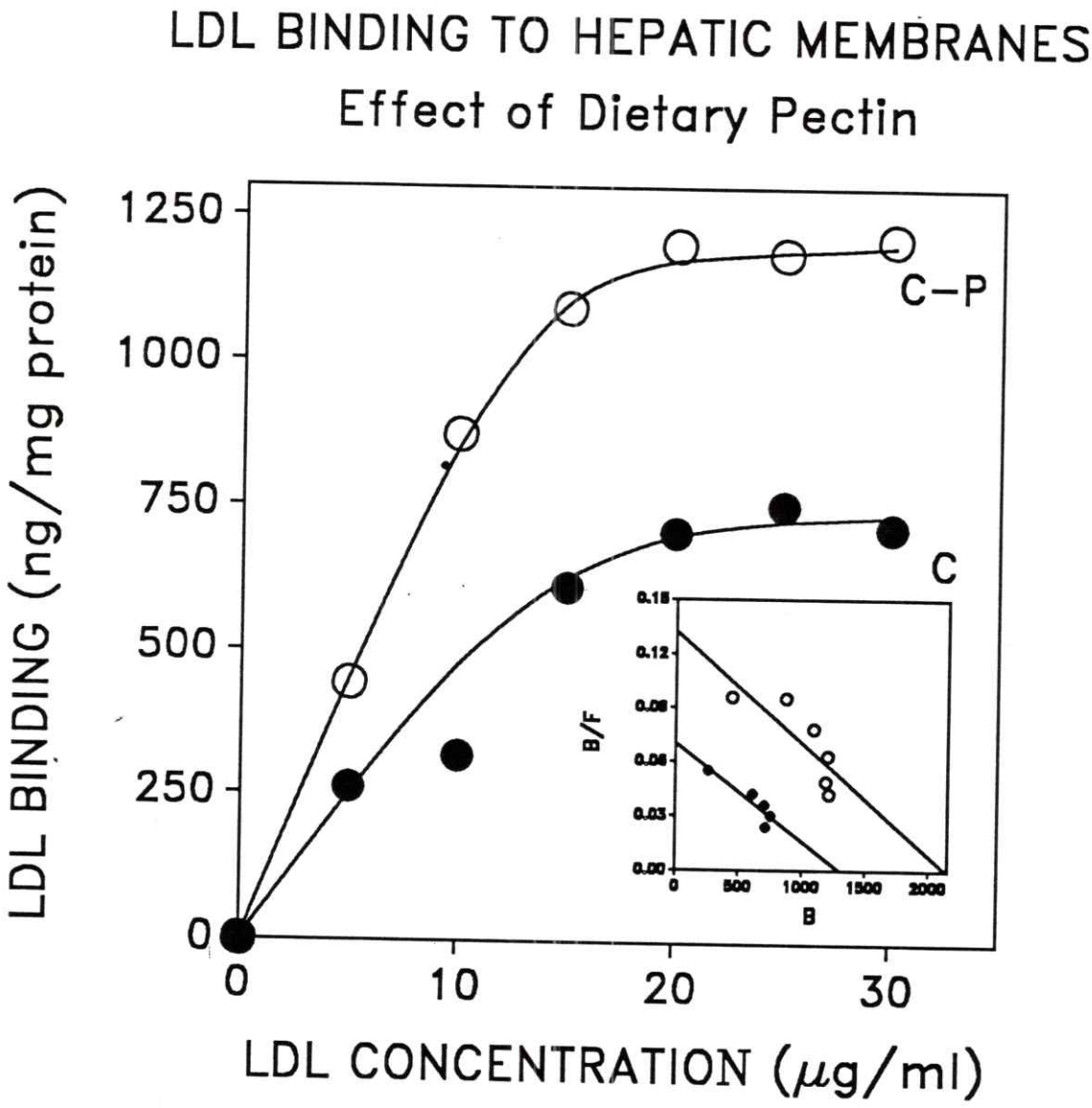

Figure 3. Binding kinetics and Scatchard plots (inset) for receptor-mediated binding of guinea pig LDL to hepatic membranes from animals fed the HC (O) or HC-P (O) diets. In the Scatchard plot (inset), B represents bound ligand (ng/mg) and B/F equals bound divided by free ligand [(ng/mg)/(μg/mL]. K_d values were 18.5 and 16.1 mg/mL, and B_{max} values were 1300 and 2137 ng/mg protein for the animals fed the HC and HC-P diets, respectively.

DISCUSSION

Effect of Prickly Pear Pectin on Plasma Cholesterol Levels and Lipoprotein Profile: In the present study, we found that prickly pear pectin had a hypocholesterolemic effect that affected both LDL and HDL fractions when fed at a concentration as low as 1% resulting in a normalization of plasma LDL and HDL cholesterol levels (10,19). Similarly, Ney et al. (6) reported a normalization of rat plasma lipoproteins from inclusion of oat bran in a hypercholesterolemic atherogenic diet; the major effect was in the VLDL fraction. A decrease in the LDL and HDL cholesterol was also reported, though apo A-I production was not affected.

The hypocholesterolemic effect of pectin was not consistent in all guinea pigs; some of the animals fed the HC-P diet had normal plasma cholesterol values of 30-40 mg/dL reported for guinea pigs fed nonpurified diets whereas other had values of 50-70 mg/dL which are typical of guinea pigs fed the HC diet (10,19). It has been found in this and other studies (19) that the plasma cholesterol levels for guinea pigs fed a high cholesterol diet range from 50 -140 mg/DL suggesting that some guinea pigs are hyperresponders to the HC diet and other are not. A similar effect was observed with the addition of pectin to the HC diet, in that some guinea pigs had a more pronounced response than others to prickly pear pectin in the presence of the high cholesterol diet. On average, an overall 26% reduction in total cholesterol and 33% reduction in LDL and HDL cholesterol were observed. Regression analysis of the relationship between plasma LDL cholesterol levels and LDL receptor binding indicated that there was a significant negative correlation ($P < 0.005$) between these two parameters. The observed correlation is consistent with the hypothesis that apo B/E receptor expression is a major determinant of plasma LDL cholesterol levels (17).

Prickly Pear Pectin Effects on LDL Density: Adding pectin to the HC diet shifted the LDL peak density from 1.040 to 1.055 g/mL (**Figure 2**) approaching values reported for guinea pigs fed nonpurified diets (1.075 g/mL). These data suggest that, in guinea pigs, consumption of pectin decreases the size and increases the density of LDL. An increase in LDL peak density also results from consumption of polyunsaturated versus saturated fat diets (9,11), and a decrease in LDL particle size results from the intake of cholestyramine (20). Because larger, less dense particles contain more cholesteryl ester (9,11), it would be predicted that pectin reduces cholesteryl ester concentrations of plasma LDL.

Pectin Effects on Hepatic Cholesterol Synthesis and Cholesterol Content: Adding 0.25% cholesterol to the diet reduced guinea pig hepatic cholesterogenesis by 90%. Values for hepatic HMG-CoA reductase activity for animals fed a nonpurified diet are 30-40 pmol/(min mg protein). Adding pectin to the cholesterol diet did not affect hepatic cholesterol synthesis since both groups of animals had similar levels of HMG-CoA reductase activity. Reports concerning the effects of oat bran and pectin on hepatic HMG-CoA reductase and hepatic cholesterol synthesis are contradictory. Addition of

soluble fiber has been reported to alter hepatic cholesterol synthesis by decreasing (21), increasing (22) or not affecting (23) HMG-CoA activity. In this study, adding pectin did not modify the levels of hepatic HMG-CoA reductase activity that were almost completely suppressed by the 0.25% cholesterol diet.

Many investigators have reported a decrease in hepatic cholesterol content (24) from the addition of pectin diets possibly due to a reduction in cholesterol absorption. In this report a similar response was observed in that prickly pear pectin significantly reduced hepatic free cholesterol and cholesteryl ester by 40 and 85% respectively. Further studies need to be done to evaluate whether prickly pear pectin decreases dietary cholesterol absorption.

Effect of Pectin on LDL Binding to Hepatic Membranes: The significant increase in LDL binding to guinea pig hepatic membranes from animals fed the HC-P diet was due to a significant increase in the expression of apo B/E receptors (B_{max}) and not to increased affinity of the receptor to LDL (K_d). Further studies are needed to investigate how dietary pectin affects receptor-mediated and receptor-independent LDL turnover in vivo in animals fed high cholesterol diets.

Comparative Effects of Cholestyramine and Prickly Pear Pectin on Cholesterol Metabolism: Pectin and the bile acid sequestrant resin cholestyramine appear to have similar mechanisms in reducing plasma cholesterol levels. Cholestyramine binds bile acids in the intestine and interrupts their enterohepatic circulation. As a result of this interruption, three enzymes are induced: Phosphatidic acid phosphatase, cholesterol 7 α-hydroxylase and HMG-CoA reductase (25). Consequently an increase in triglycerides, an increased demand for intracellular cholesterol and an increased cholesterol synthesis are expected. Similar to what has been reported for cholestyramine, prickly pear pectin may also affect cholesterol absorption, and increase bile acid excretion as has been documented for some commercial pectin sources (5).

Cholestyramine has been shown to reduce plasma and LDL cholesterol levels (26,27), decrease hepatic cholesterol content (28), decrease LDL particle size and increase LDL peak density (20); increase receptor mediated plasma clearance in rabbits (26) and humans and increase LDL binding to guinea pig hepatic membranes (27). These observations are similar to what has been reported in this study using prickly pear pectin. Contrary to reported data for rats fed a high cholesterol diet treated with cholestyramine (28), prickly pear pectin did not increase hepatic HMG-CoA reductase levels.

Summary

Adding 1% prickly pear pectin to a high cholesterol diet decreased total, LDL and HDL cholesterol levels ($P < 0.002$); did not change hepatic HMG-CoA reductase activity and significantly increased the number of hepatic apo B/E receptors ($P < 0.001$)

without modifying the receptor affinity. From these observations we conclude that the mechanisms involved in the plasma hypocholesterolemic effect of prickly pear pectin could be analogous to those reported for cholestyramine and other bile acid-binding resins in clinical and animal studies. This means that prickly pear pectin decreases dietary cholesterol absorption which results in decreased plasma cholesteral mainly LDL cholesterol which has been associated with increased risk for cardiovascular disease.

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