Inhibition of Atherosclerosis by Dietary Pectin in Microswine With Sustained Hypercholesterolemia

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Abstract Sustained hypercholesterolemia is a known risk factor for development of atherosclerosis. In animal studies, grapefruit pectin fed concurrently with a high-lipid diet inhibits hypercholesterolemia and atherogenesis. The purpose of the present study was to determine if grapefruit pectin affects cholesterol levels and atherogenesis of animals with established hypercholesterolemia. Microswine were fed an atherogenic diet to establish hypercholesterolemia. Plasma cholesterol levels rose rapidly and for 360 days were sustained at levels 6- to 12-fold the normal level. Then, half the microswine, selected at random, were fed a diet in which 3% grapefruit pectin was substituted for cellulose, and the remaining animals received the original diet. Animals were killed 270 days later, and the extent of atherosclerosis was determined. In animals with established hypercholesterolemia, pectin did not lower their cholesterol levels. However, pectin reduced the extent of atherosclerosis in both the aorta and coronary arteries. The mean surface area covered by atherosclerosis in the aorta was 13.6% in the group that did not receive pectin compared with 5.3% in the group that did receive pectin. The mean coronary artery narrowing was 45% without pectin and 24% with pectin. We conclude that pectin may have a direct beneficial effect on atherosclerosis by a mechanism independent of cholesterol levels. (***Circulation.*** **1994;89:1247-1253.)**

Key Words • atherosclerosis • diet

Hypercholesterolemia is a major risk factor in the development of atherosclerosis,[1,2] and cholesterol-lowering agents are used in selected patients to retard atherogenesis. As an alternative to the use of pharmaceutical agents, recent studies have shown that ingestion of water-soluble dietary fibers such as oat bran or pectin will lower cholesterol levels.[3-5] Pectins are a group of heterogeneous high molecular weight polysaccharides that are structural components of plant cell walls in fruits and vegetables. Pectin is composed of D-anhydrogalacturonic acid units linked through α(1-4)-glycosidic bonds forming a polygalacturonic acid with some of the carboxyl groups esterified with methanol.[6] As a human dietary supplement, citrus pectin reduces cholesterol levels[3,7-9] and lowers low-density lipoprotein (LDL) cholesterol in persons with hypercholesterolemia, and it does so without a change in lifestyle or dietary habits.[9]

In a previous study undertaken to determine if grapefruit pectin not only lowers serum cholesterol levels but also alters the development of atherosclerosis, we fed miniature swine (Pitman-Moore strain) an atherogenic diet with or without the addition of pectin. We reported that animals fed pectin for 326 days had both lower cholesterol levels and less atherosclerosis at autopsy than did animals fed the same diet without pectin.[6] In that study, pectin was fed concurrently with the atherogenic diet. Therefore, the important question remained as to whether pectin alters established hypercholesterolemia and retards the progression of atherosclerosis. We report the effect of pectin fed to microswine (Yucatan strain) after 1 year of sustained hypercholesterolemia.

Received October 1, 1993; revision accepted November 16, 1993.

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Methods

Animals and Diet

Fifteen female Yucatan microswine (Charles River Laboratory) were fed an atherogenic diet containing cellulose (Table 1, diet 1) for 390 days beginning at 5 months of age (average weight, 12 kg). After 1 year, 1 pig was killed to determine the extent of atherosclerosis, and the remaining 14 pigs were randomized into two dietary groups. One group of 7 pigs continued on diet 1, and the other group received a diet containing grapefruit pectin in the place of cellulose (Table 1, diet 2). The grapefruit pectin or cellulose was mixed into the diets at a level of 3% dry weight. All dietary components were obtained from ICN Biochemicals, except for hog finisher and grapefruit pectin. Hog finisher (3.2 kcal/g) was furnished by the University of Florida Swine Unit and contained by weight 16% protein, 4% fat, and 60% carbohydrate. The ICN vitamin fortification was formulated by ICN Biomedicals and contained all essential vitamins necessary to prevent deficiencies. Grapefruit pectin was purchased from Hercules, Inc. All daily diet portions were measured, and pigs were given a restricted feeding schedule that maintained growth rate without promoting excess weight gain. Each pig consumed its entire daily portion under observation, and food consumption for all pigs was similar and increased as the pigs matured. This investigation was approved by the Animal Care and Use Committee of the University of Florida.

Cholesterol Determinations

Blood samples were collected every 30 days in 0.15% EDTA for determination of plasma cholesterol and total triglyceride levels. A complete lipoprotein profile was determined at selected intervals. Lipids were determined enzymatically using a Sigma Diagnostic Kit (Sigma Chemical Co), with total plasma cholesterol determined by a method modified from that of Allain et al[10] and triglycerides determined by a method

TABLE 1. Composition of the Atherogenic Diets

Ingredient	Composition, %	
	Diet 1, No Pectin	Diet 2, With Pectin
Hog finisher	76.0	76.0
Lard	15.0	15.0
Casein	3.84	3.84
Cholesterol	1.0	1.0
ICN vitamin fortification	0.20	0.20
Mineral mixture USP XVII	0.96	0.96
Cellulose	3.0	None
Grapefruit pectin	None	3.0

modified from that of Bucolo and David.[11] Very-low-density lipoproteins (VLDL), LDL, and high-density lipoproteins (HDL) were separated by ultracentrifugation using the procedure of Havel et al[12] and the following density intervals as suggested by Mahley et al[13] for pigs: VLDL, <1.006; LDL, 1.006 to 1.087; and HDL, 1.087 to 1.21.

Pathological Examination

Necropsies were performed on all animals after they were killed with intramuscular ketamine HCl (Ketaset, 20 to 25 mg/kg; Bristol Laboratories) followed by 20 mL of saturated KCl solution IV (jugular) to induce cardiac arrest. The pathologists performing the examinations and determining the extent of atherosclerosis were blinded as to which animals had received pectin. The entire aorta was removed intact, opened longitudinally, pinned flat to a corkboard, and fixed in 10% neutral buffered Formalin. The vessel contour and the border of atheromatous plaques were traced onto a clear plastic overlay, from which the surface area of the aorta and the area occupied by atherosclerosis were measured by planimetry using a Hewlett Packard 911A digitizing board connected to an IBM computer with an IEEE 488 interface bus. The percentage of surface area covered by atheromatous plaques was calculated for each aorta. Meanwhile, the apex of the excised heart was removed, the atria and ventricles were stuffed with gauze, and the heart was fixed in Formalin. After fixation, the three main coronary arteries were removed intact and cut transversely at 1-cm intervals. Blocks for histology were prepared as follows: two sections of the left main coronary artery in one block, four sections each of the proximal and distal left anterior descending coronary arteries in two blocks, four sections of the left circumflex artery in one block, and three to five sections each of the proximal and distal right coronary arteries in two blocks. Microscopic sections were stained with hematoxylin and eosin, Verhoeff van Gieson's elastic stain, and Masson's trichrome for collagen and smooth muscle.[14] To quantify atherosclerotic narrowing, microscopic sections of coronary arteries were magnified ×21 with a microfiche reader and traced onto clear plastic, and dimensions were measured by planimetry. The original lumen was calculated as the area of a circle with a circumference equal in length to the internal elastic lamina[15] because normally the endothelium is closely applied to the internal elastica. The area occupied by atherosclerotic plaque was measured, and the percent luminal narrowing was calculated. Multiple transverse sections of the heart were examined grossly and microscopically for evidence of infarction.

Statistical Analysis

Longitudinal data from day 390 on were analyzed using a nonlinear mixed-effects model analysis. An exponential model of the form $y=\beta_0+e^{\beta_1}(t-390)$ was fitted for each diet. The curve for the pectin diet was compared with that for control using the tests proposed by Vonesh and Carter.[16] Such analyses were performed for y equal to total cholesterol, LDL, VLDL, HDL, and triglyceride measures, separately. The pathological data were analyzed by averaging the percent narrowing in all coronary arteries to form one measure of disease extent and using percent surface area of aorta with atherosclerosis as another

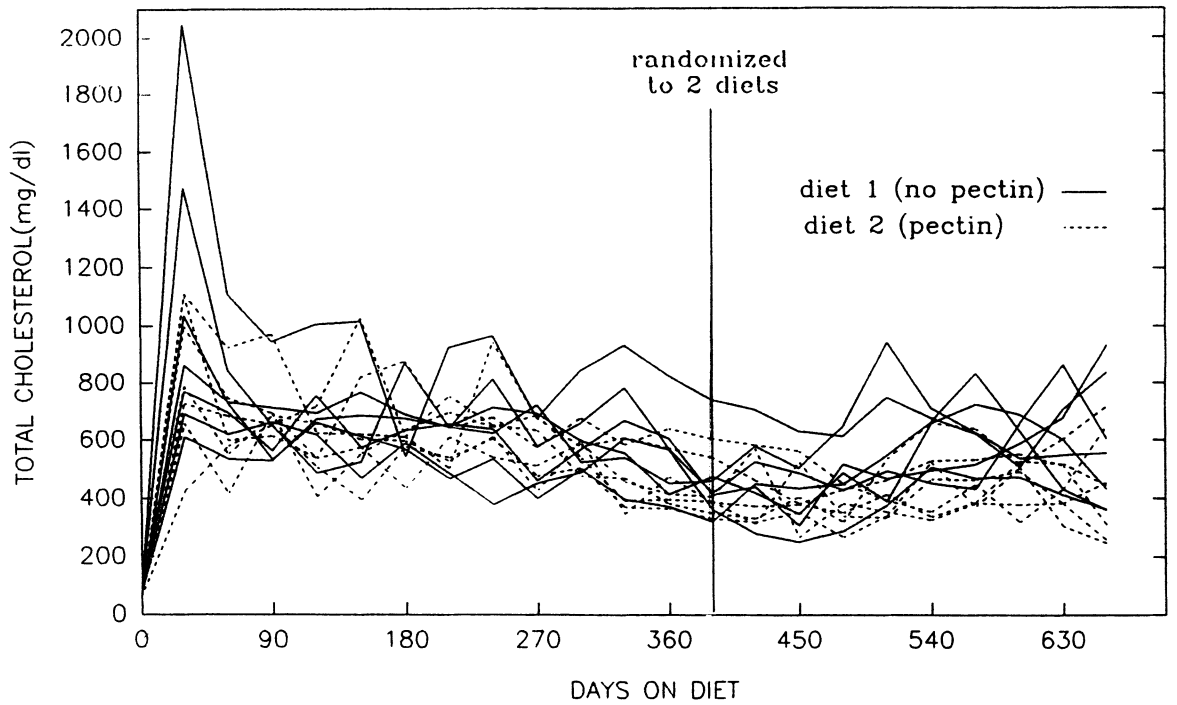

FIG 1. Plot of plasma cholesterol levels of microswine for the entire 660-day experiment. Before being randomized into two different diets (day 390), all pigs were maintained on diet 1 without pectin.

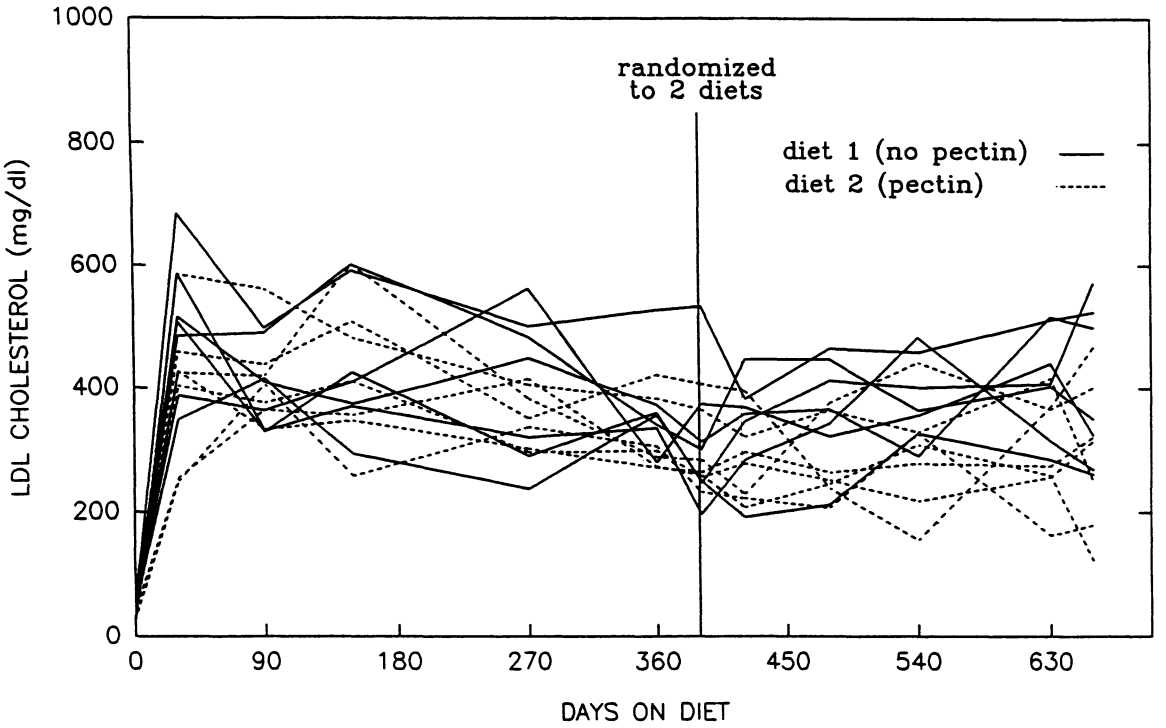

FIG 2. Plot of plasma low-density lipoproteins (LDL) of microswine for the entire 660-day experiment.

measure. Hotelling's T^2 (a multivariate test) was calculated to test simultaneously for a diet effect on the two measures. Follow-up t tests (one-sided) were then performed to evaluate the effect of diet on each measure separately. Further follow-up tests were done by performing t tests (one-sided) on the percent narrowing in the left main coronary artery and the average percent narrowing of the proximal and distal right coronary arteries. A Wilcoxon rank-sum test (one-sided) was performed on the percent narrowing in the left circumflex and the average percent narrowing of the proximal and distal left anterior descending coronary arteries. The Wilcoxon statistic was used when the measures had a large number of zeros and therefore were clearly nonnormally distributed.

Results

Fifteen microswine were fed an atherogenic diet for 390 days without pectin (diet 1) to induce hypercholes-

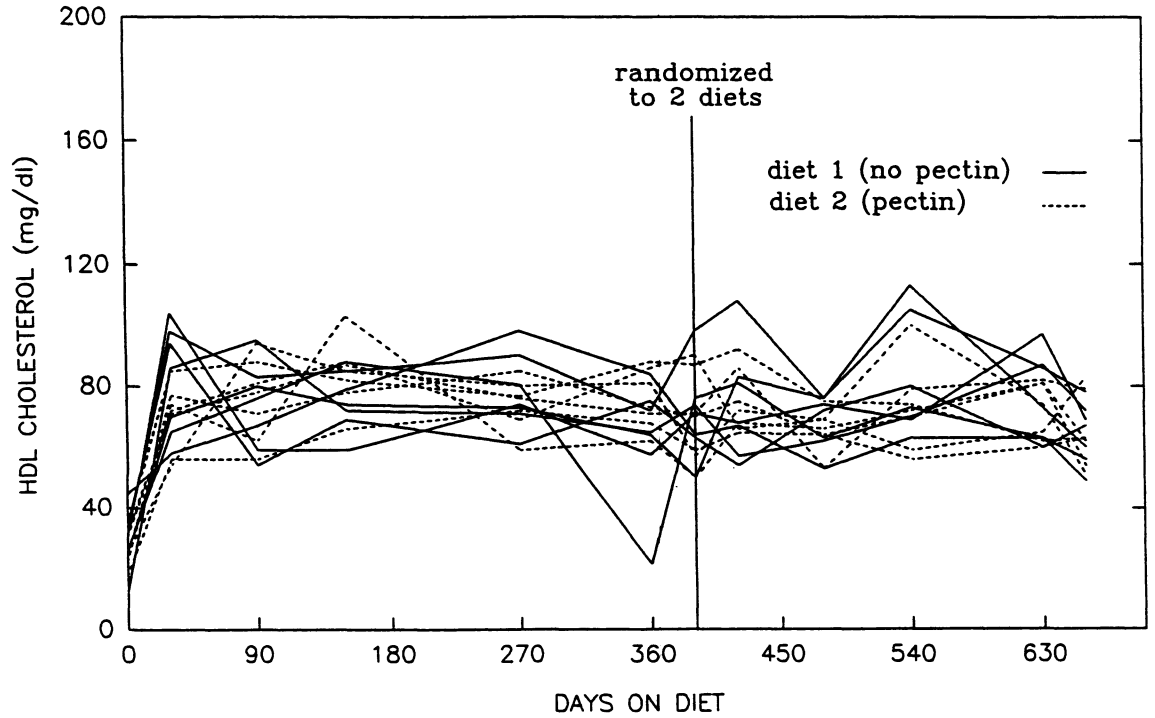

FIG 3. Plot of plasma high-density lipoproteins (HDL) of microswine for the entire 660-day experiment.

TABLE 2. Body Weight and Food Consumption of Microswine on Atherogenic Diets With or Without Pectin

	Body Weight, kg*		Food Consumption		
Day	Diet 1, No Pectin	Diet 2, Pectin Final 270 Days	Diet 1, g/d	Diet 2, g/d	kcal/d
0	12.3±1.1	11.7±0.9	185	NA	611
120	13.3±1.3	11.6±0.6	222	NA	733
240	15.8±1.3	15.1±1.1	222	NA	733
390	15.8±1.6	17.3±1.4	222	222	733
510	17.4±1.6	16.7±1.3	275	275	908
660	20.3±2.3	19.2±1.7	275	275	908

*Values are given as mean±SE.

terolemia and atherosclerosis. Total plasma cholesterol increased rapidly from a mean control level of 80 mg/dL to a maximal level of 950 mg/dL 30 days after initiation of the atherogenic diet (Fig 1). Thereafter, cholesterol levels gradually decreased to approximately 500 mg/dL. This decrease in cholesterol levels despite continued ingestion of a high-cholesterol diet has been noted in pigs[6] and is not caused by reduced dietary intake or maturation of the pigs but may reflect their adaptation to the high-fat/high-cholesterol diet. Mean LDL levels peaked at day 30 at 451 mg/dL (Fig 2) and were sustained with only a gradual decline over the course of the experiment. Although not shown, VLDL levels had a pattern similar to that of total cholesterol. HDL cholesterol increased from a mean level of 28 to 76 mg/dL with initiation of the atherogenic diet and essentially was maintained at that level (Fig 3). Mean triglyceride concentrations did not change from normal fasting levels, despite ingestion of the lipid-rich atherogenic diet.

A cholesterol elevation of the magnitude observed is known to cause atherosclerosis in miniature swine within 180 days.[6] To ensure that atherosclerosis had been induced, 1 pig was killed 390 days after being placed on the atherogenic diet. This animal had multiple atheromatous plaques in the coronary arteries and aorta. Of the 14 remaining pigs, 7 were selected randomly and continued on diet 1 without pectin, and the other 7 received the same diet with 3% grapefruit pectin replacing cellulose (Table 1, diet 2). After randomization and the start of pectin treatment, the mean cholesterol levels of the two groups of animals did not differ significantly (mean levels, 456±48 mg/dL without pectin versus 429±35 mg/dL with pectin). Furthermore, there were no significant differences in LDL or HDL levels.

TABLE 3. Reduction of Atherosclerosis by Pectin

Treatment	Abdominal Aorta, % Area Covered by Atherosclerosis	Coronary Artery, % Narrowing by Atherosclerosis
Diet 1, no pectin	13.6±6.8	45±6
Diet 2, pectin	5.3±3.2	24±5
P by multivariate test	.0240	
P by univariate *t* tests	.0054	.0095

Values are given as mean±SE.

Effect of Pectin on Sustained Hypercholesterolemia

During the next 270 days, monthly cholesterol levels stabilized for animals on both diets at levels more than threefold normal levels. Total cholesterol (Fig 1), LDL cholesterol (Fig 2), VLDL cholesterol, HDL cholesterol (Fig 3), and triglyceride levels showed no statistically significant differences between the two diets. Thus, the pectin-fed microswine had sustained hypercholesterolemia at levels comparable to those of pigs receiving an atherogenic diet without pectin. There were no differences in body weight or the amount of food consumed daily between animals receiving the atherogenic pectin diet and those receiving the atherogenic cellulose diet (Table 2).

Effect of Pectin on Atherosclerosis

At necropsy, the extent of atherosclerosis was measured in the aorta as the percent area covered by atheromatous plaque and in the coronary arteries as the percent of vessel narrowing caused by atherosclerosis. The first hypothesis tested was whether any differences existed between pectin-treated and control animals with respect to the variables in Table 3. With a multivariate test comparing all measurements of atherosclerosis (surface area of aorta covered by atherosclerosis and the degree of coronary artery narrowing), pectin significantly decreased the overall extent of atherosclerosis (Table 3). Accordingly, a follow-up test was performed to determine if pectin had a differential effect on atherosclerosis depending on anatomic site. This univariate *t* test showed that atherosclerosis was reduced significantly in both the aorta and the coronary

TABLE 4. Reduction of Atherosclerosis in Different Coronary Arteries by Pectin

	Luminal Narrowing, %		
Coronary Artery	Diet 1, No Pectin	Diet 2, Pectin	*P*
Left main	50.1±6.5	39.4±9.8	.2077 (*t* test)
Left anterior descending	21.3±7.7	2.6±1.9	.0011 (*t* test)
Left circumflex	34.4±11.8	2.3±2.1	.1253 (Wilcoxon)
Right coronary	49.8±6.3	26.6±7.7	.0487 (Wilcoxon)

Values are given as mean±SE.

arteries (Table 3). The protective effect of pectin reduced atherosclerosis by about 50% in both the aorta and coronary arteries.

Fig 4 shows representative aortas of two pectin-treated and two untreated pigs. Atherosclerotic plaques were less extensive in animals receiving pectin. Atheromatous plaques in animals receiving pectin were most severe in the abdominal aorta below the renal arteries. In animals not receiving pectin, atheromatous plaques appeared in the thoracic aorta in addition to the abdominal aorta. In the thoracic aorta, they generally were located just distal to the subclavian arteries, whereas in the abdominal aorta, they were found both above and below the renal arteries.

Having established that pectin significantly decreased overall coronary artery narrowing (Table 3), the next question was which sites might be responsible for this difference. Table 4 presents the percent luminal narrowing by atherosclerosis for different coronary arteries. Atherosclerosis was most severe in the left main and proximal right coronary arteries in both groups of animals. However, pectin reduced the extent of coronary artery narrowing in all segments and significantly reduced coronary narrowing in the left anterior descending and right coronary arteries (Table 4). Fig 5 presents representative histopathology of the coronary arteries. Fig 5A shows extensive disruption of medial elastic fibers of the left main coronary artery by atheroma in a pig that did not receive pectin. This aggressive behavior of the atheroma was not unusual (Fig 5B). Sometimes it produced an incipient aneurysm, although no coronary artery rupture occurred. In a comparison of right coronary arteries, the microswine that did not receive pectin had extensive intimal thickening with

FIG 4. Photograph of representative aortas of microswine receiving an atherogenic diet showing decreased aortic atherosclerosis in pigs receiving pectin. The left two aortas are from pigs receiving pectin (63-6II and P-83-2), whereas the right two aortas are from animals not receiving pectin. No complicated lesions (ulceration, calcification, or hemorrhage into an atheromatous plaque) were seen grossly in the aorta.

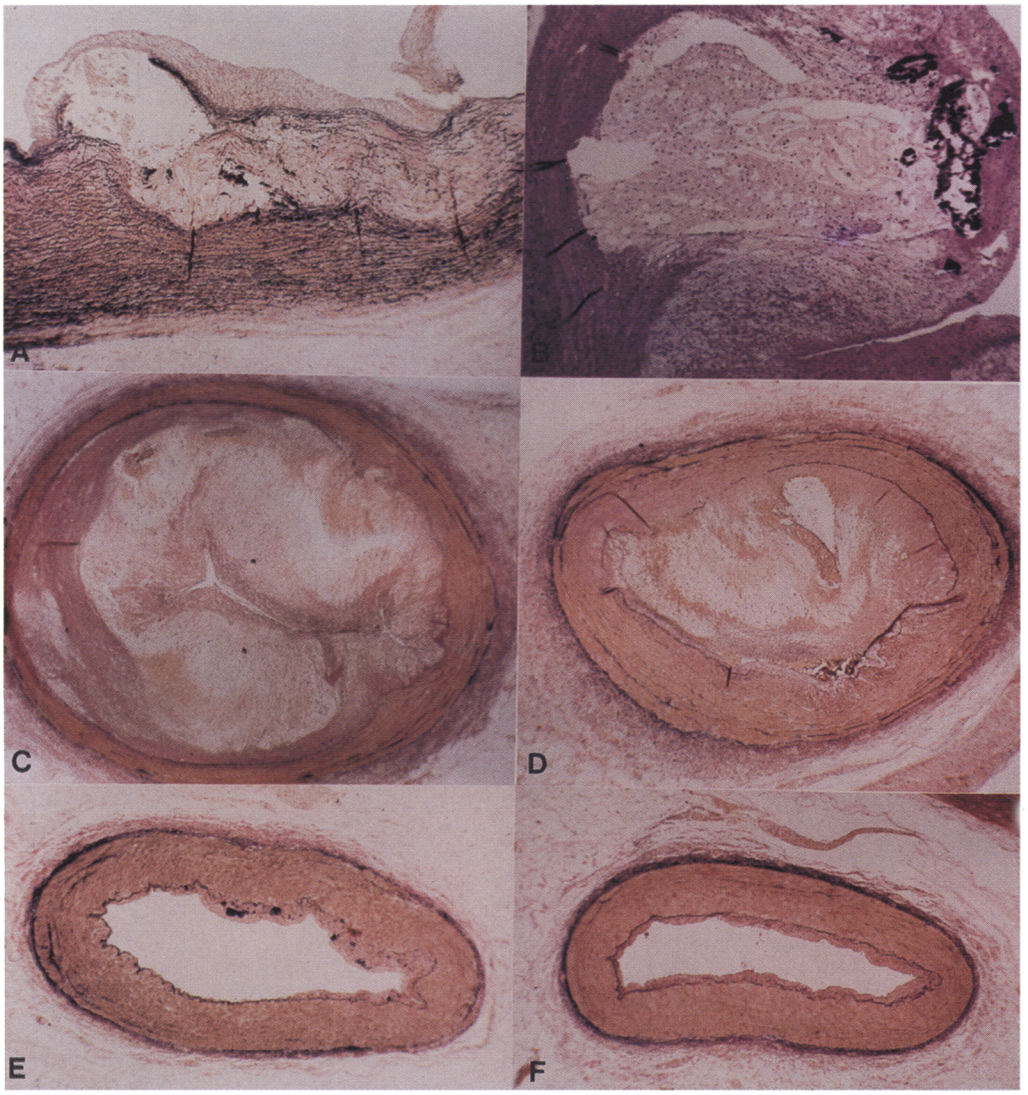

FIG 5. Photograph of representative coronary arteries of microswine receiving an atherogenic diet showing decreased coronary atherosclerosis in pigs receiving pectin. A, Left main coronary artery of an animal not receiving pectin (Verhoeff van Gieson's elastic stain, ×40). Note disruption of the abundant elastic fibers of the media by an atheromatous plaque. B, Aggressive atheroma eroding the media of the left main coronary artery of an animal not receiving pectin (hematoxylin and eosin, ×62). Note numerous foam cells and cholesterol clefts with minimal fibrous proliferation and focal calcification near the lumen. C, Right coronary artery of an animal that did not receive pectin showing extensive atherosclerosis and severe luminal compromise (×39). D, Left circumflex coronary artery of an animal that did not receive pectin showing near-total obstruction of the coronary artery lumen by atherosclerosis (×48). E, Right coronary artery of an animal treated with pectin showing minimal atherosclerosis (×62). F, Left circumflex coronary artery of an animal treated with pectin showing slight intimal thickening with virtually no luminal compromise (×62). (Sections C through F were stained with Verhoeff van Gieson's elastic stain.)

severe luminal narrowing (Fig 5C). In contrast, microswine treated with pectin had mild eccentric intimal thickening, focal calcification, and minimal luminal compromise (Fig 5E). A similar comparison is shown in Fig 5 for the left circumflex artery. Extensive atherosclerosis and severe luminal compromise were observed in the absence of pectin (Fig 5D) compared with the minimal atherosclerosis and virtual lack of luminal compromise observed in the pectin-treated animal (Fig 5F). Despite the severity of the atherosclerosis, there was no evidence of myocardial infarction either grossly or microscopically.

Discussion

Two important conclusions were derived from the present study: (1) grapefruit pectin did not significantly lower cholesterol levels or lipoprotein fractions of microswine with long-standing hypercholesterolemia, and (2) pectin either regressed atherosclerosis induced by 1 year of sustained hypercholesterolemia or interfered with lesion progression. Pectin was added as a dietary supplement only after 390 days of continuous feeding of an atherogenic diet that produced sustained hypercholesterolemia. Continued ingestion of the high-cholesterol/high–saturated fat diet with or without pectin main-

tained these cholesterol levels at values more than fivefold normal. Similarly, lipoprotein fractions (LDL, VLDL, and HDL) were increased by the atherosclerotic diet fed to all animals during the initial 390 days. The subsequent addition of pectin to the diet had no effect on these lipoprotein fractions.

Prior animal studies have demonstrated that pectin inhibits diet-induced hypercholesterolemia, including increases in LDL and VLDL, while not altering HDL.[6] In these studies, pectin was fed concurrently with the atherogenic diet. In contrast, the present study examined the effect of pectin on established hypercholesterolemia and found no lowering of cholesterol levels in pigs that continued to ingest a lipid-rich diet. Prior studies in humans with hypercholesterolemia concluded that pectin supplementation had a modest cholesterol-lowering effect,[9] reducing total cholesterol by 7.6% and LDL by 10.8%. In these human studies, pectin was given for only 4 weeks, and no attempt was made to alter diet or lifestyle. Accordingly, pectin may inhibit diet-induced hypercholesterolemia when ingested concurrently with a lipid-rich diet. However, pectin may have only a nominal cholesterol-lowering effect on chronic established hypercholesterolemia.

In contrast to its effect on cholesterol levels, the effect of pectin on atherosclerosis was dramatic. Despite nearly 2 years of sustained hypercholesterolemia, there was less atherosclerosis at necropsy in the coronary arteries and aorta of animals that had received pectin for the final 270 days. These findings are critical to the central issue of atherosclerosis prevention through dietary modification.

Very little is known about the direct effect of pectin on atherosclerosis. We attempted to measure the presence of atherosclerosis in microswine by angiography to monitor the extent of disease, but the technique was too inaccurate for research purposes.[17] Therefore, we measured the extent of atherosclerosis only at necropsy and used planimetry to record the surface area of aorta occupied by plaque or the degree of coronary artery narrowing. Accordingly, we are not able to determine if pectin actually regressed atherosclerosis in individual animals. However, we can conclude that pectin either regressed or prevented the development of atherosclerosis, because it substantially reduced the aortic area occupied by atheromatous plaques and significantly reduced coronary artery narrowing.

The ability of pectin to decrease coronary and aortic atherosclerosis was not predictable from its effect on either total cholesterol or lipoprotein fractions. In contrast to prior studies,[6] there was no correlation between plasma cholesterol levels and the extent of coronary and aortic atherosclerosis. Recent evidence suggests that atherosclerosis may be prevented by agents that do not directly affect lipid levels.[18] Although the mechanism of pectin action in reducing atherosclerosis is unknown, pectin may have a direct beneficial effect on atherosclerosis independent of cholesterol levels.

Acknowledgments

This work was supported in part by grant 4910295549212 from the Florida Citrus Commission. The authors thank Elizabeth S. Patrick for her critical review and editing of the manuscript. Special thanks are given to Dr Randy Carter and Mr Peter Geiser of the University of Florida Department of Biostatistics for their statistical analysis.

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