Effects of Dietary Pectin, Phytate, and Calcium on Selected Lipid Parameters in the Rat[1]

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ABSTRACT

For 12 wk, male weanling rats were fed a diet containing 12% of the calories from protein, 42% from fat, and 46% from carbohydrates. The diet contained 0.2% cholesterol (CH). Pectin (5 and 10%), phytate (0.31 and 0.62%), and calcium (0.4 and 0.8%) were tested with the diet (2 × 2 × 2 factorial design) to determine their effects on selected lipid parameters. During the first eight weeks of the study, pectin significantly ($P < 0.001$) affected both total (T) and high-density lipoprotein (HDL)-CH. At week 4, T-CH and HDL-CH were lowest in animals fed low pectin diets. By week 8, both of these levels were lower in rats consuming high pectin diets. By week 12, pectin affected only T-CH, through a concurrent interaction with phytate and calcium ($P < 0.01$). Pectin also had a sustained significant ($P < 0.01$–0.001) lowering effect on liver cholesterol but a variable effect on liver and serum triglycerides. Both phytate and calcium appeared to enhance pectin's lowering effect on CH.

Atherosclerosis leading to coronary heart disease (CHD) is the major cause of death in the United States (National Center for Health Statistics 1975). Elevated blood lipid levels are considered a major risk factor in the etiology of atherosclerosis (Glueck 1979, McGill 1979).

Diet appears to affect blood lipids. Most research in this area, however, has centered on the impact of fat and cholesterol on lipid parameters (Myant 1975, McGill 1979). With increasing data, which suggest that people who consume foods of primarily plant origin are much less prone to atherosclerosis, research emphasis has turned to other dietary components, such as fiber and minerals. Three of these components are pectin; the fiber-related compound, phytate; and calcium (Ca).

Jenkins et al (1975), Chen et al (1981), Nakamura et al (1982), and Judd and Truswell (1982) have all demonstrated that pectin lowers cholesterol levels. Klevay (1977) found that feeding sodium phytate caused hypocholesterolemia. The cholesterol lowering effects of Ca have been proven by Klevay et al (1979) and Thakur and Jha (1981). Furthermore, epidemiological evidence indicates an inverse relationship between hardness of water, which is primarily a function of Ca content, and the incidence of CHD (Anonymous 1967, 1968; Perry 1978; Puddu and Menotti 1980).

There is a growing body of information about the ability of these constituents to affect lipid parameters. However, because dietary constituents are seldom consumed as single entities, the purpose of this study was to examine the interaction effects of pectin, phytate, and calcium on selected lipid profiles.

MATERIALS AND METHODS

Test Components

Natural citrus, high methoxyl pectin (Hercules, Inc., Wilmington, DE), sodium phytate (Sigma Chemical Company, St. Louis, MO), and calcium sulfate (U.S. Gypsum, Southard, OK) served as the sources of test components.

Test Diets and Experimental Design

Eight diets containing low or high levels of pectin, phytate, and calcium (2×2×2 factorial design) were formulated (Table I). Diets contained 12% of the calories from protein (8% casein, 4% gluten) and 42% from fat (16% saturated, 19% monounsaturated, 7% polyunsaturated). The remaining calories were derived from carbohydrates (24% simple, 22% complex). Variables were added at the expense of simple (sucrose) carbohydrates (Table I); the diet also consisted of 0.2% cholesterol, 0.4% phosphorus (10 or 20% as phytate phosphorus), and 0.4 and 0.8% calcium. Because a relative or absolute deficiency of dietary copper, characterized by a high ratio of zinc to copper, has been implicated (Klevay 1973, 1975; Allen and Klevay 1980) as a contributory factor in CHD, all dietary components were analyzed for zinc and copper. Then, zinc oxide and copper sulfate were added, to provide 36 ppm zinc and 3.6 ppm copper.

In this study, 252 male weanling Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing an average of 51.2 g each, were used. To provide baseline data, on day zero, nine animals were sacrificed and tissues analyzed. The remaining animals were randomly assigned to eight treatment groups of 27 animals each. All animals were housed individually in mesh-bottom stainless steel cages in a controlled environment. Diet and deionized water were supplied *ad libitum*. Body weight and diet intake records were kept.

Tissue Sampling

At weeks 4, 8, and 12, nine animals per diet were fasted overnight and anesthetized lightly, and blood was withdrawn by heart puncture. The blood was allowed to clot for 1 hr and then centrifuged to obtain serum, which was frozen. Following heart puncture, the animals were sacrificed and frozen. The carcasses were thawed later and the livers removed, blotted dry, weighed, and homogenized in a Fisher Dyna-Mix homogenizer. Volumes were measured, and suitable aliquots were taken for analyses of minerals and lipids.

Analytical

Diet ingredients and test diets were analyzed for calcium and

Table I
Composition of Experimental Diets

	Low Pectin				High Pectin			
	Low Phytate		High Phytate		Low Phytate		High Phytate	
Component (g/100 g)	Low Ca A	High Ca B	Low Ca C	High Ca D	Low Ca E	High Ca F	Low Ca G	High Ca H
Pectin	5	5	5	5	10	10	10	10
Sodium phytate	0.31	0.31	0.62	0.62	0.31	0.31	0.62	0.62
Cholesterol	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
$CaSO_4 \cdot 2H_2O$	1.74	3.48	1.74	3.48	1.74	3.48	1.74	3.48
NaH_2PO_4	0.87	0.87	0.72	0.72	0.87	0.87	0.72	0.72
Others[a]	72.1	72.1	72.1	72.1	72.1	72.1	72.1	72.1
Sucrose	19.81	18.04	19.62	17.88	14.78	13.04	14.62	12.88

[a] Includes (g) casein, 11.00; gluten, 6.78; lard, 17.00; peanut oil, 3.48; corn oil, 1.68; corn starch, 28.11; vitamin diet fortification mixture (ICN Pharmaceuticals, Cleveland, OH), 2.2; trace mineral mix (Mn, 5.0 mg; Mg, 40 mg; Fe, 3.5 mg; and I, 0.015 mg in sucrose base), 1; NaCl, 0.127; KCl 0.69; zinc oxide, 0.0038; and copper sulfate, 0.0007.

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phosphorus. Phosphorus was determined colorimetrically by the standard AACC method (1977). Calcium was determined on dry-ashed (500°C, 14 hr) samples by atomic absorption spectrophotometry (Instrumentation Laboratories 1974) using an IL (Instrumentation Laboratories, Lexington, MA) model 251 spectrophotometer. Total cholesterol in the serum and liver was determined by the method of Abell et al (1952). HDL-CH was determined by Heparin-manganese selective precipitation (Tietz 1976). Triglycerides (TG) in the serum and liver were determined using the quantitative colorimetric method of Sigma (1977).

Statistical Analysis

Data were subjected to two-way analysis of variance (Snedecor and Cochran 1980). Treatments were divided according to their main and interaction effects.

RESULTS AND DISCUSSION

Diet and Cholesterol Intake of Rats

Except for pectin at week 12, none of the test variables (calcium, phytate, or pectin) or their interactions significantly influenced the diet intake and thus, the cholesterol intake of rats. At week 12, rats fed low pectin diets A–D consumed significantly ($P<0.01$) more diet and thus, more CH than did rats fed high pectin diets E–H (Table II). This higher intake, with better diet utilization efficiencies, resulted in weight gains in these rats, which were also significantly ($P<0.001$) higher (Table III).

TABLE II
Diet and Cholesterol Intakes of Rats[a]

	Diet Intake (g)						Cholesterol Intake (g)					
	4 wk		8 wk		12 wk		4 wk		8 wk		12 wk	
Diet[b]	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
A	303.9	27.4	708.8	78.0	1,100.3	54.9	0.61	0.05	1.42	0.16	2.20	0.11
B	318.4	29.3	705.5	51.8	1,092.4	67.8	0.64	0.06	1.41	0.10	2.18	0.14
C	320.7	18.4	684.2	20.1	1,061.6	80.3	0.64	0.04	1.37	0.04	2.12	0.16
D	306.8	48.9	717.7	36.6	1,085.6	45.1	0.61	0.10	1.44	0.07	2.17	0.09
E	303.7	28.3	673.4	61.6	1,053.9	78.7	0.61	0.06	1.35	0.12	2.11	0.16
F	324.3	21.1	699.9	57.1	1,040.4	80.7	0.65	0.04	1.40	0.11	2.08	0.16
G	300.0	22.4	700.4	36.9	998.1	51.2	0.60	0.04	1.40	0.07	2.00	0.10
H	306.0	33.0	730.7	68.4	1,071.6	85.1	0.61	0.07	1.46	0.14	2.14	0.17
Analysis of variance (F values)												
Pectin	0.29	···	0.05	···	7.06[c]	···	0.29	···	0.05	···	7.12[c]	···
Phytate	0.33	···	0.73	···	1.12	···	0.33	···	0.73	···	1.11	···
Pec/Phy	0.87	···	1.75	···	0.10	···	0.87	···	1.75	···	0.10	···
Calcium	0.87	···	2.70	···	1.32	···	0.87	···	2.70	···	1.34	···
Pec/Ca	0.80	···	0.25	···	0.44	···	0.80	···	0.25	···	0.45	···
Phy/Ca	2.18	···	0.59	···	3.22	···	2.17	···	0.59	···	3.22	···
Pec/Phy/Ca	0.22	···	0.39	···	0.69	···	0.22	···	0.39	···	0.69	···

[a] Values are averages ± standard deviations.
[b] See Table I for descriptions of diet.
[c] $P \leq 0.01$.

TABLE III
Body Weight Gains and Diet/Gain Ratios[a]

	Body Weight Gain (g)						Diet/Gain (Ratio)[c]					
	4 wk		8 wk		12 wk		4 wk		8 wk		12 wk	
Diet[b]	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
A	128	14	232	18	288	13	2.39	0.20	3.06	0.23	3.82	0.18
B	140	19	236	18	295	25	2.30	0.14	2.99	0.07	3.71	0.13
C	145	9	229	14	291	20	2.21	0.12	2.99	0.15	3.66	0.33
D	134	20	249	13	274	25	2.29	0.12	2.88	0.06	3.99	0.34
E	136	10	214	16	263	18	2.41	0.16	3.15	0.27	4.02	0.38
F	130	14	212	16	254	17	2.50	0.18	3.31	0.33	4.10	0.32
G	117	20	217	16	232	23	2.64	0.52	3.24	0.26	4.35	0.54
H	112	14	221	25	272	25	2.73	0.20	3.33	0.27	3.95	0.25
Analysis of variance (F values)												
Pectin	16.51[d]	···	24.67[d]	···	45.83[d]	···	21.93[d]	···	25.05[d]	···	15.10[d]	···
Phytate	1.05	···	1.78	···	1.45	···	1.40	···	0.12	···	0.86	···
Pec/Phy	6.82[e]	···	···	···	0.36	···	7.62[e]	···	1.67	···	0.06	···
Calcium	···	···	2.31	···	2.07	···	0.54	···	0.14	···	0.09	···
Pec/Ca	0.01	···	1.85	···	6.30[e]	···	0.80	···	3.61	···	2.89	···
Phy/Ca	4.41[f]	···	1.71	···	0.8	···	0.51	···	0.29	···	0.02	···
Pec/Phy/Ca	0.95	···	0.34	···	11.06[d]	···	0.51	···	0.03	···	8.59[e]	···

[a] Values are averages ± standard deviations.
[b] See Table I for descriptions of diets.
[c] Diet consumed (g)/weight gained (g).
[d] $P \leq 0.001$.
[e] $P \leq 0.01$.
[f] $P \leq 0.05$.

TABLE IV
Serum Cholesterol Levels[a]

Diet[b]	Total Cholesterol (mg/dl)						HDL Cholesterol (mg/dl)					
	4 wk		8 wk		12 wk		4 wk		8 wk		12 wk	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
A	64.6	11.3	91.5	16.3	65.2	15.4	31.8	12.9	33.3	14.8	34.9	10.1
B	75.6	9.3	99.0	10.4	62.5	13.3	39.4	15.6	24.7	8.5	33.4	10.5
C	73.4	13.7	79.4	12.6	51.0	10.6	22.6	6.9	19.0	8.1	28.5	9.9
D	75.7	12.6	57.8	7.7	55.3	8.0	34.9	16.1	27.6	9.5	27.9	10.7
E	82.6	16.8	75.9	12.3	53.4	12.2	42.2	18.7	11.5	4.5	23.7	8.2
F	99.2	15.7	79.0	8.7	66.0	16.2	54.1	19.0	20.8	6.3	24.9	4.2
G	100.9	28.7	65.8	13.6	70.2	19.5	34.7	7.9	23.6	10.8	40.1	12.5
H	86.0	21.7	58.8	9.2	54.5	20.0	46.4	13.4	15.0	8.3	26.2	10.7
Analysis of variance (F values)												
Pectin	22.03[c]	···	17.88[c]	···	0.48	···	12.12[c]	···	13.99[c]	···	1.03	···
Phytate	0.70	···	53.95[c]	···	1.24	···	4.30[d]	···	0.31	···	0.37	···
Pec/Phy	0.05	···	4.09[d]	···	3.36	···	0.01	···	3.83[d]	···	9.35[e]	···
Calcium	0.77	···	2.49	···	0.01	···	9.65[e]	···	0.01	···	2.35	···
Pec/Ca	0.47	···	0.82	···	0.11	···	0.07	···	0.01	···	1.20	···
Phy/Ca	5.64[d]	···	11.92[c]	···	2.12	···	0.10	···	0.01	···	2.16	···
Pec/Phy/Ca	1.82	···	2.82		5.84[e]	···	0.12	···	15.24[c]	···	2.76	···

[a] Values are averages ± standard deviations.
[b] See Table I for descriptions of diets.
[c] $P \leq 0.001$.
[d] $P \leq 0.05$.
[e] $P \leq 0.01$.

TABLE V
Liver Total Cholesterol[a]

Diet[b]	Values (mg/g)					
	4 wk		8 wk		12 wk	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
A	6.6	2.4	5.4	2.1	4.8	2.1
B	6.6	1.3	5.4	1.3	4.9	0.6
C	6.8	0.9	4.7	1.1	5.1	1.2
D	3.0	0.7	2.6	0.5	2.3	0.4
E	3.3	1.1	3.1	0.6	2.5	0.6
F	4.2	0.9	3.6	0.4	3.2	0.3
G	6.0	1.6	4.3	0.7	3.7	0.9
H	4.6	1.0	4.5	0.7	4.1	0.6
Analysis of variance (F values)						
Pectin	14.15[c]	···	6.29[d]	···	14.24[c]	···
Phytate	···	···	1.91	···	0.07	···
Pec/Phy	26.50[c]	···	29.09[c]	···	20.41[c]	···
Calcium	11.05[d]	···	1.91	···	2.93	···
Pec/Ca	6.80[d]	···	7.92[d]	···	14.81[c]	···
Phy/Ca	23.37[c]	···	5.77[e]	···	10.47[d]	···
Pec/Phy/Ca	1.41	···	2.35	···	6.90[d]	···

[a] Values are averages ± standard deviations.
[b] See Table I for descriptions of diets.
[c] $P \leq 0.001$.
[d] $P \leq 0.01$.
[e] $P \leq 0.05$.

Serum Total and HDL Cholesterol

For the rat, serum CH levels of 100 mg/dl or less are usually considered normal (Ranhotra et al 1977, 1978; Story et al 1981). Thus, hypercholesterolemia was not observed or, at best, was only mild in this study (Table IV). On day zero, serum T-CH levels averaged 84.7 mg/dl. Even in the absence of marked hypercholesterolemia, pectin affected serum CH levels significantly ($P < 0.001$) at weeks 4 and 8. This effect was observed at week 4 on low pectin diets, in which the average total cholesterol level of animals fed diets A–D was 72.3 mg/dl, compared to 92.2 mg/dl in animals fed high pectin diets E–H. At week 8, the effect was observed in animals fed high pectin diets E–H, in which the total cholesterol averaged 69.9 mg/dl, compared to 81.9 mg/dl in animals fed low pectin diets A–D. This hypocholesterolemic effect due to pectin has been demonstrated repeatedly (Leveille and Sauberlich 1966, Leeds and Gassull 1976, Kay et al 1978, Judd and Leeds 1981). By week 12, pectin exerted its CH-lowering effect only through concurrent interaction with phytate and calcium ($P < 0.01$). This was proven by the consistently lower serum CH levels observed in rats fed the high phytate, high calcium diets D and H.

Although total serum cholesterol values were higher in animals on some diets at weeks 4 and 8, by week 12, levels observed in animals on all diets were lower (51–70 mg/dl) than the 84.7 mg/dl observed on day zero. On diets C, D, and H, in which there was progressive or near progressive decline from the value observed on day zero, levels were 35–37% less. This is higher than the 18% decline reported by Judd and Truswell (1982), who used 15 g of high methoxyl pectin only.

On day zero, serum HDL-CH levels averaged 33.2 mg/dl. At weeks 4 and 8, pectin had a significant ($P < 0.001$) effect on serum HDL-CH. At week 4, animals on low pectin diets had HDL-CH levels averaging 32.2 mg/dl, compared to 44.5 mg/dl in animals fed high pectin diets. By week 8, animals fed low pectin diets A–D had average HDL-CH levels of 26.2 mg/dl, and those on high pectin diets E–H had average levels of 17.7 mg/dl (Table IV). These data indicate that although pectin affects both T-CH and HDL-CH, as Koo and Stanton (1981) suggest, pectin's effect on HDL-CH may be short-lived. This is further emphasized by the fact that pectin had a minimal effect on HDL-CH after week 8. In addition, unlike T-CH, which continued to decline on all diets after week 8, HDL values began to rise, exceeding the level observed on day zero. Other variables also had inconsistent effects on HDL-CH.

The effect of the dietary constituents and constituent interactions on the HDL-CH:T-CH ratio was inconsistent, but they were higher on all diets at week 12 than the 0.39 ratio observed on day zero.

Liver Weights and Cholesterol

Like body weights, liver weights were significantly ($P < 0.01$–0.001) higher in rats fed low pectin diets than in those fed high pectin diets. Other variables or interactions had inconsistent effects on liver weights.

On day zero, the average liver cholesterol was 2.7 mg/g (wet basis). Although values rose from those observed on day zero, liver

TABLE VI
Serum and Liver Triglycerides[a]

Diet[b]	Serum Triglycerides (mg/dl)						Liver Triglycerides (mg/g)					
	4 wk		8 wk		12 wk		4 wk		8 wk		12 wk	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
A	110.2	54.4	216.1	65.8	204.5	64.2	35.4	20.0	35.3	15.9	30.4	20.8
B	163.5	79.7	193.2	92.1	190.7	79.0	25.6	12.2	27.0	16.0	19.4	10.7
C	224.3	113.0	206.0	124.6	195.1	79.5	32.4	19.7	21.2	15.7	31.9	17.8
D	270.6	87.9	281.8	158.4	215.0	68.2	47.9	33.2	30.0	13.8	50.5	21.6
E	269.0	114.3	252.3	57.4	196.5	66.3	37.4	18.9	32.5	16.2	37.0	16.5
F	306.9	128.5	225.6	90.2	181.0	46.3	30.8	18.1	26.9	14.4	30.0	15.6
G	180.7	83.5	285.1	132.2	162.8	58.8	71.2	41.7	51.6	19.3	41.7	19.6
H	192.5	99.1	303.6	97.2	146.0	42.9	39.4	23.9	32.8	15.9	52.5	23.3
Analysis of variance (F values)												
Pectin	3.68[c]	···	2.75	···	3.81[c]	···	2.35	···	3.78	···	2.65	···
Phytate	0.04	···	3.44	···	0.78	···	6.38[c]	···	0.80	···	11.29[d]	···
Pec/Phy	20.30[d]	···	0.10	···	1.89	···	0.90	···	5.43[c]	···	0.09	···
Calcium	2.51	···	0.19	···	0.19	···	1.77	···	2.38	···	0.40	···
Pec/Ca	0.28	···	0.36	···	0.46	···	3.26	···	2.57	···	0.05	···
Phy/Ca	0.12	···	1.99	···	0.28	···	···	···	0.07	···	7.09[c]	···
Pec/Phy/Ca	0.04	···	0.27	···	0.33	···	4.30[c]	···	3.81	···	0.45	···

[a] Values are averages ± standard deviations.
[b] See Table I for descriptions of diets.
[c] $P \leq 0.05$.
[d] $P \leq 0.001$.
[e] $P \leq 0.01$.

T-CH levels were, at best, only slightly elevated (Ranhotra et al 1977, 1978; Story et al 1981) and as the study progressed from week 4 to 12, values declined steadily on all diets except C, in which a slight rise and then decline was observed. High pectin diets either lowered or prevented elevation of liver CH levels significantly ($P < 0.01$–0.001) and consistently (Table V). This effect due to pectin persisted in separate interactions with phytate and calcium and, at week 12, also through the combined pectin, phytate, and calcium interactions (Table V). However, because of problems inherent in cholesterol analyses, caution must be taken in interpreting T-CH and HDL-CH data even when statistically significant results are obtained.

Serum and Liver Triglycerides

Serum and liver TG levels tended to be somewhat elevated. This was observed on all diets at all sampling periods. On day zero, the average serum TG was 148.2 mg/dl. By week 4, levels ranged from 110.2 mg/dl in rats on diet A to 306.9 mg/dl in those on diet F. At week 8, values remained high, 193.2–303.6 mg/dl. Pectin had a significant ($P < 0.05$) effect at only weeks 4 and 12. At the 4-week sampling period, animals on low pectin diets had lower TG levels than those fed high pectin diets, 192.2 vs 237.3 mg/dl. The situation was reversed at week 12, when levels were lower in animals fed high pectin diets, 171.6 vs 201.3 mg/dl (Table VI).

Liver TG levels were not affected by pectin. However, at weeks 4 and 12, low phytate diets had lower ($P < 0.01$–0.001) liver TG levels than did high phytate diets (Table VI). The high animal to animal variations observed tended to overshadow the effects due to variables on both serum and liver TG levels.

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