

Influence of dietary fiber on establishment and progression of atherosclerosis in rabbits

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Rabbits were fed a semipurified diet containing 0.5% cholesterol and either 14% cellulose or a psyllium-rich cereal (PRC) which actually provided 6.2% fiber (insoluble fiber/soluble fiber:2.21/1.00). The PRC-containing diet led to significantly lower serum total cholesterol, higher high-density lipoprotein (HDL) cholesterol, and less severe atherosclerosis. In a regression study, after 2 months on the atherogenic diet rabbits were placed on cholesterol-free diets containing 6% corn oil and 14% cellulose or PRC. After 3 months serum cholesterol levels had fallen by 33% in the group fed cellulose and by 51% in the group given PRC. Liver cholesterol levels fell by 62 and 74% in the cellulose and PRC groups, respectively. The average severity of atherosclerosis had risen by 35% in the cellulose group and by only 4% in the rabbits fed PRC. This study shows that compared with 14% cellulose, 6.2% psyllium fed as part of an atherogenic diet resulted in lower serum and liver lipids and less severe atherosclerosis. (J. Nutr. Biochem. 6:509–512, 1995.)

Keywords: atherosclerosis; cellulose; cholesterolemia; fiber; psyllium; rabbits

Introduction

The influence of dietary fiber on serum or plasma lipids in man has been a topic of concern for many years; one of the more recent reviews of the subject was written by Truswell and Beynen.¹ The effects of dietary fiber on hyperlipidemia in experimental animals has been studied in a number of species, principally the rat, and this field too was the subject of a recent review.² However, there are relatively few studies relating to fiber and its role in experimental atherosclerosis. Thirty years ago a comparison of the published studies of the different effects of cholesterol-free semipurified and commercially based diets on atherosclerosis in rabbits evoked the suggestion that the difference in severity of atherosclerosis was due to the type of fiber in the diets.³ The protective effect of the residue obtained after extraction of lipids from commercial rabbit ration was shown experimentally.^{4,5} Moore,⁶ using diets containing 20% butter, found significantly less severe atherosclerosis in rabbits fed wheat straw compared with those fed cellulose. Although pectin

has been shown to reduce cholesterolemia in rabbits⁷ no direct comparison of the atherogenic effects of cellulose with other fibers is available. A comparison of atherogenic diets containing cellulose or a psyllium-rich cereal (PRC) is the basis of this report.

Methods and materials

Male, New Zealand White rabbits (2 to 2.5 kg) were used in all studies. The rabbits were maintained in individual stainless steel cages and housed in a room that was maintained on a 12-hour light/dark cycle at $22 \pm 1^\circ\text{C}$. The animals were given food and water ad libitum.

In the first study the rabbits (8/group) were fed a semipurified diet containing 14% lard and 14% cellulose (*Table 1*). The diet also contained 2% safflower oil to ensure against essential fatty acid deficiency. After analyzing the lard present in the diet for cholesterol content, we added 0.43% cholesterol to bring the cholesterol level of the diet to 0.5%. The test diet was identical to the control diet except that 14% PRC was added in place of cellulose. The total level of fiber in PRC is 44.6% so that we were actually feeding 6.2 g of fiber compared with 14% cellulose in the test diet. The ratio of insoluble/soluble fiber in PRC is 2.21:1.00. The diets were prepared to our specification by Dyets Inc. (Bethlehem, PA USA).

After 2 months the rabbits were sacrificed by barbiturate injection. Serum was analyzed for total cholesterol. Low-density

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Table 1 Composition of semipurified diet

Ingredient	%	Calorie (%)
Casein	24.0	24.0
Sucrose	24.1	24.0
Corn starch	16.0	16.0
Lard	14.0	31.5
Safflower oil	2.0	
Fiber*	14.0	
Mineral mix†	4.0	
Vitamin mix‡	1.0	
Cholesterol	0.4	
DL-methionine	0.3	
Choline bitartrate	0.2	

*Fiber was either cellulose or psyllium-rich cereal (PRC).

†In g/kg: calcium carbonate (68.6), calcium citrate (306.19), calcium phosphate, monobasic (112.8), magnesium carbonate (35.2), magnesium sulfate, anhydrous (38.3), potassium chloride (124.7), potassium phosphate, dibasic (218.8), sodium chloride (77.7), ferric ammonium citrate (15.28), cupric carbonate (0.04), manganese sulfate (0.20), aluminum ammonium sulfate (0.09), potassium iodide (0.04), sodium fluoride (0.51), chromium potassium sulfate (0.55), and zinc carbonate (1.6).

‡In g/kg of diet: thiamin HCl (6), riboflavin (6), pyridoxine HCl (40), niacin (180), Ca pantothenate (16), folic acid (2), biotin (0.2), B-12 (10), menadione sodium bisulfite (0.8), vitamin A (4,000.1.0), vitamin E (501.), vitamin D3 (1,000.1.0).

lipoprotein (LDL) cholesterol and triglyceride using commercially available kits (Sigma, St. Louis, MO USA). Livers were weighed, lipids extracted with chloroform-methanol 2:1,⁸ and the extracts analyzed for total and free cholesterol. Free cholesterol was isolated by digitonin precipitation,⁹ and cholesterol levels were estimated colorimetrically.¹⁰ Liver triglyceride levels were determined colorimetrically.¹¹ Aortas were removed, cleared of adhering tissue, and severity of atherosclerosis was graded visually using a 0–4 scale.¹²

The purpose of the second experiment was to examine effects of a cholesterol-free regimen on pre-established atherosclerosis. Thirty rabbits were fed the atherogenic cellulose-containing diet and 10 were given the PRC-rich diet. After 2 months the rabbits were bled under light anesthesia. The 30 control rabbits were segregated into three groups of 10 having the same serum cholesterol level (762 ± 8 mg/dL). One group of controls was necropsied as were the rabbits on the PRC diet. The two remaining groups of control rabbits were returned to a cholesterol-free regimen containing 24% casein, 20% cornstarch, 30.5% sucrose, 6% corn oil, 4% mineral mix, 1% vitamin mix, 0.3% DL methionine, 0.2% choline bitartrate, and 14% of either cellulose or PRC. The severity of atherosclerosis in the rabbits in the postcholesterol feeding study was assumed to be equal to that observed in the initially necropsied group. The rabbits were maintained on their respective diets for 3 months, at which time they were subject to the treatments and analyses described previously. Significance of differences was determined by *t*-test. The protocol was approved by the IUCAC of the Wistar Institute.

Results and discussion

The results of the first experiment are summarized in *Table 2*. Rabbits fed PRC has significantly lower levels of serum total cholesterol and significantly higher levels of high-density lipoprotein (HDL) cholesterol. Liver total cholesterol and triglyceride levels were 21 and 16% lower, respectively, in the PRC group. Severity of atherosclerosis in

Table 2 Necropsy data in rabbits fed diets containing 14% cellulose or PRC* (Experiment 1)

Parameter	Cellulose	PRC	<i>P</i> <
Number	6/8	8/8	
Weight gain (g)	18 ± 140	527 ± 137	0.05
Liver weight (g)	87 ± 9	95 ± 9	—
Relative liver weight (%)	2.98 ± 0.21	2.76 ± 0.20	—
<u>Serum (mmol/L)</u>			
Total cholesterol (C)	19.34 ± 0.26	16.01 ± 1.11	0.02
% HDL-C	7.1 ± 1.1	10.0 ± 0.7	0.05
Triglyceride	3.79 ± 0.67	3.47 ± 0.52	—
<u>Liver (mmol/100 g)</u>			
Total cholesterol (C)	70.65 ± 10.86	56.12 ± 6.72	—
% ester C	85 ± 2	83 ± 2	—
Triglycerides	24.06 ± 0.79	20.22 ± 1.58	—
<u>Atherosclerosis†</u>			
Aortic arch	1.42 ± 0.30	0.81 ± 0.28	
Thoracic aorta	1.10 ± 0.22	0.69 ± 0.23	

*Diet composition given in *Table 1*.

†Visually graded 0–4 scale.

the aortic arch and thoracic aortas of the PRC-fed rabbits were 43 and 37% lower than in the controls.

In the second study, total serum cholesterol was again significantly lower in the rabbits fed PRC (*Table 3*). Liver total cholesterol and triglycerides were both 10% lower in the PRC group. The severity of atherosclerosis in the aortic arch of the PRC-fed rabbits was significantly reduced. Severity of thoracic atherosclerosis was 56% higher in the control group. When we combine the results of the two studies in which we established atherosclerosis (*Table 4*) we find that PRC feeding significantly lowered serum cholesterol levels and reduced the severity of atherosclerosis.

The results of the postcholesterol feeding study are de-

Table 3 Necropsy data in rabbits fed diets containing 14% cellulose or PRC* (Experiment 2)

Parameter	Cellulose	PRC	<i>P</i> <
Number	10/10	9/10	
Weight gain (g)	256 ± 105	432 ± 175	—
Liver weight (g)	73 ± 6	66 ± 4	—
Relative liver weight (%)	3.16 ± 0.17	2.69 ± 0.10	0.05
<u>Serum (mmol/L)</u>			
Total cholesterol (C)	19.68 ± 0.36	16.11 ± 1.19	0.02
% HDL-C	12.3 ± 1.9	15.1 ± 2.4	—
Triglyceride	2.24 ± 0.36	2.47 ± 0.26	—
<u>Liver (mmol/100 g)</u>			
Total cholesterol (C)	62.32 ± 4.65	55.86 ± 4.14	—
% ester C	78 ± 1	73 ± 1	0.02
Triglycerides	18.97 ± 3.05	17.17 ± 1.69	—
<u>Atherosclerosis†</u>			
Aortic arch	2.17 ± 0.22	1.00 ± 0.29	0.01
Thoracic aorta	1.00 ± 0.24	0.44 ± 0.33	—

*Diet composition given in *Table 1*.

†Visually graded 0–4 scale.

Table 4 Necropsy data in rabbits fed diets containing 14% cellulose or PRC* combined experiments 1 and 2

Parameter	Cellulose	PRC	P
Number	(16/18)	(17/18)	
Weight gain (g)	168 ± 87	478 ± 110	0.05
Liver weight (g)	79.4 ± 5.8	78.6 ± 5.1	
Relative liver weight (%)	3.11 ± 0.14	2.73 ± 0.10	0.05
Serum (mmol/L)			
Cholesterol (C)	19.55 ± 0.26	16.06 ± 0.83	0.001
% HDL-C	10.4 ± 1.41	12.7 ± 1.43	
Triglycerides	2.82 ± 0.36	2.94 ± 0.32	
Liver (mmol/100 g)			
Cholesterol (C)	65.43 ± 4.91	55.86 ± 3.62	
% ester	80 ± 1	77 ± 2	
Triglycerides	20.89 ± 2.03	18.52 ± 1.24	
Atherosclerosis†			
Aortic arch	1.87 ± 0.20	0.91 ± 0.20	0.01
Thoracic aorta	1.03 ± 0.17	0.56 ± 0.20	

*Diet composition given in Table 1

†Visually graded, 0–4.

tailed in Table 5. In both the cellulose and PRC groups serum cholesterol was significantly lower than that seen in the initial cellulose fed group (Table 3). Liver lipids were also reduced in both groups in the postcholesterol phase, and cholesterol levels of rabbits in the PRC group were significantly lower than those in the cellulose group. It is noteworthy that the percentage of esterified cholesterol in the livers of the PRC group was significantly below that of the cellulose controls. This difference suggests increased mobilization of cholesterol.

Table 5 Influence of PRC on pre-established atherosclerosis in rabbits*†

Parameter	Cellulose	PRC	P
Number	8/10	8/10	
Weight gain (g)	236 ± 215	524 ± 138	
Liver weight (g)	98 ± 7	105 ± 9	
Relative liver weight (%)	3.49 ± 0.25	3.63 ± 0.28	
Serum lipids (mmol/L)			
Cholesterol (C)	13.16 ± 1.53	9.62 ± 1.71	0.05
% HDL-C	3.2 ± 0.6	7.0 ± 1.6	
Triglyceride	1.49 ± 0.28	1.94 ± 0.27	
Liver lipids (mmol/100 g)			
Cholesterol (C)	23.79 ± 1.81	16.29 ± 1.55	0.01
% ester C	56 ± 3	44 ± 4	0.05
Triglyceride	11.52 ± 0.45	10.89 ± 0.34	
Atherosclerosis‡			
Aortic arch	3.14 ± 0.32	2.44 ± 0.33	
Thoracic aorta	1.13 ± 0.28	0.98 ± 0.18	

*Diet: 24% casein; 20% cornstarch; 30.5% sucrose; 6% corn oil; 4% mineral mix; 1% vitamin mix; 0.3% DL methionine; 0.2% choline bitartrate; and 14% cellulose or PRC. Fed for 3 months.

†Serum cholesterol levels and severity of atherosclerosis at the beginning of this phase of the study are given in Table 3.

‡Visually graded, 0–4 scale.

Data in Table 5 must be compared with those obtained for the cellulose-fed group in the second study (Table 3). The data presented in Table 5 were obtained in rabbits whose lipid and atherosclerosis parameters were assumed to resemble those found in the cellulose-fed rabbits at the end of the cholesterol-feeding period.

Serum cholesterol levels fell by 33% in the rabbits fed cellulose ($P < 0.001$) and by 51% ($P < 0.001$) in those fed PRC. The percentage of HDL cholesterol was significantly lower in rabbits fed cellulose (by 74%, $P < 0.001$) or PRC (by 51%, $P < 0.025$). Triglyceride levels were not significantly lower. Liver cholesterol levels were reduced significantly in both the cellulose ($P < 0.001$) and PRC ($P < 0.001$) groups as was the percentage of esterified cholesterol (cellulose, $P < 0.001$; PRC, $P < 0.001$). Liver triglyceride levels were reduced by 39% in rabbits fed cellulose ($P < 0.05$) and by 45% in those fed PRC ($P < 0.02$).

The severity of atherosclerosis in the aortic arch of rabbits fed cellulose during the postcholesterol feeding period rose by 45% ($P < 0.05$) whereas it was virtually unchanged (+8%, not significant) in rabbits fed PRC. The severity of thoracic aorta atherosclerosis was increased by 13% in rabbits fed cellulose and decreased by 2% in those fed PRC. The differences were not significant.

True regression of plaques in rabbits is rare. In general the severity of lesions is increased after cessation of cholesterol feeding. Addition of unsaturated fat to the postcholesterol regimen will reduce the exacerbation of lesions but will not cause regression.^{13–18} Gupta et al.¹⁹ found that if rabbits previously exposed to cholesterol are then maintained on a cholesterol-free regimen for a sufficiently long time severity of atherosclerosis will regress to the level seen at the point when cholesterol feeding was suspended but no further. Thus after 8 weeks of an atherogenic diet, 17% of the aortic surface was involved. At this point the rabbits were returned to a cholesterol-free regimen. After 10 weeks aortic involvement had risen to 58% of surface, and 10 weeks later, when serum cholesterol levels returned to normal, involvement was 18%. Vesselinovitch et al.²⁰ have shown that administration of a hypocholesterolemic drug and oxygen after cessation of cholesterol feeding leads to significant regression of lesions. In our hands hypocholesterolemic drugs alone showed only a modest effect on pre-established lesions.^{13,15,16,18}

This study has shown that psyllium, in the form of cereal, when fed to rabbits maintained on an atherogenic regimen can significantly lower serum cholesterol levels and significantly reduce the severity of atherosclerosis. Psyllium also inhibited the exacerbation of atherosclerosis when the atherogenic stimulus was removed. The 6.2% of fiber provided by a psyllium-rich cereal was effective when compared with a diet containing 14% cellulose. The ratio of insoluble to soluble fiber in PRC is 2.21 to 1.00. It would be of interest to test higher levels of pure fibers in this system.

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References

- 1 Truswell, A.S. and Beynen, A.C. (1992). Dietary fibre and plasma lipids: Potential for prevention of hyperlipidemias. In: *Dietary Fibre—A Component of Food*. ed. T.F. Schweizer and C.A. Edwards. Springer Verlag. London, pp. 295–332.
- 2 Kritchevsky, D. and Story, J.A. (1993). Influence of dietary fiber on cholesterol metabolism in experimental animals. In: *Dietary Fiber in Human Nutrition 2nd. Ed.* ed. G.A. Spiller CRC Press. Boca Raton, FL pp. 163–178
- 3 Kritchevsky, D. (1964). Experimental atherosclerosis in rabbits fed cholesterol-free diets. *J. Atheroscler. Res.* **4**, 103–105
- 4 Kritchevsky, D. and Tepper, S.A. (1965). Factors affecting atherosclerosis in rabbits fed cholesterol-free diets. *Life Sci.* **4**, 1467–1471
- 5 Kritchevsky, D. and Tepper, S.A. (1968). Experimental atherosclerosis in rabbits fed cholesterol-free diets: Influence of chow components. *J. Atheroscler. Res.* **8**, 357–369
- 6 Moore, J.H. (1967). The effect of the type of roughage in the diet on plasma cholesterol levels and atherosclerosis in rabbits. *Br. J. Nutr.* **21**, 207–215
- 7 Berenson, L.M., Bhandaru, R.R., Rhadakrishnamurty, B., Srinivasan, S.R., and Berenson, G.S. (1975). The effect of dietary pectin on serum lipoprotein cholesterol in rabbits. *Life Sci.* **16**, 1533–1544
- 8 Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497–509
- 9 Sperry, W.M. and Webb, M. (1950). A revision of the Schoenheimer-Sperry method for cholesterol determination. *J. Biol. Chem.* **187**, 97–106
- 10 Rudel, L.L. and Morris, M.D. (1973). Determination of cholesterol using 0-phthalaldehyde. *J. Lipid Res.* **14**, 364–366
- 11 Levy, A.L. and Keyloun, C. (1972). Measurement of triglycerides using nonane extraction and colorimetry. *Adv. Automated Anal.* **1**, 487–502
- 12 Duff, G.L. and McMillian, G.C. (1949). The effect of alloxan diabetes on experimental atherosclerosis in the rabbit. *J. Exp. Med.* **89**, 611–630
- 13 Kritchevsky, D., Moynihan, J.L., Langan, J., Tepper, S.A., and Sachs, M.L. (1961). Effects of D- and L-thyroxine and of D- and L-3,5,3'-triiodo-thyronine on development and regression of experimental atherosclerosis in rabbits. *J. Atheroscler. Res.* **1**, 211–221
- 14 Vles, R.O., Buller, J., Gottenbos, J.J., and Thomasson, H.J. (1964). Influence of type of dietary fat on cholesterol-induced atherosclerosis in the rabbit. *J. Atheroscler. Res.* **4**, 170–183
- 15 Kritchevsky, D. and Tepper, S.A. (1967). Influence of D- and L-thyroxine on pre-established atheroma in rabbits. Effects of mode of administration. *J. Atheroscler. Res.* **7**, 103–110
- 16 Kritchevsky, D., Sallata, P., and Tepper, S.A. (1968). Influence of ethyl p-chlorophenoxyisobutyrate (CPIB) upon establishment and progression of experimental atherosclerosis in rabbits. *J. Atheroscler. Res.* **8**, 755–761
- 17 Guski, H. and Weiss, P. (1972). Frage der spontanen Rückbildungsfähigkeit der experimentellen Atheromatose. *Exp. Path.* **6**, 214–224
- 18 Kritchevsky, D., Kim, H.K., and Tepper, S.A. (1973). Effect of colestipol (U-26597A) on experimental atherosclerosis in rabbits. *Proc. Soc. Exp. Biol.* **142**, 185–188
- 19 Gupta, P.P., Tandon, H.D., and Ramalingaswami, V. (1971). Further observations on the reversibility of induced atherosclerosis in rabbits. *J. Path.* **105**, 229–238
- 20 Vesselinovitch, D., Wissler, R.W., Fisher-Dzoga, K., Hughes, R., and Dubien, L. (1974). Regression of atherosclerosis in rabbits. I. Treatment with low-fat diet, hyperoxia and hypolipidemic agents. *Atherosclerosis* **19**, 259–275