Effect of Cellulose and Wheat Mill-Fractions on Plasma and Liver Cholesterol Levels in Cholesterol-Fed Rats

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ABSTRACT

The effect of microcrystalline cellulose, wheat protein concentrate, and wheat mill-fractions on plasma and liver cholesterol levels in cholesterol-fed rats was studied. Though extraneous cellulose, even at 20% level in the diet, failed to prevent the elevation of plasma and liver cholesterol levels, fibrous mill-fractions (namely shorts and bran) appeared quite effective. Germ and fractions high in starch and gluten were also effective in preventing elevation of plasma cholesterol levels. Compared to rats fed casein, those fed wheat protein concentrate absorbed, and deposited in liver, less cholesterol; but their plasma cholesterol levels were elevated.

Characteristic findings of low plasma cholesterol levels and the accompanying low incidence of cardiovascular diseases in population groups consuming substantial amounts of cellulose in their diets in the form of cereals and vegetables have prompted investigations into possible hypocholesteremic effect of dietary cellulose (1-3). While some investigators (4,5), in studies with rats and humans, failed to show the hypocholesteremic effect of dietary cellulose, others (6,7) have suggested cellulose as causing such an effect. Present studies report the effect of feeding cellulose, wheat protein concentrate (WPC), and mill-fractions from wheat on the plasma and liver cholesterol levels of cholesterol-fed rats.

MATERIALS AND METHODS

Of the five experiments conducted, experiments A and B were undertaken to examine the effect of microcrystalline cellulose. In experiment C, repeated as experiment D, a casein-based diet was compared to one based on WPC (a low-fiber, high-protein flour prepared from fibrous mill-fractions). The effect of different mill-fractions was examined in experiment E.

Basal diet in experiments A and B consisted of the following: salt 446 (General Biochemicals), 3%; vitamin diet fortification mixture (Nutritional Biochemicals), 2%; sodium chloride, 1%; corn oil, 3% (10% in experiment B); casein, 10.6% (26% in experiment B); and corn starch to make 100%. Cholesterol (Fisher) and alphacel (microcrystalline cellulose from Nutritional Biochemicals) were incorporated in the basal diet (Table I) at the expense of starch. Diets offered in experiment A were premixed with water.

Diets in experiment C and D contained the following: casein, 10.6% or WPC, 71.8%; salt 446, 3%; vitamin mixture, 2%; sodium chloride, 1%; cholesterol, 1.5%; corn oil, 10% (6% in case of WPC diet, because WPC, in the amount used, contained 4% oil); and corn starch to make 100%. Protein (N X 6.25) in each diet was 10%.

In experiment E, hard red spring wheat and the resultant six mill-fractions (Fig. 1) were obtained locally (Dixie-Portland Mill). All fractions were ground to a particle size approximating that of patent flour. A total of seven diets, each containing 10% protein (N X 6.25), were formulated. These were: patent flour diet

(flour, 75.7%); farina diet (farina, 83.9%); whole-wheat flour diet (flour, 72.4%); germ diet (germ, 37.4%); bran diet (bran, 65.4%); shorts diet (shorts, 58.3%); and red dog diet (red dog, 59.5%). In addition, each diet also contained: salt 446, 3%; vitamin mixture, 2%; sodium chloride, 1%; cholesterol, 1.5%; enough corn oil to bring total oil content to 10%; and corn starch to make 100%.

Male rats (Sprague-Dawley), 9 weeks old in experiment A and 3 weeks old in other experiments, were used. All were housed individually and offered test diets and water ad libitum (8 to 10 rats per diet) for 3 weeks (5 in experiment B). At the end of the experimental period, all rats were anesthetized and blood was withdrawn by heart puncture into heparinized tubes. Livers were then removed, washed thoroughly, blotted dry, weighed, and homogenized using cold water in a Potter-Elvehjem homogenizer. In experiment B, blood was also withdrawn at the end of the first and third weeks of feeding.

For cholesterol balance studies (Table II), rats in experiment C were placed in metabolism cages from days 8 through 12 of feeding. The composite fecal samples of 5 days for each rat were dried, pulverized, and analyzed for sterol content. For each rat, total cholesterol in separated blood plasma, in liver homogenate, and in fecal samples was determined by the method of Abell et al. (8). Test diets were also analyzed for total cholesterol content. Protein and lipid contents of casein, WPC, and mill-fractions were determined by standard AOAC methods (9).

TABLE I. DIETARY CELLULOSE, PLASMA, AND LIVER CHOLESTEROL LEVELS IN CHOLESTEROL-FED RATS

	Description of Diets	Weight at Sacrifice		Total Cholesterol[a]				
				Plasma			Liver	
		Body g.	Liver g.	1 wk. mg.%	3 wk. mg.%	5 wk. mg.%	3 wk. mg./g.	5 wk. mg./g.
				Experiment A (3 weeks)				
A-1	Basal	318 ±22	15.5 ±1.4	...	92 ±8	...	1.1 ±0.2	...
A-2	Basal+1.5% cholesterol	329 ±27	16.9 ±1.8	...	92 ±6	...	6.7 ±1.5	...
A-3	A-2 + 5% cellulose	320 ±13	15.9 ±1.1	...	87 ±10	...	7.5 ±1.0	...
A-4	A-2 + 10% cellulose	322 ±17	16.2 ±1.1	...	91 ±14	...	7.4 ±0.9	...
A-5	A-2 + 20% cellulose	313 ±14	15.6 ±0.7	...	99 ±9	...	6.7 ±0.4	...
				Experiment B (5 weeks)				
B-1	Basal	278 ±17	14.8 ±1.9	71 ±14	95 ±6	87 ±10	...	2.6 ±0.2
B-2	Basal+1.5% cholesterol	275 ±12	16.7 ±0.9	104 ±16	107 ±18	107 ±13	...	9.9 ±2.0
B-3	B-2 + 5% cellulose	279 ±13	17.1 ±1.3	117 ±11	130 ±17	116 ±12	...	11.4 ±0.7
B-4	B-2 + 10% cellulose	270 ±23	17.0 ±2.2	118 ±20	152 ±19	117 ±13	...	11.5 ±1.0
B-5	B-2 + 20% cellulose	278 ±15	17.3 ±1.5	107 ±15	137 ±17	104 ±9	...	11.6 ±0.9
B-6	B-5 premixed with water	271 ±14	15.7 ±1.3	...	...	127 ±13	...	14.1 ±0.3
B-7	Same as A-5	203 ±9	12.0 ±1.1	...	...	104 ±16	...	9.5 ±1.8

[a]All values represent average ± standard deviation.

RESULTS AND DISCUSSION

The addition of cholesterol to the basal diet in experiment A increased the level of cholesterol in the liver by many times (Table I). However, contrary to earlier findings (4,6), no increase in plasma cholesterol levels resulted. It could be that during the period of dietary-induced hypercholesteremia, particularly of shorter duration, cholesterol is first deposited in the liver before plasma levels are affected. Perhaps for this reason, concurrent feeding of cellulose at increasing levels (diets A-3 through A-5) had no significant ($P > 0.05$) effect on plasma cholesterol levels.

Since conditions in experiment A differed from those of Sundaravalli et al. (6), who found a distinct hypocholesteremic effect caused by dietary cellulose, experiment B was undertaken to duplicate as closely as possible their experimental conditions. The basal diet now contained 26% casein and 10% fat, and weanling rats were fed diets for 5 weeks instead of 3. Cholesterol feeding now elevated plasma cholesterol levels significantly ($P < 0.01$) at each sampling time, but no hypocholesteremic effect of concurrent feeding of cellulose even at 20% level in the diet was again observed (Table I). In fact, feeding of cellulose elevated the plasma cholesterol levels even further (diet B-2 vs. diets B-3 through B-5), though differences between diets B-3 through B-5 were not significant ($P > 0.05$) except at week 3. Working with rats and human subjects, Wells and Ershoff (4) and Keys et al. (5) also failed to show any hypocholesteremic effect of cellulose. Feeding diet B-5 premixed with water (B-6) (as were all diets in experiment A) raised the plasma and liver cholesterol levels still further; cellulose fed at 20% level in the diet with reduced fat and protein contents (B-7) failed to show any hypocholesteremic effect.

TABLE II. EFFECT OF CASEIN AND WPC ON PLASMA AND LIVER CHOLESTEROL LEVELS AND ON CHOLESTEROL ABSORPTION IN CHOLESTEROL-FED RATS

	Casein Diet Experiment		WPC Diet Experiment	
	C	D	C	D
Body weight, g.	135 ±13	137 ±22	143 ±5	145 ±7
Liver weight, g.	7.6 ±1.0	8.3 ±2.0	8.2 ±0.6	8.7 ±0.4
Liver cholesterol,[a] mg./g.	17.2 ±5.4	20.2 ±4.2	3.3 ±0.9	4.8 ±1.1
Plasma cholesterol,[a] mg.%	82 ±16	88 ±8	120 ±13	140 ±11
	CHOLESTEROL ABSORPTION[b]			
Cholesterol intake, mg.	921.0 ±57.8	...	1121.7 ±25.6	...
Cholesterol excreted,[c] mg.	563.1 ±27.5	...	922.1 ±47.5	...
Cholesterol absorbed, %	38.7 ±3.5	...	17.8 ±4.2	...

[a]Weanling rats (ten per diet) were fed cholesterol diet for 3 weeks.

[b]Balance study done (five rats per diet) in metabolism cages.

[c]Cholesterol and other fecal sterols.

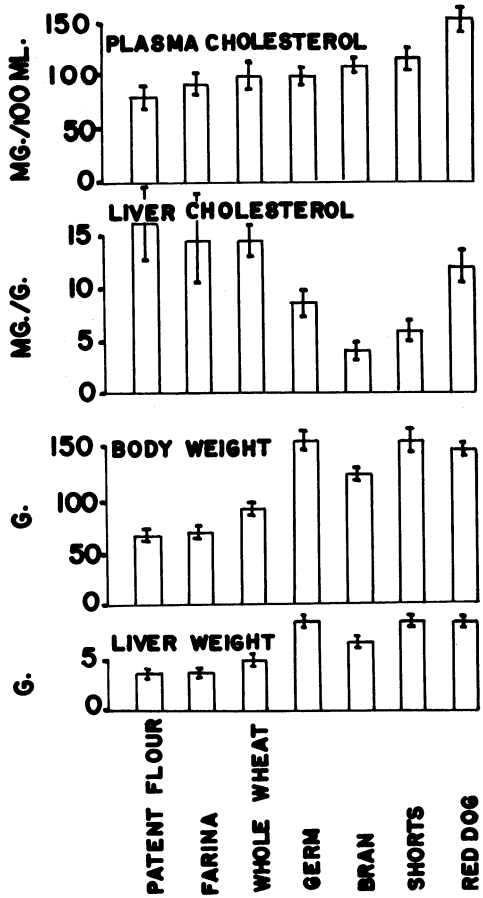

Fig. 1. The effect of wheat and resultant fractions on the plasma and liver cholesterol levels in cholesterol-fed rats.

Although dietary fats have been most extensively studied as to their effect on plasma cholesterol levels, information has become increasingly available on the role of complex carbohydrates and proteins in cereals and vegetables in lowering plasma cholesterol levels (10–14). Comparison of a casein-based diet with one based on WPC, both containing cholesterol as well, showed that while plasma cholesterol levels rose significantly ($P < 0.01$) in WPC-fed rats, they were virtually unaffected in rats fed casein (Table II). Since feeding of WPC reduced cholesterol absorption and probably caused a significant increase in fecal bile acids, the elevation of plasma cholesterol still occurring may have been caused by a decreased rate of cholesterol removal from the plasma. Highly significant ($P < 0.01$) differences observed in liver cholesterol levels in casein- and WPC-fed rats seem to support this. Present studies do not furnish information on the mechanism, obviously a complex one, regulating plasma cholesterol levels; but impairment of such a mechanism has been reported (15) in dietary deficiency of sulfur-containing amino acids.

In the milling of wheat, one or more grades of flour and five mill-fractions (Fig. 1) are produced. To identify the fraction or fractions that might be more hypocholesteremic than the others, rats were fed these fractions concurrently with cholesterol. Fractions high in starch and gluten, namely farina and patent flour, prevented elevation of plasma cholesterol levels (a function ascribed to gluten by Nath et al., 16), though liver cholesterol levels were most elevated. Some elevation in plasma cholesterol levels occurred when fibrous mill-fractions (bran and shorts) were fed, but liver cholesterol levels stayed characteristically low. This seems to suggest the fibrous mill-fractions, probably because of their high cellulose and hemicellulose content, may have depressed cholesterol absorption and/or synthesis of endogenous cholesterol, or may have even caused an increase in the metabolism of cholesterol. Kiriyama et al. (17) recently reported that the hypocholesteremic effect of crude konjac flour was completely lost when subjected to fungal cellulase activity. Whole-wheat flour and germ also showed some hypocholesteremic effect. On the contrary, red dog, which is a by-product consisting chiefly of the aleurone cells, caused a most pronounced elevation of the plasma cholesterol level as well as an appreciable increase in liver cholesterol levels. Thus, complex carbohydrates, starch, and, most importantly, the cellulose and hemicellulose in unmodified form, may possibly be the likely constituents of wheat responsible for its hypocholesteremic effect. This does not, however, rule out the possible contributory effect of constituents such as niacin, glutamine, and phytosterols, which are quite high in wheat, and are known to induce hypocholesteremia. Further studies on these lines using a more suitable model than rats are being initiated.

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