

The Interaction of Dietary Fibers and Cholesterol upon the Plasma Lipids and Lipoproteins, Sterol Balance, and Bowel Function in Human Subjects

THOMAS L. RAYMOND, WILLIAM E. CONNOR, DON S. LIN, SUSAN WARNER, MARTHA M. FRY, and SONJA L. CONNOR, *Department of Medicine and Clinical Research Center, University of Oregon Health Sciences Center, Portland, Oregon 97201, and the Clinical Research Center, University of Iowa College of Medicine, Iowa City, Iowa 52240*

ABSTRACT To identify any metabolic effects of dietary fiber upon cholesterol metabolism in man, six adult volunteer subjects were fed eucaloric cholesterol-free formula diets, with and without added dietary fiber for two 4-wk periods. A large quantity of dietary fiber was fed, some 60 g of plant cell wall material (or 16 g of crude fiber) derived from corn, beans, bran, pectin, and purified cellulose. This provided about five times the fiber intake of the typical American diet. The addition of fiber to the cholesterol-free diet did not change either the plasma cholesterol level (171 ± 21 mg/dl, SEM, to 167 ± 18) or the triglyceride (103 ± 39 to 93 ± 27 mg/dl). The excretion of both endogenous neutral steroids and bile acids were unchanged with fiber (505 ± 41 to 636 ± 75 mg/day and 194 ± 23 to 266 ± 47 mg/day, respectively). However, total fecal steroid excretion was increased 699 ± 29 to 902 ± 64 mg/day, $P < 0.025$). With fiber, intestinal transit time was decreased (59 ± 9 to 35 ± 8 h, $P < 0.005$), and both the wet and dry stool weights were greatly increased.

A second group of six subjects was fed similar diets containing 1,000 mg cholesterol derived from egg yolk. The addition of fiber to the 1,000-mg cholesterol diet did not alter either plasma cholesterol level (233 ± 26 to 223 ± 36 mg/dl) or triglyceride (102 ± 19 to 83 ± 11 mg/dl). The excretion of endogenous neutral steroids (618 ± 84 to 571 ± 59 mg/day), of bile acids (423 ± 122 to 401 ± 89 mg/day), and of total fecal steroids ($1,041 \pm 175$ to 972 ± 111 mg/day) were unchanged by fiber. The absorption of dietary cholesterol was not altered when

fiber was added to the 1,000-mg cholesterol diet (44.0 ± 3.3 to $42.9 \pm 2.5\%$). A two-way analysis of variance utilizing both groups of subjects indicated a significant ($P < 0.001$) effect of dietary cholesterol upon the plasma cholesterol concentration.

We concluded that a large quantity of dietary fiber from diverse sources had little or no effect upon the plasma lipids and sterol balance in man in spite of the fact that intestinal transit time and stool bulk changed greatly.

INTRODUCTION

Fiber has currently aroused great interest as a major dietary component which may affect the incidence of coronary heart disease in Western societies. Based upon epidemiological findings, Burkitt, Trowell, and others found a relationship between the lack of fiber in the diet and the incidence of several common disease states (2-9). These disorders included coronary heart disease, diabetes mellitus, diverticular disease and cancer of the colon, appendicitis, and dental caries. Their hypothesis was supported by strong evidence that these diseases were rarely seen in populations whose diets had a high fiber content in contrast to people of the Western world consuming a low-fiber and highly purified diet.

The fiber found in cereals, legumes, fruits, and vegetables has been a major component of the diet of man since the dawn of history. However, the fiber content of the Western diet has gradually been reduced by two technical changes: the refinement of food products and the accompanying removal of fiber, and the gradual increase in meat and fat consumption with a reciprocal decrease in foods having a high fiber content such as cereals and potatoes. An important landmark was the change in the milling process of wheat flour from stone

This report was presented at the 1976 Annual Meeting of the American Heart Association (1).

Dr. Raymond is a recipient of National Institutes of Health Postdoctoral Fellowship HL-05164.

Received for publication 24 June 1977 and in revised form 15 August 1977.

grinding to the use of steel rollers in the late nineteenth century (10, 11). Steel rollers more efficiently extracted bran from flour. Thus, the final product had a lower fiber content. In Western countries, the average daily fiber intake is only one-fifth of what it was in the mid-nineteenth century (12). The crude fiber content of the present American diet is estimated to be 2–5 g/day, whereas 18–35 g/day is reported in more primitive cultures (13).

Dietary fiber has been defined as that vegetable material resistant to digestion by the gastrointestinal tract. It is composed of several complex carbohydrates which include the hemicelluloses, cellulose and pectin, as well as lignin. The most common index of the fiber content in food utilized today is "crude fiber" (14). Crude fiber is the residue remaining after treatment with ether, boiling sulfuric acid, and sodium hydroxide. This residue consists of 10–50% lignin and 20–50% of the cellulose present in the original food. In the determination of crude fiber, all of the pectin and about 80% of the hemicelluloses are not measured, with a considerable underestimation of true dietary fiber. A better index of dietary fiber intake is the measurement of plant cell wall content as perfected by Van Soest and McQueen (15) and which we have utilized in this study.

To date there have been conflicting reports in the literature about the effects of fiber upon the plasma lipid levels (16–22). Studies with the addition of bran fiber have been largely negative in man, but some lowering of serum cholesterol levels has occurred with dietary supplements of pectin, lignin, and various gums (23–29). In a variety of animals, a hypocholesterolemic effect has been reported in some but not all with the feeding of oats, wheat straw, rice bran, soy bran, alfalfa, and various mucilaginous materials (30–34).

In view of these divergent reports and the absence of sterol balance studies in this important area of human nutrition, we designed a controlled metabolic investigation to evaluate the effects of the addition of dietary fibers from diverse sources upon plasma lipids and lipoproteins, cholesterol absorption, total intestinal transit time, stool bulk, and the fecal excretion of cholesterol and bile acids. We wished, furthermore, to measure any interaction of dietary cholesterol and fiber, so that our subjects received both high and low cholesterol diets with and without the large amounts of fiber in the diet. We hoped to provide information, then, about the metabolic effects of fiber in humans, especially in view of its possible public health importance.

METHODS

Eight adult volunteer subjects were hospitalized on a metabolic ward for the entire study. They ranged in age from 19–67 yr and in body weight from 45 to 68 kg, and had a range of plasma cholesterol levels from 167 to 317 mg/dl (Table I).

TABLE I
Description of the Experimental Subjects

Subject	Age	Sex	Weight	Height	Cholesterol	Tri-glyceride	Diagnosis*
	yr		kg	cm	mg/dl	mg/dl	
1	67	F	45	152	315	119	Ila
2	19	F	60	164	167	51	Normal
3	23	M	76	185	251	367	Iib
4	26	M	56	159	199	63	Normal
5	32	M	67	185	173	91	Normal
6	29	M	67	174	189	111	CTX†
7	67	F	67	163	227	194	Iib
8	66	M	68	172	236	138	Ila

* Lipoprotein phenotypes Ila and Iib are based upon classification in 1970 by the World Health Organization (*Bull. W.H.O.* 43: 891).

† CTX = Cerebrotendinous xanthomatosis.

None were obese and all were either normal or in good health during the course of the study. Informed consent was obtained from all subjects and from the responsible guardians for subjects 1 and 6.

The dietary protocol consisted of four eucaloric formula diets, each administered over a 4-wk period (Table II). Subjects 1 through 6 received a cholesterol-free formula diet without and with added fiber. Subjects 1, 2, and 5–8 received a 1,000-mg cholesterol formula without and with added fiber. 40% of calories were derived from fat and 15% from protein. The saturation of the fat was held constant (Iodine no. 80–85, P/S = 0.8). The plant sterol content varied from 166 to 383 mg/day and was held constant for each subject during each dietary period. Fiber was incorporated into the diet in the form of muffins. Each subject consumed nine muffins/day, except subject 1 who had reduced caloric requirement and who consumed only seven muffins/day. The sources of fiber in the diet were purified corn pericarp fiber (CPC International, Inc., Argo, Ill.), soybean hulls (Archer Daniels Midland Co., Decatur, Ill.), wheat bran (Kellogg's All Bran, Kellogg, Battle Creek, Mich.), hydroxyethylcellulose (Union Carbide Corp., New York), and pectin, n.f. (Sunkist Growers, Inc., Sherman Oaks, Calif.). This combination provided a daily intake of 16.2 g of crude fiber (35). The wet and dry ingredients of the muffins were mixed separately, blended together, and baked. The muffins were analyzed by the detergent method of Van Soest and Wine (36–38) which provides the amounts of both neutral detergent fiber (plant cell wall) and acid detergent fiber (cellulose and lignin). Lignin was precipitated from the acid detergent fiber with permanganate, and hemicellulose is expressed as the calculated difference between neutral detergent fiber and acid detergent fiber. The plant cell wall content of the diet was found to be 60 g/day, consisting of 39 g of hemicellulose, 14 g cellulose, 5 g lignin, and 2 g pectin (Table III).

Twice weekly, for the duration of the study, fasting blood samples were drawn into tubes containing disodium EDTA, 1 mg/dl. Plasma was analyzed for cholesterol and triglyceride content with the AutoAnalyzer II (Technicon Instruments Corp., Tarrytown, N. Y.) (39). Lipoproteins were separated according to the method of Havel et al. (40) in a Beckman L3-40 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.) and cholesterol and triglyceride analyses were determined on the $d < 1.006$ supernate (VLDL), and the infranate

TABLE II
Composition of the Formulas for the Different Dietary Periods*†

Period	Subjects	Cholesterol	Source of fat		Iodine value	Fatty acids		
			Whole egg yolk	Vegetable oil mixture‡		Saturated	Monounsaturated	Polyunsaturated
		mg	g		%			
I Cholesterol-free, fiber-free	1,2,3,4,5,6	<50	0	111	81	47.5	21.3	31.2
II Cholesterol-free, high-fiber	1,2,3,4,5,6	<50	0	112	83	47.0	22.3	30.7
III High-cholesterol, fiber-free	1,2,5,6,7,8	1,000	28	83	83	48.0	22.2	29.8
IV High-cholesterol, high-fiber	1,2,5,6,7,8	1,000	28	83	82	46.9	21.8	31.3

* These representative formulas are calculated for a daily intake of 2,500 Calories.

† Vitamins and minerals added to meet Recommended Dietary Allowance and remained constant throughout the study.

‡ Combination of peanut oil and cocoa butter, typically 80% and 20% for Periods I and II, and 58% and 16% for Periods III and IV.

TABLE III
The Precise Fiber Content of the High Fiber Muffin Constituents by the Detergent Analysis Method

Ingredient	Dry weight	Neutral detergent fiber	Hemi-cellulose	Cellulose	Lignin
<i>g/day</i>					
Corn kernel fiber	8.6	7.9	6.1	1.7	0.1
Soybean hulls	11.0	7.6	2.0	5.2	0.4
"All Bran"	72.1	22.9	16.7	4.5	1.8
Hydroxyethyl cellulose	3.8				
Enriched flour	84.9	3.1	3.1		
Citrus pectin	1.8				
Total	182.2	41.5	27.9	15.2	2.3

before and after precipitation with heparin and manganese chloride (LDL and HDL).¹

Complete daily stools were collected for the duration of the study and frozen until time of analysis. Stools were thawed and separated into 7-day pools. An equal amount of water was added to each 7-day pool sample and the stools were homogenized with a paint can shaker. A weighed aliquot was extracted and saponified, as modified in our laboratory (41). Neutral steroids and bile acids were separated by thin layer chromatography according to the methods of Miettinen et al. (42) and Grundy et al. (43) as modified (41). Trimethylsilyl derivatives of fecal steroids were quantitated with a Hewlett-Packard 7610 Gas Chromatograph (Hewlett-Packard Co., Avondale Div., Avondale, Pa.) fitted with a flame ionization detector and utilizing a ¼ inch-1 × 6-ft glass column packed with diatoport-S 80/100 coated with 3.8% SE-30. Oven temperature was 230°C and carrier gas flow set at 50 ml/min. The recovery of ingested β-sitosterol was utilized for assessment

¹ Abbreviations used in this paper: HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

of bacterial degradation and 5-α-cholestane was used for an analytical internal standard. An additional aliquot of each 7-day pool homogenate was dried under vacuum to 60°C to determine moisture content and stool dry weight.

Total intestinal transit time was measured in each subject two–four times during each dietary period. This was done by administering 40 radiopaque marker pellets (Portex, Wilmington, Mass.) and counting the pellets in the stool as visualized by X ray. The time at which 80% of the pellets were passed into the stool was taken to be transit time as described in the method originally described by Hinton et al. (44).

The absorption of dietary cholesterol was measured twice in subjects 1, 2, and 5–8 while consuming the 1,000-mg cholesterol formula. Each subject was fed a single radioactive meal containing 10 μCi [1, 2-³H]cholesterol and 2.5 μCi [4-¹⁴C]β-sitosterol (New England Nuclear, Boston, Mass.) along with two capsules of inert stool marker, brilliant cresyl blue. The recovery of unabsorbed dietary cholesterol in the stool over the next 7 days was measured according to Method One of Quintao et al. (45) as modified by our laboratory (46).

With each subject acting as his own control, statistical comparisons were made by utilizing the Student's paired *t* analysis. A *P* value <0.05 was considered to be a significant difference (47).

RESULTS

Subject acceptance of the diet and weight changes. All eight subjects enrolled in the study tolerated the experimental diets satisfactorily. The mean frequency of bowel movements was increased from 9 to 11/wk and no intestinal discomfort or diarrhea was experienced. Caloric balance was maintained and no changes in body weight occurred from the beginning to the end of the study.

Plasma lipids and lipoproteins. A 10–31% reduction (20.7±3.3%, SEM) in plasma cholesterol concentration occurred when subjects 1–6 were given the cholesterol-free formula diet in place of the usual American diet (Table IV). The addition of fiber to the cholesterol-free diet failed to decrease the plasma cho-

TABLE IV
Plasma Cholesterol and Triglyceride Concentrations during the Four Dietary Periods (mg/dl)

Subject	Plasma cholesterol		Plasma triglyceride	
	Cholesterol-free, fiber-free	Cholesterol-free, 60 g fiber	Cholesterol-free, fiber-free	Cholesterol-free, 60 g fiber
1	274	252	82	66
2	150	138	47	46
3	174	168	296	224
4	147	153	54	62
5	129	148	68	95
6	153	144	72	66
Mean ± SEM	171 ± 21	167 ± 18	103 ± 39	93 ± 27
Percent change		-2%		-10%
P value		>0.5		<0.5

Subject	1,000 mg cholesterol, fiber-free		1,000 mg cholesterol, 60 g fiber	
	1,000 mg cholesterol, fiber-free	1,000 mg cholesterol, 60 g fiber	1,000 mg cholesterol, fiber-free	1,000 mg cholesterol, 60 g fiber
1	356	388	70	62
2	179	153	56	50
5	199	162	94	93
6	193	176	73	63
7	230	222	176	116
8	242	241	144	111
Mean ± SEM	233 ± 26	224 ± 36	102 ± 19	83 ± 11
Percent change		-4%		-19%
P value		<0.4		<0.1

Each individual value represents the mean of the last four determinations during each dietary period.

lesterol concentration further (171 to 167 mg/dl). The plasma triglyceride concentration of the group was similarly unaffected by dietary fiber. However, subject 3 with marked hypertriglyceridemia and type II-b

phenotype had a 24% reduction in plasma triglyceride with the addition of fiber to the cholesterol-free diet.

The distribution of cholesterol and triglycerides in very low density lipoprotein (VLDL), low density lipo-

TABLE V
The Effects of Dietary Fiber upon the Plasma Lipoproteins of All Subjects

	VLDL		LDL		HDL	
	Cholesterol	Triglyceride	Cholesterol	Triglyceride	Cholesterol	Triglyceride
<i>mg/dl</i>						
Cholesterol-free diet						
Fiber-free	13.2 ± 7.1*	82.6 ± 46.6	114.6 ± 26.1	21.2 ± 3	49.4 ± 5.4	7.4 ± 2.2
High-fiber	13.2 ± 5.5	68.6 ± 31.7	103.6 ± 20.1	22.3 ± 2.9	50.4 ± 5.1	6.2 ± 0.9
P value	>0.5	>0.4	>0.2	>0.4	>0.5	>0.5
1,000 mg cholesterol diet						
Fiber-free	14 ± 3.9	66.7 ± 17.8	159 ± 22.6	27.2 ± 4.8	57.3 ± 5.2	8.3 ± 1.9
High-fiber	9.8 ± 2.2	44.8 ± 9.3	158.5 ± 86	25.8 ± 3.7	58.7 ± 5.4	6.7 ± 1.0
P value	>0.05	>0.05	>0.5	>0.2	>0.5	>0.4

* Values given are mean ± SEM.

TABLE VI
Plasma Lipid Levels in the Four Subjects Who Consumed All Four Diets*

Dietary period	Cholesterol	VLDL cholesterol	LDL cholesterol	HDL cholesterol	Triglyceride
Fiber, 60 g					
Cholesterol-free	171±27‡	8±2	105±26	55±3	68±10
Cholesterol, 1,000 mg	220±56	7±1	156±55	61±5	67±9
Percent change	+22%	-13%	+33%	+10%	+1.5%
P value	>0.1	>0.4	>0.1	>0.2	>0.5
Fiber-free					
Cholesterol-free	177±33	6±2	119±33	54±4	67±7
Cholesterol, 1,000 mg	232±42	8±2	160±34	62±5	73±8
Percent change	+24%	+25%	+26%	+13%	+8%
P value	<0.025	>0.4	<0.05	>0.4	>0.5

* Subjects 1, 2, 5, and 6.

‡ Mean±SEM.

protein (LDL), and high density lipoprotein (HDL) did not change when fiber was added to the cholesterol-free diet (Table V). The marked reduction of plasma triglyceride in subject 3 was confined to the VLDL fraction (268 to 191 mg/dl) and was not accompanied by similar changes in the other subjects.

The feeding of 1,000 mg cholesterol (equivalent to four whole egg yolks) in the second phase of the study increased the plasma cholesterol concentrations (177 to 232 mg/dl, $P < 0.025$) in subjects 1, 2, 5, and 6 (Table VI). The addition of fiber to this diet did not change either the plasma lipids or lipoproteins consistently (Table IV). The elevation in plasma cholesterol with feeding of 1,000 mg cholesterol/day to subjects 1, 2, 5, and 6 was primarily in the LDL fraction (119 to 160 mg/dl, $P < 0.05$) with a slight increase in HDL cholesterol concentration. This distribution pattern remained unchanged when fiber was added to the diet.

By utilizing the plasma lipid values for all eight subjects from all dietary periods, a two-way analysis of variance with a randomized block design was performed to test the effects of dietary cholesterol and dietary fiber as well as their interaction upon plasma lipids. Under this condition of analysis, dietary cholesterol had a highly significant effect ($P < 0.001$) upon the plasma cholesterol level. No effect occurred for dietary fiber or for the interaction of cholesterol and fiber. Similar analyses for plasma triglyceride showed no effect of cholesterol, fiber, or their interaction.

Intestinal transit time and stool output. The intestinal transit time was measured two–four times in each subject during each of the four dietary periods. The variation between tests on the same diet was often several hours, with an average coefficient of variation of 33%. The addition of fiber to the cholesterol-free formula invariably and uniformly produced a great decrease in transit times of all subjects (59 to 35 h, P

< 0.025) (Table VII). Similar findings occurred when fiber was incorporated into the 1,000-mg cholesterol diet (66 to 46 h, $P < 0.05$). Both the wet weight and dry weight of the stool were significantly increased when fiber was incorporated in both the cholesterol-free and 1,000-mg cholesterol diets.

The absorption of dietary cholesterol. The absorption of dietary cholesterol was measured in subjects 1, 2, and 5–8 during the 4th wk of both the 1,000-mg cholesterol, fiber-free diet and 1,000-mg cholesterol diet with fiber. These results are presented in Table VIII. Cholesterol absorption was similar and consistent without (44.0%) and with fiber (42.9%). These values are exactly within the range previously reported from our laboratory for cholesterol absorption in man (46). In our laboratory, repeated tests on two subjects have shown a coefficient of variation of 4.75% for this methodology.

Fecal steroid excretion. Complete stool collections were made by each subject throughout the study and

TABLE VII
The Stool Weights and Intestinal Transit Times in Subjects Fed Fiber-Free and High-Fiber Diets

Diet	Stool wet weight	Stool dry weight	Transit time
	<i>g/wk</i>		<i>h</i>
Cholesterol-free			
Fiber-free	1,236±279*	225±13	59±9
60 g fiber	1,677±245	376±63	35±8
P value	<0.025	<0.001	<0.025
Cholesterol, 1,000 mg			
Fiber-free	1,343±285	154±13	66±11
60 g fiber	2,005±149	464±25	46±9
P value	<0.001	<0.001	<0.05

* Mean±SEM.

TABLE VIII
Absorption of Dietary Cholesterol as Measured with
a Single Radioactive Meal

Subject	Dietary period	
	1,000 mg cholesterol, fiber-free	1,000 mg cholesterol, 60 g fiber
	%	
1	46.6	41.7
2	37.7	38.2
5	34.3	40.7
6	48.6	48.6
7	56.3	43.4
8	40.3	38.7
Mean±SEM	44.0±3.3	42.9±2.5
P value	NS	

analyzed as 7-day pools (Table IX). Fecal neutral steroids were quantitated as cholesterol and its bacterial metabolites. The values given have been corrected for loss of ingested β -sitosterol (46).

During the cholesterol-free, fiber-free period, the mean neutral steroid excretion in subjects 1–6 was 505 mg/day and the mean bile acid output was 194 mg/day. With the addition of fiber to the cholesterol-free diet,

the neutral sterol excretion increased to 636 mg/day and bile acids increased to 266 mg/day, but neither of these increases was significant. However, the total fecal steroid excretion increased significantly ($P < 0.025$) from 699 to 902 mg/day.

The fecal steroid excretion in the subjects fed the 1,000-mg cholesterol diet is also presented in Table IX. Both total and endogenous neutral steroid data are listed. The endogenous steroid excretion data are comparable to the data of the group fed the cholesterol-free diet. Since cholesterol was present in the diet, correction was made for unabsorbed dietary cholesterol excreted in the stool (42). During the 1,000-mg cholesterol, fiber-free diet, the mean endogenous neutral steroid excretion was 618 mg/day and the fecal bile acid output was 423 mg/day. The total endogenous fecal steroid excretion equalled 1,041 mg/day. With the addition of fiber to the 1,000-mg cholesterol diet, both neutral steroid and bile acid excretion fell slightly to 571 and 401 mg/day, respectively. Total endogenous fecal steroid excretion was 972 mg/day. These decremental changes were not statistically significant.

DISCUSSION

In this metabolic balance study, a large quantity of dietary fiber from a variety of sources was added to both

TABLE IX
The Effects of Dietary Fiber upon Fecal Steroid Excretion in Man

Dietary period	Sub- ject	Total neutral steroids*†	Bile acids*	Total fecal steroids	Dietary period	Sub- ject	Total neutral steroids	Endogenous neutral steroids‡	Bile acids	Total endogenous fecal steroids
		mg/day	mg/day	mg/day			mg/day	mg/day	mg/day	mg/day
Cholesterol- free, fiber-free	1	561	148	709	1,000 mg cholesterol, fiber-free	1	1,107	539	181	720
	2	481	225	706		2	1,186	648	212	860
	3	481	245	726		5	1,014	302	254	556
	4	379	187	566		6	1,398	796	280	1,076
	5	456	249	705		7	1,468	881	856	1,737
	6	673	108	781		8	958	541	753	1,294
Mean		505±41	194±23	699±29	Mean		1,189±84	618±84	423±122	1,041±175
Cholesterol- free, 60 g fiber	1	646	135	781	1,000 mg cholesterol, 60 g fiber	1	1,037	531	196	727
	2	921	186	1,107		2	1,087	463	250	713
	3	632	429	1,061		5	1,053	469	494	963
	4	374	329	703		6	1,288	720	183	903
	5	525	333	858		7	1,367	786	662	1,448
	6	717	182	899		8	1,078	455	622	1,077
Mean		656±75	266±47	902±64	Mean		1,152±57	571±59	401±89	972±111
P values (fiber-free vs. fiber)		NS	NS	$P < 0.025$			NS	NS	NS	NS

* Each value represents the average of the last 2 wk of each dietary period.

† Each value has been corrected for recovery of β -sitosterol.

‡ Endogenous neutral steroid excretion = total neutral steroids – unabsorbed dietary cholesterol.

|| Mean±SEM.

cholesterol-free and 1,000-mg cholesterol diets fed to human subjects. While the plasma cholesterol concentrations readily responded to the changes in dietary cholesterol, the addition of fiber to either of these diets did not affect the basal plasma cholesterol levels. Plasma triglyceride concentrations were similarly unaffected. Moreover, the distribution of cholesterol and triglyceride between the lipoproteins was also unchanged with fiber. The amounts of dietary fiber utilized in these studies were roughly equivalent to amounts which human beings would reasonably eat and which certain populations other than our own are now consuming (16 g of crude fiber/day).

The sterol balance was not greatly affected by dietary fiber. There were slight increases in both neutral sterol and bile acid output in the cholesterol-free experiment. Only when these increases were combined was statistical significance achieved for total fecal sterol excretion. This could represent increased cholesterol biosynthesis or increased excretion of steroid from the body. Since in the other phase of the study the total fecal sterol excretion did not change, the physiological significance of the statistical increase of total fecal steroid excretion with the addition of 60 g of fiber to the cholesterol-free diet remains unclear.

With regard to the high cholesterol diet, both endogenous neutral steroids and bile acids decreased slightly, albeit not significantly, during the fiber-free period. The total decrease did not attain significance for total fecal steroid excretion. Neutral steroid excretion was reduced slightly when fiber was added to the 1,000-mg cholesterol diet. Fecal bile acid excretion was increased (194 to 423 mg/day, $P < 0.1$) when compared to the group fed no cholesterol. This most likely resulted from the increased plasma pool of cholesterol substrate occurring from the high cholesterol diet. All these results indicate that the bile acid binding capacity of these fibers *in vitro* as shown by others (48, 49) is probably not of a sufficient order of magnitude to produce increases in fecal bile acid excretion.

The absorption of dietary cholesterol during the final week of the 1,000-mg cholesterol fiber-free diet was 43%. This value is within the range previously reported by us for normal and hypercholesterolemic individuals (46). Absorption of dietary cholesterol was unchanged when fiber was added to the diet, and therefore cannot account for the slight decrease in neutral steroid excretion when this group of subjects was fed the high cholesterol, 60-g fiber diet.

Proof that the dietary fiber fed had a biological effect was indicated by the great decrease in total intestinal transit time and the significant increase in both the wet and dry weight of the stools when fiber was added to both cholesterol-free and 1,000-mg cholesterol diets. These findings are in agreement with the reports of

others (50–54) but these manifest changes failed to alter the ability of the intestinal tract to absorb dietary cholesterol or enhance its excretion in the stool.

Several investigators have reported the inability of wheat bran fiber to affect plasma lipid levels. Heaton and Pomare reported a significant reduction in plasma triglyceride concentration in fourteen volunteers consuming 18–100 g of wheat bran per day (17). There were no changes in plasma cholesterol concentrations. Other sources of dietary fiber have been shown to be effective in lowering plasma lipid levels in both man and animals (23–29). Keys and co-workers demonstrated that legumes and citrus pectin lowered plasma cholesterol levels in man (28, 55). In recent reports, reduced plasma cholesterol levels have been demonstrated when volunteer subjects were fed 36 g of guar gum (23) or 15–36 g of pectin (56). These amounts were far above the amount of pectin utilized in our investigation and are equivalent to the ingestion of several dozen apples or oranges per day, an amount which cannot practically be incorporated in the current American diet.

The effects of dietary cholesterol upon the plasma lipids have been well documented in the past (57–60) but have recently been questioned (61). In this study, dietary cholesterol was tested in two ways, when it was removed from the diet as the subjects entered the study having previously been consuming the typical high cholesterol, high fat American diet. Secondly, 1,000 mg of cholesterol was added to both fiber-free and high fiber periods. The plasma cholesterol concentration increased 49 and 55 mg/dl, respectively (28.5 and 32.5% increase over the base-line cholesterol-free diets. Most of this increase occurred in LDL). This evidence further substantiated the powerful effects of dietary cholesterol upon the plasma cholesterol and LDL levels.

Although addition of dietary fiber failed to alter plasma lipid levels despite significant changes in stool transit time and stool weights, high fiber diets as described among various tribes and religious groups may conceivably be beneficial in the treatment and prevention of hyperlipidemia because natural high fiber diets frequently have other associated hypolipidemic characteristics. The rural African tribesman who naturally consumes a high fiber diet also consumes a diet which is low in cholesterol and saturated fat, low in total fat, low in meat, low in refined carbohydrate, high in bulk, and low in caloric density. Studies reported in Seventh Day Adventists (62) and collectives of vegetarian subjects (63) demonstrate lower plasma cholesterol levels than their meat-eating counterparts. Connor et al. (13) have reported low levels of plasma cholesterol (123 mg/dl) in adult Tarahumara Indians compared with the average resident of the U. S. The Tarahumara Indians consume a high fiber diet (19 g crude fiber/day), but a

diet that contains little meat and only 20% fat as compared to 40–45% fat in the typical American diet. Cholesterol intake has been calculated to be 71 mg/day as compared to 600–800 mg/day for the average American.

In our experiments we wanted to use various dietary fibers as they might occur in the natural diet of man. We tried not to use fiber as a drug, but incorporated into the diet as muffins. The amounts of cellulose, pectin, corn, bean, and wheat fibers are amounts that human beings have actually been observed to consume. Our own data in the Tarahumara Indians suggests that something on the order of 60 g of fiber, largely from corn and beans, but some from cellulose and pectin, are consumed daily by the Tarahumaras. Our stool weight and transit times during the high fiber period, furthermore, compared favorably with the Tarahumaras.

While dietary fiber probably does not play a role in the low plasma levels found in vegetarians and more primitive populations, fiber alone is probably largely responsible for the increased stool bulk, decreased transit time, and therefore possibly related to the lowered incidence of certain gastrointestinal disorders noted in the African tribesman (2, 3). To better evaluate the role of dietary fiber in these populations with low plasma lipid levels, studies of lipid and sterol metabolism need to be conducted with diets where fiber is high, but as a natural ingredient of a diet representative of the low-meat, low-cholesterol diet of these people. Our studies indicate that the simple addition of these fibers to any diet will not have any significant effects upon lipid metabolism in man.

ACKNOWLEDGMENTS

The authors wish to thank Dr. James B. Robertson and Dr. Peter J. Van Soest for the analysis of dietary fiber, Dr. Donald Wiebe for the isolation of lipoproteins, and Meg Larson for the typing of the manuscript.

This investigation was supported in part by U. S. Public Health Service grants HL-19130 and HL-06336 from the National Heart and Lung Institute and by the General Clinical Research Centers Program (RR-334 and RR-59) of the Division of Research Resources of the National Institutes of Health, and a grant from CPC International, Argo, Ill.

REFERENCES

1. Raymond, T. L., W. E. Connor, D. S. Lin, and S. L. Connor. 1976. Effects of dietary fiber upon plasma lipids, sterol balance, and bowel function. *Circulation*. 56(Suppl. II): 692. (Abstr.)
2. Burkitt, D. P., A. R. P. Walker, and N. S. Painter. 1972. Effect of dietary fiber on stools and transit times, and its role in the causation of disease. *Lancet*. II: 1209.
3. Burkitt, D. P., A. R. P. Walker, and N. S. Painter. 1974. Dietary fiber and disease. *J.A.M.A. (J. Am. Med. Assoc.)*. 229: 1068–1074.
4. Trowell, H. 1972. Crude fiber, dietary fiber and atherosclerosis. *Atherosclerosis*. 16: 138–140.
5. Trowell, H. 1972. Ischemic heart disease and dietary fiber. *Am. J. Clin. Nutr.* 25: 926–932.
6. Trowell, H. 1974. Diabetes mellitus, death rates in England and Wales, 1920–70 and food supplies. *Lancet*. II: 998–999.
7. Walker, A. R. P. 1971. Diet, bowel motility, feces composition, and colonic cancer. *S. Afr. Med. J.* 45: 337–379.
8. Painter, N. S., A. Z. Almeida, and K. W. Colebourne. 1972. Unprocessed bran in treatment of diverticular disease of the colon. *Br. Med. J.* 2: 137–140.
9. Malhotra, S. L. 1971. Dietary factors and ischemic heart disease. *Am. J. Clin. Nutr.* 24: 1195–1198.
10. Trowell, H. 1973. Dietary fiber, ischemic heart disease and diabetes mellitus. *Proc. Nutr. Soc.* 32: 151–157.
11. Jones, C. R. 1958. The essentials of the flour-milling process. *Proc. Nutr. Soc.* 17: 7–14.
12. Scala, J. 1974. Fiber, the forgotten nutrient. *Food Technol.* 28: 34–36.
13. Connor, W. E., M. C. Urban, R. W. Connor, R. W. Wallace, M. R. Malinow, and H. Casdorph. 1975. The serum lipids, lipoproteins and diet in the Tarahumara Indians of Mexico. *Circulation*. 52 (Suppl. II): 171. (Abstr.)
14. Cummings, J. H. 1973. Progress report, dietary fiber. *Gut*. 14: 69–81.
15. Van Soest, P. J., and R. W. McQueen. 1973. The chemistry and estimation of fiber. *Proc. Nutr. Soc.* 32: 123–130.
16. Walker, A. R. P., and U. B. Arvidsson. 1954. Fat intake, serum cholesterol concentration, and atherosclerosis in the South African bantu. I. Low fat intake and the age trend of serum cholesterol concentration in the South African bantu. *J. Clin. Invest.* 33: 1358–1365.
17. Heaton, K. W., and E. W. Pomane. 1974. Effect of bran on blood lipids and calcium. *Lancet*. I: 49–50.
18. Connell, A. M., C. L. Smith, and M. Somse. 1975. Absence of effect of bran on blood lipids. *Lancet*. I: 496–497.
19. Walters, R. L., I. M. Baird, P. S. Davies, M. H. Mill, B. S. Drasar, D. A. T. Southgate, J. Green, and B. Morgan. 1975. The effects of two types of dietary fiber on fecal steroid and lipid excretion. *Br. Med. J.* 2: 536–538.
20. Durrington, P., A. C. B. Wicks, and K. W. Heaton. 1975. Effect of bran on blood lipids. *Lancet*. II: 133–134.
21. Bremner, W. F., P. M. Brooks, J. L. H. C. Third, and T. D. V. Lawrie. 1975. Bran in hypertriglyceridemia: a failure of response. *Br. Med. J.* 3: 574.
22. Eastwood, M. 1969. Dietary fiber and blood lipids. *Lancet*. II: 1222–1224.
23. Jenkins, D. J., A. C. Newton, A. R. Leeds, and J. H. Cummings. 1975. Effect of pectin, guar gum and wheat fiber on serum cholesterol. *Lancet*. I: 116–117.
24. Thiffault, C., M. Belanger, and M. Pouliot. 1970. Traitement de l'hyperproteinemie essentielle de type II par un nouvel agent therapeutique, la celluline. *Can. Med. Assoc. J.* 103: 165–166.
25. Mathur, K. S., M. A. Khan, and R. D. Sharma. 1968. Hypocholesterolemic effect of Bengal Gram: A long-term study in man. *Br. Med. J.* 20: 30–31.
26. Garvin, J. E., D. E. Forman, W. R. Eideman, and E. R. Phillips. 1965. Lowering of human serum cholesterol by an oral hydrophilic colloid. *Proc. Soc. Exp. Biol. Med.* 120: 744–746.
27. Stanley, M. M., D. Paul, D. Gacke, and J. Murphy. 1973. Effects of cholestyramine, metacumil, and cellulose on fecal bile salt excretion in man. *Gastroenterology*. 65: 889–894.
28. Keys, A., F. Grande, and J. T. Anderson. 1961. Fiber and pectin in the diet and serum cholesterol concentration in man. *Proc. Soc. Exp. Biol. Med.* 106: 555–558.

29. Palmer, G. H., and D. G. Dixon. 1966. Effect of pectin dose on serum cholesterol levels. *Am. J. Clin. Nutr.* **18**: 437-442.
30. Fisher, H., and P. Griminger. 1967. Cholesterol-lowering effects of certain grains and oats fractions in the chick. *Proc. Soc. Exp. Biol. Med.* **126**: 108-111.
31. Cookson, F. B., R. Altschul, and S. Fedoroff. 1967. The effect of alfalfa feeding on serum cholesterol and in modifying or preventing cholesterol-induced atherosclerosis in rabbits. *J. Atheroscler. Res.* **7**: 69-81.
32. Moore, J. H. 1967. The effect of the type of roughage in the diet on plasma cholesterol and aortic atherosclerosis in the rabbit. *Br. J. Nutr.* **21**: 207-215.
33. Vijayagopal, P., and P. A. Kurup. 1970. Effect of dietary starches on the serum aorta and hepatic lipid levels in cholesterol-fed rats. *Atherosclerosis.* **11**: 257-264.
34. Fahrenback, M. J., B. A. Riccardi, and W. C. Grant. 1966. Hypocholesterolemic activity of mucilaginous polysaccharides in white leghorn cockerels. *Proc. Soc. Exp. Biol. Med.* **124**: 321-326.
35. Watt, B. K., and A. L. Merrill. 1963. Composition of Foods. Agricultural Handbook No. 8, U. S. Department of Agriculture, U. S. Government Printing Office, Washington, D. C. 6-67.
36. Van Soest, P. J., and R. H. Wine. 1968. Determination of lignin and cellulose in acid-detergent fiber with permanganate. *J. Assoc. Off. Agric. Chem.* **51**: 780-785.
37. Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *J. Assoc. Off. Agric. Chem.* **46**: 829-835.
38. Van Soest, P. J., and R. H. Wine. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. *J. Assoc. Off. Agric. Chem.* **50**: 49-55.
39. Rush, R. L., L. Leon, and J. Turrel. 1971. Automated simultaneous cholesterol and triglyceride determination on the AutoAnalyzer II. In *Advances in Automated Analysis—Technicon International Congress, 1970*. E. C. Barton, M. I. DuCros, M. M. Erdrich, J. E. Golin, J. B. Levine, H. Mansberg, D. W. Mayfield, P. Ohringer, D. N. Reed, C. R. Roesch, K. L. Roth, A. M. Saunders, W. Smythe, and W. A. Weldon, editors. Thurman Associates, Miami, Fla. **1**: 503-507.
40. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34**: 1345-1353.
41. Connor, W. E., D. T. Witiak, D. B. Stone, and M. L. Armstrong. 1969. Cholesterol balance and fecal neutral steroid and bile acid excretion in normal men fed dietary fats of different fatty acid composition. *J. Clin. Invest.* **48**: 1363-1375.
42. Miettinen, T. A., E. H. Ahrens, and S. M. Grundy. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6**: 411-423.
43. Grundy, S. M., E. H. Ahrens, and T. A. Miettinen. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* **6**: 397-410.
44. Hinton, J. M., J. E. Lennard-Jones, and A. C. Young. 1969. A new method for studying gut transit times using a radiopaque marker. *Gut.* **10**: 842-859.
45. Quintao, E., S. M. Grundy, and E. H. Ahrens. 1971. An evaluation of four methods of measuring cholesterol absorption by the intestine in man. *J. Lipid Res.* **12**: 221-232.
46. Connor, W. E., and D. S. Lin. 1974. The intestinal absorption of dietary cholesterol by hypercholesterolemic (Type II) and normocholesterolemic humans. *J. Clin. Invest.* **53**: 1062-1070.
47. Snedecor, G. W., and W. G. Cochran. 1967. *Statistical Methods*. Iowa State University Press, Ames, Iowa. 6th edition. 91-95.
48. Eastwood, M. A., and D. Hamilton. 1968. Studies on the adsorption of bile salts to non-absorbed components of the diet. *Biochim. Biophys. Acta.* **152**: 165-173.
49. Kritchevsky, D., and J. A. Story. 1974. Binding of bile salts *in vitro* by non-nutritive fiber. *J. Nutr.* **104**: 458-462.
50. Holmgren, C. O. R., and J. M. Mynors. 1972. The effect of diet on bowel transit time. *S. Afr. Med. J.* **46**: 918-920.
51. Crofts, T. J. 1975. Bowel transit times and diet. *Lancet.* **I**: 801.
52. Eastwood, M. A., J. R. Kirkpatrick, W. D. Mitchell, A. Bone, and T. Hamilton. 1973. Effects of dietary supplements of wheat bran and cellulose on feces and bowel function. *Br. Med. J.* **4**: 392-394.
53. Harvey, R. F., E. W. Pomare, and K. W. Heaton. 1973. Effects of dietary fibre on intestinal transit. *Lancet.* **I**: 1278-1280.
54. Walker, A. R. P. 1975. Effect of high crude fiber intake on transit time and the absorption of nutrients in South African Negro children. *Am. J. Clin. Nutr.* **28**: 1161-1169.
55. Grande, F., J. T. Anderson, and A. Keys. 1965. Effect of carbohydrates of leguminous seeds, wheat and potatoes on serum cholesterol concentration in man. *J. Nutr.* **86**: 313-317.
56. Kay, R. M., and A. S. Truswell. 1977. Effect of citrus pectin on blood lipids and fecal steroid excretion in man. *Am. J. Clin. Nutr.* **30**: 171-175.
57. Connor, W. E., R. E. Hodges, and R. E. Bleiler. 1961. The effect of dietary cholesterol upon the serum lipids in man. *J. Lab. Clin. Med.* **57**: 331-342.
58. Connor, W. E., R. E. Hodges, and R. E. Bleiler. 1961. The serum lipids in men receiving high cholesterol and cholesterol-free diets. *J. Clin. Invest.* **40**: 894-901.
59. Mattson, F. H., B. A. Erickson, and A. M. Kligman. 1972. Effect of dietary cholesterol on serum cholesterol in man. *Am. J. Clin. Nutr.* **25**: 589-594.
60. Connor, W. E., D. B. Stone, and R. E. Hodges. 1964. The interrelated effects of dietary cholesterol and fat upon human serum lipid levels. *J. Clin. Invest.* **43**: 1691-1696.
61. Slater, G., J. Mead, G. Dhopeswarkar, S. Robinson, and R. B. Alfin-Slater. 1976. Plasma cholesterol and triglycerides in men with added eggs in the diet. *Nutr. Rep. Int.* **14**: 249-260.
62. Ruys, J., and J. B. Hickie. 1976. Serum cholesterol and triglyceride levels in Australian adolescent vegetarians. *Br. Med. J.* **2**: 87.
63. Sacks, F. M., W. P. Castelli, A. Donner, and E. H. Kass. 1975. Plasma lipids and lipoproteins in vegetarians and controls. *N. Engl. J. Med.* **292**: 1148-1151.