Influence of dietary fiber from vegetables and fruits, bran or citrus pectin on serum lipids, fecal lipids, and colonic function[1-3]

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ABSTRACT The effects of dietary fiber from different sources on cholesterol metabolism and colonic function were investigated in a group of 62 young healthy volunteers under strict dietary control. All foodstuffs except for 100 kcal/day were supplied, taking into account each subject's energy needs. The subjects consumed a relatively low-fiber diet for 2.5 weeks, after which period they were divided into four groups. For the next 5 weeks one group continued on the low-fiber diet, a second group received a high-fiber diet rich in vegetables and fruits, while the diets of the third and fourth group were supplemented with citrus pectin and wheat bran, respectively. These four groups consumed on average 18, 43, 28, and 37 g dietary fiber per day, respectively. Differences in consumption of type and amount of fat, cholesterol, protein, and carbohydrates between the groups were negligible, as ascertained both by 5-day food records and by analysis of duplicate portions. The concentration of serum total cholesterol decreased in those subjects on the diet containing citrus pectin and also in those on the diet containing vegetables and fruits, by 0.34 and 0.17 mmole/liter (13 and 7 mg/dl), respectively. The addition of bran, however, caused a statistically significant increase of 0.34 mmole/liter (13 mg/dl). The amount and type of dietary fiber had no significant effect on the concentration of serum high-density lipoprotein-cholesterol. The effects on serum total cholesterol could be explained only to a small extent by changes in the excretion of fecal steroids. The high-fiber diet with vegetables and fruits as well as the diet with bran shortened the intestinal transit time by 13 and 19 hr, respectively, and enhanced feces production by 49 and 77 g wet weight per 24 hr, respectively. Pectin had no effect on colonic functions. After 5 weeks no deleterious effect on Mg^{2+} or Ca^{2+} absorption was observed. The higher intake of potassium from the diet containing vegetables and fruits was not accompanied by a lowering of blood pressure. It is concluded that—at least in short-term controlled experiments—fiber components from vegetables and fruits, in contrast with bran, have a small favorable effect on the concentration of serum cholesterol. Both a vegetables/fruits diet and a bran diet may improve colonic functions. *Am. J. Clin. Nutr.* 33: 1745–1756, 1980.

Epidemiological studies (1) have suggested that the development of atherosclerotic diseases and large-bowel disorders in western communities is linked to a low intake of dietary fiber. Dietary fiber has been defined as the plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man (2). The main components of dietary fiber are cellulose, hemicelluloses, pectic substances (polygalacturonic acid compounds) and lignin.

The dietary fiber hypothesis has been tested in many experiments with human volunteers, in which the effect of different fiber sources on serum lipids and colonic function

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were examined (3, 4). In most studies dietary fiber was added to the diet in the form of isolated components (pectin, cellulose) or fiber-rich foodstuffs such as bran. Isolated citrus pectin is known to lower the level of serum cholesterol in man in short-term trials, while bran has been reported to have no significant effect (5, 6). Fiber-rich cereal products increase the volume of feces and (marginally) shorten intestinal transit time (7), whereas the effects on colonic functions of citrus pectin are less clear.

Only a few controlled studies with natural high-fiber diets have been published. We have recently reported (4) the effects of a mixed high-fiber diet on a group of 47 healthy volunteers. Half of the dietary fiber was provided by vegetables and fruits and the rest came from bread and other cereal products. On that diet the concentration of serum cholesterol decreased on average by 0.38 mmole/liter (15 mg/dl). However, only part of this effect might be due to dietary fiber per se, as during the high-fiber period subjects consumed less fat and cholesterol and more carbohydrates, than during the low-fiber period.

The present paper describes the effects on cholesterol metabolism and colonic functions of isolated citrus pectin in comparison with the same amount of pectic substances contained naturally in fruits and vegetables, and also with a comparable amount of fiber from wheat bran.

Subjects and methods

Subjects

The volunteers were 40 male and 22 female university students, aged 18 to 28. All subjects satisfied the following criteria: *1*) apparently healthy, as judged by a detailed questionnaire; *2*) a level of serum cholesterol below 5.7 mmole/liter (220 mg/dl); *3*) serum triglycerides below 1.7 mmole/liter (150 mg/dl); *4*) diastolic blood pressure below 90 mm Hg; *5*) a percentage of body fat lower than 23% in males and 30% in females.

Body weight was recorded weekly and energy intake was adjusted when necessary, in order to avoid changes in body weight of more than 2 kg. The mean change in body weight over the experimental period was −0.2 to −0.3 kg in the control, vegetables/fruits and bran groups, and +0.3 kg in the pectin group. The subjects were asked to note in diaries illness, drug use, and departures from the diet.

Diets

All subjects consumed a relatively low-fiber diet for 2.5 weeks after which period they were divided into four groups. During the next 5 weeks, group 1 continued on the low-fiber diet; group 2 received a high-fiber diet rich in vegetables and fruits; in group 3 citrus pectin was added to the low-fiber diet, and group 4 received the low-fiber diet supplemented with wheat bran. The groups were matched for initial serum cholesterol level, energy intake, and sex. For statistical evaluation, in each group, the individual values at the beginning and the end of the experimental period were compared, using a paired two-tailed *t* test (8). Each subject thus served as his own control. In addition, analysis of variance was used to test diet and sex effects on changes in parameters over the experimental period (9). If this revealed a significant effect of diet, a Tukey range test was applied to compare group means (9).

The amount (and type) of fat, cholesterol, protein, and carbohydrates was planned to simulate an "average Netherlands diet" (10). The planned intake of total dietary fiber and pectin (polygalacturonic acid) was adjusted to each subject's energy intake (Table 3). Citrus pectin was added to the diet of group 3, so that the level of polygalacturonic acid was identical to that in the vegetables/fruits diet. Wheat bran was added to the diet of group 4, so that the level of nonpolygalacturonic acid dietary fiber was similar to that of the diet in the vegetables/fruits group. Subjects in group 3 received on average 9 g citrus pectin per day. The bran diet contained on average 38 g bran per day.

Citrus pectin (National Formulary) was obtained from Bulmer Ltd. (Hereford, England); the polygalacturonic acid and total dietary fiber content amounted to 76 and 95 g/100 g, respectively; its jellying power (11) was 233°US-SAG and the degree of esterification of the polygalacturonic acid was 78%. The pectin was incorporated into desserts made from buttermilk and fruit juices.

Commercial coarse wheat bran was produced by Meneba (Rotterdam) from a grist of 65% European and 35% North American bread wheat (*Triticum aestivum*). The water-holding capacity (12) of the unprocessed bran was 4.1 g water per g dry matter. It contained 7.5 g ash, 17.3 g crude protein and 58 g neutral detergent fiber (13) per 100 g dry matter. Most of the bran was incorporated into bread and the rest into desserts (Table 1).

Throughout the 8-week period all foodstuffs, except for 100 kcal/day, were weighed out separately for each subject, in quantities appropriate to his energy needs. **Table 1** shows the quantities of fiber-containing foodstuffs supplied to a subject with an energy intake of 2550 kcal/day. On weekdays, hot meals were prepared and served at the laboratory. Detailed instructions were given for the preparation of hot meals from ingredients supplied for the weekend, and for spending the "free" daily 100 kcal. Monotony of menus was avoided by using a large variety of fresh foodstuffs. The high intake of carbohydrates (mostly sugars) and vitamin C from vegetables and fruits on diet 2, was compensated by fruit juices, marmelade, sugar and vitamin C-concentrate in the other three groups.

Measurement of nutrient intake

Before the experiment and again during the control and experimental periods, the actual intake of nutrients was measured on 3, 2, and 5 separate days, respectively, by weighing plus questionnaire, using Netherlands food composition tables. Data for polygalacturonic acid and total dietary fiber were obtained as described below.

TABLE 1
Amounts of some fiber-providing foodstuffs supplied to a subject with an energy intake of 2550 kcal/day, and total dietary fiber and polygalacturonic acid content of these foodstuffs

	Diet				Total dietary fiber content[a]	Polygalacturonic acid content[b]
	Low-fiber	Vegetable/fruits	Citrus pectin	Bran		
	g/day				*g/100 g edible portion*	
Low-fiber bread	225	200	225		3.2[c]	0.1
Bran-bread				250	8.6[c]	0.1
Raw wheat bran				4	50.4[d]	0.1
Potatoes, cooked	200	150	200	200	3.0	0.2
Vegetables, raw[e]	75		75	75		
Cucumber					0.7	0.2
Lettuce					1.3	0.3
Cabbage					2.6	0.6
Vegetables, cooked[e]		400				
Green cabbage					3.4	0.7
Sliced beans					4.4	0.8
Carrots					2.7	0.9
Endive					5.6	0.8
Beetroots					2.8	0.4
Tomatoes		170			1.4	0.3
Apples, with skin		600			2.3	0.5
Citrus pectin			9		95	76
Planned intake of						
Dietary fiber	15	41	24	34		
Polygalacturonic acid	1	8	8	1		

[a] Analysed according to References 14 and 20 unless indicated otherwise. [b] Analysed according to References 15 and 16. [c] Estimate; the neutral detergent fiber (13) content was 2.1 for the low-fiber bread and 7.1 for the bran-bread; the unavailable carbohydrates plus lignin content (14,20) was 6.4 and 9.1, respectively. The low-fiber bread contained 2 g bran/100 g dry matter and the bran-bread 17 g/100 g dry matter. [d] According to Reference 13; analysis according to References 14 and 20 yielded the same result. [e] One type per day.

In addition, aliquot samples of each diet were chemically analysed. For each of the four experimental diets and for the control diet, all foodstuffs corresponding with the diet of a hypothetical subject consuming 2300 kcal/day were collected daily and stored at −20 C. At the end of the study the duplicate diets were homogenized, and a sample was freeze-dried and analysed for moisture, ash, crude protein, fat (17), fatty acid composition (18), cholesterol and plant sterols (19), free sugars (by gas chromatography), starch (20), Na^+, K^+ (using flame photometry), and Ca^{2+} and Mg^{2+} (by atomic absorption spectrophotometry). The total dietary fiber content of fiber-containing foodstuffs and of duplicate diets was measured as "unavailable carbohydrates plus lignin" according to the subtraction method of McCance et al. (14), as modified by Katan and Van de Bovenkamp (20), and also as "neutral detergent fiber" (13). To calculate the dietary fiber intake before the experiment, we used the above results and also figures from various authors (21–23). The pectin (polygalacturonic acid) content of the mixed diets and of samples of vegetables and fruits was analysed according to Galambos (15) and Keijbets and Pilnik (16), the method being modified by Katan and Van de Bovenkamp (20).

Analytical methods

Before the start of the study and at the end of the control and experimental period, two fasting blood samples were taken at 1-day intervals and the results were averaged. In addition, samples were taken once during weeks 2, 3, and 4 of the experimental period. The serum was analysed for total and high-density lipoprotein (HDL)-cholesterol. Serum cholesterol was measured according to Huang et al. (24) with Abell-calibrated sera (25) instead of cholesterol solutions for calibration, as described by Van der Haar et al. (26). Our laboratory is certified by the W.H.O. Collaborating Center for Research in Blood Lipids, Center for Disease Control, Atlanta, Ga. Long-term reproducibility for blind external control sera was 1.4% (coefficient of variation) and accuracy was within 1.8% of the "true" (target) values. Mn-heparin precipitation (27) was used to isolate HDL-cholesterol.

Feces were collected during the last 7 days of both periods. The stools were usually frozen within 12 hr of being passed and stored at −30 or −45 C. Mean transit time (MTT) through the gut was measured using radio-opaque pellets (28). Fecal primary (cholic acid and chenodeoxycholic acid) and secondary (deoxycholic acid and lithocholic acid) bile acids were determined by gas-liquid chromatography (29), as were cholesterol and the secondary neutral steroids (coprostanol, epicoprostanol, and coprostanone) (30). Thin-layer chromatography was omitted. Repeated determinations on a control pool of freeze-dried feces revealed an interassay variablity of 4.9% (coefficient of variation) for cholesterol and 5.1% for coprostanol. Long-term reproducibility for total bile acids was 5%. Fecal fat was measured as fatty acids after saponification (31), and electrolytes were measured after nitric acid digestion by flame photometry (Na^+, K^+) or

TABLE 2
Mean daily intake of nutrients according to individual food records[a]

	Habitual intake before experiment[b]	Control period	Experimental period			
		Low-fiber[c]	Low-fiber[d]	Vegetable/fruits[d]	Citrus pectin[d]	Bran[d]
Energy (MJ)	10.9	10.6	11.0	10.5	11.4	11.1
(kcal)	2595	2520	2610	2495	2715	2630
Protein (energy%)[e]	14	13	13	13	12	13
Vegetable	6	4	4	5	4	6
Animal	8	8	8	8	8	7
Total fat (energy%)	32	36	37	37	36	37
Saturated	14	18	19	19	18	18
Monounsaturated	11	12	12	12	11	11
Polyunsaturated	6	4	4	3	4	4
Carbohydrates (energy%)	49	48	48	48	48	48
Sugars	22	23	23	24	24	23
Polysaccharides	27	25	25	24	24	26
Alcohol (energy%)	4	2	1	1	2	2
Vitamin C (mg/1000 kcal)	46	71	71	69	56	68
Cholesterol (mg/1000 kcal)	115	141	145	143	143	136
β-Sitosterol (mg/1000 kcal)[f]		30	30	58	28	36
Sodium (mg/1000 kcal)[f]		1434	1436	1611	1482	1499
Potassium (mg/1000 kcal)[f]		1290	1296	1798	1210	1580
Calcium (mg/1000 kcal)[f]		572	583	615	548	553
Magnesium (mg/1000 kcal)[f]		119	117	134	118	232

[a] The food records were elaborated using Netherlands food composition tables supplemented with analyses of fiber-rich products. [b] Three-day records. [c] Two-day records. [d] Five-day records. [e] Percentage of daily energy intake. [f] Measured by analysis of duplicate portions, providing 2300 kcal/day.

atomic absorption spectrophotometry (Ca^{2+}, Mg^{2+}). Blood pressure was measured in the sitting position using an Elag (Köln, Germany) automatic recording sphygmomanometer, which uses phase IV (Korotkoff) as diastolic blood pressure.

Results

Nutrient intake

Table 2 shows the intake of nutrients during the study. There were only minor differences in intake of energy, protein, fat, cholesterol, carbohydrates and alcohol between the four dietary groups. Duplicate portions of foodstuffs were collected throughout the study for one imaginary person in each group. Chemical analysis of these showed a slight discrepancy with individual food records: in all four groups fat consumption was about 3 energy%[5] higher, and carbohydrate consumption about 5 energy% lower than planned.

With the vegetables/fruits diet and with the bran diet consumption of vegetable protein was 1 to 2 energy% higher than with the control diet. Vitamin C intake was about 30 mg/day lower in the pectin group than in the other three groups. Compared with the control diet, the vegetables/fruits diet and the bran diet contained about 1 g/day more potassium. The vegetables/fruits diet resulted in a slightly increased consumption of β-sitosterol.

Table 3 shows the total dietary fiber and polygalacturonic acid (pectin) content of the diets, planned and actual, as calculated from food records and by chemical analysis. Average consumption of dietary fiber amounted to 18, 43, 28, and 37 g/day in the control, vegetables/fruits, pectin and bran group, respectively; the average daily intake of polygalacturonic acid in these groups was 1.7, 7.5, 8.8, and 1.7 g/day, respectively (results of food records). It is clear that the planned differences in consumption of total dietary fiber between the four groups were indeed achieved. There was a variation of about twofold between the two analytical methods for total fiber. Analysis of duplicate portions indicated that in the vegetables/fruits and pectin groups, the consumption of polygalacturonic acid was somewhat lower than had

[5] Energy % = percentage of daily energy intake.

been planned. Both food records and chemical analysis showed that the intake of polygalacturonic acid was about 0.3 g/1000 kcal lower in the vegetables/fruits group than in the pectin group.

Serum cholesterol concentration

Concentrations of serum cholesterol throughout the experiment are recorded in **Table 4**. During the control period of 2.5 weeks the concentration of serum cholesterol increased in all four groups, because the control diet contained more fat and cholesterol than the habitual diets of the subjects (Table 1). During the 5 weeks thereafter, no significant change in serum cholesterol was observed in the control group. With the diet containing citrus pectin serum cholesterol decreased significantly ($P < 0.01$) by 0.34 mmole/liter, and with the vegetables/fruits diet it decreased by 0.17 mmole/liter (NS). In the vegetables/fruits group as well as in the pectin group, the decrease of serum cholesterol was more marked after 2 weeks than after 5 weeks ($P < 0.02$ for both groups).

Surprisingly, the bran diet caused an average *increase* in concentration of serum cholesterol of 0.34 mmole/liter at 5 weeks ($P < 0.01$). At 2 weeks the rise was not significant. Analysis of variance revealed that there was no difference between males and females in the response of serum cholesterol to the diets.

At the beginning of the study HDL-cholesterol concentration was on average 34% of total serum cholesterol. HDL-cholesterol concentration was 1.65 ± 0.43 mmole/liter (± 1 SD) in female subjects (n = 22), and 1.42 ± 0.24 mmole/liter in males (n = 40). None of the diets produced any significant effect on HDL-cholesterol levels (Table 4). There was no difference between males and females in response to the diets for this parameter.

Fecal output and intestinal transit time.

There was a remarkable variation in fecal output: in the control period fecal wet weight ranged from 10 to 210 g/day, and the percentage dry matter from 17 to 37%. Fecal weight was significantly enhanced in the vegetables/fruits group, and even more marked, in the bran group (by 49 and 77 g/day respectively; **Table 5**). However, in the vegetables/fruits group the average increase in fecal wet weight in female subjects was only 3 g/day, while male subjects showed an increase of 72 g/day. Three of the five female subjects in this group showed no change in fecal weight or a small decrease, while two females and all ten males showed an increase. On the other hand, in all 16 subjects taking bran fecal output was enhanced. In both the vegetables/fruits and the bran group the percentage dry matter in feces showed a small but significant decrease, and the number of stools produced per day increased. Inclusion of citrus pectin in the diet had no effect on fecal output.

During the low-fiber control period the 61 subjects showed a range of intestinal transit

TABLE 3
Mean daily intake of total dietary fiber and polygalacturonic acid per group

	Habitual intake before experiment	Control period	Experimental period			
		Low-fiber	Low-fiber	Vegetable/fruits	Citrus pectin	Bran
Total dietary fiber (g/1000 kcal)						
Planned		6.0	6.0	16.0	9.4	13.3
Actual; by food records	17.0	7.0	6.7	17.3	10.3	13.8
analysed as NDF[a]		9.7	6.4	12.0	11.0	13.9
analysed as unavailable carbohydrates[b]		14.8	14.9	26.5	19.8	25.0
Polygalacturonic acid (g/1000 kcal)						
Planned		0.6	0.6	3.3	3.3	0.6
Actual; by food records	1.4	0.6	0.6	3.0	3.3	0.6
by analysis[c]		1.0	0.9	2.7	3.0	1.0

[a] As neutral detergent fiber, according to Reference 13. [b] As unavailable carbohydrates plus lignin, according to References 14 and 20. [c] According to References 15 and 16.

TABLE 4
Total serum cholesterol and HDL-cholesterol per group (mean ± SD)

	Control (n = 16)	Vegetables/ fruits (n = 15)	Citrus pectin (n = 15)	Bran (n = 16)
		mmole/liter[a]		
Total serum cholesterol				
Before experiment	4.33 ± 0.68	4.18 ± 0.72	4.41 ± 0.75	4.28 ± 0.63
Change over control period	+0.36 ± 0.32	+0.37 ± 0.48	+0.37 ± 0.37	+0.37 ± 0.30
Change over experimental period				
at 2 weeks	+0.04 ± 0.40[b, c]	−0.36 ± 0.50[b, d]	−0.43 ± 0.39[d]	+0.18 ± 0.51[c]
Level of significance[e]	NS	$P < 0.02$	$P < 0.01$	NS
at 3 weeks	−0.05 ± 0.53	−0.32 ± 0.52	−0.40 ± 0.47	+0.32 ± 0.52
at 4 weeks	+0.05 ± 0.40	−0.19 ± 0.63	−0.27 ± 0.44	+0.38 ± 0.52
at 5 weeks	+0.10 ± 0.34[b, c]	−0.17 ± 0.63[b, d]	−0.34 ± 0.34[d]	+0.34 ± 0.41[c]
Level of significance[e]	NS	NS	$P < 0.01$	$P < 0.01$
HDL-cholesterol				
Before experiment	1.51 ± 0.22	1.41 ± 0.26	1.60 ± 0.46	1.49 ± 0.34
Change over control period	+0.01 ± 0.15	+0.03 ± 0.13	+0.05 ± 0.22	+0.02 ± 0.21
Change over experimental period	+0.01 ± 0.12[b]	+0.01 ± 0.15[b]	+0.02 ± 0.18[b]	+0.07 ± 0.16[b]
Level of significance[e]	NS	NS	NS	NS

[a] One mmole/liter = 38.7 mg/dl. [b–d] Common symbols indicate that responses of different groups are not significantly different from each other according to analysis of variance followed by a Turkey range test (9). [e] A paired *t* test was used to determine whether average changes per group were significantly different from 0.

TABLE 5
Fecal output and mean transit time per group (mean ± SD)

	Control (n = 16)	Vegetables/ fruits (n = 15)	Citrus pectin (n = 14)	Bran (n = 16)
Wet weight (g/24 hr)				
Control period	89 ± 54	89 ± 37	89 ± 34	89 ± 53
Change over experimental period	−1 ± 35[a]	+49 ± 44[b]	+10 ± 29[a]	+77 ± 31[b]
Level of significance[c]	NS	$P < 0.01$	NS	$P < 0.01$
Dry matter (g/100 g wet weight)				
Control period	26 ± 5	26 ± 6	25 ± 3	25 ± 5
Change over experimental period	+2 ± 4[a]	−3 ± 4[b]	+1 ± 2[a]	−3 ± 4[b]
Level of significance[c]	NS	$P < 0.05$	NS	$P < 0.01$
Frequency of stools (per 24 hr)				
Control period	0.8 ± 0.4	0.8 ± 0.3	0.9 ± 0.3	0.7 ± 0.3
Change over experimental period	0.0 ± 0.4[a]	+0.2 ± 0.3[a, b]	0.0 ± 0.4[a, b]	+0.3 ± 0.3[b]
Level of significance[c]	NS	$P < 0.05$	NS	$P < 0.01$
Mean transit time (hr)[d]				
Control period	73 ± 48	66 ± 40	59 ± 25	67 ± 28
Change over experimental period	+18 ± 39[a]	−13 ± 22[b]	+4 ± 35[a, b]	−19 ± 19[b]
Level of significance[c]	NS	$P < 0.05$	NS	$P < 0.01$

[a, b] Common symbols indicate that responses of different groups are not significantly different from each other according to analysis of variance followed by a Tukey range test (9). [c] A paired *t* test was used to determine whether average changes per group were significantly different from 0. [d] Data for 15 subjects in the control group.

times from 16 to more than 167 hr. The mean transit time was shortened by 13 hr in the vegetables/fruits group, and by 19 hr in the bran group (Table 5). No effect on transit time was found with the diet containing citrus pectin.

Fecal steroid excretion

Total excretion of bile acids and neutral steroids was on average 2.31 mmole/day at the end of the control period. Excretion of total steroids increased by 0.28, 0.48, and 0.47 mmole/day in the control, vegetables/fruits, and pectin group, respectively, while the bran group showed no change. The excretion of fecal steroids showed a peculiar sex effect, with males and females reacting to the diets in opposite ways (**Table 6**).

The average concentration of total steroids in feces amounted to 30 mmole/kg wet weight

TABLE 6
Fecal excretion of neutral and acidic steroids per group (mean ± SD)

	Control	Vegetables/ fruits	Citrus pectin	Bran
		mmole/24 hr		
Males	n = 10	n = 10	n = 10	n = 10
Neutral steroids				
Control period	1.55 ± 0.40	1.86 ± 0.64	1.73 ± 0.36	1.81 ± 0.50
Change over experimental period	−0.04 ± 0.52[a]	+0.93 ± 0.56[b]	+0.31 ± 0.34[a]	−0.09 ± 0.37[a]
Level of significance[c]	NS	$P < 0.01$	$P < 0.05$	NS
Bile acids				
Control period	0.78 ± 0.17	1.01 ± 0.42	0.77 ± 0.19	0.95 ± 0.40
Change over experimental period	+0.14 ± 0.29[a, b]	+0.03 ± 0.31[a, d]	+0.39 ± 0.21[b]	−0.22 ± 0.23[d]
Level of significance[c]	NS	NS	$P < 0.01$	$P < 0.05$
Females	n = 6	n = 5	n = 4	n = 6
Neutral steroids				
Control period	1.08 ± 0.64	1.58 ± 0.41	1.27 ± 0.79	1.11 ± 0.41
Change over experimental period	+0.56 ± 1.22[a]	−0.26 ± 0.40[a]	−0.30 ± 0.87[a]	+0.37 ± 0.29[a]
Level of significance[c]	NS	NS	NS	$P < 0.05$
Bile acids				
Control period	0.45 ± 0.36	0.71 ± 0.11	0.38 ± 0.21	0.34 ± 0.21
Change over experimental period	+0.01 ± 0.40[a]	−0.22 ± 0.26[a]	+0.20 ± 0.50[a]	+0.14 ± 0.10[a]
Level of significance[c]	NS	NS	NS	$P < 0.05$

[a, b, d] Common symbols indicate that responses of different groups are not significantly different from each other according to analysis of variance followed by a Tukey range test (9). [c] A paired t test was used to determine whether average changes per group were significantly different from 0.

TABLE 7
Fecal fat and electrolytes per group (mean ± SD)

	Control (n = 16)	Vegetables/ fruits (n = 15)	Citrus pectin (n = 14)	Bran (n = 16)
Fat (g/24 hr)				
Control period	1.6 ± 1.0	2.2 ± 1.9	1.6 ± 0.8	1.7 ± 1.1
Change over experimental period	+0.6 ± 1.1[a]	+0.7 ± 1.4[a]	+1.2 ± 1.3[a]	+1.2 ± 1.0[a]
Level of significance[b]	$P < 0.05$	NS	$P < 0.01$	$P < 0.01$
Na^+ (mmole/24 hr)				
Control period	1.0 ± 1.3	1.1 ± 0.7	0.9 ± 0.8	1.2 ± 2.0
Change over experimental period	−0.2 ± 1.2[a]	+1.2 ± 2.1[c]	+0.3 ± 1.0[a, c]	+0.5 ± 1.1[a, c]
Level of significance[b]	NS	$P < 0.05$	NS	NS
K^+ (mmole/24 hr)				
Control period	8.7 ± 4.8	9.0 ± 3.4	8.9 ± 3.7	8.2 ± 2.9
Change over experimental period	+0.2 ± 3.3[a]	+5.4 ± 4.3[c]	+0.9 + 3.9[a]	+10.7 ± 5.5[d]
Level of significance[b]	NS	$P < 0.01$	NS	$P < 0.01$
Ca^{2+} (mmole/24 hr)				
Control period	26.1 ± 12.2	27.9 ± 9.3	26.9 ± 10.7	25.3 ± 10.9
Change over experimental period	+0.9 ± 14.7[a]	+0.8 ± 6.8[a]	+2.3 ± 9.4[a]	+1.6 ± 7.5[a]
Level of significance[b]	NS	NS	NS	NS
Mg^{2+} (mmole/24 hr)				
Control period	6.7 ± 3.0	6.9 ± 2.3	7.4 ± 2.8	6.5 ± 2.9
Change over experimental period	+0.7 ± 3.2[a]	+1.0 ± 2.2[a]	+0.3 ± 2.5[a]	+6.1 ± 2.4[c]
Level of significance[b]	NS	NS	NS	$P < 0.01$

[a, c, d] Common symbols indicate that responses of different groups are not significantly different from each other according to analysis of variance followed by a Tukey range test (9). [b] A paired t test was used to determine whether average changes per group were significantly different from 0.

in the control period. This was decreased in the vegetables/fruits and bran groups by 8 and 15 mmole/kg wet weight, respectively ($P < 0.01$), while in the pectin group it was slightly increased by 3 mmole/kg wet weight ($P < 0.02$). At the end of the control period the ratio of secondary to primary bile acids was 19 ± 33. For neutral steroids this ratio

was 6.3 ± 3.9. During the experimental period no significant changes in these ratios were found in any group.

Fecal fat and electrolytes

The base-line excretion of fecal fat was on average 1.8 g/day. It increased slightly in all four groups, but the diet effects were not significantly different (**Table 7**).

The vegetables/fruits diet and the bran diet caused an increase in fecal excretion of Na^+ and K^+. With the bran diet Mg^{2+} excretion was almost doubled. Citrus pectin did not influence the fecal excretion of these electrolytes. Fecal excretion of Ca^{2+} was not affected significantly in any group (Table 7).

Blood pressure

Mean changes in systolic and diastolic blood pressure over the experimental period were of the order of 0 to 4 mm Hg in all four groups. None of them was significant, even at the 90% confidence level, except for the control group, which showed a decrease in systolic blood pressure of 5 mm Hg ($P < 0.10$).

Discussion

The physiological effects of dietary fiber appear to depend on its source and composition. In this study we investigated firstly whether the pectic substances (polygalacturonic acid) present in vegetables and fruits have the same lowering effect on serum cholesterol as isolated pectin, and secondly whether the other fiber components such as cellulose, hemicelluloses, and lignin, have the same effect on colonic functions when they are provided by vegetables and fruits as when they are provided by bran.

Control of nutrient intake

In a group of free-living subjects food intake is not easily controlled. Many of the controversies regarding the relationship between dietary factors and the concentration of serum cholesterol may originate in part from insufficient measurement or control of food intake. In this study all foodstuffs were measured out and supplied to the subjects, who were under the daily supervision of M.S.-W., H.F.F.A., J.G.C.v.J., and J.W.de J. No cash-bonus was offered, but all foodstuffs were supplied free of charge. The volunteers cooperated readily and a good relationship existed.

Measurements of nutrient intake showed that the observed effects of the diets on cholesterol metabolism and colonic functions are unlikely to have been caused by dietary factors other than the amount and type of dietary fiber. Chemical analysis of duplicate portions indicated that in all experimental diets fat consumption was higher (by about 3 energy%) and carbohydrate intake lower (by about 5 energy%) than the levels found by individual food records. Nevertheless, there were only minor differences in the intake of saturated and polyunsaturated fat, cholesterol, protein, and carbohydrates both between the four groups and between the control and experimental period. This is important as indicated in our previous publication (4).

The differences in intake of vegetable protein, vitamin C, and plant sterols between the four groups (Table 2) were almost certainly too small to have any significant effect on the concentration of serum cholesterol.

Both from duplicate portion analysis and from food records we conclude that the desired differences in total dietary fiber and pectin intake between the four groups were indeed achieved. The actual values, however, depended on the analytical methods used (Table 3). Especially in mixed diets, dietary fiber analysis is known to be problematic. The pectin intake in the vegetables/fruits group was slightly lower than planned, because the content of polygalacturonic acid in the vegetables and fruits actually eaten was lower than that of the products analysed before the study, on which the calculations were based.

Serum cholesterol concentration

The vegetables/fruits and the pectin diet resulted in a reduction in the concentration of serum cholesterol by 4 and 7% respectively, the effect of the vegetables/fruits diet not being statistically significant at 5 weeks. In both groups the effect was more marked after 2 weeks than after 5 weeks. Thus it appears that the dietary fiber from vegetables and fruits can lower serum cholesterol, but the

effect is much smaller than the well-known effects of lowering the degree of saturation and the amount of dietary fat and the amount of dietary cholesterol (32, 33).

The decrease in concentration of serum cholesterol with the diet containing citrus pectin is in agreement with earlier studies (5). The smaller effect on serum cholesterol with the vegetables/fruits diet as compared with the pectin diet, may be due to differences in the degree of esterification, molecular weight and in the physical environment of the polygalacturonic acid, but also to the slightly lower pectin intake, or to small changes in the consumption of polyunsaturated fat and cholesterol in the vegetables/fruits group in going from the control to the experimental period. Using the formulae of Keys et al. (32) or Hegsted et al. (33), we predicted that these changes would cause an increase in the concentration of serum cholesterol between 0.04 and 0.22 mmole/liter, whereas in reality a decrease of 0.17 mmole/liter was found.

Few controlled studies on the effects of vegetables and fruits on serum cholesterol and colonic function have been published. Gormley et al. (34) observed a reduction in serum cholesterol in a group of 38 men, who consumed at least two apples a day for four months; nutrient intake, however, was not controlled. Grande et al. (35) showed in a controlled study that consuming vegetables (500 kcal/day) significantly reduces the concentration of serum cholesterol, but Behall et al. (36) found no effect on serum cholesterol of a diet with vegetables and fruits. It is possible that the effects of certain types of fruits and vegetables are obscured when a mixed diet is used. In our study we used five types of vegetables which prior analysis had revealed to be rich in pectic substances (Table 1).

Inclusion of bran in the diet caused a significant increase in the level of serum cholesterol by 7%. As discussed elsewhere (37), consumption of bran has often, but not always (38), been found to be associated with a small increase in the concentration of serum cholesterol, although to our knowledge statistical significance was achieved in only one experiment (39). Discrepancies between studies with bran may be explained by concomitant changes in fat and cholesterol consumption (40), as in most cases nutrient intake has not been strictly controlled, or by differences in the type of bran used (particle size, water-holding capacity, fiber composition, etc.), or by the form in which the bran is consumed, i.e., raw or incorporated into bread.

It should be emphasized that we do not know how much time is needed for the effects of dietary fiber on serum lipids to reach an equilibrium, although it seems that the serum cholesterol levels stabilized at 5 weeks (Table 4).

Serum HDL-cholesterol

The amount and type of dietary fiber had no substantial effect on the level of serum HDL-cholesterol over the experimental period of 5 weeks. For citrus pectin and bran this confirms the results of earlier reports (41, 38), while results of controlled experiments with vegetables and fruits have not been published as far as we know. In our earlier study (4) we found a small reduction of HDL-cholesterol on the high-fiber diet. In retrospect, this effect may have been due to changes in fat and carbohydrate consumption that occurred in that experiment, rather than to the fiber itself.

Fecal excretion of cholesterol metabolites and fat

The effects of dietary factors on the level of serum cholesterol may sometimes be explained by changes in the removal of cholesterol metabolites (neutral steroids and bile acids) from the body with the feces.

The excretion of fecal bile acids and neutral steroids was increased in the male subjects of the groups taking citrus pectin or vegetables and fruits, but not in the females of these groups. In contrast, fecal steroid excretion was reduced in males in the bran group, whereas in females it was enhanced (Table 6). Sex differences in excretion of fecal steroids have not been demonstrated earlier, probably because most studies have been conducted on men. It is not clear why these differences appear, but they could not be explained by differences in fat and cholesterol consumption.

Pectin has been reported to enhance the excretion of both bile acids and neutral steroids (42, 43). The pectin dose in these studies

was 15 and 40 to 50 g/day, respectively, as opposed to about 9 g/day in this study. Results for bran are variable (44–46). In two studies steroid excretion was not changed during the period of bran feeding, but increased after the bran had been stopped (47).

From our results it seems clear that the effects on serum cholesterol can be explained only partly by changes in steroid excretion. There was no correlation between individual changes in serum cholesterol concentration and changes in excretion of bile acids or neutral steroids ($r = -0.11$ and -0.10; NS). There was also no connection between the excretion of fecal fat and the change in concentration of serum cholesterol.

It has been suggested that a high consumption of dietary fiber protects against the development of colon cancer (48). According to this hypothesis, with a high-fiber diet the conversion of primary steroids into secondary, possible (co)carcinogenic compounds is reduced, and the concentration of these products in feces is lower. The literature on this subject is conflicting (49). The concentration of fecal steroids was indeed decreased with the vetabables/fruits and the bran diet, because of fecal bulking. We found, however, no consistent changes in the ratio of secondary to primary steroids. There was a significant, but very weak correlation between the individual changes in transit time and the secondary to primary bile acid ratio ($r = 0.25$) or the secondary to primary neutral steroid ratio ($r = 0.26$; $P < 0.05$).

Colonic functions

With the vegetables/fruits diet and with the bran diet stools were significantly bulkier, wetter, and more frequent, and intestinal transit was marginally faster. These effects were more marked with the bran diet. Citrus pectin did not affect colonic functions. These effects are similar to those found in other studies (4, 7, 42, 46, 50, 51, 53).

It is not clear why three female subjects in the vegetables/fruits group showed no change in fecal weight or even a decrease, while in all males it was enhanced. Stool marker recovery indicated that this sex difference was not due to incomplete collection of stools. The normal variability of colonic functions is known to be very great, but in a group of 10 healthy females Wyman et al. (52) found no obvious changes related to the phases of the menstrual cycle. In the group consuming bran there was no difference in colonic response between males and females.

A widely differing colonic response to increased fiber intake between individuals was reported earlier (53). In spite of this, we found a strong correlation between the logarithm of fecal weight and the logarithm of transit time ($r = -0.82$).

Cummings et al. (53) found a high correlation between group means of changes in fecal weight and increase in intake of pentose-containing polysaccharides. Using Dr. D. A. T. Southgate's data for pentosan content of foodstuffs, we found a similar result for group averages ($r = 0.95$; $n = 4$). However, for individual data, the correlation was not significant ($r = 0.16$; $n = 61$). Our results thus give only weak support to the suggestion that the pentose fraction of dietary fiber is mainly responsible for the changes in stool weight.

Binding of water by fiber itself or by its fermentation products has been suggested as a mechanism for fecal bulking. The water-holding capacity of bran was found to predict the effect on stool weight (12), but the increase in stool weight in subjects consuming carrot fiber was far less than was expected from the water-holding capacity (47). Thus, fermentation products such as fatty acids or even microbial cell mass may through their own hydration and absorption capacities contribute to the effects of fiber on total mass and water content of stools (54). On the other hand, in fruits and vegetables it may be the fiber residue left after bacterial hydrolysis that is responsible for the increase in fecal weight.

Electrolyte balance and blood pressure

With the high-fiber diets fecal excretion of various electrolytes was increased, in agreement with other studies (55). The enhanced fecal output of Na^+ and K^+ on the diets with vegetables and fruits and with bran is of little importance because only approximately 1% of dietary sodium and 10% of potassium were excreted by this route. At 5 weeks the excretion of Ca^{2+} was unaffected by any of the diets. According to James et al. (56) the ability to maintain calcium balance on high-fiber diets may depend on the adaptive capacity of the colon for calcium absorption, as in the colon the microbial digestion of uronic acids to which calcium is mainly bound, will lib-

erate the calcium. The enhanced intake of Mg^{2+} on the bran diet (Table 2) resulted in an enhanced fecal excretion. Independent of the intake level about 70% of the daily intake of Ca^{2+} and 60% of dietary Mg^{2+} was recovered in the feces.

Thus, in this short-term study no deleterious effects on Mg^{2+} or Ca^{2+} absorption were observed. However, prolonged balance studies are needed to investigate the mineral binding properties of dietary fiber and to test the possiblity of long-term adaptation (55).

Although there are indications that a diet rich in vegetables and fruits will lower blood pressure due to its high potassium content (57), we observed no such effect. All our subjects had diastolic blood pressures below 80 mm Hg; it might be that a favorable effect is obtained only in hypertensives (50).

In conclusion, it appears that some of the dietary fiber components of fruits and vegetables lower concentrations of serum cholesterol, while others can improve colonic function. Bran also has a favorable effect on colonic function, but in this short-term controlled study it increased serum cholesterol. Although the favorable effect of vegetables and fruits on serum cholesterol is small compared to the known effects of dietary fat and cholesterol, in uncontrolled conditions dietary fiber may indirectly reduce the concentration of serum cholesterol by displacing fat-rich products from the menu (4).

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