Journal of Surgical Oncology 52:77–82 (1993)

The Effect of the Fiber Components Cellulose and Lignin on Experimental Colon Neoplasia

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Sixty Sprague-Dawley rats were pair-fed one of three nutritionally identical diets. One diet contained "low-fiber" (3.8% crude fiber); the others contained "high fiber" (28.7% crude fiber) composed of either cellulose or lignin. Although both "high fiber" diets had similar stool bulking effects, only the cellulose diet was associated with a reduction in 1,2-dimethylhydrazine (DMH)-induced colon neoplasms. The cellulose diet was also associated with distinct changes in the gut bacterial profile and with a lowered serum cholesterol. © 1993 Wiley-Liss, Inc.

KEY WORDS: **dietary fiber, gut bacteria, rats**

INTRODUCTION

Colorectal cancer is the second most common visceral malignancy in the United States and accounts for a major number of cancer-related deaths in developed countries [1]. Considerable interest has been focused on the role of dietary fiber in the pathogenesis of this disease, from both epidemiologic and experimental perspectives [2–7]. Using the 1,2-dimethylhydrazine (DMH) model [8–10], our group and others have demonstrated that bran-fed animals developed fewer colon neoplasms than did animals fed a low fiber diet [11,12]. In yet other studies, however, the opposite effect has been demonstrated, i.e., more tumor in animals fed bran [13,14]. Also, there is evidence that the different kinds of bran differ in their anti-tumor effect [14–16].

Bran is composed of a number of complex substances [17,18] including cellulose, hemicellulose, lignin, pectin, and guar gum. Cellulose has been the most studied fiber. Adding cellulose to animal diets has generally resulted in a reduction of experimental tumors [19–24], whereas the addition of other fiber components such as guar gum and pectin has proved ineffective [22,25]. Even though lignin is a major constituent of bran, it has received minimal attention in the colon cancer model. A single study suggests a protective effect [16].

Since bacteria appear to be linked to colon carcinogenesis [26–28], both experimentally and epidemiologically, we elected to take advantage of the strict nutritional control in this experiment and study the effect on the resident bacteria of adding different fiber components.

MATERIALS AND METHODS

Sixty Sprague-Dawley weanling rats were housed individually in wire mesh metabolic cages. The animals were treated with subcutaneous injections of DMH (Aldrich Chemical Corp., Milwaukee, WI) once a week for twenty weeks at a dose of 20 mg/kg in 0.9% saline solution at pH 7.2.

The animals were randomly divided into three dietary groups and were fed using pair-feeding techniques. The amount of food was weighed out daily in such a manner that each animal consumed the same amount of the basic diet. This diet consisted of standard rat chow (Charles River RHM 2000, Agway Inc, Syracuse, NY) and was considered to be the low fiber (LF) diet. The high fiber diets were produced by adding purified cellulose (Purified Cellulose [Alphacel], ICN Industries, Cleveland, OH) (HFC) or lignin (Purified Spruce Lignin [Indulin ATRC], Westvaco Inc., Charleston, SC) (HFL) to the low fiber diet.

Table I shows the composition of the diets. Each rat was given the same amount of basic rat chow, and each

Accepted for publication October 23, 1992.

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TABLE I. Daily Food Consumption Per Rat Per Day

	Low fiber (3.8%)	High fiber (28.7%) Cellulose	High fiber (28.7%) Lignin
Total diet	17.5 gm chow	17.5 gm chow +6.0 gm alpha	17.5 gm chow +6.0 gm ind
Fiber	0.7 gm	6.7 gm	6.7 gm
Protein	3.2 gm	3.2 gm	3.2 gm
Fat	1.6 gm	1.6 gm	1.6 gm
CHO	9.1 gm	9.1 gm	9.1 gm
Ash	1.2 gm	1.2 gm	1.2 gm
Water	1.7 gm	1.7 gm	1.7 gm
KCAL	80.5	80.5	80.5

TABLE II. Biochemical Profile (Mean ± SD)*

Diet	LF	HFC	HFL
Serum albumin g/dL a	3.39 ± 0.41	3.35 ± 0.42	3.50 ± 0.22
Serum glucose mg/dL b	140.8 ± 40.7	139.7 ± 53.9	152.6 ± 12.2
Serum cholesterol mg/dL c	74.4 ± 7.4	64.0 ± 6.9	75.9 ± 6.7

*Differences between groups for a and b not significant for c; LF compared to HFC, $P < .01$; HFC compared to HFL, $P < .005$; LF compared to HFL, not significant (Student's t test).

portion was completely consumed. It should be noted that the cellulose and lignin components are non-nutritive and simply dilute the chow. The animals were fed their respective diets for three weeks before DMH injections were begun. Forty-eight hour stool collections were done periodically, and the stool weight and total number of pellets were recorded. Transit times were measured by gavage-feeding carmine-red dye and recording the first appearance of dye in the stool.

The animals were killed by cervical dislocation at weeks 29–30 of the experiment. Complete necropsies were performed on all animals. On the day of necropsy, 5 mL of blood was taken from each animal and used to measure serum albumin, glucose, and cholesterol. The Technicon SMAC system was utilized for this test. Statistical analysis of this data was carried out using the Student's t test and χ^2 analysis.

Each tumor was resected, embedded in paraffin, and stained with hematoxylin and eosin. Adenomas and carcinomas were distinguished by using criteria similar to those set forward by Madara et al. [29].

At the time of necropsy, one-gram specimens of stool were taken from the ceca of eight animals in each group and added to sterile containers containing 9 mL of thioglycollate broth. The exact weight of the added feces was calculated, and thioglycollate broth was added to bring the dilution of feces to the nearest convenient decimal dilution. A Lidwell phage applicator with calibrated loops was used to deliver 0.005 mL of each dilution to the surface of the media in a uniform inoculum. All inoculations were performed in triplicate. A number of standard media were used, and incubation was carried out in standard form. Thirteen bacterial groups were examined. Colonies were counted, and statistical comparison of log bacterial numbers per gram of feces was carried out by two-tailed Student's t test.

RESULTS

The results of the pair-feeding experiments revealed that, although the two HF-fed groups ingested a greater volume of food with the same nutritional value as the LF-fed group, weight gain was identical in the two fiber groups. Serum albumin levels of all three groups were not significantly different. Serum cholesterol was significantly reduced in the HFC animals compared to the LF ($P < .01$) or HFL group ($P < .005$) (Table II).

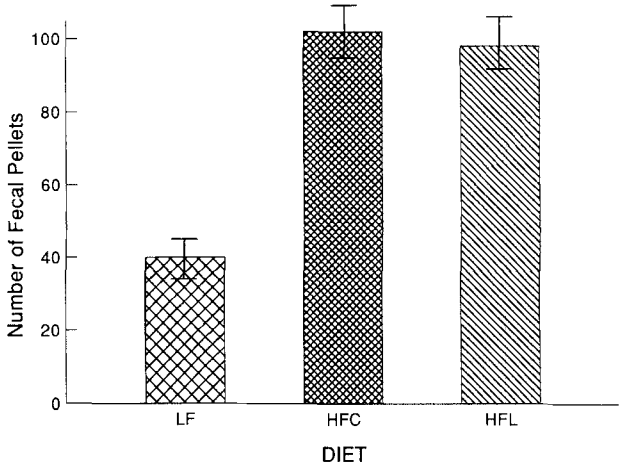

Fig. 1. Average number of fecal pellets per 48-hour collection (mean ± SD). Low-fiber diet (LF) compared to high-fiber-cellulose diet (HFC) or high-fiber-lignin diet (HFL). $P < .001$ (Student's t test).

Stool weights and the frequency of stool pellets (Fig. 1) were greater in both "high-fiber" groups when compared to the "low-fiber" group ($P < .001$).

Animals fed the LF diet had a significantly longer transit time (Fig. 2) than did the cellulose group ($P < .01$). The transit time of the lignin-fed animals is not shown, because detection of the marker was difficult due to the dark color of the stool.

None of the animals died of tumor or other disease prior to the end of the experiment. Necropsy examination revealed numerous tumors of the large and small bowel. These were polypoid, sessile, or ulcerating in nature. They varied in size from 1 to 10 mm. Tumors occurred in the proximal small bowel, ascending colon, and descending colon. Histology demonstrated both adenomas and adenocarcinomas (Fig. 3). The latter varied in the degree of differentiation.

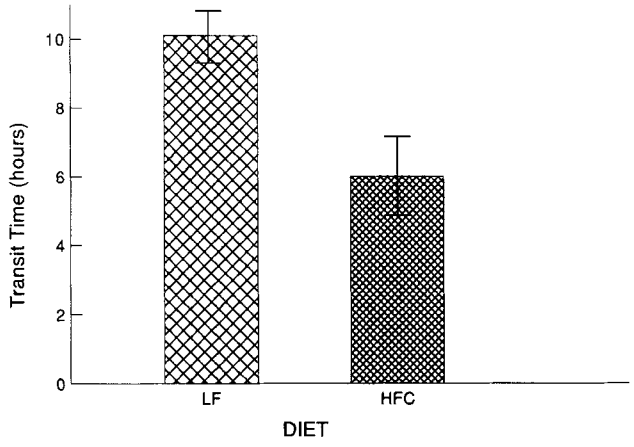

Fig. 2. Average transit time (mean ± SD). Low-fiber diet (LF) compared to high-fiber-cellulose diet (HFC), $P < .001$ (Student's t test).

The percentage of animals developing tumors (both large and small bowel) is shown in Figure 4. The LF group had the greatest risk for tumor development (75%), followed by the HFL group (65%) and then the HFC group, which had the least risk (33%) (LF vs. HFL, $P > .5$; LF vs. HFC, $P < .025$). When only colon tumors are considered (Fig. 5), 27% of the HFC group developed tumors, whereas 60% of both the LF and HFL groups developed tumors ($P < .05$). The number of tumors per animal is summarized in Table III. Within each group, there is marked variation from animal to animal, although the differences are not significant at the 5% level. However, the fewest tumors per animal occurred in the HFC group. Histology of the tumors revealed that the proportion of carcinomas to adenomas was similar in each group. Also, when comparing groups, we found no difference in the distribution of tumors greater than four mm in their greatest dimension. These findings are summarized in Table IV.

Sections taken through the prominent lymphoid follicles in the ascending colon revealed some microscopic foci of neoplasia. In addition, several well-differentiated squamous cell carcinomas of the external auditory canal were found.

Table V lists the bacterial groups consistently isolated from the fecal specimens and shows their respective colony counts in log form. Groups such as *Clostridia*, anaerobic *cocci*, and *Staphylococcus aureus* were not consistently isolated. Each dietary group is compared statistically to the other two in Table VI, and the direction of change is indicated for each bacterial group. Of particular interest are the increase in coliform in the HFC group when compared with the other two groups, and the lack of difference between the HFL and LF groups, because these two groups had more tumors than the former group and were similar to each other.

DISCUSSION

After lung cancer, colon cancer is the most common malignancy in the US and represents an important cause of mortality. There is a tremendous variation world-wide in the incidence of colon cancer. For example, the incidence in white men in Connecticut is 34.1 per 100,000, whereas the incidence in native Kuwaiti men is .2 per 100,000 [30]. Observations in central Africa led Burkitt to postulate that "high-fiber" diets had a bulking effect leading to more rapid intestinal transit. This bulking effect, he suggested, might lead to dilution and more rapid excretion of carcinogens [31]. Epidemiologic studies have shown a 62% reduction in mortality from colon cancer in Seventh Day Adventist males when compared to other white American males [32]. Seventh Day Adventists adhere to a strict high-fiber diet, as do Mormons, who also have a lower incidence of colon cancer [33]. While the epidemiologic relationship between high dietary-fiber intake and low colon-cancer incidence has been apparent for some time, it is only recently that evidence of fiber's efficacy in the clinical setting has emerged. Alberts et al. showed that wheat bran decreased the growth of rectal adenomas in patients with familial polyposis [34]. There is, in addition, evidence that wheat bran reduces fecal mutagenicity [35]. Not all studies, however, support the protective role of bran [13,14]. The subject is further complicated by the fact that there are several types of bran, and there is evidence that these types differ in effect. For example, wheat bran is much more protective than is corn bran [14–16]. Further, each type of bran is made up of a number of individual components, including cellulose, hemicellulose, pectin, lignin, and guar gum. The difficulty, then, is identifying which fiber component is the active agent in reducing colon neoplasia.

How important is the bulking effect of dietary fiber? The fiber component pectin has been shown to have no bulking effect and has demonstrated no protective effect in the colon cancer model [22,25]. In our study, both lignin and cellulose had similar stool-bulking effects (Fig. 1). In spite of this, only the cellulose-fed animals were protected against tumor induction (Figs. 4 and 5). This suggests that bulk and transit times may play secondary roles in colon carcinogenesis and that the type of fiber is important. That stool bulking may be of lesser importance is further supported by studies demonstrating that DMH-treated animals fed the bulking agent Metamucil were not protected from colon carcinogenesis [36].

Cellulose is probably the most widely studied of the fiber components and has generally been associated with a reduction in chemically-induced bowel cancer [19–24]. There is some evidence that this effect is dose-related [23]. Only a single study has examined lignin, the other major crude fiber component of bran. Reddy et al. [16] demonstrated fewer 3,2'-dimethyl-4-aminobiphenyl

Fig. 3. 1,2-Dimethylhydrazine (DMH)-induced colon adenocarcinoma.

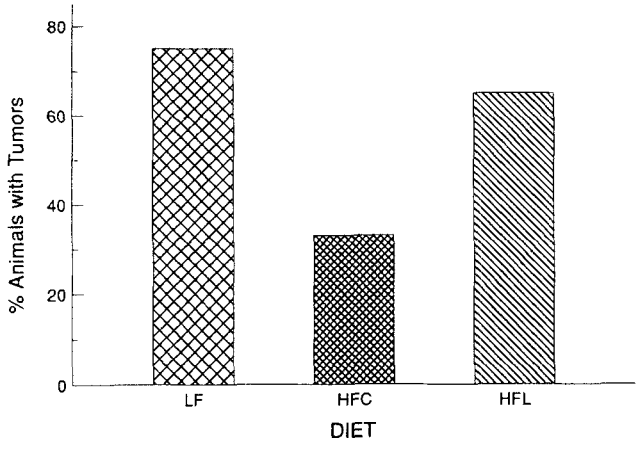

Fig. 4. Percentage of animals with tumors (large and small bowel included). Low-fiber diet (LF) compared to high-fiber-cellulose diet (HFC), $P < .025$, or high-fiber-lignin diet (HFL), $P > .5$ (Chi square).

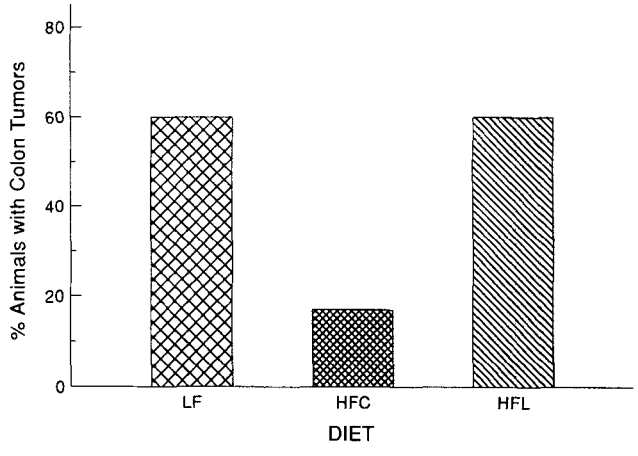

Fig. 5. Percentage of animals with colon tumors. Low-fiber diet (LF) and high-fiber-lignin diet (HFL) compared to high-fiber-cellulose diet (HFC), $P < .05$ (Chi square).

(DMAB)-induced intestinal tumors in animals fed a 7.5% lignin diet. In vitro, lignin acts as an absorbent for many organic substances including the potently carcinogenic nitrosamines and bile salts. This characteristic sharply contrasts lignin to cellulose, a poor binder [37]. Bile acids have been shown to act as co-carcinogens [29,38,39], and cholestyramine, a strong binder of bile acids [40], has been associated with increased tumor formation [41], possibly by interrupting the entero-hepatic circulation and delivering an increased amount of bile acid to the colon where it can have its co-carcinogenic action. Reddy et al. [16] showed increased daily output of fecal bile acid in animals fed lignin. In this experiment, the lack of protection afforded by lignin may be due to an effect similar to that of cholestyramine offsetting any protective tendency afforded by its bulking effect. Unfortunately, we do not have data on the fecal bile acids in the cellulose- and lignin-fed animals. Part of the explanation for the different anti-neoplastic effects of these two fiber components may relate to differential effects on stool bile acid metabolism. Further study is clearly needed, but our data would appear to support the contention that wheat

TABLE III. Tumor Profile Per Animal (Mean ± SD)*

Diet	LF	HFC	HFL
Total tumors per animal a	1.15 ± 0.85	0.66 ± 1.24	1.40 ± 1.54
Colon tumors per animal b	0.80 ± 0.74	0.44 ± 0.95	1.05 ± 1.20

*For all comparisons P is not significant (Student's t test).

TABLE IV. Tumor Profile*

Diet	LF	HFC	HFL
% carcinoma/total tumors a	60 (14/23)	75 (9/12)	71 (20/28)
% of animals with tumors >4 mm b	45 (9/20)	22 (4/18)	35 (7/20)
% of animals with colon tumors >4 mm c	25 (5/20)	17 (3/18)	25 (5/20)

*Numbers in parentheses are absolute values. Differences for a, b, or c are not significant (Chi square).

TABLE V. Bacterial Colony Log Counts*

	Diet		
Bacteria	LF	HFC	HFL
Coliforms	3.2 ± 2.06	5.5 ± 1.1	3.2 ± 1.5
Enterococci	5.1 ± .096	5.5 ± 0.58	4.7 ± 0.40
Lactobacilli	9.1 ± 0.42	8.6 ± 0.42	9.0 ± 0.34
AT lactobacilli	7.4 ± 1.0	8.7 ± 0.39	7.8 ± 0.52
ATAN lactobacilli	8.7 ± 0.64	8.4 ± 0.41	9.0 ± 0.43
Bifidobacteria	7.4 ± 0.61	7.2 ± 0.51	7.2 ± 0.49

*Bacterial colony counts/gm of cecal stool (mean log ± SD). Five to eight animals used for each value. See Table VI for P values.

TABLE VI. Cecal Bacterial Profile, P Values*

	Diet		
Bacteria	HFC LF	HFC HFL	LF HFL
Coliforms	↑<0.02	↑<0.01	NS
Enterococci	NS[a]	↑<0.01	NS
Lactobacilli	↓<0.05	NS	NS
AT lactobacilli	NS	NS	NS
ATAN lactobacilli	NS	↓<0.02	NS
Bificobacteria	NS	↓ 0.02	NS
Bacteroides	NS	NS	NS

*Statistical comparison of log bacterial numbers. See Table V for mean values (Two-tailed Student's t test).
[a]NS, not statistical.

bran consists of a number of different substances which exert opposing effects on colon neoplasia.

Burkitt's hypothesis attaches considerable importance to the effect of fiber on the bacteria on the colon [32]. He suggests that low-fiber diets leads to "fecal arrest" with resultant alterations in the bacterial population. This study attempts to define the effect of purified fibers on the fecal flora in an experimental setting where nutritional parameters have been rigidly controlled. Tables V and VI show that cellulose and lignin affected the gut bacteria in different ways. It is striking that the two experimental groups with a high incidence of intestinal tumors (LF and HFL) exhibited no statistical difference in their bacterial profiles (Table VI). Only the cellulose group exhibited distinct changes.

Bacteria are believed to play a role in colon carcinogenesis [26]. Researchers have found that populations with a high incidence of colon cancer also have higher stool *Bacteroides* counts. These bacteria, in turn, have been shown to be metabolically more active in degrading bile salts [27]. Such degraded bile salts are present in greater concentration in the stools of high risk groups [28] and have been shown to act as co-carcinogens in the experimental setting [29]. The significance of the bacterial changes induced by the cellulose diet is uncertain, but it appears to indicate that gut bacteria play a potential role in the induction of these tumors.

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