Acta Medica et Biologica
Vol. 45, No. 2, 69-73, 1997

Effects of Dietary Factors (Fat and Fiber) on Experimental Colon Carcinogenesis and Cancer Growth in Rats

Kazuhiko ENDO, Katsuyoshi HATAKEYAMA, *Jun SOGA and **Terukazu MUTO

The First Department of Surgery, *College of Biomedical Technology and **Office of the University President, Niigata University School of Medicine, Niigata, Japan

Received June 13 1996; accepted April 7 1997

Summary. **The effects of dietary fat and fiber on colonic carcinogenesis and tumor growth in rats were assessed. Over a period of 30 weeks, four groups of 10 animals were fed diets with: high fat and high fiber content; high fat content and no fiber; no fat and high fiber content; or no fat and no fiber. Five weeks after starting these diets, each group was divided into two subgroups, one receiving 10 weekly injections of the carcinogen 1, 2-dimethylhydrazine (DMH); (20 mg/kg/week). The other (control) subgroups recieved no DMH. At 30 weeks the rats were killed and autopsies were performed. Results showed cancers were limited to rats receiving DMH. The average numbers of cancers per rat were 4.8±2.6 for the high fat and fiber group; 6.2±4.6 for the high fat, no fiber group; 2.3±1.5 for no fat, high fiber; and 2.2±0.8 for no fat, no fiber. High-fat animals tended to have more DMH-induced cancers than no-fat animals. Aggregate cancer volume per rat was smallest for the no-fat, high-fiber diet.**

Key words—1,2-dimethylhydrazine (DMH), experimental colon cancer, dietary factors, fat, fiber.

INTRODUCTION

Colon cancer incidence is high in Europe and the US and low in Asia, Africa, and South America.[1-3] Epidemiologic studies have related this difference to enviromental rather than genetic factors, and particularly to eating habits.[4-6] In recent years the incidence of colon cancer in Japan has increased rapidly along with the westernization of eating habits, specifically, the consumption of more animal fat and less fiber.[3,7] With this in mind, we sought to examine the effects of dietary fat and fiber in enhancing experimental carcinogenesis and on tumor volume.

Correspondence: Kazuhiko Endo M.D., Akita General Hospital, Minatochuo 4-5-45, Tsuchisaki, Akita 011, Japan.

MATERIALS AND METHODS

Animals and diets

Forty 8-week-old male Wistar rats (Charles River Japan, Kanagawa), each weighing 250 to 280 g, were divided into four groups of 10 placed on one of the following diets (Table 1): Group I, high-fat, high-fiber (25% beef tallow, 27% cellulose); Group II, high-fat, no-fiber (25% beef tallow); Group III, no-fat, high-fiber (27% cellulose); and Group IV, no-fat, no-fiber diet supplied by Oriental Yeast Industry, Tokyo, Japan. Crude protein, minerals, and vitamins were comparable in all four diets, and animals had free access to their diets and to water. Institutional and National Research Council guidelines for the care and use of laboratory animals were followed. There were no significant differences between groups in amount of food consumed.

Administration of carcinogen

After the first 5 weeks, each group was divided equally into "a" and "b" subgroups. While "b" subgroups served as controls, animals in the "a" subgroups were given the carcinogen 1, 2-dimethylhydrazine (N,N′-dimethylhydrazine dihydrochloride, or 1, 2-dimethylhydrazine (DMH); Nakarai Chemicals, Kyoto). DMH was dissolved in 0.02 mM ethylenediamine tetraacetic acid (EDTA) and injected subcutaneously at 20 mg/kg of body weight into the femoral region once a week for 10 consecutive weeks

Table 1. Dietary composition

	Crude nutrient content (adjusted to 10% water content)			
	Group I High fat High fiber	Group II High fat No fiber	Group III No fat High fiber	Group IV No fat No fiber
Moisture (%)	10	10	10	10
Crude fat (%) (Beef tallow)	25.0	25.0	0	0
Crude fiber (%) (Cellulose)	27.0	0	27.0	0
Crude protein (%)	14.5	14.5	14.5	14.5
Nitrogen-free extractives (%)	20.3	47.3	45.3	72.3
Ash (%)	3.2	3.2	3.2	3.2
Total (%)	100	100	100	100
kcal/100 g	364	472	239	347

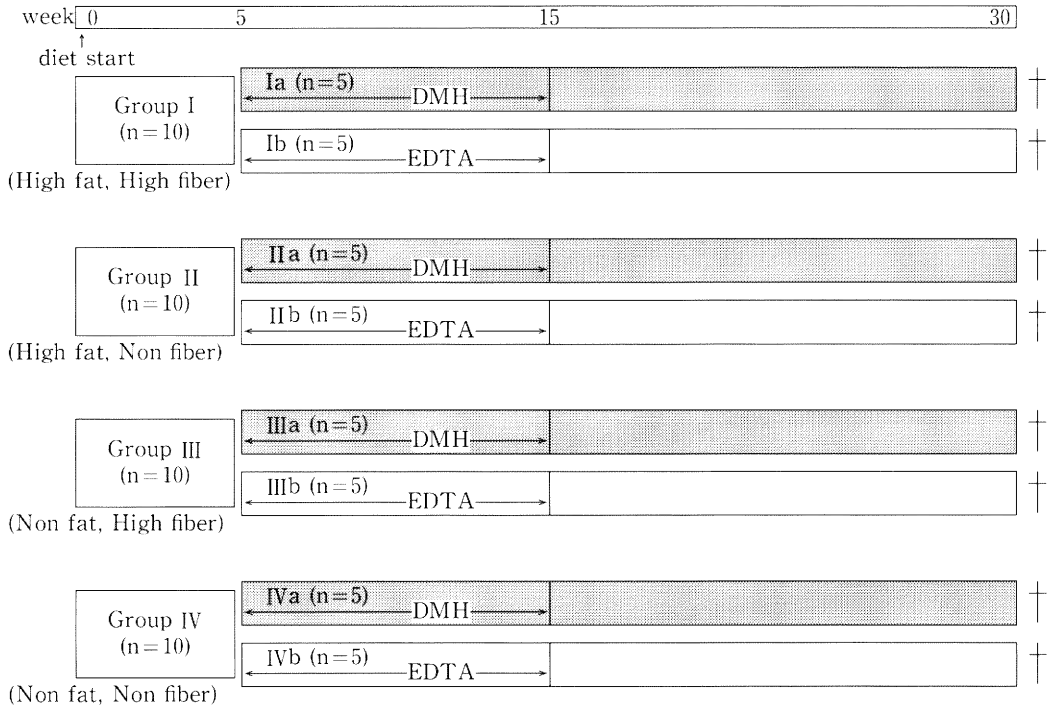

Fig. 1. Experimental protocol. DMH, 1,2-dimethylhydrazine; EDTA, ethylenediamine tetraacetic acid; †, autopsy.

(Fig. 1). The "b" controls were injected instead with comparable volumes of EDTA solution.

Histopathological examination of colon cancer

All animals were kept on their diets for 30 weeks, after which they were killed under ether anesthesia and subjected to autopsy. Abdominal organs were examined grossly. The entire large intestine was removed, including the perianal skin. After a longitudinal incision was made along the mesenteric attachment, the large intestine was fixed in 10% formalin.

The large intestine then was divided into the cecum (C); the proximal colon (P), where the mucosal folds had a herringbone configration; the mid-colon (M), representing the proximal half of the remaining specimen; and the distal colon (D). Length, width, and height were measured for all grossly identifiable cancerous lesions. Tumors then were paraffin-embedded, sectioned at 4 μm, stained with hematoxylin and eosin (HE), and examined microscopically. Histopathologic examination was conducted in accordance with the rules for managing colon cancer.[8] Cancer volume (cm^3) was calculated using the formula $v=\pi hr^2$ where v=volume, h=height, and r=(length+width)/4.[9]

For each rat, the volumes of individual tumors were totaled.

Statistical analysis

All values were expressed as mean±SD. Group comparisons initially were made using the analysis of variance (ANOVA) and then examined by multiple comparison of mean values (Duncan). Differences were considered statistically significant when p was less than 0.05.

RESULTS

Extracolonic findings

No hepatic metastasis or peritoneal tumor dissemination was observed in any group during laparotomy. One animal in each of the subgroups IIa and IVa had developed duodenal cancer in addition to colon caner. Two animals in each subgroups Ia and IIa had developed tumors of the external ear. No control "b" animals had tumors.

Colon cancers

No significant differences were noted in the amount of food consumed between groups. All colon tumors were cancers; none were adenomas. Except for one no fat, high-fiber animal in subgroup IIIa, all animals receiving DMH developed colon cancer, so that the incidence of colon cancer did not differ significantly among DMH groups. To exclude what may have been a chance outcome in one animal, a conservative approach was taken and the rat in subgroup IIIa developing no tumors was excluded from data analysis. In contrast, none of the control animals developed colon cancer. No differences were appreciated in gross mucosal findings among the control groups.

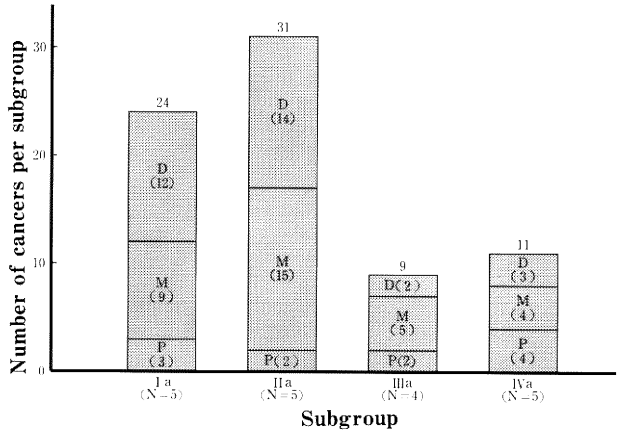

Fig. 2. Cancers by subgroup and colonic segment.
D, distal colon; M, mid-colon; P, proximal colon. No cancers occurred in the cecum (C).

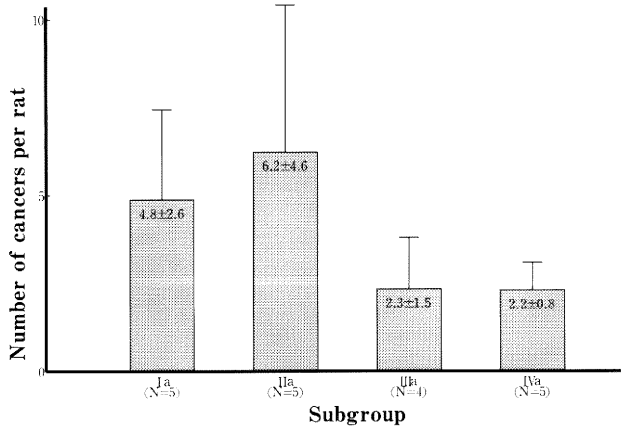

Fig. 3. Cancers per rat (mean±SD).
Differences were not statistically significant (NS).

Table 2. Cancer number and total cancer volume per subgroup

Subgroup	Cancer number	Cancer volume (cm^3)
Ia	24	1.72
IIa	31	3.92
IIIa	9	0.23
IVa	11	1.69

Each value represents the total for five animals.

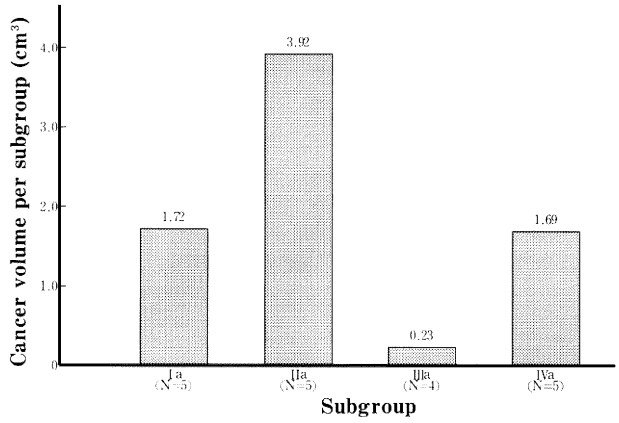

Fig. 4. Total cancer volume (cm^3) per subgroup.

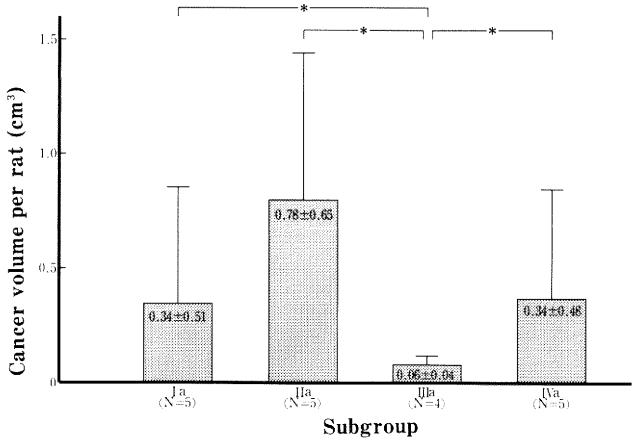

Fig. 5. Cancer volume per rat (cm^3, mean±SD). * $P<0.05$.

Table 3. Cancer number and volume per rat

Subgroup	Cancer number/rat	Cancer volume/rat
Ia	4.8±2.6	0.34±0.51
IIa	6.2±4.6	0.78±0.65 *
IIIa	2.3±1.5	0.06±0.04 *
IVa	2.2±0.8	0.34±0.48 *

All data is given as mean±SD. Volumes represent the sum of individual tumor volumes in cm^3 for each rat, averaged within the subgroup, * $P<0.05$.

Number and location of colon cancers for groups (Fig. 2)

The total number of cancerous lesions per group was highest in subgroup IIa (31), followed by subgroups Ia (24), IVa(11), and IIIa (9) (Table 2). In terms of location, no tumors occurred in the cecum (C) in any group. The most common sites were the mid-colon (M) to the distal colon (D). There were no differences in lesion distribution among the groups (chi square test).

Number of colon cancers per rat (Fig. 3)

The average number of cancerous lesions per rat was 4.8±2.6 in subgroup Ia, 6.2±4.6 in IIa, 2.3±1.5 in IIIa, and 2.2±0.8 in IVa (Table 3). The high-fat subgroups (Ia, IIa) tended to have more cancers per rat than the no-fat groups (IIIa, IVa).

Colon cancer volume per group (Fig. 4)

Total cancer volume (cm^3) per group was highest in subgroup IIa (3.92), followed by Ia (1.72), IVa (1.69), and IIIa (0.23) (Table 2). Total cancer volume was comparable between the high-fat, high-fiber-diet subgroup (Ia) and the no-fat, no-fiber-diet animals (IVa), but less in the no-fat, high-fiber subgroup (IIIa).

Average colon cancer volume per rat (Fig. 5)

Average cancer volume (cm^3) per rat was 0.34±0.51 in subgroup Ia, 0.78±0.65 in IIa, 0.06±0.04 in IIIa, and 0.34±0.48 in IVa (Table 3). Cancer volume per rat was significantly lower in the no-fat, high-fiber subgroup IIIa than in the other three "a" subgroups.

DISCUSSION

Increased fat consumption augments the liver secretion of bile acids as amino acid conjugates. When the secreted, conjugated bile acids enter the large intestine, anaerobic bacteria deconjugate them, forming free primary bile acids. Primary bile acids then are changed to secondary bile acids by 7α-dehydroxylase released by the anaerobes.[10] Many studies have demonstrated that secondary bile acids—particularly deoxycholic acid (DCA) and lithocholic acid (LCA)—promote colon cancer.[11-14] In contrast, high-fiber diets increase fecal volume, which dilutes the fecal concentration of bile acids and of enzymes released by intestinal flora.[15,16] Fiber also accelerates the intestinal transit of feces, reducing contact time

between secondary bile acids and intestinal mucosa,[17,18] thus suppressing colon cancer formation. In this study, the number of colon cancers per tumor-bearing rat tended to be higher in high-fat groups (Ia, IIa) than in no-fat groups (IIIa, IVa), though without reaching a statistical significance. However, the tumor volume per rat was basically comparable in the high-rat, high-fiber-diet subgroup (Ia) and the no-fat, no-fiber-diet group (IVa); (Table 3).

Based on the incidence of cancer and the number of cancers per animal, most previous reports have concluded that fat promotes colonic carcinogenesis and that fiber suppresses it. If we assume that the number of cancerous lesions reflects an initiation effect and cancer volume a promotion effect, the high-fat groups likely were affected by an initiation effect and the no-fiber groups by a promotion effect. More generally, high-fat diets would appear to promote carcinogenesis by strengthening initiation effects, while high-fiber diets suppress carcinogenesis by weakening those factors promoting the growth of established tumors.

REFERENCES

1) Doll R: The geographical distribution of cancer. *Br J Cancer* **23:** 1-8, 1969.
2) Wynder EL, Shigematsu T: Environmental factors of cancer of the colon and rectum. *Cancer* **20:** 1520-1561, 1967.
3) Hirayama Y: Epidemiological changes in colon cancer and future prospects. *Jpn J Clin Med* **39:** 2006-2016, 1981. (in Japanese)
4) Wynder EL, Reddy BS: Diet and cancer of the colon. *Curr Conc Nutr* **6:** 55-71, 1977.
5) Drasar BR, Irving D: Environmental factors and cancer of the colon and breast. *Br J Cancer* **27:** 167-172, 1973.
6) Reddy BS, Hedges AR, Laakso K: Metabolic epidemiology of large bowel cancer. *Cancer* **42:** 2832-2838, 1978.
7) Tajima K, Ishiguro T, Tominaga H: Trends in colon cancer in Japan. *Igaku no Ayumi* **122:** 398-406, 1982. (in Japanese)
8) Japanese Research Society for Cancer of the Colon and Rectum. Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus. (5th ed.) Kanehara Shuppan, Tokyo 1994, p 49-58.
9) Miyoshi T, Ohara T, Morioka M: Tissue growth and progress of 1,2-dimethylhydrazine-induced colon cancer in rats. *J Jpn Assoc Gast Dis* **75:** 284-293, 1978. (in Japanese)
10) Nakamura K, Makino K: Metabolism and transport of bile acids—Recent findings. *Rinsho Shokaki Naika* **5:** 1945-1950, 1990. (in Japanese)
11) Hill MJ: The role of colon anaerobes in the metabolism of bile acids and steroids, and its relation to colon cancer. *Cancer* **36:** 2387-2500, 1975.
12) Reddy BS, Watanabe K: Effect of cholesterol metabolites and promoting effects of lithocholic acid in colon carcinogenesis in germ free and conventional rats. *Cancer Res* **39:** 1521-1524, 1979.
13) Cohen BI: Effect of cholic acid feeding on N-methyl-N-nitrosourea-induced colon tumors and cell kinetics in rats. *J Ntal Cancer Inst* **64:** 573-578, 1980.
14) Narisawa T: Various promoters of carcinogenesis and their characteristics: promoters of colon carcinogenesis. *Saishin-Igaku* **37:** 1762-1765, 1982. (in Japanese)
15) Prizont R: Influence of high dietary cellulose on fecal glycosidases in experimental rat colon carcinogenesis. *Cancer Res* 44: 557-561, 1984.
16) Shimizu Y, Nakazawa S: Effects of dietary factors and fecal bile acids on DMH-induced colon carcinogenesis in rats. *J Jpn Assoc Gast Dis* **82:** 2554-2561, 1985. (in Japanese)
17) Burkitt DP, Walker ARP, Painter NS: Effect of dietary fibre on stools and transit-time, and its role in the causation of disease. *Lancet* **12:** 1408-1411, 1972.
18) Kritchevsky D: Diet, nutrition, and cancer—the role of fiber. *Cancer* **58:** 1830-1836, 1986.