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## Inability of Low- or High-Fat Diet to Modulate Late Stages of Colon Carcinogenesis in Sprague-Dawley Rats<sup>1,2</sup>

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### Abstract

The main objective of the present proposal was to investigate the effect of feeding a low- or high-fat diet in the early and late stages of colon carcinogenesis. Sprague-Dawley male rats were injected with azoxymethane (20 mg/kg/week) for 2 weeks. One week later they were randomly allocated to eat a low-fat (4% beef tallow + 1% corn oil) or a high-fat (18.6% beef tallow + 4.7% corn oil) diet (LF or HF). After 10 weeks of feeding, 10 animals per group were killed, and their colons were evaluated for tumors. The remaining animals in each group were divided further into LF and HF groups. The four experimental groups consisted of groups receiving LF or HF diet throughout the study (LF-LF or HF-HF) and the groups fed LF or HF diet for the first 10 weeks, then assigned the alternate diet for the remainder of the duration (LF-HF or HF-LF). By week 26, the remaining animals were killed, and their colons were evaluated for the number, location and size of tumors. The tumor incidence in the HF-HF and HF-LF groups were higher than the LF-LF and LF-HF groups (81.6 and 84.8% versus 71.4 and 60.0%). Tumor multiplicity ranged from  $1.86 \pm 0.26$  to  $2.54 \pm 0.33$  in all groups. The average size of tumors and total tumor area/rat were affected significantly by the time at which the diet was fed. Average size and total tumor area in the animals fed HF diet during early stages (HF-HF and HF-LF) were significantly higher than those fed the LF diet during the early stages. Late intervention by specific diets did not affect tumor outcome. Sequential enumeration of aberrant crypt foci of different growth features representing early preneoplastic stages corroborated the findings of the tumor outcome. It was concluded that early preneoplastic stages were more sensitive than their advanced counterparts to the dietary interventions of the present study.

### Introduction

The concept that diet plays an important role in the etiology and prevention of colon cancer has been receiving a great deal of attention by cancer researchers, health professionals, and the public. Epidemiological and experimental studies have provided substantial evidence to support the concept that dietary fats (level and type) play important roles in the pathogenesis of colon cancer (1-5). Animal studies allow the systematic examination of hypotheses linking diet to the etiology and prevention of colon cancer. The role of dietary fat in the pathogenesis of colon cancer has been investigated previously. However, most of these studies have used experimental protocols in which animals are injected with a carcinogen (one, two, or several injections) while they receive the test diet, or they receive the test diet 1 or 2 weeks after the last carcinogen injection. In these studies, the dietary treatments are continued throughout the experimental duration, and the tumor outcome is assessed at the end of the study. These studies

have demonstrated that dietary fat is an important modulator of colon tumorigenesis. However, to our knowledge, it is not known whether dietary fat (either the level or type), modulates the disease process by affecting the early and/or late stages of colon carcinogenesis. In addition, it is not known whether preneoplastic lesions exhibit a phenotype during their developmental stages that resist modulation by diet. The elucidation of this relationship is important if dietary intervention is to be used in, or recommended to, adult individuals with high risk for developing the disease, who presumably already harbor preneoplastic lesions in their colons.

Our study assessed the effect of a low or high beef tallow diet in the early and late stages of colon carcinogenesis in Sprague-Dawley male rats. The findings of the present study demonstrated that the tumor-modulating effect of a low or high beef tallow diet was established very early on, and that intervention of the disease process by these diets during later stages was ineffective in modulating the tumor outcome. The findings of the present study provide evidence for the first time in support of the notion that colonic preneoplastic lesions exhibit an established phenotype that resists dietary modulation. These findings stress the need to investigate the amenability of preneoplastic stages to growth regulation by a nutrient or a cancer-preventive agent in future studies. Information derived from such studies would be important in formulating cancer prevention strategies.

### Materials and Methods

**Animals.** Sprague-Dawley male rats were purchased from the Central Animal Care Facility (University of Manitoba) and cared for according to the guidelines of the Canadian Council on Animal Care. Animals were housed in wire meshed stainless steel cages (two or three rats/cage) and had free access to food and water.

**Diet.** The experimental diets were formulated based on the nutritional requirement of laboratory rats as recommended by the American Institute of Nutrition (6, 7) with slight modifications. A low beef tallow diet contained 5% fat, whereas the high beef tallow diet contained 23.5% fat. The main sources of fat were corn oil and beef tallow at a ratio of 1:4. Corn oil was added to ensure essential fatty acid content was adequate in the diet. Additional fat was added at the expense of an isocaloric amount of carbohydrate (Table 1).

**Experimental Design.** Rats were injected with AOM<sup>4</sup> (Sigma Chemical Company, St. Louis, MO) in saline (15 mg/kg s.c./week) twice. One week after the second injection, they were allocated to the LF or HF beef tallow diet group. After 10 weeks of dietary treatment (12 weeks after the first injection of AOM), 10 rats per group were killed, and their colons were evaluated for macroscopic tumors. The remaining animals in each group were subdivided further into two groups. One group continued to receive the same diet, whereas the second group was allocated to the alternate diet. Therefore, there were four diet groups, which are referred to as LF-LF, HF-HF, LF-HF, and HF-LF. The LF-HF and HF-LF are the groups that received the alternate diet after week 12 (Fig. 1). Tumor incidence was assessed in 30-35 animals/group.

**Assessment of Tumors.** All animals were killed (number of animals killed/group/day was similar among groups) between weeks 24 and 26, and their

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<sup>4</sup> The abbreviations used are: AOM, azoxymethane; HF, high fat; LF, low fat.

Table 1 Diet composition (% w/w)

	LF	HF
Casein	20.0	23.5
Dextrin	15.0	17.6
Dextrose	50.0	23.5
Corn oil	1.0	4.7
Beef tallow	4.0	18.6
Solka floc	5.0	5.9
DL-Methionine	0.3	0.4
Choline bitartrate	0.2	0.2
AIN-76A mineral mix	3.5	4.1
AIN-76A vitamin mix	1.0	1.2
Linoleic acid	0.68	3.29

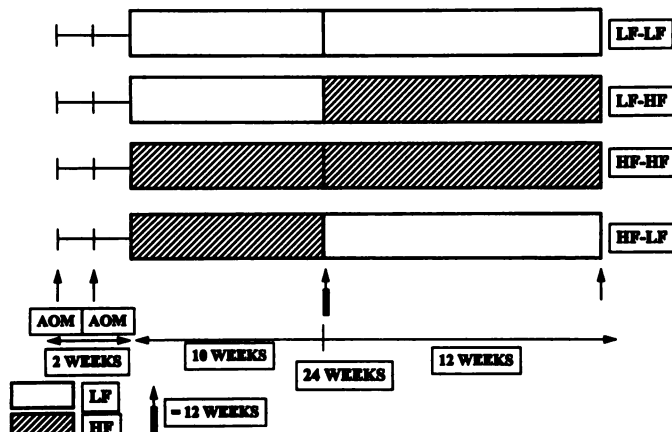


Fig. 1. A schematic presentation of the experimental protocol.

colons were excised from the rectal to the cecal end, flushed with PBS, cut open along the longitudinal axis, and palpated for tumors. The location and size (width and length) of tumors were recorded. Suspected growths were processed for histology, and their histopathological classification was ascertained as described previously (8). Tumor incidence (percentage of animals with tumors), number of tumors/tumor-bearing rats, and the average size of tumors/tumor-bearing rat were recorded. Growth characteristics of tumors as affected by dietary treatments were analyzed by group as well as by rat. This approach allows assessment of the sensitivity of each animal and group to the tumor-modulating ability of the diet.

Tumor parameters assessed were as follows (Table 2): tumor incidence (percentage of total animals with tumors); tumor multiplicity (average number of tumors per tumor bearing rat); average tumor size ( $\text{mm}^2$ ) per tumor bearing rat; average tumor size/group (average size of all tumors in a group); and tumor burden (average of the total tumor area in each tumor-bearing rat). The last three parameters were calculated as follows:

Average tumor size per tumor-bearing rat

$$= \frac{\text{Total of average size (mm}^2\text{) of tumor in each tumor-bearing rat in the group}}{\text{Number of tumor-bearing rats in the group}}$$

$$\text{Average tumor size/group} = \frac{\text{Total size (mm}^2\text{) of all tumors in the group}}{\text{Number of tumors in the group}}$$

Tumor burden

$$= \frac{\text{Total of total area occupied by tumors in each tumor-bearing rat in the group}}{\text{Total number of tumor-bearing rats in the group}}$$

## Results

The body weights of the animals were affected by the level of fat. At week 12, the body weights for the LF and HF groups were  $513 \pm 8$  g and  $604 \pm 10$  g, respectively. After the intervention by week 24, among the four groups, the group fed the high beef tallow diet throughout the experimental duration (HF-HF) had the highest

average body weight, and the group fed the low beef tallow diet throughout (LF-LF) had the lowest body weight. Body weight was in the order HF-HF > HF-LF > LF-HF > LF-LF ( $685 \pm 21$ ,  $639 \pm 16$ ,  $623 \pm 14$ , and  $607 \pm 15$ , respectively). After 10 weeks of dietary treatments, 10 animals were killed in each group to determine the incidence of tumors in the two groups. Only one animal in the HF group had one macroscopic tumor. At week 24, no significant difference was observed in tumor incidence among the groups, although there was a trend for the HF groups to have more tumors than the LF groups; i.e., tumor incidence was in the order HF-LF (84.8%) > HF-HF (81.6%) > LF-LF (71.4%) > LF-HF (60.0%). In the HF-HF and HF-LF groups, 30% of the total tumors were classified as adenocarcinomas, whereas in the LF-LF and LF-HF groups, 22% of total tumors were classified as adenocarcinomas. These differences were not significant. Tumor multiplicity, representing the average number of tumors/tumor-bearing animal in each group, was similar. It was noteworthy that the number of tumors per tumor-bearing rat was higher in the HF-LF group than the other groups. The average size of tumors per tumor bearing rat was in the order of HF-HF > HF-LF > LF-HF > LF-LF. The difference between the HF-HF and LF-LF groups was significant. Tumor burden, the total tumor area per tumor-bearing rat, was in the order of HF-LF > HF-HF > LF-LF > LF-HF (Table 2).

Distribution of tumors along the length of the colon is shown in Table 3. About 30–40% of the total tumors were present in the regions A and B compared with 60–70% in regions C and D. In regions C and D, the distribution of tumors in the LF-HF group tended to be similar to that in the HF-HF group, whereas the distribution of tumors in the HF-LF group tended to be similar to that in the LF-LF

Table 2 Tumor parameters as affected by dietary treatments

	HF-HF	HF-LF	LF-HF	LF-LF
Total number of rats	31	33	35	35
Tumor incidence (%)	81.6	84.8	60.0	71.4
Tumor multiplicity	$2.04 \pm 0.31^a$	$2.54 \pm 0.33$	$1.86 \pm 0.26$	$2.15 \pm 0.29$
Average tumor size ( $\text{mm}^2$ /tumor-bearing rat)	$20.8 \pm 5.6$	$16.8 \pm 5.5$	$13.0 \pm 4.2$	$8.0 \pm 1.5$
Average tumor size ( $\text{mm}^2$ /group)	$21.2 \pm 8.0$	$18.9 \pm 1.6$	$14.9 \pm 3.4$	$10.2 \pm 2.6$
Tumor burden	$36.0 \pm 10.2$	$37.6 \pm 10.7$	$18.9 \pm 4.8$	$19.1 \pm 4.9$

<sup>a</sup> Values are the means  $\pm$  SDs. The test diets were initiated 1 week after the last injection of AOM. HF-HF, group fed a high beef tallow diet throughout the experimental duration; LF-LF, group fed a low beef tallow diet throughout the experimental duration; HF-LF, group fed a high beef tallow diet for 10 weeks and then fed a low beef tallow diet for 12 weeks; LF-HF, group fed a low beef tallow diet for 10 weeks and then fed a high beef tallow diet for 12 weeks. To assess the effects of feeding HF or LF during early stages, LF-HF and LF-LF groups or HF-LF and HF-HF groups were combined, respectively. To assess the effects of feeding LF or HF during late stages, LF-HF and HF-HF or HF-LF and LF-LF were combined, respectively.

Table 3 Percentage of distribution of tumors along the length of the colons as affected by dietary treatments

	Colon segment (cm length)				
	A (0–4) <sup>a</sup>	B (4–8)	C (8–12)	D (12–16)	E ( $\geq 16$ )
HF-HF	17.3	17.3	36.5	26.9	1.9
HF-LF	19.7	14.0	43.7	16.9	4.2
LF-HF	10.3	30.8	30.8	28.2	0
LF-LF	10.5	24.6	49.2	12.2	1.8

<sup>a</sup> A to D represent 16 cm of the unfixed colon, 4-cm segments each starting from the rectal end. A is the most distal segment. E is the proximal region colonic segment, representing the proximal region ( $\geq 16$  cm) harboring tumors. The segment E had  $\leq 3$  tumors/group. The test diets were initiated 1 week after the last injection of AOM. HF-HF, group fed a high beef tallow diet throughout the experimental duration; HF-LF, group fed a high beef tallow diet for 10 weeks and then fed a low beef tallow diet for 12 weeks; LF-HF, group fed a low beef tallow diet for 10 weeks and then fed a high beef tallow diet for 12 weeks; LF-LF, group fed a low beef tallow diet throughout the experimental duration.

Table 4 Tumor parameters as affected by feeding a low and high beef tallow diet during early and late stages of colon carcinogenesis<sup>a</sup>

	Early feeding			Late feeding		
	HF	LF	P	HF	LF	P
Tumor multiplicity	1.91 ± 0.22 <sup>b</sup>	1.34 ± 0.18	0.0440	1.84 ± 0.21	1.34 ± 0.18	0.092
Average tumor size (mm <sup>2</sup> )/tumor bearing rat	15.7 ± 3.30	6.40 ± 1.40	0.0085	10.25 ± 2.4	11.4 ± 2.70	0.746
Tumor burden	31.9 ± 6.40	12.2 ± 2.40	0.0035	22.9 ± 5.00	20.2 ± 4.70	0.707

<sup>a</sup> n = 30–35 rats/group.

<sup>b</sup> Values are the means ± SD. The test diets were initiated 1 week after the last injection of AOM. HF-HF, group fed a high beef tallow diet throughout the experimental duration; HF-LF, group fed a high beef tallow diet for 10 weeks and then fed a low beef tallow diet for 12 weeks; LF-HF, group fed a low beef tallow diet for 12 weeks and then fed a high beef tallow diet for 12 weeks; LF-LF, group fed a low beef tallow diet throughout the experimental duration. To assess the effects of feeding HF or LF during early stages, LF-HF and LF-LF groups or HF-HF and HF-LF groups were combined, respectively. To assess the effects of feeding LF or HF during late stages, HF-HF and LF-HF or HF-LF and LF-LF were combined, respectively.

group. Dietary fat and time were important variables and had a significant effect on the number of tumors, the size of the tumors, and tumor area/rat in each group (Table 4). Early feeding with LF or HF significantly affected these parameters, whereas late feeding with these dietary treatments had little effect.

## Discussion

The main finding of the present study is that both a low beef tallow and a high beef tallow diet were ineffective in modulating the growth of preneoplastic lesions in their late stages of development. The tumor-modulating effect of these diets was established only during earlier stages of carcinogenesis. The LF-LF group had a lower tumor incidence and smaller tumors than the HF-HF group, which was consistent with the findings of others (9). The novel finding was that the intervention of the disease process by the LF or the HF diet during the later stages of carcinogenesis did not affect the overall tumor outcome. This suggests that the effect of the LF or HF diet at earlier time points is crucial in determining the disease burden.

Distribution of the number and size of tumors along the length of the colon was used to ascertain information on the sensitivity of the colonic regions to carcinogen and diet, and to gain further insight into the disease process. It is noteworthy that differences exist among different regions of the colon with respect to number of tumors. This observation supports the notion that colonic regions respond differently to AOM and to dietary manipulation. Higher numbers of tumors appearing in regions C and D than in regions A and B suggest that either more preneoplastic lesions were initiated or that a higher proportion of initiated lesions progressed into macroscopic tumors in regions C and D than in regions A and B. The distribution of tumors in regions A and B were similar for the groups LF-LF and LF-HF, and HF-HF and HF-LF. This finding suggested that, possibly, the preneoplastic lesions in regions A and B, which represented the distal 8-cm segment of the colon, were more resistant to dietary influences imposed upon them during later stages. A similarity in the distribution of tumors between LF-HF and HF-HF groups and HF-LF and LF-LF groups in regions C and D, but not in regions A and B, suggest that preneoplastic lesions in regions C and D were responding to dietary treatment introduced at later time point. Although statistically not significant, it is noteworthy that the HF-LF group had more tumors per tumor-bearing rat than the HF-HF group. The increase in the number of tumors in the HF-LF group was mainly due to the rapid appearance of small adenomas. An observation similar to this was reported in a study in which the reoccurrence rate of polyps was found to be higher in individuals consuming a LF, high-fiber diet than those consuming more fat and less fiber (10). It is important to note that, because the number of tumors/tumor-bearing rats in the LF-HF group was lower than in the LF-LF group, the average tumor size was higher in the LF-HF group. Higher number of tumors in the HF-LF group than in the HF-HF group resulted in lower average tumor size in

the HF-LF group compared to that of the HF-HF group. This explanation is supported by the fact that the total tumor area per tumor-bearing rat was similar for the LF-HF and LF-LF groups and for the HF-HF and HF-LF groups. Additional studies are in progress to quantify aberrant crypt foci in the various colonic regions at different time points to determine whether colonic regions are different from each other in their response to late dietary intervention and if there is an alteration in the number of aberrant crypt foci with varying growth characteristics.

The findings on the average size of tumors per group asserts the notion that the effect of diet on the growth of tumors was established very early on during the disease process. The most plausible explanation as to why the HF or LF diets were unable to modulate tumor growth during the later stages is that once preneoplastic lesions reach a specific growth state, they exhibit growth autonomy (established phenotype) and are less amenable to growth modulation by the nutritional status of the host. The HF diet appears to provide a growth-promoting environment, which selects a few lesions in their primal stages early in the study, and impels them to reach the specific autonomous state within a few weeks of feeding. However, in the LF environment, preneoplastic lesions require considerably longer to reach an autonomous state. The shorter time required for preneoplastic lesions to reach an autonomous state in the HF group than those in the LF group is consistent with the presence of larger tumors in the HF group than in the LF group. This is schematically depicted in Fig. 2.

This concept presents the view that preneoplastic lesions must first reach an autonomous state, after which their growth rate is a function of time. Whether this mechanism or pathway is operational under a variety of conditions or is specific to the experimental conditions of the present study remains to be seen. It is also important to consider

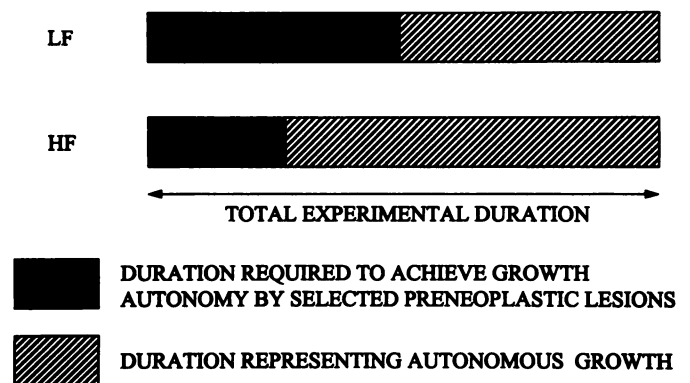


Fig. 2. A hypothetical scheme to explain the effect of a LF and a HF diet in eliciting an established phenotype (autonomous state). This scheme suggests that some preneoplastic lesions acquire an established phenotype earlier in the HF group than in the LF group. Therefore, the lesions with the established phenotype in the HF group have a longer duration for autonomous growth than those with an established phenotype in the LF group.

that the fatty acid composition of dietary fat is important in eliciting tumor-modulating effects and may modulate late stages of colon carcinogenesis.

Epidemiological studies have positively associated the intake of a high saturated fat diet, and negatively associated the intake of fish oil or olive oil with the incidence of colon cancer. Animal studies have demonstrated that beef tallow and corn oil promote colonic tumor incidence and tumor burden, whereas fish oil and olive oil do not (4). The plausible mechanism(s) by which these fats may alter tumor growth include a multitude of biological responses, altered signal transduction pathways, and the level and type of eicosanoids derived from different families of unsaturated fatty acids. Several studies have alluded to the fact that specific eicosanoids may be involved in mediating the growth and metastatic potential of cancer cells (11, 12). Most populations exhibit parallel risks for colorectal cancer and coronary heart disease (13). There have been suggestions that these two chronic illnesses also share environmental factors that could be involved in the etiology of these diseases (13). A strong positive association has been reported between the prevalence of adenomatous polyps and atheromatous plaque in Hawaiian Japanese men (13). Several similarities in the pathogenesis of these two chronic illnesses and the findings that precursor lesions of atheromatous plaque are initiated very early in life (14) lead to speculation that lesions representing the preadenomatous stages are also initiated very early in life. Therefore, reduction in fat intake very early in life may be a more useful cancer-preventative strategy than intervention later in life.

In our study, the dietary fat used was beef tallow, which consists mainly of saturated fatty acids. Therefore, it is possible that our findings are specific to saturated fat. Other types of fat may elicit different responses on the early or later stages of colon carcinogenesis (4, 11, 12). This should be scrutinized in the future. An important concept emerging from the present study is that early and later stages of the disease process are biologically different in their responses to growth modulators. Therefore, should the cancer preventative efficacy of a chemical agent or diet not be investigated at the different stages of carcinogenesis? This question is pivotal to the concept of cancer

prevention in high-risk individuals and to the basic understanding of the multistep nature of the disease process.

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