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A Western-style diet induces benign and malignant neoplasms in the colon of normal C57Bl/6 mice

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Decreased dietary intakes of calcium, vitamin D and folic acid have been suggested as risk factors for human colon cancer. We previously fed a Western-style diet (WD) containing reduced calcium, vitamin D and increased fat content to normal C57/Bl6 mice: hyperproliferation, hyperplasia and whole crypt dysplasias developed in the colon following WD administration. Utilizing the same diet, we now also decreased the levels of several nutrients that are required for biochemical reactions involving methyl group inadequacy, i.e. folic acid, methionine, choline and vitamin B_{12}. Dietary levels of these nutrients were reduced to nutrient-density levels approximating those consumed by large segments of human Western populations. This further modification of the WD resulted in adenoma and carcinoma development in normal mouse colon ($P < 0.04$ compared with AIN-76A diet). The results indicate, for the first time, that a semi-purified rodent diet designed to mimic the human Western diet can induce colonic tumors in normal mice without carcinogen exposure.

Introduction

Inadequate intakes of calcium and vitamin D in human diets have been suggested as risk factors for colorectal cancer. Many epidemiologic, rodent and human studies have been carried out that indicate adverse effects of low calcium and vitamin D dietary availability on colonic epithelial cells (reviewed in ref. 1). A large randomized clinical trial further demonstrated reduction of sporadic colonic adenoma recurrence in human subjects with increased dietary calcium intake (2).

In previous preclinical studies we have measured the effects of dietary calcium and vitamin D in several organs. Dietary intakes of these nutrients were reduced to nutrient density levels (amount per kcal of diet) found in human Western diets (3), combined with increased nutrient-density levels of dietary fat (4–11). In short-term studies in normal rodents (up to 20 weeks of feeding) this 'Western-style diet' (WD) induced hyperproliferation and hyperplasia of colonic epithelial cells (4), which were inhibited by increasing dietary calcium or vitamin D (5,6); this WD also induced mammary epithelial cell hyperproliferation and hyperplasia (7–9), and hyperproliferation in exocrine pancreas (9,10) and prostate gland (9,11). In a long-term study (104 weeks) in normal mice on WD, whole colonic crypt dysplasias occurred; however, no adenomas or adenocarcinomas developed (12). In further studies of *Apc 1638N*, *ApcMin* and *Mcc* mice carrying targeted mutations that led to intestinal and colonic neoplasia, the WD increased the neoplastic lesions (13–15), whereas supplemental dietary calcium alone decreased tumor formation in *Apc1638N* mice (unpublished data).

In the study reported here, we considered additional human nutritional factors whose dietary intakes are believed to contribute to colon cancer in Western populations (1,16). We chose for further study in this model nutrients required for biochemical reactions that involve methyl-donor availability, i.e. folic acid, methionine, choline and vitamin B_{12}, the biological significance of which is further described in (16–18). These dietary components have essentially equal nutrient-density level requirements (3) in both human and rodent diets. The most widely studied of these nutrients is folic acid which provides one-carbon moieties for purine and thymidylate synthesis and for transfer to methionine to form *S*-adenosylmethionine, a critical methyl-donor involved in a variety of methylation reactions including DNA methylation. In particular, dietary folate depletion has been implicated in increased risk for the development of human and experimental rodent colon cancer (16,19–24).

Abbreviations: *Apc*, adenomatous polyposis coli; kcal, kilocalories; NWD, new Western-style diet; WD, Western-style diet.

Materials and methods

Mice and diets

Mice. A total of 90 C57Bl/6 mice at 3–4 weeks of age of both sexes were obtained from the Jackson Laboratories (Bar Harbor, ME, USA). They were housed 10 animals per cage, with males and females separated, in cages with a wire bottom to prevent coprophagia. On arrival all these mice were fed a standard AIN-76A control diet, given water *ad libitum* and kept on a 12 h light/dark cycle in a controlled temperature and humidity room. At 6 weeks of age these mice were randomly divided into three diet groups and fed with one of three diets: (i) AIN-76A diet (control); (ii) new Western-style diet 1 (NWD1); (iii) new Western-style diet 2 (NWD2) which were continued until the experiment was completed. The body weights of all mice were recorded every other week. Mice from each diet group were scheduled to be killed at 12 and 18 months after feeding the diets.

Characteristics of the NWD1 and 2. The diets used in the study were based on the semi-purified mouse diet of the American Institute of Nutrition (AIN-76A) (25). The basic AIN-76A diet was used as the control diet. The NWD1 had an increase in fat content, and reduced calcium, vitamin D and fiber; it also had reduced levels of methyl-donor nutrients (folic acid, methionine and choline) decreased to nutrient-density levels approximating those consumed by large segments of human Western populations (Table I).

The NWD2 had the same nutrient composition as NWD1 except for increased levels of calcium and vitamin D (Table I).

Design of diet composition of NWSD. The levels of decreased calcium and vitamin D in NWD1 were the same as in our previous WD studies (4–13), and the levels of calcium and vitamin D in NWD2 were increased to the nutrient-density equivalents of the maximum levels permitted in US daily intakes. These levels are higher than the nutrient-density levels of calcium and vitamin D in the basic AIN-76A diet (Table I). Thus, NWD1 and NWD2 both represent low and high nutrient-density equivalent human intakes of calcium and vitamin D, respectively. Fiber was reduced from 5% in AIN-76A control diet [equivalent to ~25 g in a human 2000 kcal (500 g dry weight) diet] to 2%, equivalent to ~9 g in a human 2000 kcal diet.

Table I. Diet compositions

Ingredients, % (wt) or amount (wt)	Control A		Elevated Ca and vit. D NWD2
	IN-76A	NWD1	
Fat (corn oil), %	5 (13)	20 (40)	20 (40)
Calcium, mg/g	5 (2700)	0.5 (220)	7.0 (3000)
Vitamin D_3, IU/g	1 (600)	0.11 (50)	2.3 (1000)
Phosphorus (PO_4), mg/g	4 (2200)	3.6 (1600)	3.6 (1600)
Fiber (cellulose), %	5 (25)	2 (9)	2 (9)
Folic acid, μg/g	2 (1100)	0.23 (100)	0.23 (100)
dl methionine, %	0.3	–	–
I cysteine, %	–	0.3	0.3
Choline bitartrate, %	0.2	0.12	0.12
kcal/g (approximate)	3.6	4.5	4.5

Numbers in the table are in terms of units, weight or percent of diet by weight for that specific ingredient per gram of diet. Numbers in parenthesis in the table are the calculated equivalents, based on nutrient density, in a daily human 2000 kcal diet (500 g/dry weight) in terms of the units used in the 'Ingredient' column. Thus, calcium (Ca) is in mg, vitamin D (vit. D) in IU, folic acid in μg, etc. Exceptions are fat, which is '% calories' in the parenthesis, and fiber, which is grams per day in human diets. The low levels in NWD1 are largely based either on the lower quartile (e.g. calcium), or the average of the human intake on a 2000 kcal daily diet converted by nutrient-density principle to the rodent diet. Part of the total calcium is supplied, particularly in NWD1 and NWD2 by the calcium content of the casein. The kcal/g includes an approximate correction for moisture content of ingredients (e.g. 15% of casein). Due to increased nutrient density of NWD diets, derived from the high fat content, all essential nutrients (protein, vitamins other than vitamin D and folate, and minerals other than calcium and phosphorus) are present at 20% higher levels in NWD1 and NWD2 than in the AIN-76A diet, added at the expense of sucrose to give approximately equivalent nutrient densities of these essential nutrients in all of these diets.

Three methyl-donor co-factors, folic acid, choline and methionine, were reduced in content in both NWDs. Folic acid in humans essentially comes from dietary sources. In rodents, coprophagy of microbiologically produced folic acid and vitamin B_{12} in the colon may supply an appreciable amount of folic acid and vitamin B_{12}. To reduce the folic acid and vitamin B_{12} supply from fiber fermentation in the colon to approximate that of humans, the mice fed NWDs were housed in cages with wire-bottoms to prevent coprophagy. The supplemental methionine was replaced with cysteine in the NWDs to decrease the supply of methyl-donors as in AIN-93 diet (26). It should be noted that appreciable methionine is still present from the high casein content of all of the diets (25). Choline was also reduced almost one-half in the NWDs compared with AIN-76A, based on nutrient density, to approximate the intakes in human Western populations.

Details of the NWDs used in our studies are as follows:

(i) *Choice of the fat used*. All the short-term studies previously performed with WD used corn oil as the source of fat, similar to the reference AIN-76A diet from which the WD was derived (4–11). In our previous long-term study of 2 years duration we compared WD compositions prepared with corn oil or American Blend Fat, the latter formulated to simulate the blend of fat intake in the average US human diet, and found similar results (12). Therefore, corn oil was used in the present study as the fat source (Table I).

(ii) The levels of *calcium and vitamin D* used in the NWD formulations were the same as used in the previous WD formulations. The rationale and details of design calculations of the levels of fat, calcium and vitamin D were fully described previously (3,4).

(iii) *Total dietary fiber* of adults in the US is estimated to be ~12 g/2000 kcal diet (27,28). The 'dry' weight of a 2000 kcal diet is ~500 g, so the fiber content is ~2.4% of the adult US diet. The recommendations for adequate dietary fiber intake are reviewed (see ref. 27), and range from 18 to 35 g/day (4–7% of a 2000 kcal diet). The AIN-76A diet has 5% fiber, usually a wood cellulose that is fermentable, although qualitatively different from the blend of dietary fibers in the human diet. In the NWD diet, the fiber was therefore reduced to 2%, equivalent to ~9 g /day on a human 2000 kcal diet, based on nutrient density (3), to approximately mimic the relative human dietary fiber intake (Table I).

(iv) *Folic acid*. Dietary folate intake in young women in the US and Canada is estimated as ~200 μg/day and ~250 μg/day for men (29). However, the folate in food is estimated to be only 50% bioavailable (29), whereas synthetic folate (added to food and used in preparation of animal feed diets such as AIN-76A and NWD) is 85% bioavailable. Therefore, we considered the human diet as containing ~100 μg synthetic folic acid equivalent per 2000 kcal of diet (or = 0.05 μg/kcal of diet) (Table I). The NWD diet is ~4.5 kcal/g, so this led to the use of 0.23 μg/g of diet in NWD diets (Table I). The folate in AIN-76 control diet is 2 μg/g of diet (3.6 kcal/g), or ~0.56 μg/kcal of diet, and would be equivalent, on a nutrient-density basis, to ~1100 μg/day of folic acid (synthetic) in a human 2000 kcal daily diet. In terms of the relative bioavailability of synthetic to food folate, this suggests that the synthetic folic acid content of AIN-76A could be the equivalent, based on nutrient density, of $1.7 \times 1100 = 1780$ μg of dietary food equivalents (DFEs) (29). However, using only the calculations based on the properties of synthetic folic acid, the nutrient density of folic acid in NWD formulations is only about one-tenth that of AIN-76A, and represents the largest single dietary alteration in the NWD composition. It should be noted that since January 1, 1998 cereal grain products in the US have been fortified with synthetic folic acid, estimated to add ~80 μg/day to the average adult intake (29). However, our NWD formulations were designed before folic acid food fortification occurred, and we have not yet changed the NWDs. It would be interesting if the current folic acid food fortification leads to a significant reduction in colon cancer incidence in the US.

(v) *Methionine* dietary requirements for laboratory animals and humans usually are combinations of the total of the methionine and cysteine in the diet, e.g. the total sulfur containing dietary amino acids. With adequate methyl transfer co-factors in the diet (i.e. folic acid, vitamin B_{12} and vitamin B_6), normal enzyme systems allow ready interchange between cysteine and methionine *in vivo* (17). The estimated amino acid human dietary daily requirement for the combination of methionine and cysteine is ~15 mg/kg body weight, or ~900 mg/day on a 2000 kcal diet or ~0.2% of the 500 g human diet (31). The AIN-76A diet contains 0.3% dl of added methionine to the cysteine plus methionine content of the casein used as the sole protein source in AIN-76A diet. To reduce the methionine content of the rodent diet without lowering the total cysteine plus methionine requirement, we substituted 0.3% added L-cysteine for the 0.3% dl methionine of the AIN-76A diet, as suggested for older rodents in AIN-93 diets (26) (Table I).

(vi) *Choline* human dietary requirement for adults is ~500 mg/day, or ~0.25 mg/kcal of diet. Dietary intake, as free choline and phospatidyl choline is estimated to be over 700 mg daily in the US (18). The level used in the NWD formulations provide 0.12% of choline, or 1.2 mg choline/g of NWD composition, or ~0.26 mg choline/kcal in the NWDs (Table I).

(vii) *Vitamin B_{12}* (cobalamin) estimated daily dietary requirements for adults is ~2 μg (30). Because a generous intake of animal foods is common in the US and Canada (rich sources of vitamin B_{12}), the median intake in the US is estimated to be 4–5 μg, and 4–7 μg in Canada (31). At 4 μg/day in a 2000 kcal diet, this is 0.002 μg vitamin B_{12} per kcal of the human diet. AIN-76A diet is prepared with 25 μg/kg of diet, or 0.025 μg/g of diet, or 0.007 μg vitamin B_{12} per kcal of diet. This is also the level in NWD formulations, as a 20% excess of essential nutrients is added to WD and NWD formulations to compensate for the higher caloric density of these diets compared with the AIN-76A diet. Thus no changes in vitamin B_{12} level were made in the WD and NWD formulations to be equivalent to human diets. It was estimated that US and Canadian human diets were more than adequate in vitamin B_{12} content, and similarly the AIN-76A diet was also adequate.

Bromodeoxyuridine labeling and tissue collection

The mice were injected intraperitoneally with bromodeoxyuridine (BrdU, from Sigma, St Louis, MO) in amounts of 20 μg/g of body weight 1 h before necropsy. The gastrointestinal tract and internal organs were removed. Representative tissue segments were taken from the distal colon, fixed in 10% neutral buffered formalin and 80% ethanol overnight and transferred to 95% ethanol. The remaining gastrointestinal tract was opened longitudinally, except for the stomach, which was opened along the greater curvature. The gastrointestinal tract and all internal organs removed were examined for tumors under a dissecting microscope. Size and location of tumors found in the gastrointestinal tract and other organs were recorded and tumors were taken for processing and stained with hematoxylin and eosin. Histopathologic diagnosis for gastrointestinal tumors was made based on the World Health Organization's classification (13).

Immunohistochemical assay of cell proliferation

Colonic epithelial cells in DNA synthesis (S phase) can be visualized in these mice with an immunohistochemical preparation using the avidin–biotin-peroxidase technique, as widely used in our previous studies (6–11). Tissues used for BrdU study were fixed in ethanol. A specific monoclonal antibody to BrdU (Becton-Dickinson, San Jose, CA, USA) was added to deparaffinized

Table II. Tumor incidence in the intestinal tract and in other organs of normal C57Bl/6 mice fed NWD for 18 months

Diet group with tumors in other organs	*n*	Number (%) of mice with intestinal tumors	Number (%) of mice with tumors in other organs
AIN-76A	10	0 (0%)	0 (0%)
NWD1	12	5 (42%)[a]	1 (8%)
NWD2	12	2 (17%)	2 (17%)

n, number of mice studied; NWD1, new Western-style diet (low calcium, vitamin D, folic acid and methyl-donor availability); NWD2, new Western-style diet (the same as NWD1 except for increased calcium and vitamin D). Statistical results were from Fisher exact probabilities test: compared with AIN-76A diet: [a]$P < 0.04$.

tissue sections at 4°C overnight. The secondary antibody to mouse, avidin–biotin-peroxidase complex (Vector, CA, USA) was then applied to the slides after washing in phosphate-buffered saline (PBS) (pH 7.4). Color developed in diaminobenzidine solution, counterstained with hematoxylin, dehydrated in graded ethanol, cleared in xylene and mounted with permount. BrdU positive cells were stained brown and identified under a light microscope. Fifty crypt columns, well oriented were evaluated for each mouse. The total number of colonic epithelial cells and number and position of BrdU positive cells were recorded. The data were entered into an LI 5 software program developed in our laboratory, and resultant group data were generated for comparisons.

Statistical analysis

The Fisher exact probabilities test, Student *t*-test, Mann–Whitney test, and binomial test were used to compare data from different diet groups. Differences were considered significant when *P*-value was <0.05.

Results

Mice, both male and female, were killed on schedule ~12 (30 mice) and 18 months (34 mice) after feeding was begun. No tumors were found in the mice after 12 months of feeding. After 18 months of feeding, tumors developed both within and outside of the gastrointestinal tract of the C57Bl/6 mice and are reported below. Results on male and female mice in each group were pooled.

Body weights

The mice fed NWD1 and NWD2 began to gain more body weight than the AIN-76A controls after 1 month of feeding which gradually increased during the study ($P < 0.01$ for each comparison). These results are similar to previous studies of WDs in mice and are probably related to the increased dietary fat content (5,6). In contrast, previous studies of WDs in rats did not show significant increases in weight gain (3).

Tumor development

After feeding NWD1 for 18 months five of 12 (42%) of the mice developed tumors in the intestine; most of the tumors and all of the tumor types were located in the large intestine (Tables II and III). In the NWD2 group, 17% of mice developed tumors located in both large and small intestine. The tumor incidence in the NWD1 group was significantly higher than in the control AIN-76A group ($P < 0.04$), and was increased but not significantly in the NWD2 group (Table II). In the large intestine of both NWD groups all tumors were located in the cecum adjacent to the proximal colon. The number and histological types of these tumors are given in Table III and include: one early invasive carcinoma, four tubulovillous adenomas, two tubular adenomas and three flat adenomas (Figure 1). There were no gastrointestinal tumors in the control mice fed AIN-76A diet.

Table III. Histologic types of tumors in normal C57Bl/6 mice after 18-month of feeding of NWD

Diet	Tumors in the intestinal tract	Tumors in other group
NWD1	One early invasive CA Two flat adenomas Two tubular adenomas	One non-Hodgkin's lymphoma
NWD2	One tubulovillous adenoma[a] One flat adenoma	One non-Hodgkin's lymphoma One bronchio-alveolar carcinoma

Abbreviations: CA, adenocarcinoma.
[a]One tubulovillous adenoma was found in the small intestine; all other intestinal tumors were in the large intestine.

Tumors also were seen in both NWD groups outside of the gastrointestinal tract: one tumor (8%) and two (17%) in NWD1 and NWD2 mice, respectively. These included two non-Hodgkin's lymphomas involving mesenteric lymph nodes and one bronchio-alveolar carcinoma of the lung (Table III). No tumors outside of the gastrointestinal tract were observed in AIN-76A control mice.

Measurements of cell proliferation in flat mucosa of the colon

In mice fed the NWD1 diet for 12 months the number of colonic epithelial cells per crypt column significantly increased compared with mice fed the control AIN-76A diet (20.05 ± 0.39 versus 18.05 ± 0.49 (mean ± SEM), $P < 0.05$); and increased after 18 months (17.61 ± 1.37 versus 13.89 ± 0.73, $P < 0.01$). In NWD2 mice, non-significant increases were seen compared with controls. Other proliferative parameters did not significantly change except for the number of BrdU-labeled cells per colonic crypt column in the NWD2 group: this was lower than in the NWD1 group after 12 months of feeding (1.04 ± 0.06 versus 1.25 ± 0.06, $P < 0.05$).

The NWD2 diet, containing much higher calcium than NWD1 diet, reduced the hyperplasia (cells per colonic crypt column) and proliferation (BrdU-labeled cells per crypt column) compared with NWD1. This is similar to results reported previously with WD diet (high fat, reduced calcium and vitamin D) when dietary calcium was increased (5,6). The tumor incidence (Table II) decreased after calcium addition to NWD-1 but not significantly.

Focal hyperplasia in the liver

After feeding the NWDs with reduced methyl-donor co-factor availability, the livers were enlarged and were light yellow in color. Small nodules 1–2 mm in diameter were observed on the surface of the liver in several mice in the NWD groups after 12 and 18 months of feeding. One of 10 (10%) mice in the NWD1 group developed focal hyperplasias in these hepatic nodules after 12 months of feeding and 1 of 12 (8%) of mice developed nodules after 18 months of feeding in the NWD1 group (Table IV). In the NWD2 group, 20% of mice developed nodules after 12 months of feeding and 42% of mice after 18 months. However, the number of visible nodules in each liver was low, and ranged from one to four. Microscopically, most liver cells outside the nodules showed cytoplasm to be vacuolated or clear, the nodules had no capsule and there was no obvious compression of adjacent normal liver tissue. Nodules were composed of cells that were eosinophilic, basophilic, vacuolated, clear or mixed. Mitotic figures could be seen in some nodules. Hyperplasias of small bile ducts were also observed in some of the nodules. Small nodules also were seen microscopically that might represent an early stage of

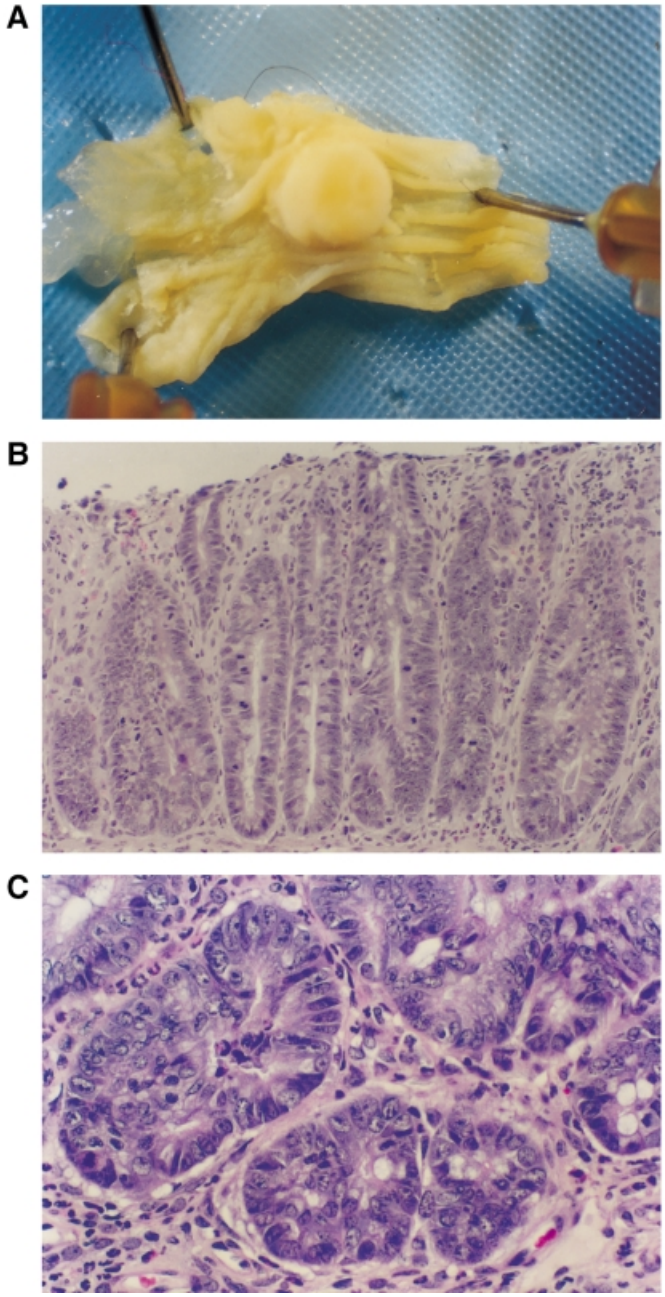

Fig. 1. Tumors developing in the large intestine of normal C57Bl/6 mice fed NWD1 for 18 months. (**A**) Gross tumor elevated above the surface of the colonic mucosa. (**B**) Histological features of a flat adenoma showing parallel arrangements of neoplastic crypts with erosion on the surface of the tumor. The tumor cells were in moderate dysplasia. Hematoxylin and eosin 200× original magnification. (**C**) Histological features of a tubular adenoma showing branching tubular elements. Hematoxylin and eosin, 400× original magnification.

nodule development. Focal hyperplasias or other abnormalities were not seen in mice fed the AIN-76A control diet. These findings differ from previous results obtained after feeding a choline-methionine deficient diet (32–35): our model did not develop focal necrosis, cirrhosis or hepatocellular carcinoma, and liver nodules developed only at an older age.

Discussion

To the best of our knowledge this study is the first report of the dietary induction of colon cancer in normal rodents without the presence of a chemical carcinogen or targeted mutation. The tumors produced included tubulovillous and tubular flat adenomas, an early invasive carcinoma and were mainly in the large intestine (Table III). In previous studies Choi *et al.* (19) have shown biochemical changes in the colon of rats maintained on a diet with inadequate folate intake. Liver, but not intestinal, tumors have developed in rats with severe choline and methionine deficiency (32–35). In our study the induction of colonic neoplasms by modifying a standard AIN-76A semi-purified diet, with essentially the same basic ingredient components as the concurrently prepared AIN-76A control diet, strongly suggests the absence of any unexpected colonic carcinogenic agents in the diets.

Table IV. Focal hyperplasias in the liver of C57Bl/6 mice fed NWD

Diet	12 months on diet		18 months on diet	
	n	number (%) of mice with focal hyperplasia	*n*	number (%) of mice with focal hyperplasia
AIN-76A	10	0 (0%)	10	0 (0%)
NWD1	10	1 (10%)	12	1 (8%)
NWD2	10	2 (20%)	12	5 (42%)

n, number of mice studied.

Our previous WDs also were modified from the AIN-76A diet with decreased calcium, vitamin D and increased fat content at nutrient-density levels consumed by Western populations. The earlier diets induced proliferative, hyperplastic and dysplastic abnormalities in colonic epithelial cells (4,6,12) but not colonic adenomas or adenocarcinomas, whereas the colonic lesions induced here by the NWDs progressed further to gross adenomas and carcinoma.

The modifications in the NWDs involved reducing components of the diet that supply methyl-donor and co-factor groups for numerous biochemical reactions occurring in DNA and other cell constituents. The precise molecular basis for this intestinal cancer induced with dietary inadequacy of methyl-transfer co-factor and donor groups remains to be clarified. Plausible mechanisms through which this might occur have been described, including reduction in *S*-adenosylmethionine formation and alteration in DNA methylation (20), uracil misincorporation into DNA with disruption of DNA integrity and repair (21) and chromosome breakage (22). It is relevant here that Choi *et al.* (19) have shown that inadequate dietary folate impairs DNA excision repair in the rat colon in the absence of any chemical carcinogen. Kim *et al.* (23) also have reported that dietary folate depletion alone causes DNA strand breaks to occur within mutation-prone exons of the p53 gene in rat colonic cells, and increased folate supplementation inhibited intestinal polyps in *Min* mice (36).

The induction of liver cancer in rats by severe dietary choline and methionine deficiency with elevated dietary fat has been well studied previously (32–35); however, tumor development in the large intestine has not been reported. Compared with these previous studies, an important difference in the composition of the NWDs, is our reduction of the dietary methionine and choline level in the AIN-76A control diet, only to the nutrient density estimated for the average US human diet and not to a level of frank deficiency (Table I). This led to moderate hepatic abnormalities that occurred in a few mice, developing at a later duration and less severely than those after feeding choline-methionine deficient diets (32–35).

It is important to note in NWD2 that the increased levels of calcium and vitamin D were insufficient to prevent the development of tumors in the colon. Therefore, the dietary factors noted above, other than calcium, vitamin D and increased fat content, appear to be of major importance in this diet-induced rodent model of colonic carcinogenesis. Because of these findings we are currently testing the NWDs with high individual dietary levels of choline, methionine, folate or fiber individually as potential inhibitors of NWD1 diet-induced colon cancer in mice utilizing far larger sample size in each group, and we are initiating studies to explore metabolic pathways and related mechanisms through which decreasing methyl-donor or co-factor availability might induce the extensive cellular abnormalities noted.

Thus, our results indicate that the NWD1 diet, essentially using human nutrient-density equivalents of increased dietary fat, coupled with reduced calcium, vitamin D, folate, choline, methionine and fiber, induced adenomas and carcinoma in the colon of C57Bl/6 mice without carcinogen exposure or targeted mutations. Generally, these nutritional risk factors correlate with recognized human dietary risk factors for colon cancer. The results of this study suggest dietary folate to be a highly important nutrient, as it required the largest reduction from the AIN-76A dietary level to achieve the human nutrient density used in the NWD formulation.

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