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Formulation of the Total Western Diet (TWD) as a Basal Diet for Rodent Cancer Studies

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ABSTRACT: Rodent cancer studies typically use defined diets with nutrient profiles optimized for rodent health. However, a defined rodent diet that represents typical American nutrition in all aspects, including calorie sources and macro- and micronutrient composition, is not yet available. Thus, a nutrient density approach was used to formulate the new Total Western Diet (TWD) based on NHANES data for macro- and micronutrient intakes. The TWD has fewer calories from protein and carbohydrate sources and twice that from fat as compared to the AIN-93 diet. The new diet contains more saturated and monounsaturated fats, less polyunsaturated fat, fewer complex carbohydrates, and twice the level of simple sugars. The TWD includes less calcium, copper, folate, thiamin, and vitamins B_6, B_{12}, D, and E, but much more sodium. This newly devised diet that better represents typical American nutrition will be highly useful for studies employing animal models of human disease, including cancer.

KEYWORDS: *Total Western Diet, rodent diets, micronutrients, macronutrients, American nutrition*

INTRODUCTION

Approximately one-fourth of all deaths in countries with a Westernized lifestyle are attributed to cancer.[1] The Western dietary pattern is characterized by high intakes of red and processed meats, sweets, fried foods, and refined grains, whereas a more balanced diet replaces these foods with fruits and vegetables, legumes, fish, poultry, and whole grains. In case-controlled and cohort studies the typical Western diet is associated with significantly higher rates of colorectal cancer compared to a balanced diet.[2] Environmental factors may contribute approximately 70% of this risk.[3−5] Colorectal cancer is the third most commonly diagnosed cancer in both men and women in the United States, with 142 570 estimated new cases in 2010; this disease is also the second most common cause of cancer-related deaths, with 51 370 expected deaths last year.[6] Colon cancer affects primarily those over the age of 50 and has the highest incidence in whites and African-Americans.

The typical Western diet is characterized by inexpensive, highly processed foods that are rich in calories, but low in some essential micronutrients such as minerals and vitamins. As most micronutrients are acquired through the diet, consumption of energy-dense, nutrient-poor foods may result in micronutrient intakes below Recommended Daily Allowances (RDAs). RDAs are formulated to prevent deficiency diseases in the U.S. population. However, some evidence suggests that chronic low intakes of micronutrients can negatively affect metabolic processes without triggering the physical manifestation of acute deficiency.[7] For example, a recent report released by the U.S. Department of Agriculture Agricultural Research Service estimated that only one-third of Americans consume adequate calcium and vitamin D and less than half consume adequate magnesium.[3] Also, 90% of Americans do not consume sufficient vitamin E, 30% enough vitamin C, and 12% adequate zinc compared to the Estimated Average Requirement (EAR) values for these micronutrients.[8] Although these low nutrient intakes do not trigger symptoms of acute deficiency, other adverse health effects from chronic low dietary exposure are possible, including increased risk or acceleration of chronic, degenerative diseases such as cancer, cardiovascular disease, and diabetes.

Rodent cancer studies generally use purified diets that were established using micronutrient profiles optimized for growth and fertility, such as the AIN diets formulated by the American Institute of Nutrition.[9,10] One rationale given for the development of these diets was to provide researchers with a nutritionally adequate diet that would allow for standardization of studies among laboratories. However, these diets do not reflect the nutrient density of either macronutrients or micronutrients in the average U.S. diet and thus may obscure some of the contributions of the overall Western dietary pattern to the development of cancer, both within individuals and potentially across generations.

Many strategies have been employed by researchers working with rodents to recapitulate the main features of the Western dietary pattern. For example, in early studies investigating diet-

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Table 1. Formulation of the Total Western Diet (TWD) Based on Energy Density

	NHANES[a]				
	10th	mean	90th	AIN-93G[b]	TWD
macronutrient (mg/kcal)					
total fat (mg)	24.6	37.8	69.1	17.9	37.8
saturated (mg)	7.7	12.7	16.9	2.7	12.7
monounsaturated (mg)	9.4	13.9	20.3	4.3	13.9
polyunsaturated (mg)	4.9	7.9	10.1	11.5	7.9
n6 PUFA (mg)	5.1	7.1	10.4	10.2	7.1
n3 PUFA (mg)	0.4	0.7	1.1	1.3	0.7
cholesterol (mg)	106.3	133.3	173.9	n/a	133.3
total carbohydrates (mg)	80.2	123.7	183.1	154.1	123.7
complex carbohydrates (mg)	38.6	58.4	87.0	124.0	58.4
simple sugars (mg)	38.2	58.0	87.0	30.1	58
dietary fiber (mg)	4.4	7.3	11.8	12.8	7.3
total protein (mg)	26.9	37.7	52.2	45.4	37.7
micronutrient (unit/kcal)					
minerals					
calcium (μg)	253.6	457.0	772.0	1282.1	457.0
phosphorus (μg)	415.9	626.6	909.7	769.2	626.6
potassium (μg)	810.6	1212.1	1730.4	923.1	1212.1
sodium (μg)	1071.0	1608.7	2327.5	266.4	1608.7
magnesium (μg)	88.9	133.8	195.7	129.7	133.8
iron (μg)	4.5	7.1	11.1	9.0	7.1
zinc (μg)	3.8	5.6	8.2	7.7	5.6
copper (μg)	0.4	0.6	0.9	1.5	0.6
selenium (ng)	33.8	48.3	73.9	38.5	48.3
vitamins					
niacin (μg)	7.9	11.5	16.8	7.7	11.5
vitamin B_6 (μg)	0.6	0.9	1.4	1.8	0.9
thiamin (μg)	0.6	0.8	1.2	1.5	0.8
riboflavin (μg)	0.7	1.0	1.6	1.5	1.0
folate (mg)	0.2	0.3	0.4	0.5	0.3
vitamin K (ng)	22.9	42.9	85.0	192.3	42.9
vitamin B_{12} (ng)	1.4	2.5	4.5	6.4	2.5
vitamin A (mIU)	501.0	977.3	1824.2	1025.6	977.3
vitamin D (mIU)	36.2	88.9	194.7	256.4	88.9
vitamin E (mIU)	3.5	5.6	8.9	19.2	5.6
choline (μg)	89.9	147.3	235.7	263.6	147.3

[a]Determined by dividing daily intake values (both sexes) from NHANES tables by 2070 kcal/day. Nutrient density of the NHANES 50th percentile were used to formulate the TWD. No data are available in NHANES for chloride, manganese, iodine, pantothenic acid, biotin, or ultratrace minerals. Thus, levels from the AIN-93G diet were used. [b]Mouse daily kcal intake based on mice consuming 2.5 g/day of AIN-93G, which has a reported energy density of 3.8 kcal/g for a total average calorie intake of 9.5 kcal/day. On the basis of this intake and nutrients supplied by the AIN-93G diet,[10] nutrient density calculations were determined.

induced obesity, animals were fed a "cafeteria diet" from which the animals could select from an array of highly palatable foods such as cookies, candy, cheese, and processed meats.[11] A diet composed of these foods is high in salt, sugar, and fat, all of which are associated with the Western dietary pattern. However, this experimental strategy suffers from the fact that the dietary exposure is not consistent across animals or time and is not necessarily reproducible across experiments due to food selection variability. An alternative option is to employ a commercial "Western diet" formulation, such as the atherogenic diet, which is formulated with 21% butterfat, 34% sucrose, and 0.2% cholesterol.[12] This particular diet is popular with researchers utilizing apolipoprotein E (*Apoe*) knockout animals to investigate atherogenesis.[13] Although this diet is effective in inducing an atherogenic phenotype, it is not truly reflective of the Western dietary pattern with respect to its sugar content, fatty acid profile, or levels of micronutrients. Commercial Western diets have also been developed for the study of obesity; these diets typically contain 45 or 60% of energy as fat and differ from the AIN diets primarily in their high lard and sucrose contents.[11] Although these high-fat diets are effective in producing obesity in rodents,[13] they are extreme in their sugar and fat compositions when compared to a typical Western dietary pattern.[11]

Another strategy that has been used in designing animal diets to reflect U.S. consumption patterns is based on the concept of nutrient density. According to Newmark,[14] translation of dietary requirements between species is difficult, especially when large differences in the metabolic rates exist, as is the case with rodents and humans. However, expression of each nutrient in terms of the energy content of the diet (usually in mass per kcal) is a straightforward way to translate diet composition between species. Importantly, this approach accounts for different nutrient needs in animals with differing metabolic

rates. Newmark and colleagues have used this strategy to investigate the role of select nutrients in colon cancer and have termed their formulation the New Western Diet (NWD).[15,16] In early studies, they employed a nutrient density approach to manipulate the composition of the AIN-76A to reflect U.S. diets. In this initial work, dietary fat and phosphate contents were increased, whereas amounts of calcium and vitamin D were reduced.[15] In subsequent studies, Newmark and collaborators have extended the same nutrient density approach to selected nutrients such as folate, methionine, cysteine, choline, and fiber.[16] Among the nutrients they have investigated, calcium and vitamin D appear to be most protective against colon tumor incidence and multiplicity. Despite the simplicity and effectiveness of the nutrient density approach, to date, no research groups have attempted to apply this method for all macro- and micronutrients in a defined rodent diet.

We believe that there is a definite need for the development of a rodent diet with a well-defined macro- and micronutrient composition that recapitulates typical Western nutritional patterns in all respects. Such a diet would be optimal for rodent colon cancer studies investigating the role of specific micronutrients or other bioactive food components for changing colon cancer risk in typical American diets. Moreover, such a diet would be highly useful for researchers investigating the impact of typical U.S. nutrition in animal models of a wide variety of human diseases, including other cancers, obesity, diabetes, and cardiovascular disease.

MATERIALS AND METHODS

The Total Western Diet (TWD) for rodents was formulated using the principle of nutrient density. This process entailed defining a Western diet based on average American nutrient intakes and translating the human diet into one suitable for rodents. As a model for a typical Western diet, we selected the average (50th percentile) daily intake levels for all reported nutrients for individuals >2 years old from the National Health and Nutrition Examination Survey *What We Eat in America* for the years 2007−2008,[17] the most recent years for which data are available. The average nutrient consumption data allowed us to normalize the daily intake to calories consumed to establish a nutrient density measure (mass of nutrient/kcal/day). The NHANES survey contains information on total energy intake, macronutrient ratios (protein, fat, and carbohydrates) and macronutrient sources (complex carbohydrates versus simple sugars and fatty acid composition), as well as information on most essential micronutrients.

After determinion of an average intake for each nutrient from the NHANES data, the next step in formulating the rodent diet required translation of the human nutrient intake information to a diet suited for rodents using the nutrient density approach. Table 1 shows the nutrient density value for each component of the TWD and AIN-93G diet. First, the relative contribution of total carbohydrates, fats, and protein sources to total calories consumed at the NHANES 50th percentile was determined; these ratios were then used to formulate the basal TWD. The carbohydrate content of the TWD was further portioned into either simple or complex carbohydrates to match NHANES data. The NHANES data set is particularly powerful in that intake values for individual fatty acids are reported. Thus, the fat portion of the TWD was devised using a diverse set of fat sources to match the fatty acid profile reported in NHANES at the 50th percentile. Of the macronutrient components, protein content is the most similar between the AIN-93G diet and the newly devised TWD, as casein and L-cystine are the primary protein sources in both diets. However, to match the total caloric contribution of protein to the NHANES data set, the TWD contains 15.4% of total energy from protein compared to 18.8% in the AIN-93G diet. The nutrient density for each noncaloric dietary component was calculated as the quotient of the daily intake of each nutrient divided by the average caloric intake per day from NHANES (eq 1). The nutrient density for each component was then translated to the TWD as follows, assuming a nutrient density of 4.4 kcal/g diet for the TWD diet:

$$\frac{\text{unit nutrient/day}}{2070\ \text{kcal/day}} \times \frac{4.4\ \text{kcal}}{\text{g diet}} \times 1000 = \text{unit nutrient/kg diet} \quad (1)$$

"Unit nutrient/day" is the average daily nutrient intake, and 2070 kcal/day is the average daily caloric intake reported at the NHANES 50th percentile. Equation 2 provides an example application of this nutrient density approach for calcium, for which the NHANES 50th percentile intake is 946 mg/day.

$$\frac{946\ \text{mg calcium/day}}{2070\ \text{kcal/day}} \times \frac{4.4\ \text{kcal}}{\text{g diet}} \times 1000 = 2010\ \text{mg calcium/kg diet} \quad (2)$$

RESULTS AND DISCUSSION

With respect to the macronutrient content, the TWD contains fewer calories from protein and carbohydrates and approximately twice that from fat (see Table 2). On a nutrient density basis, the TWD contains more saturated and monounsaturated fats, but less polyunsaturated fats. Because the TWD was formulated using a diverse set of fat sources to match patterns of fat consumption as reported in NHANES (Table 2), the TWD is much more diverse in terms of fatty acid composition when compared to AIN-76 and -93 diets (Table 3), which contain exclusively either corn or soybean oil. Unlike the TWD, the AIN diets contain no fatty acids with chain lengths shorter than 14 carbons. Moreover, the TWD contains substantially more palmitic, stearic, and oleic acid but much less linoleic acid than the AIN diets. Several rodent colon cancer studies have

Table 2. Comparison of Macronutrient Sources in the Total Western Diet (TWD) to AIN-76A and AIN-93G Basal Diets[a]

macronutrient	AIN-76A	AIN-93G	TWD
carbohydrates			
corn starch	150	398	230
maltodextrin		132	70
sucrose	500	100	261
cellulose	50	50	30
kcal (% of total)	67.7	60.1	54.5
proteins			
casein	200	200	190
L-cystine		3	2.85
DL-methionine	3		
kcal (% of total)	20.8	18.8	15.4
fats			
soybean oil		70	31.4
anhydrous milk fat			36.3
olive oil			28.0
lard			28.0
beef tallow			24.8
corn oil	50		16.5
holesterol			0.40
kcal (% of total)	11.5	17.2	34.5

[a]Values are g/kg diet for all components. Absent values indicate that the corresponding component is not present in that diet.

Table 3. Comparison of Fatty Acid Composition of the Total Western Diet (TWD) to AIN-76A and AIN-93G Basal Diets[a]

fatty acid	AIN-76A	AIN-93G	TWD
C4:0 (butyric)			0.8
C6:0 (caproic)			0.4
C8:0 (caprylic)			0.4
C10:0 (capric)			0.7
C12:0 (lauric)			1.1
C14:0 (myristic)	0.5		3.2
C16:0 (palmitic)	11.4	10.5	20.1
C16:1 (palmitoleic)	0.2		1.7
C18:0 (stearic)	2.2	4.4	9.7
C18:1 (oleic)	27.4	22.6	38.4
18:2 (linoleic)	56.4	54	20.7
C18:3 (linolenic)	1.0	7.0	2.0
kcal (% of total)	6.8	7.7	10.4
ratio of n6:n3	56.4	7.7	10.4
ratio of polyunsaturated:saturated fat	4.1	4.1	0.6

[a]Values are g/kg diet for all fatty acids. Absent values indicate that the corresponding fatty acid is not present in that diet. Fatty acids reported in NHANES that provide <0.1% of daily kcal were excluded from the TWD, including erucic acid, stearidonic acid, arachadonic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid.

Table 4. Mineral and Vitamin Profiles of the Total Western Diet (TWD) Compared to the AIN-93G Basal Diet[a]

micronutrient	AIN-93G	TWD
minerals (mg/kg diet)		
calcium	5000	2011
phosphorus	3000	2757
sodium	1019	7078
potassium	3600	5333
magnesium	507	589
iron	35	31
zinc	30	25
copper	6	2.6
selenium	0.15	0.2
vitamins (unit/kg diet)		
thiamin (mg)	5	3.5
riboflavin (mg)	6	4.4
niacin (mg)	30	50.6
pyridoxine (mg)	6	3.9
folate (mg)	2	1.3
vitamin B_{12} (μg)	25	11
vitamin A (IU)	4000	4300
vitamin D (IU)	1000	391
vitamin E (IU)	75	24.6
vitamin K (μg)	750	189
choline (mg)	1027	648

[a]No data are available in NHANES for chloride, manganese, iodine, ultratrace minerals, pantothenic acid, and biotin. Levels from the AIN-93G diet were used for these nutrients to formulate the TWD.

shown that dietary fat profiles affect carcinogenesis[18−21] and that corn oil, the sole fat source of the AIN-76 diet, may enhance colon cancer in rodent models.[22,23]

Despite the lower overall contribution of carbohydrates to nutrient density, the TWD contains half the complex carbohydrates and twice the level of simple sugars as the AIN-93G diet and approximately 60% of the dietary fiber. The contribution of simple versus complex carbohydrates is important to the TWD formulation as some evidence suggests that simple carbohydrates may increase colon carcinogenesis in rodent models.[24,25] Dietary fiber has been studied extensively using rodent colon cancer models. In the Chemoprevention of Colorectal Cancer Database compiled by Corpet and colleagues,[26−28] 119 studies are listed that have investigated the effects of dietary fiber on colon cancer in rodent models. Generally, the studies in this database show a decrease in colon cancer as dietary fiber is increased in the rodent diet. Thus, the lowered fiber content of the TWD may increase carcinogenesis compared to the AIN-93G diet.

In terms of minerals, the TWD contains significantly less calcium and copper and significantly more sodium than the AIN-93G diet (Table 4). The TWD is lower in most vitamins than the AIN-93G diet, except for niacin and vitamin A (Table 4). Several vitamins and minerals implicated in colon carcinogenesis are at least 2-fold lower in the TWD compared to the AIN-93G diet, including calcium, copper, vitamin B_6, thiamin, folate, and vitamins B_{12}, D, and E (Figure 1).[16,29−43]

Although the concept of formulating basal rodent diets based on nutrient density is not novel, the TWD differs from previous diets such as the New Western Diet formulated by Newmark and Lipkin in several important ways. First, they based dietary vitamin D and calcium levels on reported human intakes that were exceedingly low and, thus, not typical of the broader population.[15] The TWD reflects average intakes for all macro- and micronutrients. As a result, the TWD is not necessarily extreme in the level of any given nutrient, but rather reflects the overall dietary pattern of the United States. Moreover, when a nutrient density approach is used to translate human intakes to rodent diets, none of the nutrient levels in the new TWD are misrepresentative of U.S. nutrition as all nutrient values are well within the 10th−90th percentile of reported American intakes (Figure 1). Alternatively, as illustrated in Figure 1, several components of the AIN-93G diet are provided at levels far below the NHANES 10th percentile, including total fat, saturated fat, monounsaturated fat, simple sugars, sodium, and niacin. Conversely, many components of the AIN-93G diet are provided at levels above the NHANES 90th percentile, including total polyunsaturated fat, n3 polyunsaturated fat, complex carbohydrates, calcium, choline, copper, thiamin, folate, and vitamins B_6, B_{12}, D, and E.

A number of foods, micronutrients, and bioactive food components have been identified using rodent models of carcinogenesis that may help reduce cancer risk, including chemicals present in certain fruits, vegetables, and whole grains. However, many individuals consume a diet that is deficient in these food items and the beneficial micronutrients they provide; the typical Western diet is emblematic of this problem. For example, consumption of vegetables and micronutrients such as folate, vitamins B_{12} and B_6, n3 polyunsaturated fatty acids, calcium, vitamins D, C, and E, and selenium have been linked to decreased risk of colon cancer in humans.[38,44−48] However, basal rodent diets used in cancer studies are typically optimized for these nutrients, and the contribution of specific dietary interventions may be masked in rodent colon cancer models. For instance, consumption of methionine and other cofactors for the *S*-adenosylmethionine (SAM) pathway, such as folate, vitamin B_{12}, and choline, is necessary for maintaining the epigenome.[49] In animal studies, diets inadequate in methyl donors have been associated with increased risk of liver, colon,

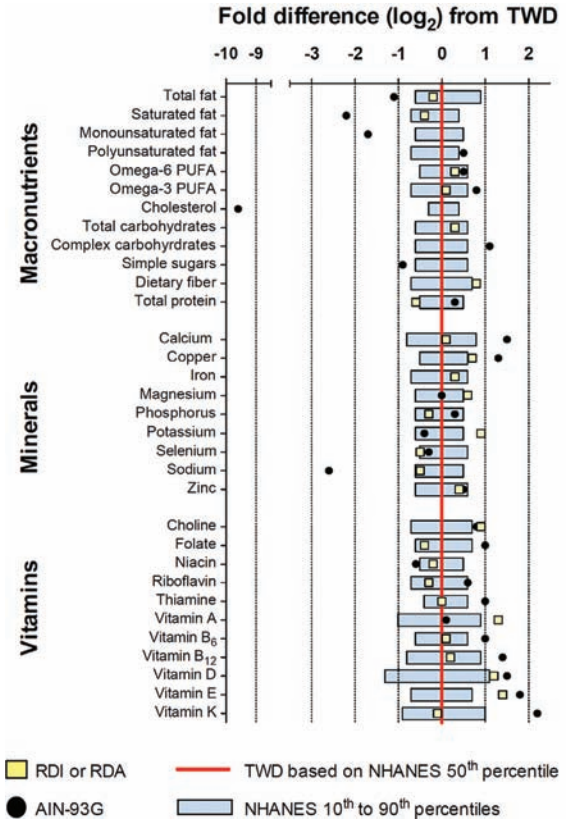

Figure 1. Energy density-normalized macro- and micronutrient comparisons of the TWD versus the AIN-93G diet in relation to intakes reported in NHANES and RDI/RDA values. The relationship (calculated as a log2-fold difference) between TWD (red line) and the range of intakes for the typical American diet (10th–90th percentiles, blue bar) is shown for each diet component. Also shown are the energy density-normalized amounts of each nutrient in the AIN-93G diet (black circles) and the normalized RDI or RDA value (yellow squares, adult >2 years, not considering pregnant or lactating women).

pancreas, and esophageal cancer in rats exposed to chemical carcinogens (reviewed in ref 50). Conversely, recent papers show that direct supplementation with SAM protects against carcinogen-induced colon cancer in rats.[51] Epidemiological evidence also points to a potential critical role of dietary methyl donors in cancer prevention.[52−55] Additionally, deficiencies in vitamins B_6 and B_{12}, both components of the methyl donor metabolism pathway, have been associated with increased risk of colon cancer.[38,56] The TWD contains substantially less folate, vitamin B_{12}, vitamin B_6, and choline compared to AIN diets, and levels of these micronutrients in the AIN-93 diet are in excess of the 90th percentile for U.S. intakes, when normalized by energy density (Table 1; Figure 1). Because the availability of methyl donors plays a significant role in colon carcinogenesis, it stands to reason that basal diets used in rodent colon cancer investigations should emulate typical American intakes of nutrients involved in one-carbon metabolism.

The rationale for formulating the TWD was based on the need for rodent diets to mimic typical American intakes of macro- and micronutrients to more precisely investigate dietary chemoprevention strategies in rodent models of human disease. Chronic micronutrient deficiencies common in American diets may cause metabolism changes that obstruct certain metabolic pathways, placing the individual at greater risk for chronic disease.[8] Whereas metabolic processes may compensate for periodic dietary shortages of micronutrients, the long-term effects of inadequate nutrient intake are unknown. Moreover, high caloric intake could reasonably be expected to exacerbate chronic micronutrient deficiencies by stressing metabolic pathways in which these nutrients act as cofactors. Focused in vitro and in vivo studies with specific micronutrients such as vitamins E, B_6, and B_{12} as well as folate, iron, and zinc indicate that deficiencies of these micronutrients can result in DNA damage and/or oxidative lesions as well as mitochondria dysfunction (reviewed in ref 7).

Although many studies have investigated the health effects of chronic low consumption of single micronutrients, information regarding the impact of chronic low intake of multiple micronutrients on disease outcome is lacking, especially in the context of a typical Western diet. In the papers discussed above, researchers employed rodent models and standard formulated diets that were generally balanced with respect to macro- and micronutrient levels to optimize animal health. It is critical to note, however, that these optimally formulated rodent diets are not relevant to most human diets, especially for at-risk populations that frequently consume energy-dense, nutrient-poor foods. We believe that this new TWD for use in typical rodent colon cancer investigations may fill a critical void that is not addressed by using optimal basal diets that have little relevance to American dietary patterns.

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REFERENCES

(1) Boyle, P.; Langman, J. S. ABC of colorectal cancer: epidemiology. *BMJ* **2000**, *321* (7264), 805−808.

(2) Meyerhardt, J. A.; Niedzwiecki, D.; Hollis, D.; Saltz, L. B.; Hu, F. B.; Mayer, R. J.; Nelson, H.; Whittom, R.; Hantel, A.; Thomas, J.; Fuchs, C. S. Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer. *JAMA, J. Am. Med. Assoc.* **2007**, *298* (7), 754−764.

(3) Jemal, A.; Siegel, R.; Ward, E.; Hao, Y.; Xu, J.; Thun, M. J. Cancer statistics, 2009. *CA Cancer J. Clin.* **2009**, *59* (4), 225−249.

(4) Wiseman, M. The second World Cancer Research Fund/ American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. *Proc. Nutr. Soc.* **2008**, *67* (3), 253−256.

(5) Doll, R.; Peto, R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* **1981**, *66* (6), 1191−1308.

(6) American Cancer Society. *Cancer Facts and Figures, 2009*; Atlanta, GA, 2009.

(7) Ames, B. N. Increasing longevity by tuning up metabolism. To maximize human health and lifespan, scientists must abandon outdated models of micronutrients. *EMBO Rep.* **2005**, *6* Spec. No., S20−S24.

(8) Ames, B. N. Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (47), 17589−17594.
(9) American Institute of Nutrition. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. *J. Nutr.* **1977**, *107* (7), 1340−1348.
(10) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C. Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123* (11), 1939−1951.
(11) Gajda, A. M. High fat diets for diet-induced obesity models, http://www.researchdiets.com/OSD/DIDM/obesity.htm.
(12) Plump, A. S.; Smith, J. D.; Hayek, T.; Aalto-Setala, K.; Walsh, A.; Verstuyft, J. G.; Rubin, E. M.; Breslow, J. L. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* **1992**, *71* (2), 343−353.
(13) Jawien, J.; Nastalek, P.; Korbut, R. Mouse models of experimental atherosclerosis. *J. Physiol. Pharmacol.* **2004**, *55* (3), 503−517.
(14) Newmark, H. L. Nutrient density: an important and useful tool for laboratory animal studies. *Carcinogenesis* **1987**, *8* (7), 871−873.
(15) Newmark, H. L.; Yang, K.; Lipkin, M.; Kopelovich, L.; Liu, Y.; Fan, K.; Shinozaki, H. A Western-style diet induces benign and malignant neoplasms in the colon of normal C57Bl/6 mice. *Carcinogenesis* **2001**, *22* (11), 1871−1875.
(16) Newmark, H. L.; Yang, K.; Kurihara, N.; Fan, K.; Augenlicht, L. H.; Lipkin, M. Western-style diet-induced colonic tumors and their modulation by calcium and vitamin D in C57Bl/6 mice: a preclinical model for human sporadic colon cancer. *Carcinogenesis* **2009**, *30* (1), 88−92.
(17) U.S. Department of Agriculture; Agricultural Research Service; Beltsville Human Nutrition Research Center; Food Surveys Research Group (Beltsville MD) and; U.S. Department of Health and Human Services; Centers for Disease Control and Prevention; National Center for Health Statistics (Hyattsville MD) *What we eat in America, NHANES*, 2005−2006.
(18) Fujise, T.; Iwakiri, R.; Kakimoto, T.; Shiraishi, R.; Sakata, Y.; Wu, B.; Tsunada, S.; Ootani, A.; Fujimoto, K. Long-term feeding of various fat diets modulates azoxymethane-induced colon carcinogenesis through Wnt/β-catenin signaling in rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2007**, *292* (4), G1150−G1156.
(19) Rao, C. V.; Hirose, Y.; Indranie, C.; Reddy, B. S. Modulation of experimental colon tumorigenesis by types and amounts of dietary fatty acids. *Cancer Res.* **2001**, *61* (5), 1927−1933.
(20) Wasan, H. S.; Novelli, M.; Bee, J.; Bodmer, W. F. Dietary fat influences on polyp phenotype in multiple intestinal neoplasia mice. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94* (7), 3308−3313.
(21) Mai, V.; Colbert, L. H.; Berrigan, D.; Perkins, S. N.; Pfeiffer, R.; Lavigne, J. A.; Lanza, E.; Haines, D. C.; Schatzkin, A.; Hursting, S. D. Calorie restriction and diet composition modulate spontaneous intestinal tumorigenesis in Apc(Min) mice through different mechanisms. *Cancer Res.* **2003**, *63* (8), 1752−1755.
(22) Kohno, H.; Yamaguchi, N.; Ohdoi, C.; Nakajima, S.; Odashima, S.; Tanaka, T. Modifying effect of tuna orbital oil rich in docosahexaenoic acid and vitamin D3 on azoxymethane-induced colonic aberrant crypt foci in rats. *Oncol. Rep.* **2000**, *7* (5), 1069−1074.
(23) Dommels, Y. E.; Heemskerk, S.; van den Berg, H.; Alink, G. M.; van Bladeren, P. J.; van Ommen, B. Effects of high fat fish oil and high fat corn oil diets on initiation of AOM-induced colonic aberrant crypt foci in male F344 rats. *Food Chem. Toxicol.* **2003**, *41* (12), 1739−1747.
(24) Wang, B.; Bobe, G.; LaPres, J. J.; Bourquin, L. D. High sucrose diets promote intestinal epithelial cell proliferation and tumorigenesis in APC(Min) mice by increasing insulin and IGF-I levels. *Nutr. Cancer* **2009**, *61* (1), 81−93.
(25) Poulsen, M.; Molck, A. M.; Thorup, I.; Breinholt, V.; Meyer, O. The influence of simple sugars and starch given during pre- or post-initiation on aberrant crypt foci in rat colon. *Cancer Lett.* **2001**, *167* (2), 135−143.
(26) Corpet, D. E.; Pierre, F. Point: From animal models to prevention of colon cancer. Systematic review of chemoprevention in min mice and choice of the model system. *Cancer Epidemiol. Biomarkers Prev.* **2003**, *12* (5), 391−400.
(27) Corpet, D. E.; Tache, S. Most effective colon cancer chemopreventive agents in rats: a systematic review of aberrant crypt foci and tumor data, ranked by potency. *Nutr. Cancer* **2002**, *43* (1), 1−21.
(28) Corpet, D. E.; Pierre, F. How good are rodent models of carcinogenesis in predicting efficacy in humans? A systematic review and meta-analysis of colon chemoprevention in rats, mice and men. *Eur. J. Cancer* **2005**, *41* (13), 1911−1922.
(29) Ames, B. N. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. *Mutat. Res.* **2001**, *475* (1−2), 7−20.
(30) Bruce, W. R.; Furrer, R.; Shangari, N.; O'Brien, P. J.; Medline, A.; Wang, Y. Marginal dietary thiamin deficiency induces the formation of colonic aberrant crypt foci (ACF) in rats. *Cancer Lett.* **2003**, *202* (2), 125−129.
(31) Davis, C. D. Low dietary copper increases fecal free radical production, fecal water alkaline phosphatase activity and cytotoxicity in healthy men. *J. Nutr.* **2003**, *133* (2), 522−527.
(32) Davis, C. D.; Feng, Y. Dietary copper, manganese and iron affect the formation of aberrant crypts in colon of rats administered 3,2′-dimethyl-4-aminobiphenyl. *J. Nutr.* **1999**, *129* (5), 1060−1067.
(33) Davis, C. D.; Johnson, W. T. Dietary copper affects azoxymethane-induced intestinal tumor formation and protein kinase C isozyme protein and mRNA expression in colon of rats. *J. Nutr.* **2002**, *132* (5), 1018−1025.
(34) Geter, D. R.; Moore, T. M.; George, M. H.; Kilburn, S. R.; Allen, J. W.; Nelson, G. M.; Winkfield, E.; DeAngelo, A. B. Tribromomethane exposure and dietary folate deficiency in the formation of aberrant crypt foci in the colons of F344/N rats. *Food Chem. Toxicol.* **2005**, *43* (9), 1405−1412.
(35) Komatsu, S.; Isobe, M.; Yanaka, N.; Kato, N. A high-fat diet enhances the inhibitory effect of dietary vitamin B6 on colon cell proliferation in mice. *Oncol. Rep.* **2005**, *14* (1), 265−269.
(36) Komatsu, S.; Watanabe, H.; Oka, T.; Tsuge, H.; Kat, N. Dietary vitamin B6 suppresses colon tumorigenesis, 8-hydroxyguanosine, 4-hydroxynonenal, and inducible nitric oxide synthase protein in azoxymethane-treated mice. *J. Nutr. Sci. Vitaminol. (Tokyo)* **2002**, *48* (1), 65−68.
(37) Komatsu, S. I.; Watanabe, H.; Oka, T.; Tsuge, H.; Nii, H.; Kato, N. Vitamin B-6-supplemented diets compared with a low vitamin B-6 diet suppress azoxymethane-induced colon tumorigenesis in mice by reducing cell proliferation. *J. Nutr.* **2001**, *131* (8), 2204−2207.
(38) Larsson, S. C.; Orsini, N.; Wolk, A. Vitamin B6 and risk of colorectal cancer: a meta-analysis of prospective studies. *JAMA, J. Am. Med. Assoc.* **2010**, *303* (11), 1077−1083.
(39) Newmark, H. L.; Lipkin, M.; Maheshwari, N. Colonic hyperplasia and hyperproliferation induced by a nutritional stress diet with four components of Western-style diet. *J. Natl. Cancer Inst.* **1990**, *82* (6), 491−496.
(40) Newmark, H. L.; Lipkin, M.; Maheshwari, N. Colonic hyperproliferation induced in rats and mice by nutritional-stress diets containing four components of a human Western-style diet (series 2). *Am. J. Clin. Nutr.* **1991**, *54* (1 Suppl.), 209S−214S.
(41) Newmark, H. L.; Huang, M. T.; Reddy, B. S. Mixed tocopherols inhibit azoxymethane-induced aberrant crypt foci in rats. *Nutr. Cancer* **2006**, *56* (1), 82−85.
(42) Shivapurkar, N.; Tang, Z.; Frost, A.; Alabaster, O. Inhibition of progression of aberrant crypt foci and colon tumor development by vitamin E and β-carotene in rats on a high-risk diet. *Cancer Lett.* **1995**, *91* (1), 125−132.
(43) Yao, K.; Latta, M.; Bird, R. P. Modulation of colonic aberrant crypt foci and proliferative indexes in colon and prostate glands of rats by vitamin E. *Nutr. Cancer* **1996**, *26* (1), 99−109.

(44) Forte, A.; De Sanctis, R.; Leonetti, G.; Manfredelli, S.; Urbano, V.; Bezzi, M. Dietary chemoprevention of colorectal cancer. *Ann. Ital. Chir.* **2008**, *79* (4), 261−267.
(45) Kim, Y. S.; Milner, J. A. Dietary modulation of colon cancer risk. *J. Nutr.* **2007**, *137* (11 Suppl.), 2576S−2579S.
(46) Pufulete, M. Intake of dairy products and risk of colorectal neoplasia. *Nutr. Res. Rev.* **2008**, *21* (1), 56−67.
(47) Kune, G.; Watson, L. Colorectal cancer protective effects and the dietary micronutrients folate, methionine, vitamins B6, B12, C, E, selenium, and lycopene. *Nutr. Cancer* **2006**, *56* (1), 11−21.
(48) Roynette, C. E.; Calder, P. C.; Dupertuis, Y. M.; Pichard, C. n-3 polyunsaturated fatty acids and colon cancer prevention. *Clin. Nutr.* **2004**, *23* (2), 139−151.
(49) Van den Veyver, I. B. Genetic effects of methylation diets. *Annu. Rev. Nutr.* **2002**, *22*, 255−282.
(50) Rogers, A. E. Methyl donors in the diet and responses to chemical carcinogens. *Am. J. Clin. Nutr.* **1995**, *61* (3 Suppl.), 659S−665S.
(51) Guruswamy, S.; Swamy, M. V.; Choi, C. I.; Steele, V. E.; Rao, C. V. *S*-Adenosyl L-methionine inhibits azoxymethane-induced colonic aberrant crypt foci in F344 rats and suppresses human colon cancer Caco-2 cell growth in 3D culture. *Int. J. Cancer* **2008**, *122* (1), 25−30.
(52) Kim, Y. I. Folate and carcinogenesis: evidence, mechanisms, and implications. *J. Nutr. Biochem.* **1999**, *10* (2), 66−88.
(53) Prinz-Langenohl, R.; Fohr, I.; Pietrzik, K. Beneficial role for folate in the prevention of colorectal and breast cancer. *Eur. J. Nutr.* **2001**, *40* (3), 98−105.
(54) Choi, S. W.; Mason, J. B. Folate and carcinogenesis: an integrated scheme. *J. Nutr.* **2000**, *130* (2), 129−132.
(55) Friso, S.; Choi, S. W. Gene-nutrient interactions and DNA methylation. *J. Nutr.* **2002**, *132* (8 Suppl.), 2382S−2387S.
(56) Friso, S.; Choi, S. W. The potential cocarcinogenic effect of vitamin B12 deficiency. *Clin. Chem. Lab. Med.* **2005**, *43* (10), 1158−1163.