



# Why Do African Americans Get More Colon Cancer than Native Africans?<sup>1-3</sup>

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## Abstract

The incidence of colorectal cancer (CRC) is dramatically higher in African Americans (AAs) than in Native Africans (NAs) (60:100,000 vs. <1:100,000) and slightly higher than in Caucasian Americans (CAs). To explore whether the difference could be explained by interactions between diet and colonic bacterial flora, we compared randomly selected samples of healthy 50- to 65-y-old AAs ( $n = 17$ ) with NAs ( $n = 18$ ) and CAs ( $n = 17$ ). Diet was measured by 3-d recall, and colonic metabolism by breath hydrogen and methane responses to oral lactulose. Fecal samples were cultured for 7- $\alpha$  dehydroxylating bacteria and *Lactobacillus plantarum*. Colonoscopic mucosal biopsies were taken to measure proliferation rates. In comparison with NAs, AAs consumed more ( $P < 0.01$ ) protein ( $94 \pm 9.3$  vs.  $58 \pm 4.1$  g/d) and fat ( $114 \pm 11.2$  vs.  $38 \pm 3.0$  g/d), meat, saturated fat, and cholesterol. However, they also consumed more ( $P < 0.05$ ) calcium, vitamin A, and vitamin C, and fiber intake was the same. Breath hydrogen was higher ( $P < 0.0001$ ) and methane lower in AAs, and fecal colony counts of 7- $\alpha$  dehydroxylating bacteria were higher and of *Lactobacilli* were lower. Colonic crypt cell proliferation rates were dramatically higher in AAs ( $21.8 \pm 1.1\%$  vs.  $3.2 \pm 0.8\%$  labeling,  $P < 0.0001$ ). In conclusion, the higher CRC risk and mucosal proliferation rates in AAs than in NAs were associated with higher dietary intakes of animal products and higher colonic populations of potentially toxic hydrogen and secondary bile-salt-producing bacteria. This supports our hypothesis that CRC risk is determined by interactions between the external (dietary) and internal (bacterial) environments. J. Nutr. 137: 175S–182S, 2007.

## Introduction

Colorectal cancer is the most common gastrointestinal cancer and the second leading cause of cancer death in the United States (1,2). African Americans (AAs)<sup>6</sup> have the highest incidence and mortality rates for colon cancer among ethnic populations in the United States (3), the incidence being ~15% higher and the mortality 40% higher in AAs than in Caucasian Americans (CAs) (1). The observation that colon cancer is extremely rare in

native African (NA) populations, <1 case per 100,000 population compared with 65 per 100,000 for AAs (1), suggests that differences in environment influence carcinogenesis. This is consistent with Doll and Peto's (4) suggestion that over 90% of gastrointestinal cancers are determined by environmental influences such as diet. Burkitt (5) was the first to describe the low incidence of colon cancer in NAs, ascribing it to the traditional staple diet that was high in unrefined cereals and, therefore, fiber content. However, we showed that the modern African diet is highly refined and low in fiber (6,7), and yet there has been no dramatic increase in colon cancer incidence. In a study comparing NAs and Caucasian Africans, who have cancer rates similar to Americans, we examined the dietary differences that may have accounted for the low cancer rate in NAs and found that many of the recognized protective nutrients, namely antioxidant vitamins (C, A, and E) and calcium, were surprisingly lower in NAs (7). However, meat and animal fat consumption was also much lower in NAs, supporting the epidemiologic and experimental evidence that perhaps the most powerful determinants of colon cancer risk are the levels of meat and animal fat intake (8–10). In further field studies, we noted that breath methane concentrations, a product of colonic fermentation by methanogenic bacteria, were markedly elevated in African populations, raising the intriguing possibility that differences in colonic bacterial flora may account for the differences (11–13). This was

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<sup>6</sup> Abbreviations used: AA, African American; CA, Caucasian American; CRC, colorectal cancer; 7DHC, 7- $\alpha$ -dehydroxylating *Clostridia*; LOH, loss of heterozygosity; NA, native African; TE, Tris-EDTA.

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consistent with experimental evidence from Cummings's group (14) that hydrogen-producing bacteria may injure the mucosa, whereas methanogens detoxify the hydrogen by combining it into methane. They also provided evidence that a diet high in meat, and therefore sulfur, would inhibit methanogens and stimulate sulfur-reducing bacteria, which also produce toxic metabolites such as hydrogen sulfide (15). The importance of gut bacteria may also be linked to a high-fat diet, as this stimulates the hepatic production of bile acids. Bile acids stimulate growth in the colon of *Clostridia*, which convert primary to secondary bile acids, and secondary bile acids were shown to be carcinogenic (16–20).

We therefore hypothesized that the higher risk of colon cancer in AAs results from differences in the colonic milieu, which consists of substances derived from the environment, such as nutrients, and the bacterial flora, which are also influenced by the environment. To investigate this hypothesis, we performed studies in samples of the AA population, the NA population in South Africa, and, as a westernized control, the CA population, focusing on dietary intakes, colonic bacterial metabolism, and colonic mucosal health and proliferation.

## Methods

**Study populations.** For the American part of the study, the protocol was reviewed and approved by the Institutional Review Boards of the Virginia Commonwealth University and the University of Pittsburgh. For the African study, the protocol was reviewed and approved by the Medical University of Southern Africa Medical Ethics and Safety Committee.

There were 3 study populations. For groups 1 and 2 (AAs and CAs), healthy male and female volunteers, aged 50–60 y, were recruited by advertisements, approved by our institutional review board, placed in public and community centers. This age group was selected because the risk of cancer increases appreciably after age 50. Subjects with a history of gastrointestinal disease or surgery or antibiotic use during the previous 8 wk were excluded because this might influence colonic bacterial metabolism. For group 3 (NAs), equal numbers of urban and rural healthy male and female volunteers aged 50–60 y were recruited in the same way from urban Pretoria and the rural regions of Gauteng. The same exclusion criteria were applied.

**Experimental design.** The study consisted of investigations into dietary intake, blood testing, colonic bacterial metabolism, and a colonoscopy to collect colonic contents and to examine colonic mucosal health. After informed consent was obtained, American subjects were admitted to the General Clinical Research Center for a 2-d stay. In the African study, participants were admitted to GaRankua Hospital, Gauteng (urban), or Tintswalo Hospital in Limpopo Province (rural) of South Africa. The dietary inquiry and colonic metabolism studies were conducted on the first day, and the colonoscopy was conducted on the second day. After the colonic metabolism studies, subjects were given a normal meal and a snack at night before they fasted from midnight until the colonoscopy in the morning. To sample colonic contents, a rapid colon evacuation was performed 3 h before the colonoscopy by consumption of 2 L of Go-Lytely preparation (polyethylene glycol 3500) within 30 min.

**Dietary assessment.** A dietitian experienced in dietary analysis took a 3-d dietary recall from each participant in both centers. Estimated quantities were converted into basic nutrient quantities by a computerized software program based on foods commonly consumed by the population. For Americans, the Nutrition Data System for Research Software, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN (database version 4.02–30, released July 1998) was used. For Africans, similar software based on local food analysis was used (21). Although it would have been optimal to use the same software program for all populations, many commonly used

African foods are not precisely analyzed by American software programs (21). Consequently, local programs were used for each population.

**Colonic bacterial metabolism and gut transit rates.** After an overnight fast, breath hydrogen and methane concentrations were measured before the ingestion of 10 g lactulose drink and then again every hour for 6 h while fasting continued (12). Lactulose is a disaccharide carbohydrate that is resistant to human intestinal digestion but fermentable by colonic bacteria, forming hydrogen and methane, which are absorbed and excreted in the breath. The time from ingestion to the appearance of hydrogen and methane in the breath provides a measure of small intestinal transit, whereas the quantities of hydrogen and methane excreted in breath are related to the biological mass of bacteria forming hydrogen and methane in the colon.

**Colonic contents.** After an overnight fast, volunteers were given 2 L of polyethylene glycol orally, and the first (solid) bowel movement was collected. A 10-g stool sample was then divided into aliquots and mixed with 10 mL of an oxygen-free glycerol solution and transported to the laboratory in an anaerobic plastic bag. The stool samples were stored in an anaerobic jar (Anaeropack System 2.5-L jar, Mitsubishi Gas) containing 2 gas packs (Pack-Anaero), an oxygen indicator strip, and Drierite desiccant (Becton Dickinson) at  $-20^{\circ}\text{C}$  until analysis (22).

**Colonoscopy.** After colonic evacuation, the subjects underwent colonoscopic examination to exclude the presence of disease and to obtain mucosal biopsies from the cecum, transverse colon, and sigmoid colon for measurement of epithelial proliferation rate and genetic mutations associated with colon cancer. Cell cycle staging will be determined by Ki-67 staining techniques (7).

**Breath hydrogen and methane.** End-expiratory breath samples were collected in airtight aluminum foil bags before and every hour for 6 h after consumption of a drink containing 10 g lactulose. Concentrations of hydrogen and methane in the breath samples were measured at the completion of the study using gas chromatography (Quintron Instruments) (13). The instrument was recalibrated using standard gases before each run of samples.

**Fecal bacterial analysis.** A common method for the culture and extraction of bacterial DNA was used for identification and quantification of 7 $\alpha$ -dehydroxylating *Clostridia* (7DHC) and *Lactobacillus plantarum* in a similar way to that described by Ridlon et al. (22). Two grams of the stored stool sample was mixed in 8 mL modified brain heart infusion medium in a sterile Hungate tube. Serial dilutions (1:10) were then performed in additional Hungate tubes containing 9 mL medium. Culture tubes were incubated at  $37^{\circ}\text{C}$  for 24–48 h until turbid; 2 mL of each dilution was placed in screw-top Bead Beat tubes (Biospec) and centrifuged at  $13,600 \times g$  for 60 s. The pellet was resuspended; washed in sterile Tris-EDTA (TE) buffer (pH 7.6); resuspended in TE buffer; and treated with lysozyme, proteinase K, and 5% SDS. Glass beads (200  $\mu\text{L}$ ; Biospec) were added with buffered phenol, and the tube was shaken using a minibead beater (Biospec). After a cleaning step with phenol-chloroform-isoamyl alcohol, DNA was precipitated with sodium acetate and absolute ethanol at  $-20^{\circ}\text{C}$  overnight. After centrifugation and drying using a vacuum centrifuge, the DNA pellets were resuspended in TE buffer. DNA was quantified using spectrophotometry. PCR, using baiCD primers for 7DHC and pREV and planF (Sigma-Genosys) for *L. plantarum*, was performed on DNA extracted from serial stool cultures. We used 200 ng DNA with a modified BD Titanium Taq PCR kit using DMSO (Sigma) and BSA and 0.5  $\mu\text{mol/L}$  of each baiCD primer. PCR conditions and cycling details were as previously described (23). Positive and negative control reactions were included with each batch of samples analyzed. A standard PCR mixture containing 200 ng 7DHC or *L. plantarum* genomic DNA instead of sample was used as a positive control, and the negative control contained water instead of sample. PCR was performed with initial denaturation at  $94^{\circ}\text{C}$  for 3 min, 30 cycles of denaturation at  $94^{\circ}\text{C}$  (30 s), annealing at  $56^{\circ}\text{C}$  (10 s), elongation at  $72^{\circ}\text{C}$  (30 s), and final extension at  $72^{\circ}\text{C}$  for 5 min. PCR products were separated by electrophoresis on 2% agarose gels in 1%  $1 \times$  Tris-acetate

EDTA and stained with ethidium bromide (24). Calculation of concentration of bacteria was from the highest dilution level in which a PCR product could be detected on electrophoresis (23). This calculation was based on an initial 1:2 dilution, then a 1:5 dilution, followed by serial 1:10 dilutions.

**Colonic epithelial cell proliferation measurement.** Colonic mucosal biopsies were initially stored in 10% buffered formalin and then embedded in paraffin; 5- $\mu$ m sections were stained with Ki-67 antigen antibodies (25–27). The mouse monoclonal antihuman Ki-67 (Dako, MIB-1) was used at 1:100 dilution after antigen retrieval using CC1 solution (Ventana). The immunohistochemical stains were performed with the Ventana autostainer Benchmark  $\times 7$ . The proliferation pattern was assessed in tissue sections after immunostaining for Ki-67. The total number of labeled and unlabeled nuclei were scored in at least 4 well-oriented crypts, displaying the full length of colonic crypts ( $\approx 500$  cells), and were used to assess proliferation. The proliferative index of the mucosa was calculated by dividing the number of labeled cells by the total number of cells and expressing the results as a percentage.

**Mucosal and blood DNA genotyping analysis.** DNA was extracted from colonic mucosa sections of paraffin-embedded tissues collected in lysis buffer or from peripheral blood using the DNEasy kit (Qiagen) according to the manufacturer's instructions. Fluorescently labeled primers for APC/5q (D5S346) were used for PCR. PCR products were analyzed by capillary electrophoresis using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and Genescan computer software for fragment analysis (Applied Biosystems). The PCR products from blood were analyzed to determine whether the D5S346 locus was informative (heterozygous, 2 peaks). The colonic tissue was then examined for allelic imbalance and loss of heterozygosity (LOH) by comparing the amplification profile with that of blood from the same individual. PCR amplifications were performed with the BAT26 oligonucleotide primer set for microsatellite instability testing (28). Microsatellite instability was scored if the colonic DNA showed amplification of additional fragments of different size from that seen with amplification from blood DNA from the same individual.

**Statistical analysis.** The significance of group differences in dietary intake, breath tests, fecal bacterial counts, and colonic mucosal epithelial proliferation was initially assessed using a mixed-model ANOVA. An unspecified within-subject covariance structure was assumed. If an overall difference was found, comparisons were made across each response using appropriate post hoc tests. Transformations of the responses were used if the residuals from the model were not normally distributed. Results are generally expressed as group mean value followed by standard error in brackets.

## Results

**Demographics.** Table 1 summarizes the results showing that there were no significant differences in age, sex, or body mass among the 3 groups. Blood pressure and hemoglobin measurements were also similar; 31% of AAs and 36% of CAs gave a family history (first-degree relative) of colon cancer in comparison with none of the NAs.

**Dietary measurements.** AAs consumed more protein ( $P = 0.005$ ), fat ( $P < 0.0001$ ), animal protein ( $P = 0.006$ ), saturated fat ( $P < 0.0001$ ), and cholesterol ( $P = 0.007$ ) than NAs (Table 1). Red meat was eaten more commonly by AAs. For example, during the 3-d recall, all AAs had eaten red meat at least once whereas only 2 of the 17 NAs had consumed red meat, and the chief source of meat was unskinned chicken. Cornmeal, in the form of refined white maize meal, was eaten daily by all NAs either as the meal itself or combined with vegetable and meat flavorings. NAs also consumed less calcium ( $P < 0.0001$ ),

**TABLE 1** Demographics and dietary measurements

	Native Africans	African Americans	Caucasian Americans
Age, y	55.2 (0.7)	53.2 (0.7)	55.9 (2.0)
BMI, kg/m <sup>2</sup>	28.0 (1.2)	30.5 (3.0)	28.9 (1.3)
Pulse, b/min	73 (3.0)	69 (3)	75 (4)
BP systolic, mm Hg	141 (6)	134 (4)	125 (6)
diastolic	82 (3)	82 (3)	75 (2)
Diet energy, kcal/d	1669 (160)**	2650 (230)	2695 (227)
Diet carbohydrate, g/d	282 (28)	312 (27)	301 (27)
Total diet protein, g/d	58 (4)*	94 (9)	108 (9)
Diet animal protein, g/d	26 (3)**	51 (5)	59 (13)
Total diet fat, g/d	38 (3)***	114 (11)	114 (13)
Diet sat fat, g/d	9 (0.7)***	35 (4)	33 (4)
Diet cholesterol, mg/d	165 (18)*	300 (36)	324 (43)
Diet fiber, g/d	17 (2)	20 (1.5)	23 (2.5)
Diet folate, $\mu$ g/d	201 (22)**	480 (47)	526 (50)
Diet calcium, mg/d	228 (27)**	833 (99)	1049 (112)
Diet iron, mg/d	7.1 (0.5)**	18.3 (2)	18.9 (1.8)
Diet vitamin C, mg/d	48 (15)**	198 (22)	159 (20)
Diet vitamin A, $\mu$ g/d	630 (162)*	1466 (194)	1642 (253)
Diet zinc, mg/d	6.7 (0.5)**	14 (1.5)	15 (1.5)
Blood hemoglobin, g/L	131 (10)	138 (3)	141 (3)
Fasting breath hydrogen, ppm	10.8 (1.8)	17.4 (2.4)	13.9 (3.3)
Fasting breath methane, ppm	33.9 (8.9)**	5.0 (2.0)	10.9 (3.9)

\*  $P < 0.05$ , \*\*  $P < 0.005$ , \*\*\*  $P < 0.0001$  vs. African Americans, ANOVA with post hoc Bonferroni-Dunn correction.

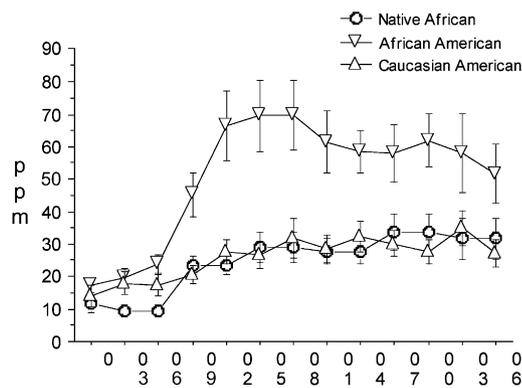
vitamin A ( $P = 0.005$ ), vitamin C ( $P < 0.0001$ ), and niacin ( $P < 0.0001$ ), and there was no difference in fiber intake ( $P = 0.2$ ). There were no significant dietary differences between AAs and CAs.

**Colonoscopic findings.** Less pathology was observed during colonoscopy of NAs compared with both groups of Americans (Table 2). In particular, only one 2-mm adenomatous polyp was found and removed in the Africans compared with 4 in the AAs, 3 measuring  $> 5$  mm and pedunculated, and 3 in CAs. Diverticulosis and hemorrhoids were also more frequent in Americans. No cancers were found.

**Colonic bacterial metabolism.** Fasting breath methane concentrations were significantly higher in NAs compared with both groups of Americans (Table 1). Levels remained elevated throughout the lactulose test. Figures 1 and 2 illustrate the group differences in breath hydrogen and methane responses to lactulose. The time taken for hydrogen to increase was not significantly different, suggesting similar small intestinal transit. Hydrogen concentrations were significantly higher during the

**TABLE 2** Colonoscopic findings

	Native Africans (n = 18)	African Americans (n = 17)	Caucasian Americans (n = 17)
Polyps			
Hyperplastic	1	3	3
Adenomatous	1	4	3
Diverticulae	0	3	7
Hemorrhoids	2	11	6
Melanosis	0	0	2



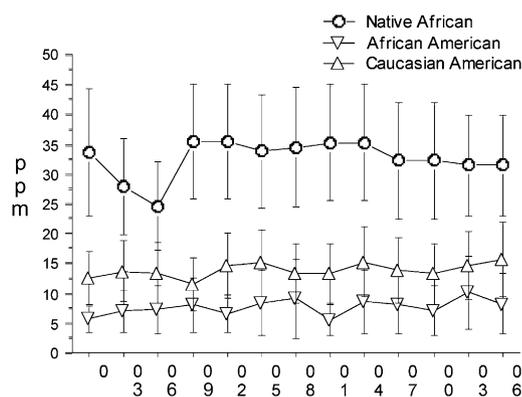
**Figure 1** Breath hydrogen response to 10 g oral lactulose. Small intestinal transit was similar in the 3 groups (60 min), but the response was highest in African Americans. ppm, parts per million.

6-h test in AAs ( $50.9 \pm 2.6$  ppm,  $P \leq 0.0001$  ANOVA with post hoc Bonferroni-Dunn correction) than in NAs ( $24.7 \pm 1.4$  ppm) and CAs ( $25.9 \pm 1.2$  ppm). Breath methane concentrations were significantly higher in NAs ( $32.5 \pm 2.5$  ppm;  $P < 0.0001$ ) than in AAs ( $7.6 \pm 1.2$  ppm) and CAs ( $13.8 \pm 1.4$  ppm). Methanes were also significantly higher in CAs than AAs ( $P = 0.01$ ).

**Colonic bacterial flora.** CAs had the highest counts of 7DHC bacteria followed by AAs (Table 3). Counts were significantly lower in NAs compared with all Americans ( $P = 0.02$ ) and CAs ( $P = 0.01$ ). There were no significant differences for *Lactobacillus* count among the 3 groups, although the numbers were lowest in AAs.

**Colonic epithelial proliferation rates.** Proliferation rates were significantly different among the 3 groups studied (Figs. 3 and 4). In the sigmoid colon, NAs had a mean Ki-67 index of 1.6% compared with 17.2% for AAs and 18.6% for CAs ( $P < 0.0001$  by ANOVA and Bonferroni Dunn correction). Similar differences were seen in the transverse colon (5.6% compared with 26.1% and 24.9%, respectively;  $P < 0.0001$ ) and in the ascending colon (2.8% compared with 21.9% and 24.1%, respectively;  $P < 0.0001$ ).

**Mucosal and blood DNA polymorphisms.** As a pilot investigation, 5 subjects were randomly selected from each of the 3 groups for analysis. No abnormalities were found in NAs



**Figure 2** Breath methane response to 10 g lactulose. Fasting and fed methane concentrations were persistently higher in native Africans and lowest in African Americans.

**TABLE 3** Group mean (SE) results of the fecal bacterial counts

Bacterial Species	Bacterial counts/g stool		
	Native Africans	African Americans	Caucasian Americans
<i>7α DH Clostridia</i>	714 (714)*	$9.2 \times 10^4$ ( $7 \times 10^4$ )	$2.4 \times 10^6$ ( $1.2 \times 10^6$ )
<i>Lactobacillus</i> sp.	$1.0 \times 10^6$	$1.3 \times 10^4$ ( $1.2 \times 10^4$ )	$2.7 \times 10^6$ ( $1.4 \times 10^6$ )

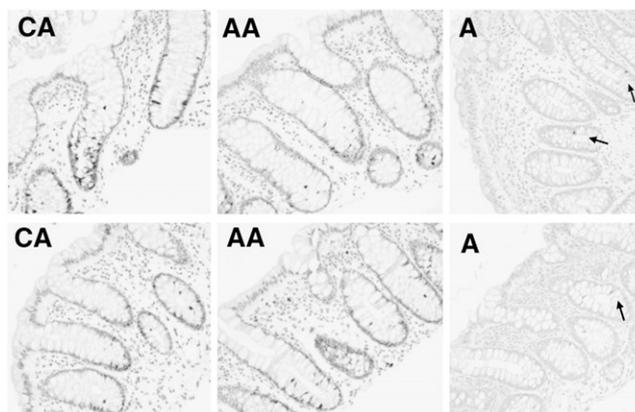
\*  $P < 0.05$  ANOVA.

or CAs. However, 2 AA brothers with a family history of colon cancer had genomic instability in the D56346 locus near the APC gene (Fig. 5). Interestingly, one was obese with a normal colonoscopy other than hemorrhoids, whereas the other was found to have a 1-cm adenomatous polyp that was removed successfully by snare.

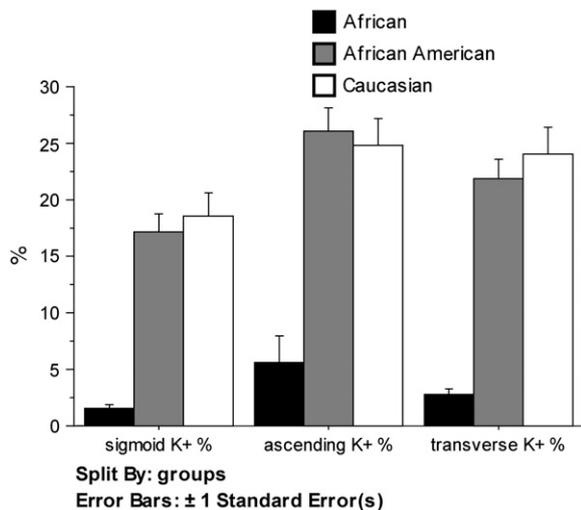
## Discussion

Why do AAs get more colon cancer than NAs? The answer is that they live in different environments. In this study we focused on dietary differences and variations in the intracolonic environment that have been found either epidemiologically or experimentally to influence cancer risk. Our results suggest that the significantly higher dietary intakes of animal protein and fat and the higher colonic colonization with hydrogen-producing bacteria and lower activity of colonic methanogenic bacteria help explain the higher risk of colon cancer in AAs.

Strong epidemiologic and experimental evidence shows that a diet high in red meat and animal fat is associated with increased colon cancer risk. In a prospective study of 88,751 healthy women aged 34–59 y (the Nurses Health Study), Willett et al. (8) reported dietary questionnaire information on cancer-free subjects and the 150 incident cases of colon cancer detected within the cohort. Their analysis showed that, after adjustment for total energy intake, animal fat was positively associated with cancer risk, and those who frequently ate beef, pork, and lamb were at a 2.5 times increased risk. Furthermore, processed meats and liver were also associated with increased risk, whereas fish and skinless chicken were associated with decreased risk. Remarkably similar findings were recently published by Norat et al. (9) from a database of 478,040 men and women from 10 European countries (the EPIC study) who were enrolled when free of



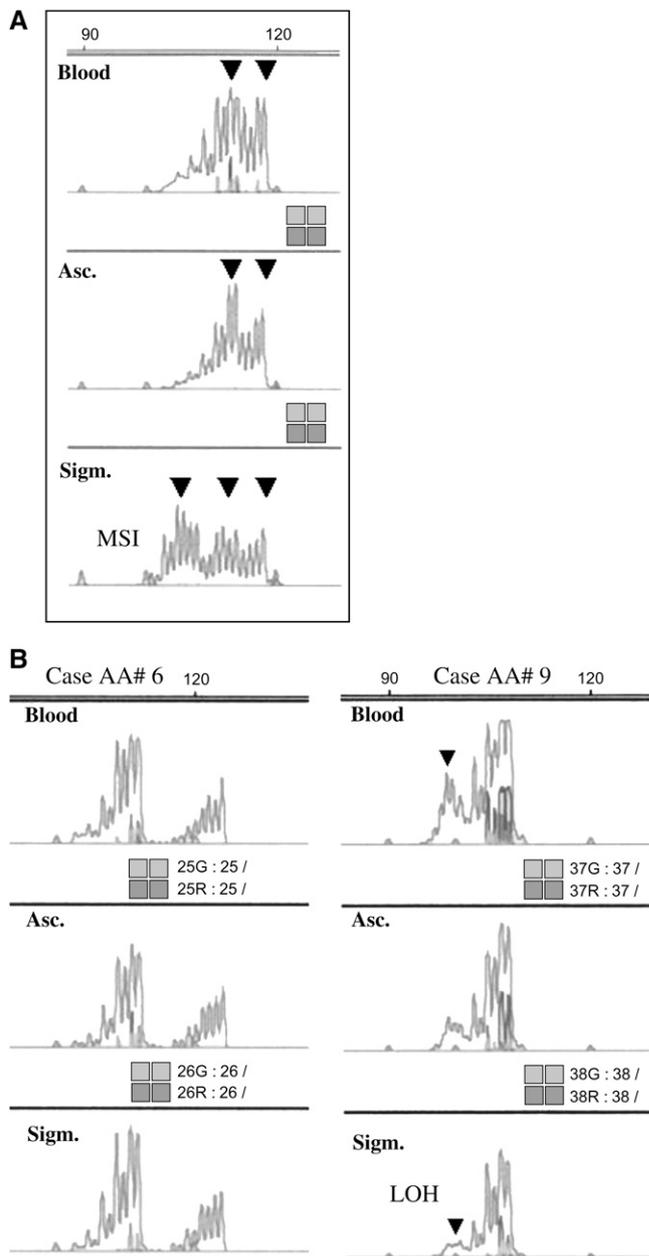
**Figure 3** Ki-67 staining of proliferating cells in the colonic epithelial crypts identified in black, showing numerous dividing cells in the African Americans (AA) and Caucasian Americans (CA) but only a few in the native African (A) section.



**Figure 4** Colonic epithelial proliferation rates among the 3 groups. Rates were severalfold higher in both groups of Americans compared with native Africans ( $P < 0.0001$ ).

cancer between 1992 and 1998; 1329 incident cases of colon cancer were identified. Colorectal cancer risk was positively associated with a dietary history of consumption of red and processed meat and inversely associated with fish. In a systematic review of the 13 published prospective cohort studies published at that time, Sandhu et al. (10) found that there was a significant 12–17% increased risk of colon cancer associated with an increased consumption of 100 g meat per day. Red meat may have a particularly strong effect because of its content of saturated fat, protein, heme, and iron, and browned meat may increase risk further because of the formation of heterocyclic amines (8,9,29). Like Africans, the Japanese traditionally ate a low-meat-protein diet and had low colon cancer rates (30,31). However, a dramatic recent increase in colon cancer was associated with westernization of their diet, which now contains more animal proteins and fat (30). Migration of Japanese to the United States has resulted in increased colon cancer (32), a remarkably similar situation to that of migrant Africans. A wealth of experimental studies support the role of both animal protein and fat in stimulating colonic epithelial cell proliferation and tumorigenesis (8,9,16,17,19,20,33,34).

The significantly lower colon cancer risk in our NA sample was verified not only by published colon cancer incidence rates but also by the surrogate markers of cancer risk we used, namely the low epithelial cell proliferation rate and the much healthier physical appearance of the mucosa during colonoscopy. The low epithelial proliferation rates in NAs (i.e., 1.6% vs. 17.2% for AAs) were particularly striking. The difference was unlikely to have been caused by technical factors, such as transportation from Africa to the United States for measurement, because similar differences were observed between Caucasian Africans and NAs in earlier studies of ours performed in Africa, where we used both Ki-67 and BrDU cell cycle markers (7). Numerous studies have supported the use of measurements of cellular proliferation as biomarkers of cancer risk (25–27), and some studies have shown that increased Ki-67 indexes in patients with colon cancer predict a higher probability of recurrence and diminished survival (35,36). The remarkably pristine condition of the colons in our African volunteers further supports our impression that NA colons were, in general, far healthier than those of age-matched Americans.



**Figure 5** Microsatellite instability at the D5S346 locus, near the *APC* gene (A). DNA from the sigmoid colonic mucosa shows amplification of 3 fragment clusters, whereas DNA from the blood and ascending colon from the same patient show only 2 amplification clusters. Case AA#6 with no LOH detected in the ascending or sigmoid colonic mucosa as compared with amplification from blood of the same patient (B, left); case AA#9 demonstrated LOH at the D5S346 locus with greatly reduced amplification of 1 of the alleles in the colon as compared with DNA from blood of the same individual (B, right).

The differences between AAs and CAs were relatively small, suggesting that what we were really looking at was a comparison between African and westernized living, supporting the conclusion of Doll and Peto (4) that ~90% of gastrointestinal cancers are determined by environmental rather than genetic factors. However, it must be stressed that AAs and native South Africans do not belong to the identical genetic pool. Although most AAs originated from the same West African (Bantu) populations, there has been considerable intermarriage since their arrival in America. It remains possible, however, that the marginally higher incidence of colon cancer in AAs compared with CAs may also be related to colonic bacterial differences, as the breath

hydrogen production relative to methane production was significantly higher in the AA group. The reason for this difference is unclear from the present investigations but may be related to more subtle differences in fiber and calcium intake, the significance of which needs to be studied in larger samples of the population. For example, although we were unable to detect significant differences in meat consumption between AAs and CAs, national statistics show that AAs have higher intakes of beef and pork than CAs (37), which would then be associated with higher intakes of saturated fat. Other lifestyle factors, such as exercise and obesity may also influence risk; our AA population sample had higher BMIs.

Our findings, although preliminary, support our hypothesis that environmental cancer risk is determined by the interaction between diet and colonic bacterial metabolism. From the investigations of others, we know that not only does the cancer risk vary between Americans and Africans but so do the colonic flora. For example, Moore and Moore (38) examined the bacterial flora contained in stool samples from populations with high (including U.S. Caucasians) and low cancer risk (including South African blacks) and noted that concentrations of *Bacteroides* and *Bifidobacterium* were positively associated, and *Lactobacillus* and *Eubacterium aerofaciens* were negatively associated, with colon cancer risk. We have evidence that diets high in fat can stimulate bacterial colonization by *Clostridiae* (39), whereas a diet high in dietary sulfur can stimulate sulfur-reducing bacteria (14,15). A diet high in fat and cholesterol stimulates the synthesis and enterohepatic circulation of primary bile acids, such as cholic acid. A fraction of the primary bile acids escapes the enterohepatic circulation in the intestine and reaches the colon, where it stimulates the growth of 7DHC, enabling them to convert primary bile acids into secondary bile acids. There is considerable experimental evidence that these secondary bile acids, such as deoxycholic acid and chenodeoxycholic acid, are cytotoxic to colonic epithelial cells as well as mutagenic with antiapoptotic properties (18,19,40). Although the precise mechanism has not been defined, the process may involve activation of a  $\beta$ -catenin signaling pathway (20). Support for the bile acid hypothesis in humans was also obtained from the observation that fecal and serum deoxycholic acid levels were increased in colon cancer patients (16). The beneficial effects of fiber, calcium, and *Lactobacillus* on the colon were suggested to be a consequence of the ability of these nutrients to bind and reduce the concentration of secondary bile acids in fecal water (29,41).

The bile salt mechanism is an example of how colonic bacteria can modify luminal contents to produce toxic products. Overgrowth of *Clostridia* can, in itself, lead to mucosal damage. Many studies showed that commercial antibiotics adversely affect colonic function (20), the extreme example being pseudomembranous colitis, where suppression of the normal flora with overgrowth of *C. difficile* leads to necrosis and sloughing of the entire epithelial lining. In lesser forms, patients suffer chronic diarrhea, which may take months to resolve. It should also be noted that far more NAs than Americans are antibiotic naive, a factor that might contribute to the long-term health of their colons. A recent study from Europe showed that treatment with probiotics and prebiotics can be effective in resolving the antibiotics-associated diarrhea (42). For example, *L. planatarum* is active after oral ingestion, colonizing the colon, displacing the pathogenic organisms, and restoring normal colonic function and metabolism (43). *Lactobacillus* can protect membranes against secondary bile salt toxicity (44), and *L. planatarum* was shown to be nearly always present in African colons but rarely in American colons (45). Although we found that *Lactobacillus*

populations were higher in NAs than AAs, the differences were not significant, and bacterial counts were numerically highest in CAs. Travel from Africa, despite the tight precautions on storage under temperature-controlled anaerobic conditions, may have impaired the culture potential of our fecal bacteria. Anaerobic culture and isolation of the different colonic bacterial species is notoriously difficult (22).

Meat protein can also theoretically lead to chronic colonic mucosal injury through interaction with "bad" colonic bacteria. Meat is a rich source of dietary sulfur (through its high content of sulfur amino acids), and dietary sulfur promotes the growth of sulfur-reducing bacteria (e.g., *Desulfovibrio vulgaris*), which outcompetes methanogenic bacteria for hydrogen produced by other bacterial species (15). However, unlike methanogenic bacteria, which prevent toxicity of hydrogen by binding it into methane, they elaborate hydrogen into hydrogen sulfide, which, over and above its objectionable odor, impairs cytochrome oxidase, tissue metabolism, mucus formation, and DNA methylation (46). Colonic overgrowth with sulfur-reducing bacteria was shown in patients with ulcerative colitis (46), another condition that is rare in NAs and increases colon cancer risk. We did not study the other mechanisms whereby a high-meat diet may increase colon cancer risk, namely the effects of heme, iron, and heterocyclic amines. Human studies provided evidence that a diet high in red meat or processed meat increases endogenous nitrosation because of its content of heme iron and that the nitrosation increases colon cancer risk (33,34). Polycyclic aromatic hydrocarbons and heterocyclic amines, which are potent carcinogens in experimental animals, are produced when red meat is cooked at high temperatures, for example, during grilling and barbecuing (47). The American diets were richer in both protein and iron.

The modern-day African diet is not high in fiber content, and therefore fiber cannot be claimed to explain the low colon cancer rate. When Burkitt (5) made his landmark observations in the 1950s, the populations he studied in East Africa subsisted on home grown vegetables rich in fiber. Today, most Africans live on highly refined corn meal, which is low in fiber. However, corn meal is consumed daily in large quantities as the staple, and ~20% of its starch is resistant to intestinal digestion (48). Resistant starch enters the colon and behaves like soluble fiber, stimulating bacterial metabolism. The bacteria have a symbiotic association with the host as they salvage undigested carbohydrate by converting it to short-chain fatty acids that form the chief energy source for the colonic epithelium (49). One of these fatty acids, butyrate, has important anti-proliferative, antiapoptotic, and antineoplastic properties (50,51) and thus may reduce cancer risk. Unfortunately, another product of bacterial metabolism, hydrogen gas, is toxic to the epithelium and in high concentrations can inhibit cellular metabolism and NAD regeneration (14). In excess it can cause the clinical disease pneumatosis intestinalis (52). Consequently, high fermenters, such as ruminants, have adapted by replacing hydrogen-producing bacteria with methane producers (e.g., *Methanobrevibacter smithii*), which convert 4 molecules of hydrogen into 1 molecule of methane, a nontoxic gas. In the same way, it is reasonable to suggest that the colonic flora of Africans has adapted to minimize the effects of hydrogen by excreting it as methane, which is less toxic (14). It is tempting to suggest that our AA group has lost this adaptation and thus have higher rates of hydrogen and lower rates of methane production in response to a standard dose of resistant sugar.

Earlier studies of ours highlighted the apparent paradoxical finding of low dietary intakes of the so-called colon

cancer-protective nutrients, such as the antioxidant vitamins A and C as well as fiber and calcium, which lead us to suggest that the low cancer risk resulted from their low dietary intake of animal protein and fat and relatively high intake of carbohydrate in the form of corn meal (6,7,53,54). In sharp contrast, the diet of our American subgroups was sufficient in antioxidants, fiber, and calcium but also high in protein, saturated fat, and cholesterol, again supporting our view that the deleterious effects of animal products on colonic metabolism override the potentially beneficial effects of other protective nutrients.

In conclusion, our study provides evidence that environmental colonic carcinogenesis is far more complex than commonly appreciated and that risk is determined by the overall lifetime balance between environmental factors that promote and those that aggravate mucosal health. This helps explain the apparent failure of many interventional studies that provided short-term recognized protective nutrients as supplements to a basic diet containing aggravating factors. Our studies should be seen as a first attempt to unravel some of these interactions. However, we have examined only a few of the 400+ bacteria that cohabit the colon, only the tip of the iceberg. Many more as yet undiscovered nutrient-bacteria interactions undoubtedly contribute to colonic mucosal health and colon cancer risk. However, our study confirms the USDA figures that suggest Americans, and particularly AAs, consume excess quantities of animal protein and fat (38) and lead us to the conclusion that a healthier lifestyle that includes less meat and more fruit, vegetables, grains, and exercise should be beneficial not only for the colon but also for general health.

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