Published in final edited form as:

Appl Physiol Nutr Metab. 2019 February ; 44(2): 127–132. doi:10.1139/apnm-2018-0181.

Switching to a fibre-rich and low-fat diet increases colonic folate contents among African Americans

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Abstract

How dietary patterns impact colonic bacterial biosynthesis of vitamins and utilization by humans is poorly understood. The aim of this study was to investigate whether a reciprocal dietary switch between rural South Africans (traditionally high fibre, low fat) and African Americans (Western diet of low fibre, high fat) affects colonic folate synthesis. Colonic evacuants were obtained from 20 rural South Africans and 20 African Americans consuming their usual diets at baseline. Thereafter, rural South Africans were provided with a Western diet (protein 27%, fat 52%, carbohydrate 20% and fibre 8 g/d) for two weeks. African Americans were provided with a high fibre, low fat diet (protein 16%, fat 17%, carbohydrate 63% and fiber 43 g/d) for 2 weeks. Colonic evacuants were again collected at the end of the intervention. Folate in 3-hour evacuants was measured by microbial assay. No difference in colonic folate content at baseline between groups was observed. The high fibre low fat diet consumed by African Americans during the intervention produced a 41% increase in mean total folate content compared with baseline values ($p=0.0037$). No change was observed in rural South Africans consuming a Western diet. Mean total folate content of colonic evacuants was higher among African Americans at the end of the dietary switch ($3107 \pm 1811 \mu\text{g}$) compared to rural South Africans ($2157 \pm 1956 \mu\text{g}$) ($p=0.0409$). In conclusion, consistent with animal studies, switching from a Western diet to one higher in fibre and lower in fat can be expected to result in greater colonic folate content.

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Keywords

folate; fibre; gut microbiome; fat; colon; cancer

Introduction

Folate by way of its role in transferring one-carbon units is required for nucleotide biosynthesis which is necessary for DNA and RNA biosynthesis and repair (IOM 1998). Folate also serves as a co-factor for re-methylation of homocysteine to produce methionine. Methionine may be used for protein synthesis or can be converted to S-adenosylmethionine (SAM) which facilitates methylation reactions in the body. Depending on severity and duration, suboptimal folate intake may increase the risk of anemia, colorectal cancer and neural tube birth defects (IOM 1998; Malouf and Grimley Evans 2008; De-Regil et al. 2015). The folate status of Canadians has shifted over the past 50 years from one where a significant proportion of the population was at risk of folate deficiency to one where folate deficiency is virtually absent and arguably non-physiologically high levels are now present in sub-populations (Colapinto et al. 2011; Plumptre et al. 2015). Animal studies show excessive intakes of synthetic folic acid accelerate pre-existing tumor progression and alter DNA methylation, embryonic development, and can produce greater adiposity and negative metabolic effects in offspring (Szeto et al. 2008; Pickell et al. 2011; Mikael et al. 2013; Aleliunas et al. 2016). A better understanding of the “input side” of folate nutrition will help set dietary and folate supplement recommendations that strike the right balance between known benefits and possible risks.

Until recently the contribution of folate from the gut microbiome has not been considered on the input side of folate balance. Unlike humans, many colonic bacteria species can synthesize folate (Rossi et al. 2011). The quantity of folate in the colon can exceed dietary intake and data from controlled feeding studies in animal models and stable isotope studies in humans suggest that it is at least partially absorbed (Kim et al. 2004; Asrar and O'Connor 2005; Aufreiter et al. 2009; Lakoff et al. 2014). Using data generated from delivering isotopically labelled folate ($^{13}\text{C}_5$]5-formyltetrahydrofolate) to the colon of adults via pH-sensitive caplets and measurements of total folate in the aqueous fraction of colonic evacuates, we proposed that 322 to 396 μg of folate could potentially be absorbed across the colon each day (O'Keefe et al. 2009; Lakoff et al. 2014). To put this amount of folate in perspective, the estimated average requirement for folate for adults is 320 $\mu\text{g}/\text{d}$ (IOM 1998). In controlled animal studies, colonic bacterial folate biosynthesis and subsequently blood folate levels were shown to be altered by the quantity and type of dietary fibre or inclusion of human milk solids (source of the oligosaccharides) (Keagy and Oace 1984; Semchuk et al. 1994; Krause et al. 1996; Thoma et al. 2003). However, there is a paucity of data on what impact, if any, dietary manipulation has on bacterial biosynthesis of folate in the colon of humans.

Chronic dietary folate deficiency associated with low red blood cell levels is common in some rural African communities despite low rates of colorectal cancer known to be inversely related to dietary folate intake (O'Keefe et al. 1999; O'Keefe et al. 2009; Burr et al. 2017).

Our studies of colonic evacuants from rural South Africans, similar to that of African- and Caucasian Americans, suggest high rates of microbial production of folate in the colon (O'Keefe et al. 2009). We hypothesized that the discord between low dietary folate intakes and low colon cancer rates may be explained by the high topical or microenvironment levels of bacterially synthesized folate found in the colon and its impact on mucosal DNA regulation. The aim of this study, then, was to investigate whether the colonic folate content in humans (African Americans) can be manipulated by exchanging a typical Western diet (high fat, low fibre) for two weeks with one traditionally consumed by rural South Africans (high fibre, low fat) (Burkitt 1973; De Filippo et al. 2010).

Materials and methods

Subject and diet

This present study utilized data and frozen colonic evacuants (-80°C) collected as part of a published study that examined whether differences in fat and fibre intake as a result of a dietary pattern switch between African Americans and rural South Africans yielded reciprocal responses in mucosal biomarkers and microbial composition associated with the risk of colon cancer (O'Keefe et al. 2015). Detailed description of the feeding trial and colonoscopies are available in the original publication. Briefly, a group of age and sex-matched African Americans ($n=20$) from Pittsburgh, PA USA and rural South Africans ($n=20$) from rural Kwazulu-Natal (Empangeni) South Africa were recruited through local advertisements and provided with compensation for their participation. Individuals were eligible if they were 40–65 years of age, had a body mass index (BMI) between 18–35 kg/m^2 and were otherwise healthy. Potential subjects were excluded if they had: any form of cancer, recent gastrointestinal surgery, a chronic gastrointestinal disease or condition affecting gastrointestinal function, a renal, hepatic or bleeding disorder, consumed antibiotics or other medications, prebiotics and probiotics that could affect bacterial biosynthesis or folate metabolism 12 week prior to enrollment (O'Keefe et al. 2015).

The study consisted of two phases that included a home environment phase (day 0 - day 14) and a dietary intervention phase (day 15 - day 29). All subjects started with the two-week home environment phase during which time they consumed their usual diet. Three-day dietary recalls were collected on a subset of subjects ($n=12$ in rural South Africans and $n=10$ in African Americans) to verify their anticipated dietary pattern. On day 0 of the home environment phase following an overnight fast, subjects drank 2L of bowel wash-out solution (polyethylene glycol) over 30 minutes. The evacuants expelled over the subsequent 3 hr were collected, weighed, homogenized, aliquoted and frozen at -80°C .

Diets were individually cooked and consumed by participants under close supervision during the intervention phase (days 15 – 29). They were designed using dietary data previously collected from African Americans and rural South Africans by our group (O'Keefe et al. 1999; O'Keefe et al. 2007). During the intervention phase, rural South Africans consumed a Western diet which provided 30–35 kcal/kg of their ideal body weight. The macronutrient distribution of this diet (as a % total energy) was 27% protein, 52% fat and 20% carbohydrate with 8 g/d fibre. Typical foods provided included hotdogs, fries, steak and hamburgers. During the intervention phase, African Americans were provided with a

high fibre, low fat rural South African diet that contained 30–35 kcal/kg of their ideal body weight and 16% protein, 17% fat and 63% carbohydrate with 43 g/d fibre. The intervention diets were designed using dietary data previously collected on samples of African Americans and rural South Africans from the same communities by the study team (O’Keefe et al. 1999; O’Keefe et al. 2007). Foods included in this intervention diet included “meilie meal” (somp and beans) with corn kernels and sugar beans, white potatoes, bread, cabbage, mango, banana, vegetable oil and small portions of animal-based protein (e.g. eggs and chicken). Sample menus for both diets and how nutrient intakes were estimated can be found in the on-line Supplementary material for the original publication (O’Keefe et al. 2015). All meals during the intervention were prepared using a 3-day rotation menu and consumed by African Americans at the University of Pittsburgh Clinical Translational Research Center and by rural South Africans at a local rural lodging facility in South Africa. Subjects were encouraged to consume all the foods that were provided; however, leftovers were weighed. Dietary intakes were determined by 3-day recalls for African Americans and recalls and home visits for the rural South Africans at baseline and observation of the diets fed during the intervention (e.g. amount offered from the standardized menus minus plate waste). Nutrient intakes were calculated using the USDA Nutrient Database Standard and local South African food composition values. Colonic evacuates were collected, homogenized and stored as described above until analysis.

Analysis of colonic evacuates

Folate in colonic evacuates was extracted using the tri-enzyme digestion method (Hyun and Tamura 2005). Thereafter, folate concentrations were measured by the microbial assay using 5-methyltetrahydrofolate to generate the standard curve (Molloy and Scott 1997). Results are expressed as the concentration ($\mu\text{g/mL}$) and total folate (μg) found in the 3-hour evacuates. Certified pig liver (13.3 mg folate/kg, Pig Liver, BCR 487, IRMM, Geel, Belgium) was used to assess the accuracy and reproducibility of the assay. We found the average folate concentrations of the pig liver reference standard was 12.4 ± 0.9 mg/kg with an inter-assay CV of 7.5% ($n=4$).

Statistical analysis

Differences between African American and rural South Africans for baseline variables were assessed using Mann-Whitney tests. The differences in dietary composition between groups and changes in volume of 3-hour evacuates or dietary composition between the home and intervention phases within groups were analyzed using PROC MIXED (SAS 9.4, SAS Institute, Cary NC). The impact of diet on total folate and the folate concentration of colonic evacuates during the home and intervention phases was similarly assessed using PROC MIXED (SAS 9.4, SAS Institute, Cary NC). The sample size for the parent study was calculated to enable detection of statistically significant differences in epithelial proliferation (Ki 67), production of butyrate, breath hydrogen and methane between groups at an $\alpha=0.05$ and 80% power, using effect sizes and variations reported in earlier work (O’Keefe et al. 1999; O’Keefe et al. 2007) and unpublished pilot data. Unless otherwise indicated results are expressed as mean $\pm$ standard deviation of the mean (SD). A p -value < 0.05 was considered statistically significant.

Results

Subject characteristics

There was no difference in the mean age of African Americans or rural South Africans (Table 1). While African Americans were heavier and taller than the rural South Africans ($p < 0.05$), there was no difference in BMI between groups. The intervention diet was well-tolerated and the body weight of each subject at the end of the intervention remained within 2 kg of their baseline weight.

Dietary intakes

Of the sub-set of participants where dietary intakes were assessed during the home environment phase, as anticipated, mean ($\pm$ SD) dietary intakes of rural South Africans were lower in fat and soluble fibre but higher in carbohydrate, insoluble fibre and total dietary fibre, compared to African Americans ($p < 0.05$) (Table 2). During the intervention phase, African Americans consumed lower amounts of protein, fat but higher amounts of carbohydrate, soluble fibre, insoluble fibre, total dietary fibre and folate, compared to rural South Africans consuming a Western diet ($p < 0.0001$) (Table 3).

Colonic folate contents in evacuates

There were no statistically significant differences in the mean volume of 3-hour evacuates either between groups at baseline or at the end of the feeding intervention or within each group between baseline and the end of the intervention. The mean volumes of the 3-hour evacuates at baseline were 1.74 ± 0.36 and 1.62 ± 1.02 L for rural South Africans and African Americans, respectively ($p = 0.74$). After the dietary switch, the mean volumes were 1.68 ± 0.31 and 1.46 ± 0.52 L for rural South Africans and African Americans, respectively ($p = 0.078$).

There was no difference in the mean folate contents in colonic evacuates of rural South Africans and African Americans at baseline, whether expressed as concentration or as total content (concentration $\times$ evacuant volume) (Figure 1 and 2). The Western diet intervention did not affect the folate concentration nor total folate contents in 3-hour colonic evacuates of the rural South African subjects. However, the high fibre low fat intervention diet increased both the folate concentration (1.5 ± 1.3 vs 2.3 ± 1.3 $\mu\text{g/mL}$; $p = 0.0003$) and total folate contents (2197 ± 2483 vs 3107 ± 1811 μg ; $p = 0.0037$) above baseline in African Americans. In addition, the African Americans group had higher folate concentrations (2.3 ± 1.3 vs 1.3 ± 1.2 $\mu\text{g/mL}$; $p = 0.0045$) and total folate contents (3107 ± 1811 vs 2157 ± 1956 μg ; $p = 0.0409$) compared to rural South Africans post-intervention.

Discussion

Results from the present study show that African Americans switched to a diet high in fibre and low in fat (43 g/d fibre, 17% total energy as fat) for two weeks had higher colonic folate contents compared to their baseline Western diet (15 g/d fibre, 36 % of total energy as fat). As reported previously, this dietary switch also led to reciprocal changes in specific colonic microbes and increased saccharolytic fermentation and butyrogenesis (O'Keefe et al. 2015).

We speculate that changes in colonic folate contents observed herein were due, at least in part, to increased bacterial biosynthesis of folate in the colon. The biomass of the microbiota in the distal intestine is tremendous and estimated to surpass 10^{11} cells per gram. Hence the colonic microbiota have significant capacity to synthesize large quantities of metabolites including short-chain fatty acids and vitamins. Unlike humans who lack the enzymatic machinery to condense para-aminobenzoic acid with dihydropterin pyrophosphate to produce folate, it is estimated that 43% of bacterial species in the colon are able to synthesize folate (Magnusdottir et al. 2015). Folate is secreted into the lumen in its bioavailable monoglutamylated form. It is known fermentable substrates, such as dietary fibre, that escape digestion in the small intestine, serve as a fuel source for colonic bacteria, resulting in increased total bacterial load (Stephen 1994; Gibson and Roberfroid 1995; Singh et al. 2017). The increased saccharolytic fermentation and butyrogenesis observed in this sample of African Americans previously following the two week high fibre low fat dietary switch is consistent with the aforementioned understanding (O'Keefe et al. 2015). Evidence, primarily from animal models, suggests that as total bacterial load increases so does net folate production in the colon (Krause et al. 1996; Aufreiter et al. 2011). It is unclear how much of this folate is absorbed or utilized by the colonic mucosa. Data from rodent studies demonstrate alteration of blood folate levels with manipulation of bacterial biosynthesis of folate in the colon; however these studies are confounded by the fact that rodents are coprophagic and hence folate produced in the colon could be absorbed across the small intestine (Keagy and Oace 1984; Semchuk et al. 1994; Krause et al. 1996; Thoma et al. 2003). Using stable isotopes of folate quantitatively delivered to colon by either colonoscopy or enteric coated caplets, we previously demonstrated that folate is absorbed across the colon in humans (Aufreiter et al. 2009; Lakoff et al. 2014). In a cross-sectional study of young women pre-folic acid fortification of the food supply in Canada, we observed for each 1gram increase in nonstarch polysaccharide in the diet, a 1.8% increase in serum folate concentration was observed, even after controlling for dietary folate and supplemental folic acid intake ($P < 0.001$) (Houghton et al. 1997).

As summarized in a recent systematic review, in addition to the impact on total bacterial load, the quantity and type of fibre and fat consumed may also impact microbial diversity and composition (Singh et al. 2017). While the change in gut microbiota related to folate production was not assessed in the systematic review, the portfolio of the evidence suggests that fibre, prebiotics and resistant starch increase bacterial abundance *Bifidobacteria*; many strains of the latter are potent producers of folate (Deguchi et al. 1985; Pompei et al. 2007; Magnusdottir et al. 2015). Further, authors of the systematic review reported consumption of low fat diets similarly leads to increased fecal abundance of *Bifidobacterium*.

There could be a number of explanations why we did not see differences in colonic contents in rural South Africans and African Americans at baseline or between rural South Africans at baseline and rural South Africans at the end of the two-week feeding intervention. Of note, the absence of a difference in colonic folate content between rural South Africans and African Americans at baseline reported in the present study is consistent with previous findings (O'Keefe et al. 2009). It is possible that bacterial biosynthesis of folate is higher in rural South Africans but utilized by the colonic mucosa or within the splanchnic bed. Colonic folate may serve as a local or topical source of folate that may suppress neoplastic

changes contributing to the lower colorectal cancer rate in rural South Africans despite suboptimal dietary folate intakes. Observation of a greater production of short-chain fatty acids at baseline in rural South Africans suggesting higher levels of saccharolytic fermentation compared to African Americans would be consistent with this hypothesis (O'Keefe et al. 2015). An alternative explanation of why there were no differences in colonic folate levels at baseline is that diets were not sufficiently different in fibre or fat to generate such a difference. For example, the net difference in total fibre content between the African Americans and rural South Africans at baseline was 13 g/d whereas at the end of the feeding intervention it was 35 g/d. Likewise, the differences in total fat content in the diets was larger between the two groups during the intervention diet than they were at baseline. In addition, a 2-week intervention period may have been too short for rural South Africans whose fibre intake was decreased by 20 g compared to an increase of 28 g in the African Americans to show an effect on colonic folate content.

There are a number of limitations of this study. While recent evidence confirms that changes in dietary fibre composition can alter gut microbiome within a day, it is unknown whether the changes in colonic folate production among African Americans consuming the high fibre, low fat diet would be sustained longer-term (Wu et al. 2011; David et al. 2014). A second acknowledged limitation of this study was that the baseline and intervention diets differed in their folate content. While it is generally believed that majority of dietary folate is absorbed in the small intestine (IOM 1998), we cannot discount the possibility that some of the differences in colonic folate contents were due to differences in dietary folate intake. Although mean folate intake was higher among African Americans compared to rural South Africans during intervention phase and seemingly higher than in their baseline diet, mean differences in colonic folate contents greatly exceeded these differences in dietary intake. Though mean volume of the 3-hour colonic evacuations did not differ by group or between baseline and intervention periods, differences in transit time as a result of the fibre content of diets may have influenced the time available for folate absorption and hence the folate content of colonic evacuations (Shinnick et al. 1989). While reported in animal studies, the Institutes of Medicine concluded the presence of fibre per se does not appear to impact folate absorption in humans (IOM 1998). Finally, only a fraction of resistant starch is included in the total fibre intakes reported in the present study. Corn meal was consumed daily in large quantities as a staple in both the baseline diet of rural South Africans and in the intervention diet of African Americans. It has been shown that approximately 20% of the starch in corn is resistant to digestion (Segal et al. 1991). Resistant starch enters the colon and behaves much like soluble fibre, stimulating bacterial metabolism (O'Keefe et al. 2007). The impact of resistant starch on the colonic folate content in our study cannot be fully assessed.

In summary, this study provides the first direct evidence in humans that colonic folate contents can be enhanced by a reduction in fat and an increase in fibre consumption in healthy adults normally consuming a Western diet characterized by high fat and low fibre content. We propose that the change in colonic contents is likely due, at least in part, to upregulation of folate production by the microbiota in the colon. Additional research, however, will be required to ascertain whether the observed changes after a two week intervention are sustained longer-term or are transitory. Further future research will also

need to address how fibre and fat and may affect factors influencing the folate content in the colon aside from bacterial biosynthesis such as transit time. Given the size of the colonic pool of folate and evidence of significant folate absorption across the colon, future exploration of how an individual's dietary folate requirement may be affected by the size and composition of the gastrointestinal microbiota is warranted.

References

- Aleliunas RE, Aljaadi AM, Laher I, Glier MB, Green TJ, Murphy M, et al. 2016 Folic Acid Supplementation of Female Mice, with or without Vitamin B-12, before and during Pregnancy and Lactation Programs Adiposity and Vascular Health in Adult Male Offspring. *J Nutr.*
- Asrar FM and O'Connor DL 2005 Bacterially synthesized folate and supplemental folic acid are absorbed across the large intestine of piglets. *J. Nutr. Biochem.* 16(10): 587–93. [PubMed: 16081276]
- Aufreiter S, Gregory JF, 3rd, Pfeiffer CM, Fazili Z, Kim YI, Marcon N, et al. 2009 Folate is absorbed across the colon of adults: evidence from cecal infusion of (13)C-labeled [6S]-5-formyltetrahydrofolic acid. *Am. J. Clin. Nutr.* 90(1): 116–23. [PubMed: 19439459]
- Aufreiter S, Kim JH, and O'Connor DL 2011 Dietary oligosaccharides increase colonic weight and the amount but not concentration of bacterially synthesized folate in the colon of piglets. *J. Nutr.* 141(3): 366–72. [PubMed: 21270368]
- Burkitt DP 1973 Epidemiology of large bowel disease: the role of fibre. *Proc. Nutr. Soc.* 32(3): 145–9. [PubMed: 4211989]
- Burr NE, Hull MA, and Subramanian V 2017 Folic Acid Supplementation May Reduce Colorectal Cancer Risk in Patients With Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis. *J Clin Gastroenterol.* 51(3): 247–253. [PubMed: 26905603]
- Colapinto CK, O'Connor DL, and Tremblay MS 2011 Folate status of the population in the Canadian Health Measures Survey. *CMAJ.* 183(2): E100–6. [PubMed: 21149516]
- David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. 2014 Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* 505(7484): 559–63. [PubMed: 24336217]
- De-Regil LM, Pena-Rosas JP, Fernandez-Gaxiola AC, and Rayco-Solon P 2015 Effects and safety of periconceptional oral folate supplementation for preventing birth defects. *Cochrane Database Syst. Rev.*(12): CD007950. [PubMed: 26662928]
- De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. 2010 Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *PNAS.* 107(33): 14691–6. [PubMed: 20679230]
- Deguchi Y, Morishita T, and Mutai M 1985 Comparative studies on synthesis of water-soluble vitamins among human species of bifidobacteria. *Agric. Biol. Chem.* 49(1): 13–19.
- Gibson GR and Roberfroid MB 1995 Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125(6): 1401–12. [PubMed: 7782892]
- Houghton LA, Green TJ, Donovan UM, Gibson RS, Stephen AM, and O'Connor DL 1997 Association between dietary fiber intake and the folate status of a group of female adolescents. *Am. J. Clin. Nutr.* 66(6): 1414–21. [PubMed: 9394694]
- Hyun TH and Tamura T 2005 Trienzyme extraction in combination with microbiologic assay in food folate analysis: an updated review. *Exp. Biol. Med.* 230(7): 444–54.
- IOM 1998 Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline. Washington, DC: National Academy Press.
- Keagy PM and Oace SM 1984 Folic acid utilization from high fiber diets in rats. *J. Nutr.* 114(7): 1252–9. [PubMed: 6330320]
- Kim TH, Yang J, Darling PB, and O'Connor DL 2004 A large pool of available folate exists in the large intestine of human infants and piglets. *J. Nutr.* 134(6): 1389–94. [PubMed: 15173401]

- Krause LJ, Forsberg CW, and O'Connor DL 1996 Feeding human milk to rats increases Bifidobacterium in the cecum and colon which correlates with enhanced folate status. *J. Nutr.* 126(5): 1505–11. [PubMed: 8618150]
- Lakoff A, Fazili Z, Aufreiter S, Pfeiffer CM, Connolly B, Gregory JF, 3rd, et al. 2014 Folate is absorbed across the human colon: evidence by using enteric-coated caplets containing ¹³C-labeled [6S]-5-formyltetrahydrofolate. *Am. J. Clin. Nutr.* 100(5): 1278–86. [PubMed: 25332326]
- Magnusdottir S, Ravcheev D, de Crecy-Lagard V, and Thiele I 2015 Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. *Front. Genet.* 6: 148. [PubMed: 25941533]
- Malouf R and Grimley Evans J 2008 Folic acid with or without vitamin B12 for the prevention and treatment of healthy elderly and demented people. *Cochrane Database Syst. Rev.*(4): CD004514. [PubMed: 18843658]
- Mikael LG, Deng L, Paul L, Selhub J, and Rozen R 2013 Moderately high intake of folic acid has a negative impact on mouse embryonic development. *Birth Defects Res. Part A Clin. Mol. Teratol.* 97(1): 47–52. [PubMed: 23125102]
- Molloy AM and Scott JM 1997 Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. *Methods Enzymol.* 281: 43–53. [PubMed: 9250965]
- O'Keefe SJ, Chung D, Mahmoud N, Sepulveda AR, Manafe M, Arch J, et al. 2007 Why do African Americans get more colon cancer than Native Africans? *J. Nutr.* 137(1 Suppl): 175S–182S. [PubMed: 17182822]
- O'Keefe SJ, Kidd M, Espitalier-Noel G, and Owira P 1999 Rarity of colon cancer in Africans is associated with low animal product consumption, not fiber. *Am. J. Gastroenterol.* 94(5): 1373–80. [PubMed: 10235221]
- O'Keefe SJ, Li JV, Lahti L, Ou J, Carbonero F, Mohammed K, et al. 2015 Fat, fibre and cancer risk in African Americans and rural Africans. *Nat. Commun.* 6: 6342. [PubMed: 25919227]
- O'Keefe SJ, Ou J, Aufreiter S, O'Connor D, Sharma S, Sepulveda J, et al. 2009 Products of the colonic microbiota mediate the effects of diet on colon cancer risk. *J. Nutr.* 139 (11): 2044–8. [PubMed: 19741203]
- Pickell L, Brown K, Li D, Wang XL, Deng L, Wu Q, et al. 2011 High intake of folic acid disrupts embryonic development in mice. *Birth Defects Res. Part A Clin. Mol. Teratol.* 91(1): 8–19. [PubMed: 21254354]
- Plumtre L, Masih SP, Ly A, Aufreiter S, Sohn KJ, Croxford R, et al. 2015 High concentrations of folate and unmetabolized folic acid in a cohort of pregnant Canadian women and umbilical cord blood. *Am. J. Clin. Nutr.* 102(4): 848–57. [PubMed: 26269367]
- Pompei A, Cordisco L, Amaretti A, Zanoni S, Matteuzzi D, and Rossi M 2007 Folate production by bifidobacteria as a potential probiotic property. *Appl. Environ. Microbiol.* 73(1): 179–85. [PubMed: 17071792]
- Rossi M, Amaretti A, and Raimondi S 2011 Folate production by probiotic bacteria. *Nutrients.* 3(1): 118–34. [PubMed: 22254078]
- Segal I, Walker AR, Naik I, Riedel L, Daya B, and de Beer M 1991 Malabsorption of carbohydrate foods by urban blacks. *S. Afr. Med. J.* 80(11–12): 543–5. [PubMed: 1745937]
- Semchuk GM, Allen OB, and O'Connor DL 1994 Folate bioavailability from milk-containing diets is affected by altered intestinal biosynthesis of folate in rats. *J. Nutr.* 124(7): 1118–25. [PubMed: 8027864]
- Shinnick FL, Hess RL, Fischer MH, and Marlett J 1989 Apparent nutrient absorption and upper gastrointestinal transit with fiber-containing enteral feedings. *Am. J. Clin. Nutr.* 49(3): 471–5. [PubMed: 2538050]
- Singh RK, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, et al. 2017 Influence of diet on the gut microbiome and implications for human health. *J. Transl. Med.* 15(1): 73. [PubMed: 28388917]
- Stephen AM 1994 Whole grains--impact of consuming whole grains on physiological effects of dietary fiber and starch. *Crit. Rev. Food. Sci. Nutr.* 34(5–6): 499–511. [PubMed: 7811380]
- Szeto IM, Aziz A, Das PJ, Taha AY, Okubo N, Reza-Lopez S, et al. 2008 High multivitamin intake by Wistar rats during pregnancy results in increased food intake and components of the metabolic

syndrome in male offspring. *Am J Physiol Regul Integr Comp Physiol.* 295(2): R575–82. [PubMed: 18525008]

Thoma C, Green TJ, and Ferguson LR 2003 Citrus pectin and oligofructose improve folate status and lower serum total homocysteine in rats. *Int. J. Vitam. Nutr. Res.* 73(6): 403–9. [PubMed: 14743543]

Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. 2011 Linking long-term dietary patterns with gut microbial enterotypes. *Science.* 334(6052): 105–8. [PubMed: 21885731]

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Figure 1. Mean ($\pm$ SD) colonic folate concentrations ($\mu\text{g}/\text{mL}$) in a sample of rural South Africans ($n=20$) and African Americans ($n=20$) consuming their usual home diet (baseline) and an intervention diet, under supervision. The baseline diet for rural South Africans was high in fibre and low in fat whereas the baseline diet for African Americans was low in fibre and high in fat. The intervention diet for rural South Africans was low in fibre and high in fat whereas the intervention diet for African Americans was high in fibre and low in fat.

Figure 2.

Mean ($\pm$ SD) total colonic folate contents (μ g) in a sample of rural South Africans ($n=20$) and African Americans ($n=20$) consuming their usual home diet (baseline) and an intervention diet, under supervision. The baseline diet for rural South Africans was high in fibre and low in fat whereas the baseline diet for African Americans was low in fibre and high in fat. The intervention diet for rural South Africans was low in fibre and high in fat whereas the intervention diet for African Americans was high in fibre and low in fat. Total folate content was calculated from the folate concentration found in the colonic evacuates multiplied by the total volume expelled during the three hour collection period.

Table 1.

Baseline characteristics of the study population.*

	Rural South Africans (n=20)	African Americans (n=20)
Sex	M=10/F=10	M=11/F=9
Age (y)	54.8 ± 4.5	55.6 ± 3.6
Weight (Kg)	73.1 ± 14.8	87.1 ± 17.9 [†]
Height (cm)	163.5 ± 9.8	172.5 ± 10.7 [†]
Body mass index (Kg/m ²)	27.7 ± 6.7	29.4 ± 3.6

* Average ± SD

[†] p<0.05

Table 2

Usual home (baseline) diet consumed by rural South African and African Americans *

	Rural South Africans (n=12)	African Americans (n=10)
Energy (kcal/d)	2403 ± 483	2454 ± 863
Total protein (g/d)	71 ± 31	92 ± 30
Total fat (g/d)	45 ± 27	98 ± 41 [†]
Total carbohydrate (g/d)	391 ± 69	292 ± 100 [†]
Total dietary fibre (g/d)	28 ± 14	15 ± 6 [†]
Insoluble fibre (g/d)	26 ± 14	9 ± 3 [†]
Soluble fibre (g/d)	3 ± 3	6 ± 3 [†]
Folate (µg/d)	368 ± 354	504 ± 296

* Average ± SD

[†] p<0.05

Table 3.

Intervention diet consumed by rural South Africans and African Americans *

	Rural South Africans (n=20)	African Americans (n=20)
Energy (kcal/d)	2375 ± 474	2216 ± 406
Total protein (g/d)	160 ± 31	87 ± 16 [†]
Total fat (g/d)	137 ± 27	42 ± 7 [†]
Total carbohydrate (g/d)	120 ± 24	349 ± 73 [†]
Total dietary fibre (g/d)	8 ± 2	43 ± 5 [†]
Insoluble fibre (g/d)	7 ± 1	40 ± 5 [†]
Soluble fibre (g/d)	2 ± 0	4 ± 1 [†]
Folate (µg/d)	424 ± 85	772 ± 134 [†]

* Average ± SD

[†] p<0.0001