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Diet, Microbiota, And Dysbiosis: A ‘Recipe’ For Colorectal Cancer

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Abstract

The food we consume feeds not only us, but also a vast and diverse community of microbiota within our gastrointestinal tract. In a process of symbiotic co-evolution, the gut microbiota became essential for the maintenance of the health and integrity of our colon. The advent of next-generation DNA sequencing technology and metabolic profiling have, in the recent years, revealed the remarkable complexity of microbial diversity and function, and that the microbiota produce a wide variety of bioactive products that are not only active at the mucosal surface, but also absorbed and circulated throughout the body, influencing distant organ health and function. As a result, several microbiota compositional patterns and their associations with both health and disease states have been identified. Importantly, a disturbed microbiota-host relationship, termed *dysbiosis*, is now recognized to be the root cause for a growing list of diseases, including colorectal cancer (CRC). There is mounting *in-vitro* and *in-vivo* evidence to suggest that diet selects for the microbiota composition and several health promoting and deleterious effects of diet are, in fact, mediated by the microbiota. Recent findings of the feasibility of dietary fiber to boost the colonic microbial synthesis of anti-proliferative and counter carcinogenic metabolites, particularly butyrate, underscores the prerequisite of dietary modification as a key measure to curb the pandemic of CRC in westernized countries. Better understanding of the diet-microbiota interplay and large-scale studies to evaluate the efficacy of dietary modification and gut microbiota modulation in reversing dysbiosis and restoring health could offer novel preventative and/or therapeutic strategies against westernized diseases, which are now considered the chief threat to public health.

Introduction

A large and diverse community of microbiota comprising about a 100 trillion microbes, termed the microbiome, inhabits human gastrointestinal tract [1]. The microbiome thrives on the undigested dietary residues in the intestinal lumen and in return yields a wide array of metabolites, termed the metabolome, by conducting a robust network of intricate metabolic functions. The intestinal commensal bacteria resist proliferation of pathogenic organisms by competing for nutrition and producing bactericidal factors, play a crucial role in the immunomodulation of the gut associated lymphoid tissue, reinforce gut mucosal defense barrier, and synthesize vitamins such as biotin, folic acid and vitamin-K serving an excellent paradigm of symbiosis [2-5]. Colon, rich in microbiota present at a concentration of 10^{12} cfu/mL, can now be perceived as a 'new' metabolic organ, with its functional potential matching that of the liver [6].

In the present '-omic' era, state-of-the-art techniques such as high throughput gene sequencing and polymerized chain reaction (PCR) of conserved regions of microbial 16S rRNA have enabled identification of the predominantly 'unculturable' anaerobic gut microbiota [7]. Advances in the fields analyzing the genomic content of the gut microbiota (metagenomics); elucidating their metabolic functional potential through metabolite profiling (metabolomics); and studying their interactions and influence on our health and disease (metabonomics) have given us a new perspective of the role of microbiota in our health and disease [7, 8].

After being passed on maternally and shaped by factors such as breast-feeding, maternal skin and environmental contact, the gross microbiota composition and distribution of an

individual remains quite resilient [9, 10]. Interestingly, fundamentally distinct microbiomes have been identified among people of different origin that could be categorized into three human fecal ‘enterotypes’ based on the abundance of one of three genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3). [11]. However, microbiome is a dynamic entity that is affected by several factors besides diet such as exposure to antibiotics, and gastrointestinal diseases and surgery that can disturb the composition and metabolic activity of gut microbiota resulting in a state of disturbed host-microbiota homeostasis, termed *dysbiosis* [12, 13]. In addition, several studies have shown that microbiome changes in composition and diversity as we age, but specific or consistent patterns have not been recognized to date. However, the existing evidence supports a heightened inflammatory state in the elderly hypothesized to be secondary to a decline in gut immune system (‘immunosenescence’) and perpetuation of a chronic low grade inflammation (‘inflamm’aging’) in association with enrichment of facultative anaerobes or pathobionts and decrease in symbiotic species such as *Faecalibacterium prauznitzii* that have anti-inflammatory effect [14, 15]. Dysbiosis has been identified as the root cause for a growing list of medical problems including allergy and autoimmune disorders, irritable bowel syndrome, inflammatory bowel disease, diabetes, obesity, *Clostridium difficile* diarrhea, and colon cancer [16-21].

Research in this field had produced a wealth of information in the recent years and several reviews have summarized a plethora of observational, *in-vitro*, and *in-vivo* studies describing the role of the microbiota in health and disease [22]. In this review, we will focus on the interplay between diet and microbiota with regards to its influence on risk of CRC.

Colorectal cancer- association with diet and microbiota

Colorectal cancer (CRC) is the most common gastrointestinal cancer, and ranks globally as the third most common cancer by incidence, and fourth most common in mortality, with over a million people affected annually, nearly half of who die based on GLOBOCAN 2012 estimates [23]. Over 90% of CRC cases are sporadic, where a complex interplay between genetic and environmental factors determines neoplastic transformation to colorectal carcinogenesis. Seminal analyses by Doll and Peto of the geographic variation in CRC and its association with diet composition in the 80's had shown that over 90% of the gastrointestinal cancers could be attributed to dietary habits [24]. Alcohol, smoking, obesity, and inflammatory bowel disease are also recognized risk factors of CRC. Importantly, dietary factors like higher red and processed meat consumption and deficiency of fiber, calcium, vitamin D and folate are well-recognized factors associated with a higher CRC risk [25]. Western nations bear up to 60% of the global disease burden, which has been attributed to the high red meat, high fat, and low fiber content of their diet [26, 27].

The link between diet and colon cancer is perhaps best exemplified by migration studies. Japanese residents traditionally had a low incidence of CRC, but within one generation of change to a western diet after migration to Hawaii, their incidence increased to levels similar to local Hawaiians [28]. In keeping with a microbiota-mediated explanation for the geographic variation of CRC, fundamentally distinct microbiota composition has been identified in different populations. De Filippo *et al* noted that the gut microbial composition of European children had higher Enterobacteriaceae representation, whereas the rural African children who consumed

higher fiber diet had higher Bacteroidetes and lower Firmicutes counts [29]. We confirmed that these differences persisted into adulthood. In our studies into reasons why colon cancer is most common in African Americans (65:100,000) in the USA, and rare in rural Africans (<5:100,000), we showed that the genotype Prevotella was predominant in native Africans while Bacteroides was in African Americans (Figure 1) [30].

Recent research has produced a wealth of information highlighting the key role of microbiota in mediating the dietary risk of CRC. The fact that colon has the highest microbial concentration and cancer rates along the gastrointestinal tract combined with the observation from gnotobiological studies such as detection of colonic adenocarcinoma in 70% of conventionalized, but not the germ-free TCRbeta and p53 double-knockout (TCRbeta^{-/-} p53^{-/-}) mice, accentuates the crucial role of microbiota in causing CRC [31]. Metagenomic studies have now identified specific bacterial species that colonize the tumor as well as non-tumor colonic sites and characterized individualized oncogenic microbiome [21]. Association of *Streptococcus gallolyticus* (previously *S. bovis*) with CRC was one of the earliest, although the causative link has not been established [32]. In one study, fecal samples of CRC patients had reduced butyrate-producing *Eubacterium rectale* and *Faecalibacterium prausnitzii* and increased populations of *Enterococcus faecalis*, when compared with healthy volunteers [33]. High abundance of *Fusobacterium necroforum*, an oral pathogen causing periodontitis, was recently identified in the CRC tumor tissue compared to normal mucosa [34]. Whether this represents cause or effect remains unknown, but as one potential causative mechanism, its adherence to colonic epithelium through FadA adhesion has been shown to stimulate of E-cadherin/ β -catenin signaling, a mechanism known to promote carcinogenesis [35]. Sobhani *et al* compared the fecal microbiota

composition in endoscopic mucosal biopsies between healthy subjects and CRC patients [36]. They noted significant higher fecal populations of the Bacteroides-Prevotella group in CRC patients with identification of increased representation of IL-17 immunoreactive cells in the healthy subjects, suggesting immunological functional consequences from the dysbiosis.

Overall, review of multiple studies reporting associations between specific microbiota and healthy, adenoma, and CRC populations suggests that there is substantial evidence to date suggestive of microbial role in CRC although further research is warranted to establish firm causative links [37]. Moreover, it seems clear that it's not one specific microorganism that is responsible for CRC, but an abundance of a group of bacteria whose detrimental actions surpass those of the beneficial commensals. A recent systematic review of 31 original human and animal studies on association between microbiota and CRC found conclusive evidence of role of microbiota and dysbiosis in CRC [38]. It was noted that certain bacteria (such as Fusobacteria, Alistipes, Porphyromonadaceae, Coriobacteridae, Staphylococcaceae, Akkermansia spp. and Methanobacteriales) were consistently augmented, while some other (such as Bifidobacterium, Lactobacillus, Ruminococcus, Faecalibacterium spp., Roseburia, and Treponema) were underrepresented in CRC. Of note, reduced butyrate and elevated amino acid metabolites were identified throughout colonic carcinogenesis.

Substantial *in-vitro* and *in-vivo* evidence supports the role of microbiota in causing DNA damage and chromosomal instability that lead to mutations responsible for carcinogenesis. Microbiota contribute to neoplastic transformation of the colonic epithelium by instigating a state of chronic inflammation mediated by signaling pathways such as induction of Toll-like receptors, up-

regulation of cyclooxygenase-2 (COX-2), and activation of mitogen-activated protein kinases (MAPK) that promote epithelial proliferation and genetic mutations [39, 40]. Also, microbiota implement multiple other mechanisms including biotransformation of dietary pro-carcinogens, production of reactive oxygen and nitrogen species, and genotoxins [41]. Contrarily, cancer-protective factors such as folate and biotin that are essential for DNA synthesis and repair are also synthesized by microbiota, such as *Bifidobacterium* [42].

Mechanisms of diet-microbiota mediated CRC risk

The principal dietary components, i.e., carbohydrates, protein, and fat are essential for the energy and metabolism as well as structural maintenance and repair of all aspects of human functioning. However, their disproportionate consumption or aberrant metabolism predisposes to dysbiosis. Generation of pro-inflammatory and toxic metabolites detrimental to colonic mucosa surmounting the anti-inflammatory defensive metabolites seems to be an essential precursor for carcinogenesis. Here, we will discuss the role of the well-recognized diets and microbiota implicated in either aggravating (red meat and fat) or mitigating (fiber) CRC risk (Figure 2).

1. Fiber

Working in East Africa in the 50's, Burkitt noted the association between the traditional African diet, which contained 50-100g fiber/day, and absence of non-inflammatory bowel diseases, particularly colon cancer, being one of the first to suggest the protective role of fiber [43]. Up to 20% of potentially digestible starch plus resistant starches enter the colon and undergo saccharolytic fermentation by most members of the microbiota, but especially *Bacteroides* spp.,

Lactobacillus spp., and Bifidobacterium spp. [44]. This process yields end-products such as short-chain fatty acids (SCFAs); gases such as carbon dioxide (CO₂), hydrogen (H₂), methane (CH₄); and ethanol. Acetate, propionate, and butyrate constitute the three major SCFAs.

Whilst all three SCFAs have health-promoting effects on the colonic mucosa, butyrate is the most potent with respect to cancer protection. It is produced predominantly by Clostridia clusters XIVa and IV of the genus Firmicutes [45]. Butyrate is the chief energy source for colonocytes and regulator of epithelial proliferation [46]. Multiple potential mechanisms have been identified suggesting the protective action of butyrate against colorectal carcinogenesis [47]. Butyrate exhibits anti-proliferative activity by activation of the apoptosis cascade and arresting the growth of tumors by histone hyperacetylation [48]. It suppresses tumors by potentiating p53 gene expression and transforming growth factor- β (TGF- β) signaling, and increasing the immunogenicity of cancer cells [49, 50]. Its anti-inflammatory properties are mediated by suppressing nuclear factor-kB activation, a transcription factor controlling the expression of genes encoding proinflammatory responses and inflammatory mediators like tumor necrosis factor- α (TNF- α) and nitric oxide [51, 52]. On the other hand, acetate and propionate are largely absorbed systemically and play key roles in glucose and lipid metabolism [53, 54].

2. Red and Processed Meat

Red meat and processed meat contain an array of procarcinogenic constituents that act either directly or via their colonic end-metabolites [55]. High protein and heme content of the red meat have been shown to be deleterious to the colonic mucosal health. Proteolytic fermentation of the

meat residues by the colonic microbiota such as *Bacteroides* spp., and *Clostridium* spp. generates inflammatory and procarcinogenic end products such as nitrosamines, branched-chain fatty acids, phenolic (phenols and p-cresol) and indolic compounds from aromatic amino acids [56]. Heme has been shown to cause colonocyte injury, inhibit apoptosis, and enhance crypt hyperplasia and thereby promoting colonic epithelial proliferation [57].

In addition, several carcinogenic chemicals such as polycyclic-aromatic hydrocarbons (PAH) and heterocyclic amines (HCA) are formed when cooking meat at very high temperatures and importantly through bacterial enzymatic transformation via decarboxylation of aromatic amino acids and N-nitrosation [58, 59]. Their metabolic derivatives can cause DNA base alkylation and formation of DNA adducts which predispose to mutations and carcinogenesis [60].

Red meat is also rich in sulfur containing amino acids, which promote the growth of sulfur-reducing bacteria (SRBs, e.g., *Desulfovibrio vulgaris*) that convert the H₂ gas produced during saccharolytic fermentation into an inflammatory and genotoxic end-product, hydrogen sulfide (H₂S) [61]. H₂S impairs cytochrome oxidase, inhibits mucin synthesis, suppresses butyrate utilization, and promotes methylation of DNA by generating free radicals [62]. Also, SRBs suppress the growth of methanogens such as Methanogenic archaea (e.g., *Methanobrevibacter smithii*) that detoxify the H₂ into non-toxic methane gas that may be exhaled in the breath [63].

3. Fat

In a murine model, Devkota *et al* have noted that a high fat diet (37% of total calories) increased hepatic synthesis of sulfur-rich taurine conjugates of bile acids that promoted the growth of

Bilophila wadsworthia in the colon, which generated H₂S causing acute colitis in II10 *-/-* mice [64]. High dietary fat stimulates secretion, enterohepatic circulation, and colonic escape of primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDA) [65]. Subsequently, high colonic concentrations of CA and CDA promote 7- α hydroxylating Clostridial spp. that carry out dehydrogenation, sulfation, and 7 α -dehydroxylation yielding the secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA) [66]. Secondary bile acids have been shown to be anti-apoptotic and genotoxic via oxidative stress through generation of reactive oxygen species and suppression of p53 response to DNA damage causing cellular proliferation [67]. Furthermore, Bernstein *et al* demonstrated that the addition of secondary bile acids to the drinking water of rats induced spontaneous carcinogenesis that could be blocked by simultaneous administration of antioxidants [68].

The role of Diet and Microbiota in the mediation of CRC risk

There is substantial *in vitro* and *in vivo* evidence to suggest that dietary patterns shape the dynamic composition and diversity of colonic microbiota. In a series of studies, we tested our hypothesis that microbiota mediate the dietary risk of CRC by analyzing the dietary patterns, colonic mucosal biopsies, fecal and colonic microbiota (measured by colonic evacuation), breath contents of hydrogen and methane, colonic secondary bile acids and SCFAs concentrations among subjects belonging to different CRC risk populations, African Americans (high risk, incidence of ~65:100,000), Caucasian Americans (moderate risk, incidence ~40:100,000), and native Africans in rural South Africa (low CRC incidence of ~5:100,000) [26, 30, 42, 69, 70]. African Americans were noted to consume significantly ($p < 0.01$) higher protein (94 +/- 9.3 g/d vs. 58 +/- 4.1 g/d) and fat (114 +/- 11.2 vs. 38 +/- 3.0 g/d), meat, saturated fat, cholesterol, as

well as higher ($p < 0.05$) calcium, vitamin A, and vitamin C, and similar amount of fiber intake when compared to native Africans [42]. And they had significantly higher breath H_2 , fecal colony counts of 7-alpha dehydroxylating bacteria, and colonic crypt cell proliferation rates – a biomarker of cancer risk. On the other hand, native Africans who consumed a diet high in complex carbohydrates and resistant starch had higher breath methane and fecal colony counts of Lactobacilli, colonic SCFAs and butyrate concentrations when compared with Caucasian Americans and African Americans. And, the American groups had a significantly higher colonic content of 2-methylvalerate, a branched-chain fatty acid derived from protein fermentation [70]. Interestingly, native Africans, despite their lower dietary consumption of folate, vitamin B12, and biotin, had high colonic contents, indicating *de novo* synthesis by the microbiota. In terms of dietary fat intake and fecal secondary bile acids, African Americans had 3-4 times higher ($p < 0.05$) colonic LCA, DCA, and CA content than the native Africans, but similar when compared to Caucasian Americans [70]. We have already discussed the major phylogenetic differences in fecal and colonic microbiota above, with African Americans conforming to ‘enterotype 1’ and Africans to ‘enterotype 2’, with greater abundances of starch degraders, such as *Dialister*, *Oscilispiera*, *Succinovibrio*, *Xylanibacter*, and butyrate producers [30]. Dietary factors seem to play a key role in shaping the gut microbiota as evidenced by strong association of long-term consumption of protein and animal fat rich diet with Bacteroides predominance (enterotype 1) and that of carbohydrates with Prevotella predominance (enterotype 2) [71]. In a study comparing the fecal microbiota, the rural African children were noted to have a unique abundance of bacteria belonging to the genii Prevotella and Xylanibacter (absent in their European counterparts) that had genes for fermentation of cellulose and xylan hypothesized to be a consequence of high fiber diet in order to allow maximal metabolic energy extraction from

ingested plant fiber [29]. In keeping with the earlier findings, most of our adult rural South African subjects rural were also found to have predominance of *Prevotella* and starch degraders (*Succinivibrio* and *Oscillospira*) reflecting their high consumption of plant based polysaccharide diet. Equally impressive was the differences in diversity of fecal methanogenic archaea (MA) and SRB. Native Africans were found to have a higher proportion and diversity of MA and distinct SRB populations when compared their American and European counterparts [72]. We concluded that it was the colonic *milieu* rather than an individual microbe, metabolite, nutrient, or component, that determines the overall colon cancer risk [73].

Dietary intervention to modulate colonic microbiota and influence CRC risk

Dietary changes have been shown to result in swift shifts in microbial composition. In a germ-free mouse model colonized with human microbiota, switching the diet from a low fat to high fat altered the microbial diversity within a single day [74]. In healthy volunteers, David et al examined how dietary change altered human gut communities within 5 days when the diet was switched from a ‘plant-based’ (rich in grains, legumes, fruits and vegetables) to an ‘animal-based’ (rich in meats, eggs, and cheese) regimen [75]. The animal-based diets increased the abundance of microorganisms such as *Bilophila wadsworthia*, which is linked to dietary fat, bile acids, and mucosal inflammation and decreased the levels of saccharolytic Firmicutes. In their 10-day controlled-feeding study on effect of diet on human fecal microbiota, Wu *et al* used diet inventories and 16S rDNA sequencing of fecal microbiota and noted the enterotype clustering of *Bacteroides* predominantly in subjects on protein and animal fat rich diets and *Prevotella* in those on carbohydrate and simple sugar rich diets [71]. Interestingly, changes in microbiome composition were identified when switched between high-fat/low-fiber or low-fat/high-fiber

diets, but the enterotype clustering remained stable suggesting it takes long-term dietary changes to allow microbial adaptation and significant shift in their compositions.

In summary, we have substantial human and experimental evidence that change to a western diet explains the increase in colon cancer. However, westernization results in changes in many other aspects of the environment that might also increase exposure to luminal carcinogens. In an attempt to investigate the role of diet alone, we switched the diets of Americans and rural Africans for a 2 week period and measured the changes in the fecal and colonic microbiota, their metabolites, and inflammatory and cancer biomarkers in colonic biopsies. Global analysis of the microbiota was done using 16S rRNA gene phylogenetic microarray (Human Intestinal Tract Chip (HITChip)), which covers over 1,000 of the currently known bacterial species from the human intestine and has been demonstrated to provide highly concordant results concerning the microbiota composition when compared to 16S or metagenome sequencing [76]. HITChip allows deep profiling of phylotypes at high resolution, down to <0.1% relative abundance; corresponding to a duplicated set of 100,000 pyrosequencing reads per sample with very high reproducibility (>98%) and at considerably lower cost [77-79]. In general, targeted analysis of microbes known to be related to the area of interest is best pursued first, followed by complete microbial genome sequencing (e.g. 'shotgun' sequencing) which can identify, or predict the presence of unknown microbes and their metabolic potentials [80]. The choice of sequencing method, which is evolving daily, very much depends upon budget, as the newer techniques can cost as much as \$1000 per sample, making them inappropriate for epidemiological studies. Much of the development is now dependent on advanced computerized integration of the massive amounts of data generated from these techniques, and so the cost of interpretation will exceed the

analytical costs. For our study functional gene analysis was performed using real-time quantitative PCR. Fecal short-chain fatty acids were measured by gas chromatography and bile acids by liquid chromatography-mass spectrometry.

All volunteers were housed for the 2 weeks, African Americans in our Clinical Translational Research Center, Africans in a rural lodge, and their meals were cooked on site and delivered under strict supervision [27]. The rural South Africans were fed a western-style diet (51% fat, 27% protein, 20% carbohydrate, 3 g fiber/1000 kcals), and African Americans were fed a traditional African style diet (17% fat, 15% protein, 68% carbohydrate, 21 g fiber/1000 kcals). On switching to a high fiber/low fat diet, the butyrate increased by 2.5-fold and secondary bile acids decreased by 70% in African Americans. On the other hand, switching to low fiber/high fat diet in native Africans resulted in reduction of colonic butyrate content by 50% and increased in secondary bile acids by 400%. Similarly, in our functional gene analysis to capture the functional ability of the microbiome (as a whole as several microbes can share the same functional ability), native Americans who initially had greater expression of the *bcoA* gene that codes for the enzyme butyryl-CoA:acetate CoA-transferase, which is responsible for the last step in butyrate synthesis and *mcrA* gene for the enzyme responsible for methanogenesis at baseline, had a reduction in these gene expressions after the diet switch. And, *baiCD* gene reflecting the 7- α -dehydroxylating enzyme responsible for secondary bile acid production was low initially and increased on higher fat diet after switch. Reciprocal changes were noted among the AA. Most importantly, these reciprocal changes in aspects of the microbiome and metabolome were associated with reciprocal changes in mucosal biomarkers of cancer risk: namely mucosal epithelial proliferation rate (Ki67 staining) and immunohistochemical markers of inflammation

(CD3⁺ intraepithelial lymphocytes and CD68⁺ lamina propria macrophages), which were suppressed in Americans by the ‘African’ diet, and increased in Africans by the ‘western’ diet (Figure 3). These findings are exciting as they show not only that colonic microbial metabolism responds rapidly to dietary modification, but also that these microbial-metabolic changes are accompanied by changes in mucosa known to increase or decrease the susceptibility to neoplastic change and cancer risk within 2 weeks.

Conclusion and Future direction

Our knowledge of the microbiota-host interactions and their impact on health outcomes is still in infancy as we are transitioning our research efforts from ‘observing the association(s)’ to ‘understanding the causation’. The new ‘organ status’ of the microbiota has attracted great attention as a target for prophylactic or therapeutic intervention. Interestingly, a recent study has identified 20 microbial gene markers that differentiated patients with adenomatous polyps and cancer from healthy control subjects [81]. The study also validated 4 of those microbial genes that were enriched in the microbiome of patients, in ethnically different (Danish, French, and Austrian) cohorts, with early stage (stage I-II) CRC highlighting the potential role of fecal biomarkers for earlier diagnosis of CRC. There has been tremendous enthusiasm in the field of ‘functional foods’ with promotion of ‘prebiotics’ (indigestible carbohydrates that enhance the population or activity of ‘beneficial’ probiotic intestinal microorganisms), ‘probiotics’ (microorganisms ingested to populate the intestine for health benefits), and ‘synbiotics’ (combination of pre- and probiotics). While their health benefits seem intuitive and the supportive *in-vitro* evidence is promising, it is doubtful that the benefits of prebiotics and probiotics can outweigh those of a normal ‘balanced’ diet that our genome evolved together with.

Legends

Figure 1. Distinct Colonic Microbial Composition of African Americans (high CRC risk group) and Native rural South Africans (Low CRC risk group)

Microbial composition was dominated by *Bacteroides* in the African Americans, which indicated that they belonged to enterotype 1, and was dominated by *Prevotella* in the Native rural South Africans, which categorized them as enterotype 2 (10). AA, African American; CRC, Colorectal cancer; NA, Native African.

Adapted with permission from “Junhai Ou et al. Am J Clin Nutr 2013;98:111-120, American Society for Nutrition.”

Figure 2. Dietary risk of CRC is mediated by dysbiosis of gut microbiota and their metabolites.

Dietary fiber/complex carbohydrates promote saccharolytic fermentation yielding anti-inflammatory and antiproliferative SCFAs, such as butyrate, whereas, red meat generates inflammatory and genotoxic metabolites by promoting proteolytic fermentation, H₂S production from its sulfur-rich amino acid content, and exposing colonic mucosa to other carcinogenic constituents such as heme, nitrosamines, HCA, and PAH. High dietary fat promotes excess primary BA secretion and their conversion to pro-carcinogenic secondary bile acids (LCA, DCA). Dysbiosis, an imbalance between the ‘protective’ and ‘detrimental’ microbiota composition and their metabolic end-products results determines the risk of CRC. BA (Bile acids), BCFA (Branched-chain fatty acids), CH₄ (methane), DCA (Deoxycholic acid), HCA (Heterocyclic amines), H₂ (Hydrogen), H₂S (Hydrogen sulfide), LCA (Lithocholic acid), PAH (Polycyclic aromatic hydrocarbons), Short-chain fatty acids (SCFAs).

(Original work)

Figure 3: Colonic mucosal immunohistochemistry of proliferative and inflammatory biomarkers

Immunohistochemical analysis of colonic mucosal biopsies taken at colonoscopy with their associated quantitative analysis on the right panel. Decreased expression of the cancer biomarker- Ki67 staining of epithelial crypt cells (a) and inflammatory biomarkers- CD3⁺ staining (b) and CD68⁺ macrophages in the lamina propria (c) in an African American (the first and second panels) and reciprocal changes in a native/rural African (the third and 4th panels left to right) before and after dietary switch. The bar graphs on the far right summarize the group mean±S.E. results in 20 African Americans and 12 rural Africans. The two-tailed Mann-Whitney *U*-test was used for comparisons for non-paired samples and the Wilcoxon rank sum test for paired samples, with Bonferroni correction for multivariate comparisons. Triangles indicate significant ($P<0.05$) baseline differences and stars indicate significant changes induced by diet switch.

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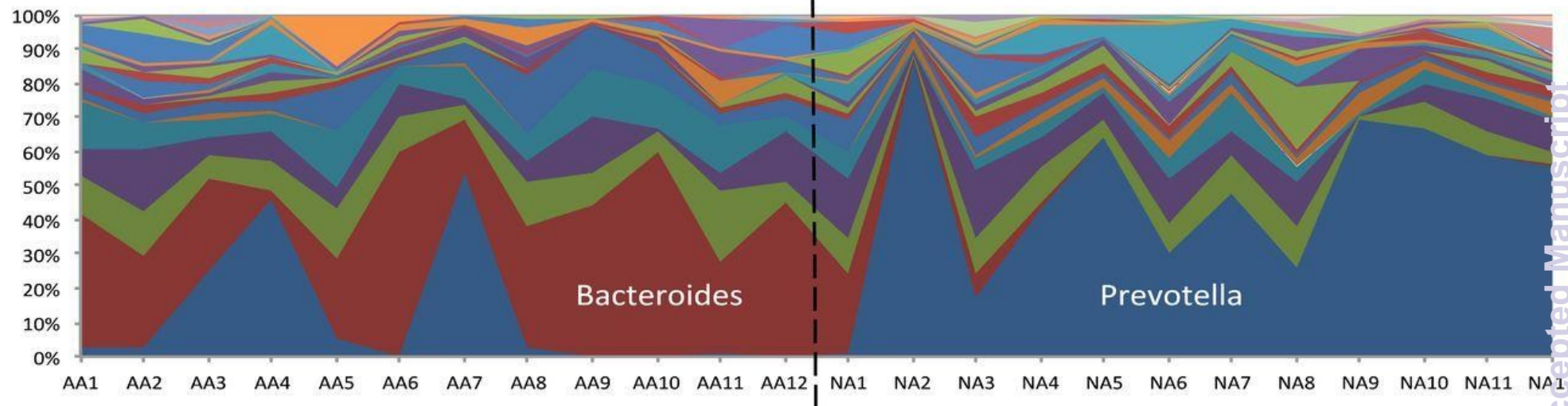
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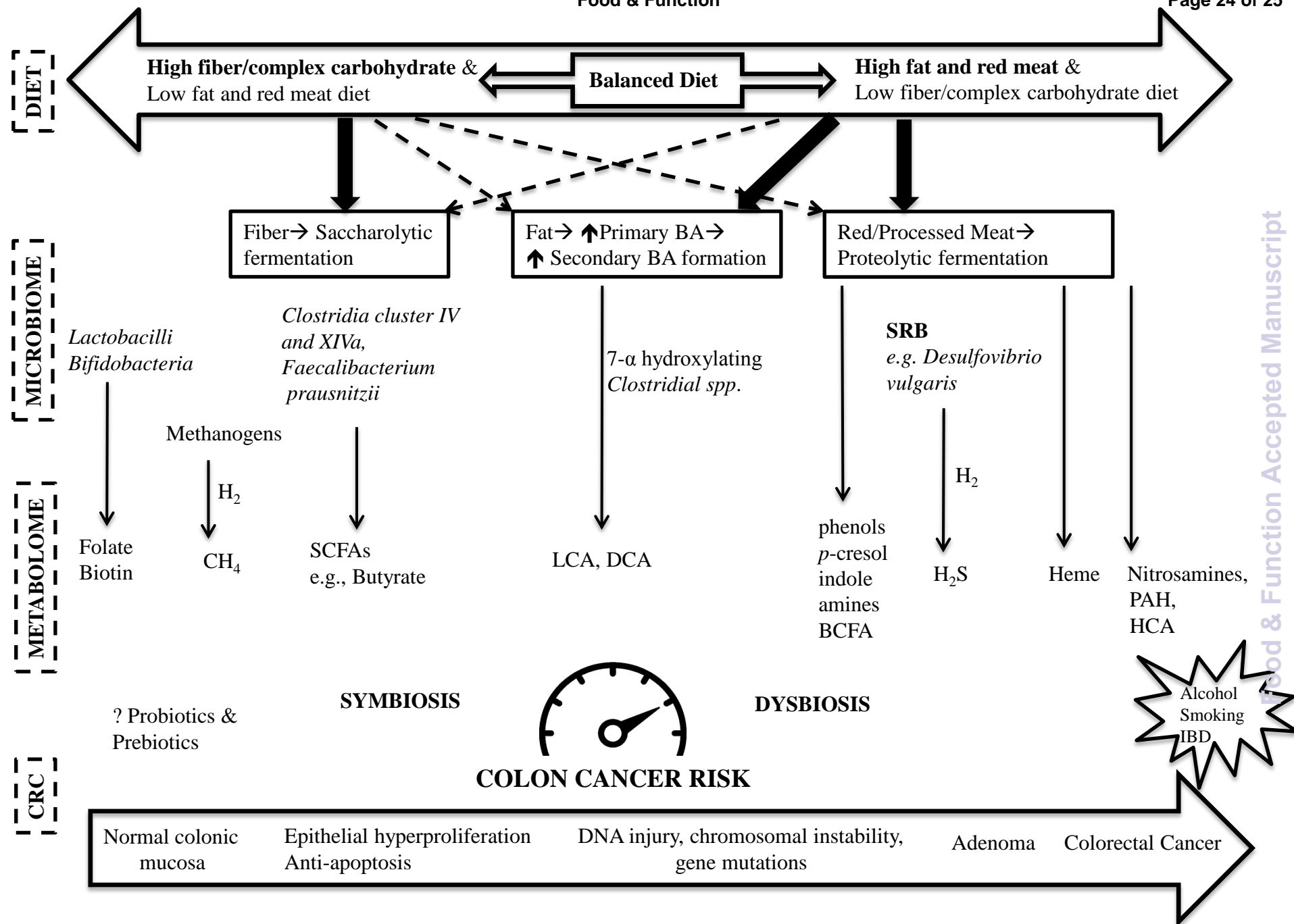


Bacteroides

Prevotella

- | | | | | |
|----------------------------|----------------------------|-----------------------|-----------------------------|----------------------------|
| ■ Prevotella | ■ Bacteroides | ■ Other Clostridiales | ■ Other Ruminococcaceae | ■ Other Lachnospiraceae |
| ■ Other Prevotellaceae | ■ Roseburia | ■ Oscillibacter | ■ Dialister | ■ Other Bacteroidales |
| ■ Other Porphyromonadaceae | ■ Other Enterobacteriaceae | ■ Ruminococcus | ■ Coprococcus | ■ Other Veillonellaceae |
| ■ Other Firmicutes | ■ Other Bacteroidetes | ■ Faecalibacterium | ■ Parabacteroides | ■ Sutterella |
| ■ Sporobacter | ■ Blautia | ■ Other Clostridia | ■ Dorea | ■ Catenibacterium |
| ■ Klebsiella | ■ Succinivibrio | ■ Eubacterium | ■ Other Incertae Sedis XIII | ■ Other Burkholderiales |
| ■ Acetivibrio | ■ Other Coriobacteriaceae | ■ Fusobacterium | ■ Oxalobacter | ■ Other Betaproteobacteria |
| ■ Megasphaera | ■ Kluyvera | ■ Anaerovorax | | |

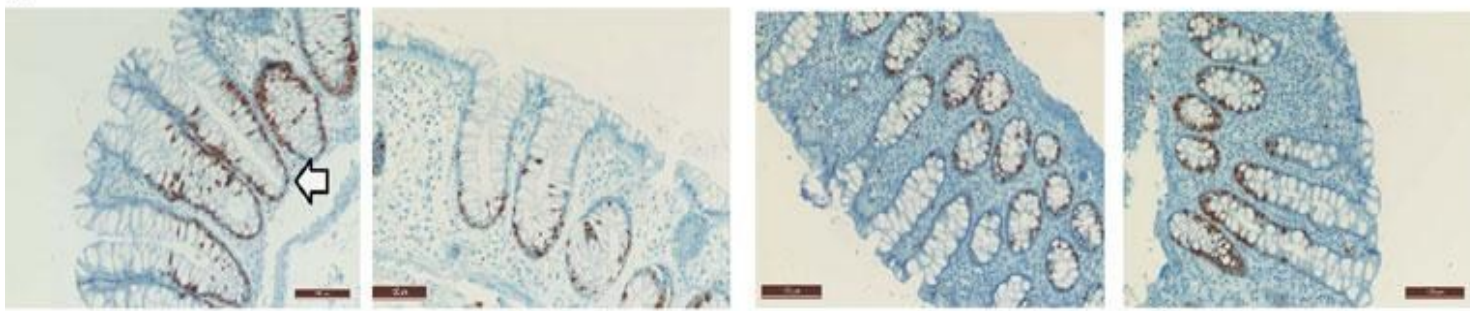
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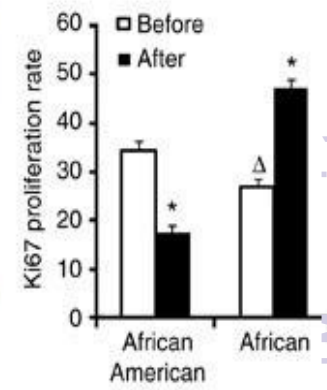
African American

Native /rural African

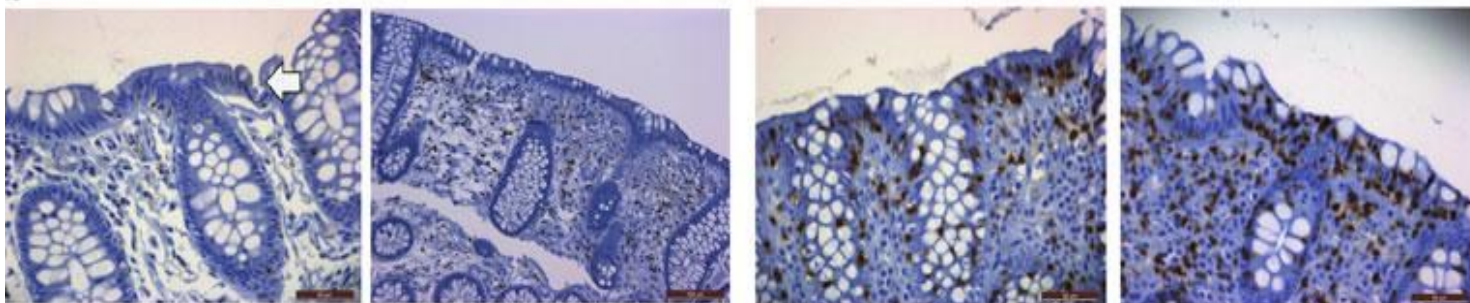
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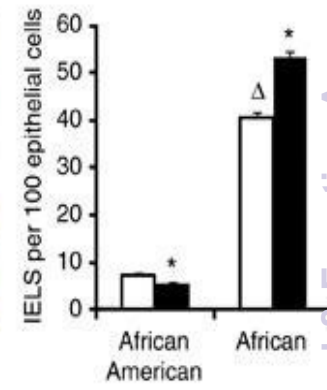
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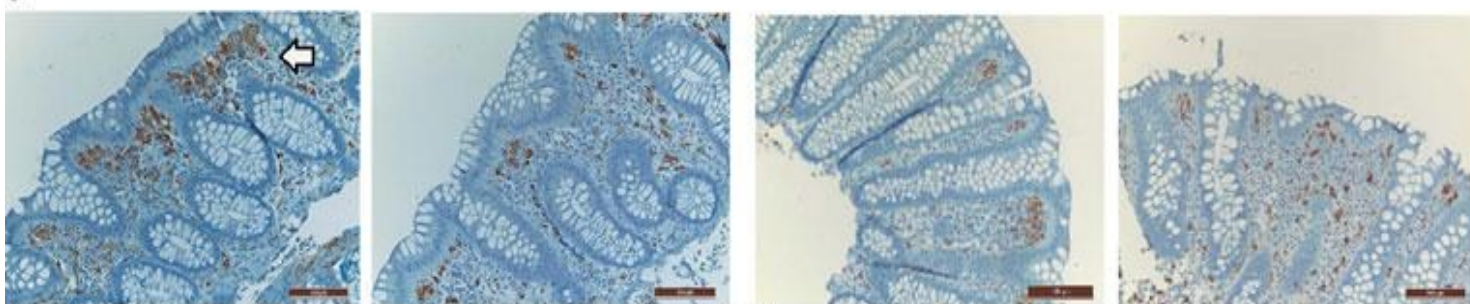
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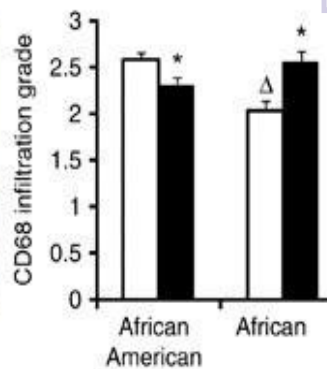
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