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Diet, microorganisms and their metabolites, and colon cancer

Stephen J. D. O'Keefe

Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh, S853 Scaife Hall, 3550 Terrace Street, Pittsburgh, Pennsylvania 15261, USA.

Abstract

Colorectal cancer is one of the so-called westernized diseases and the second leading cause of cancer death worldwide. On the basis of global epidemiological and scientific studies, evidence suggests that the risk of colorectal cancer is increased by processed and unprocessed meat consumption but suppressed by fibre, and that food composition affects colonic health and cancer risk via its effects on colonic microbial metabolism. The gut microbiota can ferment complex dietary residues that are resistant to digestion by enteric enzymes. This process provides energy for the microbiota but culminates in the release of short-chain fatty acids including butyrate, which are utilized for the metabolic needs of the colon and the body. Butyrate has a remarkable array of colonic health-promoting and antineoplastic properties: it is the preferred energy source for colonocytes, it maintains mucosal integrity and it suppresses inflammation and carcinogenesis through effects on immunity, gene expression and epigenetic modulation. Protein residues and fat-stimulated bile acids are also metabolized by the microbiota to inflammatory and/or carcinogenic metabolites, which increase the risk of neoplastic progression. This Review will discuss the mechanisms behind these microbial metabolite effects, which could be modified by diet to achieve the objective of preventing colorectal cancer in Western societies.

Colorectal cancer is the second most common cancer in women and the third in men worldwide, with slightly increased case numbers in men[1]. In 2012, 1,361,000 new cases were reported, which approximates to 10% of the total worldwide cancers — around half will die from the cancer[1]. The geographical variation in colorectal cancer incidence is remarkable, as illustrated by the latest WHO data[1] (FIG. 1). Colorectal cancer is one of the so-called westernized diseases with the highest incidence rates in North America, Australia, New Zealand and Europe (all >40 cases per 100,000) and lowest in rural Africa (<5 cases per 100,000) and Asia (variable). However, at the extreme upper end of incidence rates are the Alaska Native people, with reported rates exceeding 100 cases per 100,000 (REF 2). This degree of variation, taken together with migration studies and experimental studies, which will be discussed, provide compelling evidence that environmental factors rather than genetic dysfunction are responsible for the development of colorectal cancer.

Genome-wide association studies have identified 14 loci harbouring common variants that influence the risk of developing colorectal cancer[3], but it took the inclusion of >9,000 cases and 9,000 controls before a statistically significant interaction between one of these loci and

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the intake of processed meat was found[4]. By contrast, significant associations between dietary factors and cancer risk have been demonstrated with much smaller numbers. The World Cancer Research Fund's 2010 Continuous Update Report based on the systematic review and meta-analysis of 43 cohort or randomized controlled trials graded the evidence of linking dietary fibre with a decreased risk of colorectal cancer, and red and preserved meat with increased risk, as "convincing" — the strongest grade assigned[5–7]. Spurred by this analysis, the WHO issued a warning on the cancer risk of excessive consumption of meat and processed meat[8]. Other dietary factors, most notably fruit and vegetables, fish oils and calcium are associated with reduced risk[6]. Over three decades ago, Doll and Peto[9] estimated from the epidemiological evidence of the time that >90% of gastrointestinal cancers were diet-driven. Although there is debate that this figure is too high for some gastrointestinal cancers — for example, in the stomach other environmental factors such as *Helicobacter pylori* have a role[10] — experimental evidence will be provided to support the proposal that diet is the chief driver in the observed 20-fold geographical variation of sporadic colon cancer. The strength of environment is further illustrated by Le Marchand's classic studies of Japanese immigrants to Hawaii who, within one generation, suffered a change in colon cancer incidence from the low rate of the Japanese native population to the high rate of Hawaiian natives[11]. The excitement of these analyses is that, unlike the cancer risk attributable to inherited genetic make-up, the risk due to these dietary factors is readily modifiable by the power of education and the modern media.

Mechanisms of colorectal cancer

As summarized by Nosho *et al.*[12], colorectal carcinogenesis is a heterogeneous process with a differing set of somatic molecular alterations influenced by diet, environmental and microbial exposures and host immunity. However, not everybody who eats a high-meat, high-fat, low-fibre diet develops colorectal cancer, and not everybody who eats a balanced diet rich in fruits, vegetables and coarse grains is protected from the disease. The vast majority of colorectal cancers are not inherited but sporadic and what a person eats can have a profound effect on the initiation, promotion and progression of the neoplastic process. The rate of carcinogenesis is determined by the penetrance of the genetic defect and by the aggressiveness of the environmental insult. Evidence indicates that colorectal cancer arises from a stepwise disturbance of the composition of the gut microbiota, induced by food components or diet, plus genetic alterations in oncogenes and tumour-suppressor genes. For example, most colorectal cancers harbour mutations in the gene encoding APC, a tumour suppressor that regulates Wnt signalling by controlling the levels of β-catenin, which then regulates downstream inflammatory, cell cycle and proliferative pathways[13]. Mechanistic studies have shown that mutant *Apc* mice spontaneously develop adenomatous polyps[14,15]. However, experimental studies have shown that microbial fermentation products (such as butyrate) and microbial activated phytochemicals (such as polyphenols) have a wide range of antineoplastic effects that counteract tumorigenic signalling pathways and mount epigenetic mechanisms such as histone deacetylase inhibition that promote apoptosis, suppress proliferation and arrest neoplastic transformation[16–22] (FIG. 2).

Gut microbiota and colorectal cancer risk

Gut microbiota can influence the development of colorectal cancer in several ways. Inflammation is a necessary trigger but inflammation alone, or the presence of bacteria or bacterial metabolites alone, is not enough to promote tumorigenesis. Instead, complex inter-relationships with the gut microbiota, inflammation, host genetics and other environmental factors are needed for the progression of colorectal tumours[23]. Microorganisms can induce chronic inflammation in a number of ways: adherence to the epithelium; activation of an immune response through binding to Toll-like receptors and/or activating regulatory T (T_{reg})cells; synthesis and secretion of cytotoxic biomolecules or metabolites; or by translocation into the body[24,25]. The critical role of the gut microbiota in colorectal cancer is shown by the association between microbial abundance and cancer incidence. For example, neoplastic transformation is most common in the distal gut where the microbiota are most dense, and carcinogenesis in experimental animals is linked to the presence or absence of microorganisms[26,27]. Although great effort has been expended searching for a single causative organism — as in *H.* pylori-associated gastric ulceration and cancer — the evidence to date points to a shift in composition and activity of the gut microbiome, creating a microclimate that promotes inflammation, proliferation and neoplastic progression. For example, a review of 31 original articles on the role of colon microbiota in colorectal cancer performed in humans and animals observed that, despite differences in methodology, some bacteria were consistently augmented (including Fusobacteria, *Alistipes*, Porphyromonadaceae, Coriobacteridae, Staphylococcaceae, *Akkermansia* spp. and Methanobacteriales), whereas others were consistently diminished in colorectal cancer *(Bifidobacterium, Lactobacillus, Ruminococcus, Faecalibacterium* spp., *Roseburia* and *Treponema*)[28]. In addition, levels of some microbial metabolites (such as nitrogenous compounds) were consistently elevated, whereas others (such as butyrate) were decreased throughout colonic carcinogenesis[28].

In another study, faecal samples from three patients with colorectal cancer and three healthy individuals as controls were transplanted into germ-free mice in which cancer was chemically promoted[29]. The rate of tumour generation was associated with microbial composition. The taxa most positively associated with tumour burden were members of Gram-negative *Bacteroides, Parabacteroides, Alistipes* and *Akkermansia,* whereas Gram-positive Clostridiales, including multiple members of *Clostridium* group XlVa, were negatively correlated with tumours[29]. Furthermore, metagenomic analysis inferred a negative correlation between tumour count and the potential for butyrate production, and a positive correlation between tumour count and the capacity for host glycan degradation, suggesting a role for mucus degraders in tumorigenesis[29].

The application of the compositional pattern of the faecal microbiota in the prediction of adenoma and carcinoma has also been examined in 60 patients and 30 healthy controls[30]. This analysis revealed both an enrichment and depletion of several bacterial populations associated with adenomas and carcinomas. Combined with known clinical risk factors of colorectal cancer (such as BMI, age and race), data from the gut microbiome improved the ability to differentiate between healthy, adenoma and carcinoma clinical groups relative to risk factors by 50-fold.

Similar conclusions were reported by a European study based on faecal samples from a mixed sample of healthy individuals (colonoscopy negative), patients with adenoma, and patients with confirmed colorectal cancer from France, Germany, Denmark and Spain[31]. The Gram-negative phyla of Fusobacteria and, to a lesser extent, Proteobacteria were substantially increased in patients with cancer, whereas Gram-positive Actino bacteria were decreased[31]. Bacteroidetes and Firmicutes were enriched and depleted, respectively, in patients with colorectal cancer. In addition, meta-genomic sequencing of faecal samples was used to identify taxonomic markers that distinguished patients with colorectal cancer from tumour-free controls and found that pattern differences were able to predict colorectal cancer at a sensitivity similar to that of the usually used faecal occult blood test[31]. When the two tests were combined, detection sensitivity increased by 45%. On the basis of these findings, carcinogenesis was proposed to result from a metabolic shift of the gut microbiota from the normal fibre degradation to utilization of host carbohydrates and amino acids, accompanied by an increase in lipopolysaccharide metabolism.

Despite the substantial support for carcinogenesis being linked to an overall disturbance in microbial balance and integration, accumulating evidence suggests a predominant role of *Fusobacterium* spp. in the neoplastic process; biopsy material from adenomas and adjacent normal mucosa in 19 patients showed 48% positivity for *Fusobacterium* in tumour tissue, which was higher than in the normal tissue[32]. Using the $Apc^{Min/+}$ mouse model of intestinal tumorigenesis, *Fusobacterium nucleatum* was shown to increase tumour multiplicity and recruit tumour-infiltrating myeloid cells that can promote tumour progression[32]. Other studies have shown that *F. nucleatum*-high colorectal cancer tissues were inversely associated with the density of $CD3^+$ T cells[33] and had a strong association with microsatellite instability and CpG island methylator phenotype-linked cancers[34]. These examples demonstrate the complex interactions between microorganisms, immunity, genetic predisposition and colorectal cancer.

Also noteworthy is the evidence for the potential of individual microorganisms to induce colitis and colorectal cancer in experimental animals[35]. The microorganismstudied was the human commensal enterotoxigenic *Bacteroides fragilis* (ETBF), a subset of *Bacteroides fragilis* distinguished by the secretion of a specific enterotoxin, which has been associated with ulcerative colitis and colorectal cancer in humans[36]. First, ETBF infection in mice was shown to cause a brief, acute colitis characterized by rapid and robust colonic activation of STAT3, which induced a type 17 T helper cell immune response and evolved into chronic colitis[35]. Second, ETBF colonization of multiple intestinal neoplasia in mice induced numerous IL-17A-dependent distal colon adenomas[37]. Third, the persistence of the ETBF infection has been shown to be essential for tumour formation, as antibiotic therapy prevented tumorigenesis[37]. Finally, fascinating evidence is emerging on the ability of gut microorganisms to cross the mucosal or blood barrier and establish unique micro-biomes within solid body organs, such as breast tissue[38], seminal vesicles[39] and placenta[40]. These findings might explain the association between dysbiosis and the use of antibiotics and the increased risk of cancers of the colon, gastric, oesophageal, pancreatic, laryngeal, breast and gallbladder[41]. In a qualitative survey of the breast microbiota DNA, the bacterium *Methylobacterium radiotolerans* was relatively enriched in tumour tissue, whereas the bacterium *Sphingomonas yanoikuyae* was enriched in paired normal tissue[42]. Further

research is needed to ascertain whether these microorganisms can influence carcinogenesis by direct attachment to organ cells or whether it is through the production of toxic metabolites.

The burning question remains whether the discussed microbial associations with cancer are cause or effect. For example, tumorigenesis might produce inflammation, ulceration and necrosis, changing the microenvironment and the growth conditions for different microorganisms. For the remainder of this Review, the experimental evidence supporting a major role of microbial metabolites in initiating, promoting and advancing colorectal cancer will be examined.

The role of diet

Food is a complex mixture of thousands of bioactive molecules, many of which are modified by preservation, cooking methods, digestion, metabolism by the host and the luminal gut microbiota. Although evidence suggests that an excess of nutrients such as protein and fat might be inflammatory and therefore might potentiate neoplastic change[43], the evolutionary interaction between food and genes is in the preservation of mucosal health — from what we know of the process of evolution, the dietary needs of every organism are genetically determined. Some have argued that our health will be better served by the diet that first established *Homo sapiens* in the Palaeolithic era in Africa, a period that lasted from ~2.5 million to 11,000 years ago[44,45]. With regard to the colon, an ideal microbiota probably evolved in tandem with the development of our colon. Although the changes in diet following the Industrial Revolution provided more food for growth, the diet became progressively depleted in colonic food, namely fibre, leading to a shift in gut microbiota metabolism that does not provide for colonic mucosal health.

Advances in dental microwear and stable isotope technology have shed light on the composition of foods consumed by early hominins *(Australopithecus)* and our *Homo* ancestors[46], providing evidence that dietary consumption was diverse, from *Paranthropus robustus* consuming fleshy fruits and soft young leaves (browsers), to *P. boisei* living on a diet rich in tropical grasses and sedges (grazers). Coprolite analyses from prehistoric deposits in caves in Southwest Arizona dating back 14,000 years[47] have indicated that the diet of early *Homo sapiens* was predominantly high in complex carbohydrate and fibre. Furthermore, dental microwear and stable isotope analyses have hinted at unexpected diversity and complexity in early hominin diets, but have concluded that their diets were most likely dominated by plant foods[48]. Although these analyses provide no direct information on the quantity of meat and fat consumed, they do suggest that the diet throughout our evolution has been consistently rich in high-fibre foods. Thus, it is reasonable to propose that the appearance of colorectal cancer as one ofthe so-called westernized diseases is a consequence of the progressive diminution in the consumption of fibre-rich foods and the subsequent dysbiosis of our gut microbiota. Studies[49–51] have shown that colorectal cancer remains rare in semi-urbanized agricultural-commercial communities in Africa, who continue to consume >50 g of fibre per day, indicating that we do not have to return to hunter-gatherer status to avoid colorectal cancer, we just need to pay more attention to the nutritional needs of the colon. The concern is that our extraordinary

ability to adapt to the environment and increase our lifespan has not enabled us to identify the phenotypic identity of colorectal cancer until it is too late.

Colonic nutrition

As the undigested dietary residues that enter the colon and provide an energy source for the microbiota are chiefly complex carbohydrate fibres, mutualism will be best served by a diet containing sufficient fibre. The fermentation of fibre releases short-chain fatty acids (SCFAs), principally butyrate, propionate and acetate, which are essential for colonic mucosal health and otherwise unavailable from the diet (small quantities might be ingested but are absorbed by the small intestine). Much of the original work on the ability of the colonic microbiota to ferment fibre for their own metabolic needs, with the subsequent production of luminal SCFAs, was performed by the laboratories of Macfarlane[52,53], Cummings[54] and Englyst[54]. Roediger was the first to demonstrate that butyrate and not glucose was the preferred energy source of colonocytes, thus establishing the foundation for the now well-recognized symbiotic relationship between colonic microorganisms and colonic mucosal health[55].

Saccharolytic fermentation

Fermentation of complex carbohydrate or starch involves numerous intermediate steps and multiple enzyme pathways contributed by different microorganisms[45,46] (FIG. 3). The initial fermentation of carbohydratethat escaped digestion in the small intestine is followed by the utilization and cross-sharing of metabolites by different members of the microbiota, then finally the synthesis of SCFAs[56]. The most abundant SCFAs are butyrate, propionate and acetate. Although the molar ratios of these SCFAs vary with the composition of the microbial fermenters and the fibre source, the relative magnitude of the quantities are acetate > propionate > butyrate, which is important in considering the role each of the SCFAs has on mucosal and body homeostasis[57,58]. Reducing the carbohydrate content of the diet from the usual 52% to 4%, and increasing the protein content from 13% to 30%, caused a reduction in the proportion of butyrate measured in faecal samples from 16% to 8% in patients with obesity[59]. This finding was associated with a change in microbiota composition, with a reduction in the abundance of butyrate-producing bacteria related to *Roseburia* spp. and *Eubacterium rectale.*

Although butyrate is unique in its role as the chief energy source for the colonic mucosa, acetate and propionate share many of the same anti-inflammatory and antineoplastic properties of butyrate[60]. Furthermore, because acetate and propionate are less metabolized by the mucosa, more is absorbed to have systemic effects that influence weight gain, obesity and the metabolic syndrome[61]. Importantly, acetate suppresses appetite and enhances lipogenesis[62], and propionate suppresses cholesterol synthesis[63–65]. Quantitatively, saccharolytic fermentation usually salvages ~150–250 kcal per day, but in malabsorptive conditions such as the short bowel syndrome, it can salvage up to ~1,000 kcal per day[66].

Proteolytic fermentation

Quantitatively, proteolytic fermentation is smaller than saccharolytic fermentation, as protein digestion and absorption by the small intestine is more efficient (>95%[67]) and the quantities

of protein consumed in the diet are generally lower than those for carbohydrate, unless diets such as the Atkins or Banting diet are being followed. However, there is a substantial endogenous supply of protein derived from epithelial desquamation, secretions and mucus, and microorganisms have an important role in recycling body nitrogen and amino acids[68]. Protein fermentation differs from saccharolytic fermentation in that, in addition to producing many of the same SCFAs from the carbon skeleton, it also releases potentially toxic nitrogenous and sulfur metabolites, such as ammonia, amines, nitrates, nitrites and hydrogen sulfide[68]. One study suggested that protein fermentation might have health benefits, demonstrated by the presence of a unique bacterium, *Intestinimonas* strain AF211, belonging to *Clostridium* cluster IV (Lachnospiraceae) of the Firmicutes phylum in 50% of healthy volunteers[69]. Culture, isotope labelling and metagenomic analysis indicated that this microorganism contained all the enzymatic pathways for converting lysine into butyrate. However, this catabolic pathway also generated ammonia.

$$C_6H_{14}O_2N_2 + 2H_2O \rightarrow C_4H_8O_2 + C_2H_4O_2 + 2NH_3$$

Ammonia can be absorbed and recycled in transamination pathways in the liver and can be taken up and detoxified by probiotic *Lactobacilli;* however, in high concentrations, ammonia interferes with cell metabolism, is cytotoxic and induces inflammation and epithelial proliferation[70]. In rats, it was shown that ammonia interacted with *N*-nitroso compounds to induce colorectal cancer[71].

Many of the protein fermentation metabolites can be taken up by other microorganisms and synthesized into activated carcinogens. For example, amines and nitrates can be used by facultative and anaerobic colonic bacteria to catalyse the formation of *N*-nitrosamines, which are among the most potent experimental procarcinogens[72,73]. The clinical relevance of this finding was shown in a study in which eight healthy volunteers were assigned a diet containing 60 g or 600 g meat in a randomized crossover study. Those on the high-meat diet had threefold higher faecal concentrations of nitrosamines than those who consumed a low-meat diet[74].

Hydrogen sulfide is produced by sulfate-reducing bacteria in response to sulfur compounds derived from the diet, intestinal secretions and cell desquamation, or sulfur-rich bile acids such as taurine. Experimental studies have concluded that hydrogen sulfide generated by these microorganisms is pro-inflammatory[75] and genotoxic at physiological concentrations[76]. However, exciting new studies in rats have shown that endogenous mucosal hydrogen sulfide increases ulcer healing and is anti-inflammatory[77,78]. Furthermore, the same investigative team demonstrated that a hydrogen-sulfide-releasing form of the NSAID naproxen was more effective than naproxen alone in suppressing tumour formation in the azoxymethane (AOM)-induced mouse intestinal adenoma model[79]. This finding opens up a new, potentially safer approach to colon cancer chemoprevention than aspirin, without the added risks of gastrointestinal bleeding. These divergent findings might be explained by confusing association with cause. For example, is the finding of increased colonic hydrogen sulfide production or defective detoxification of hydrogen sulfide in

ulcerative colitis[80] an appropriate finding after all, possibly representing an enhanced drive towards ulcer healing rather than the cause of inflammation? Clearly, more work is needed in this area.

Protein fermentation also results in the release of aromatic amino acids, which in turn are metabolized to phenols and *p*-cresol before being excreted in the urine as potential biomarkers of protein consumption[68]. Although the evidence for their carcinogenic potential in humans is limited, experimental studies have shown an ability to damage cellular structure and increase permeability[81]. Theoretically, these changes could increase the susceptibility of the colonic epithelium to luminal carcinogens. Despite these experimental findings, there is no evidence that high-protein diets increase the risk of colon cancer in humans. This finding is probably explained by the fact that protein is never eaten on its own and that the other components of the diet outweigh any deleterious effects protein metabolites might have on mucosal health[68]. For example, studies in humans have shown that fibre supplementation reduces feacal ammonia and *p*-cresol[82], and increases the colonic microbial uptake of nitrogenous metabolites[83]. Additionally, the administration of synbiotics in a variety of human studies consistently reduced protein fermentation products and the genotoxicity of faecal water[68]. Furthermore, symbiotic supplementation *(Lactobacillus rhamnosus* GG, *Bifidobacterium lactis* BB12 and inulin) was associated with less DNA damage and reduced mucosal proliferation rates in patients who had undergone colonic polypectomy[84], supporting the view that cancer risk is determined by the balance between mucosa health-promoting and inflammatory or carcinogenic precursors. Strong evidence (further discussed in a following section) also suggests that the metabolic and carcinogenic effects of high-meat diets can be ameliorated by the simultaneous consumption of fibre in the form of resistant starch[85].

Butyrate

Diminished faecal butyrate levels might not only be a biomarker of cancer risk, but also cancer progression and severity[22]. Clinical studies have revealed that, compared with healthy controls, individuals with advanced colorectal cancer have diminished butyrate-producing bacteria and lower levels of SCFAs[86–88].

A wealth of experimental evidence (reviewed else-where[24,25,89]) has demonstrated the inhibitory effect of butyrate on tumorigenesis through multiple mechanisms (FIG. 2). Critical actions include its anti-inflammatory and immunomodulatory effects[90,91], downregulation of the key canonical Wnt signalling pathway linked to colonic carcinogenesis[15], inhibition of proliferation and migration of neoplastic cells, restriction of tumour angiogenesis, induction of apoptosis and the promoted differentiation of neoplastic colonocytes[92–98]. Butyrate has an important role in reinforcing the mucosal defence barrier by enhancing the expression of mucin-encoding genes and the induction of trefoil factors, heat shock proteins, antimicrobial peptides and transglutaminase activity[99–102]. Finally, evidence also exists that the powerful actions of butyrate on the maintenance of colonic mucosal health are at least partly mediated via the colonic T_{reg}-cell activation of FOXP3 and IL-10 expression[103]. In an elegant series of studies based on specific pathogen-free mice, gnotobiotic altered Schaedler-floracolonized mice and germ-free mice, SCFA was shown to

regulate the size and function of the colonic T_{reg} pool[103]. This process protected against colitis, possibly through interactions between free fatty acid receptor 2 (also known as G-protein coupled receptor 43 or GPR43) induction, the direct activation of histone deacetylase inhibition, and upregulation of IL-10 gene expression.

In one clinical study, the 5-year history of fibre consumption and faecal SCFA concentrations in 344 patients with advanced adenomas was compared to 344 healthy, aged-matched individuals as controls[87]. Fibre intake was significantly (P <0.05) reduced in patients with colorectal adenoma, as were levels of faecal SCFAs. Microbiota composition and quantities were measured by pyro-sequencing and PCR in 47 volunteers from each group, and principal component analysis showed distinct differences in the faecal microbiota communities of the two groups. *Clostridium, Roseburia* and *Eubacterium* spp. were significantly (P <0.001) less prevalent in the advanced adenoma group than in healthy individuals, whereas *Enterococcus* and *Streptococcus* spp. were more prevalent in the adenoma group. Interestingly, butyrate and recognized high-butyrate-producing bacteria were more prevalent in healthy controls consuming a high-fibre diet than in patients with colorectal adenoma consuming a high-fibre diet, suggesting colonic butyrate deficiency due to low-fibre intake or diminished butyrate-producing bacteria promotes neoplasia.

Investigating mechanisms linking fibre intake to neoplasia, a pathway involving oncogenic microRNA (miRNA; oncogenic miR-17–92a cluster) biogenesis was unravelled, through which butyrate might suppress the proliferation of human cancer cell lines[86,104]. First, miR-92a levels were established as sevenfold higher in sporadic human colorectal cancer tissue than in adjacent normal tissue. Second, the addition of butyrate to a human cancer cell line was found to reduce the levels of primary miR-17–92a, precursor and mature miR-92a. This finding was associated with inhibition of the key *MYC* oncogene and enhanced CDKN1C (also known as p57) expression, causing suppression of proliferation and an increase in apoptosis of cancer cells, respectively. The same effect was produced by the addition of other histone deacetylase inhibitors, namely suberoylanilide hydroxamic acid and valproic acid, indicating that the mechanism was principally epigenetic. An increasing number of experimental and human studies have supported the suggestion that the chief mechanism behind the ability of butyrate to suppress proliferation and cancer risk is its capacity to normalize the function of oncologic miRNA clusters (oncomirs)[85,105–107]. For example, in one randomized controlled crossover study, healthy volunteers were given either a diet high in red meat (300 g per day) or a diet high in red meat plus a resistant starch (fibre) supplement for 4 weeks[85]. The diet high in red meat increased expression of oncogenic miRNA (miR-17–92 cluster) in rectal mucosa and cell proliferation, in association with a decrease in miR-17–92 target gene transcript levels[85]. Remarkably, all these effects were negated by increasing butyrogenesis through fibre supplementation (40 g of butyrylated high-amylose-maize starch per day, of which 60% is resistant).

Experimental evidence for an alternative carcinogenesis mechanism has been provided, based on the ability of SCFAs produced from fibre fermentation to bind to the SCFA receptor GPR43 in the colon[22]. In the AOM-dextran sodium sulfate (DSS) colitis-associated colon cancer rat model, a supplementation of the diet with resistant starch decreased tumour multiplicity and adenocarcinoma formation[22]. Evidence that the mechanism involved

increased butyrogenesis was provided by the associated increases in microbial butyrate-producers (*Ruminococcus* and *Bifidobacterium*) and increased SCFA production. Furthermore, the increased butyro-genesis was associated with increased expression of the SCFA receptor GPR43 mRNA, which triggered reduced inflammation via inhibition of cyclooxygenase (COX)-2, nuclear factor-κB (NF-κB), TNF and IL-1β expression, as well as cell proliferation[22].

A threshold effect of butyrate.—That butyrate can paradoxically stimulate mucosal proliferation under certain conditions has long been recognized. This effect can be explained by the mixed action of butyrate on cell growth: as the chief energy supply it will stimulate growth, as an antineoplastic agent it will suppress growth in a phenomenon termed the 'butyrate paradox'. Evidence for the paradox includes the observation that increased butyrogenesis stimulates epithelial growth through its energy provision under starved or atrophic conditions, whereas enhanced butryogenesis under conditions of excess growth suppresses proliferation and cancer risk[108]. Experimental studies have shed light on the mechanisms behind the paradox, reporting that the quantity of butyrate produced might be critical. Low colonic crypt concentrations of butyrate (0.5 mM) had no histone deacetylase inhibitory effect because all the butyrate was used for cellular energetics, whereas the excess from higher quantities (5 mM) was able to spill into the nucleus and act as a histone deacetylase inhibitor, increasing differentiation and apoptosis, and suppressing proliferation of cancerous colonocytes[109]. Furthermore, in gnotobiotic mouse models colonized with wild-type or mutant strains of a butyrate-producing bacterium, fibre was demonstrated to have a potent tumour-suppressive effect but in a microbiota and butyrate-dependent manner[110]. However, it is not surprising to find studies that do not fit well with the idea that butyro-genesis suppresses carcinogenesis. For example, one study showed that a low-carbohydrate diet reduced key luminal butyrate-producing microorganisms (such as Clostridia cluster XIVa) and butyrate production in a colon cancer mouse model[111]. Surprisingly, this finding was associated with a reduction of colon cancer development. Backed up by cell culture studies, the authors proposed that the gut microbiota stimulate colorectal cancer by providing butyrate that drives aberrant proliferation or β-catenin activity, increasing the risk of neoplastic transformation. However, what the metabolic requirements of the colonic epithelium are in this nonphysio-logical model, and whether they were met or exceeded, is unknown.

Good evidence that excessive fermentation rates are as harmful as low rates also exists. In goats, a highgrain (65%) diet has been shown to result in sloughing of the surface layer epithelium, intercellular tight junction erosion, cell mitochondrial damage and upregulation of IL-2 and IFNγ mRNA expression in the colonic mucosa. These effects were in association with increased microbial fermenters, increased acidity and increased SCFA production[112]. The condition is also well recognized in cattle when dietary, animal or environmental factors contribute to abnormal, excessive flow of fermentable carbohydrates from the small intestine into the colon, resulting in distention, diarrhoea and severe mucosal injury[113]. Furthermore, it is important to note that humans also can have a similar condition. Massive loss of the small intestine, a condition termed short bowel syndrome, results in excessive carbohydrate delivery to the colon. In this condition, the colonic microbiota

perform an essential function of increasing fermentation with salvage of up to ~1,000 carbohydrate calories per day[66]. However, disturbance of the microbiota composition can lead to an overgrowth of D-lactate-producing organisms and potentially lethal D-lactic acidosis, as this form of lactate cannot be metabolized by humans[114].

The importance of a balanced diet

From the evidence discussed, and the mechanisms summarized in FIG. 2, the emerging picture is one in which the gut microbiota behave as a community wherein intermicrobial interactions strive to produce a metabolic phenotype that supports colonic health and function. From evolutionary considerations, the gut microbiota have a genetically determined need for food residues derived from a healthy balanced diet.

Provision of an imbalanced diet leads to disturbance in structure and function of the gut microbiota, with unopposed production of metabolites that can induce inflammation and proliferation that increase the risk of neoplasia. Critically, the inflammatory and proliferative effects induced by meat and bile acids, as well as the associated DNA damage and mutations, can be prevented by the simultaneous consumption of resistant starch[85,107,115–119]. This finding makes teleological sense as we are all omnivores.

Phytochemicals

Phytochemicals are a diverse group of micronutrients that are synthesized by plants and have remarkable antioxidant and antineoplastic effects in experimental models. Together with fibre, they account for the anticarcinogenic properties of fruits, vegetables and grains, or high-fibre foods. Studies on fermentation products have shown that their antineoplastic effects are additive to those of SCFAs[16,120]. As they are complex glycosylated molecules, considered xenobiotics and mostly stored within plant cells, phytochemicals have little influence on the upper gastrointestinal tract with 95% unabsorbed by the small bowel and entering the colon[121]. Within the colon, microbial fermentation releases these compounds and specific bacteria metabolize many of them into more bioactive metabolites. This process results in the accumulation of metabolites in the millimolar range at the surface of the mucosa, amplifying biological potency[121]. A good example of the relationship between plant-cell breakdown, fibre metabolism and phytochemical release is given by a study that reported that the feeding of a high-protein, low-carbohydrate and low-fibre diet to human volunteers resulted in reduced levels of key butyrate-producers *(Roseburia* and *Eubacterium rectale),* a decrease in the proportion of butyrate in faecal SCFA concentrations, and greatly reduced concentrations of free phenolic acids in the gut[122]. A forward and backward relationship exists between the metabolites and the gut microbiota, such that the specific microorganisms that metabolize phytochemicals, such as *Escherichia coli, Bifidobacterium* sp., *Lactobacillus* sp., *Bacteroides* sp. and *Eubacterium* sp. are stimulated by the increased concentrations of phytochemical metabolites[121,123]. As different polyphenols affect different bacteria, their effect on the gut microbiota composition is variable. The ability of phytochemicals to suppress Gram-positive bacteria could be due to the ability of the metabolites to bind to membranes of specific bacteria and trigger the release of hydrogen peroxide, which induces cell-wall breakdown and lysis[124,125].

A wealth of experimental studies (reviewed elsewhere[120,121,126]) have shown that polyphenols, particularly those derived from grapes, tea, coffee and cocoa or chocolate, have powerful anti-inflammatory, antiproliferative and tumour-inhibiting properties in the colon. Mechanistic studies have suggested that these actions are mediated by modification of eicosanoid synthesis, downregulation of the inflammatory cascade (COX-2, NF-κB, AP-1, TNF, IL-6 and VEGF), regulation of DNA synthesis, and induction of luminal detoxifying enzymes (β-glucuronidase, β-glucosidase, β-galactosidase, mucinase and nitroreductase). In this way, phytochemicals complement many of the actions of SCFAs illustrated in FIG. 3, strengthening support for a balanced diet containing high-fibre fruits and vegetables.

This synergism explains why the effects of total saccharolytic fermentation products on cell proliferation were greater than that due to SCFAs alone[16,120]. Unfortunately, because of practical difficulties in measuring mucosal levels of phytonutrients in humans, there are few physiological studies on the relative effects of phytonutrients on mucosal metabolism and cancer risk. Estimates that have been obtained are generally based on metabolites in urine[124]. For example, a randomized controlled trial of dates (seven per day for 3 weeks), a rich source of fibre and polyphenols, showed a reduction in the genotoxicity of faecal water in normal healthy individuals in the absence of changes in specific faecal microbiota (including bifidobacteria and high butyrate-producers) and in SCFAs[127]. This finding suggests that either the observed increase in gut transit masked SCFA changes, or that polyphenols were more responsible for the antineoplastic changes. Unfortunately, no biological measurements of polyphenol metabolism were made and so the mechanism remains unexplained.

In the AOM-DSS rat model of colitis-associated colorectal cancer, the protective effects of resistant starch on adenoma and carcinoma formation were not seen when a high-polyphenol-containing green tea supplement (main component epigallocatechin-3-gallate) was added to the diet instead of resistant starch[22]. The dose of green tea given was estimated to be equivalent to 3–4 cups per day but, again, no biological measurements were made. In cell cultures, the inhibitory effects of polyphenols have been well demonstrated. For example, the ability of polyphenols and their metabolites, namely ellagitannin metabolites, at concentrations found in the colon following the consumption of pomegranates and walnuts, were shown to have strong antiproliferative effects in cancer cell lines. Using Caco-2 cells and primary tumour cells from a patient with colon cancer, mixtures of ellagitannin metabolites, ellagic acid and gut-microbiota-derived urolithins reduced the number and size of colonospheres and aldehyde dehydrogenase activity, indicating the potential for phytochemicals to control colon cancer chemoresistance and relapse[128]. This finding followed earlier studies reporting that urolithin metabolites were anti-inflammatory in human colonic fibroblasts[129] and strongly antiproliferative in human colorectal cancer (Caco-2) and normal (CCD18-Co) cell lines[130].

The antineoplastic potential of black raspberries has been studied for many years[131,132], identifying anthocyanins (cyanidin-3-O-glucoside, cyanidin-3-*O*-rutinoside and cyanidin 3-*O*-2G-xylosylrutinoside) as the most potent anticarcinogenic constituents, whereas ellagitannins have been identified as the key components of tumour suppression in red raspberries. These studies have shown that extracts suppressed colon tumour formation by

up to 70% in the AOM rat cancer model. Studies in tumorigenic cell lines suggest that the mechanism involves the inhibition of proliferation, induction of apoptosis and the modulation of the expression of COX-2 and inducible nitric oxide synthase[132]. Randomized controlled trials of black raspberries are now being performed in patients with a high risk of colon and oesophageal cancer. Preliminary findings of a phase 1b controlled clinical trial in 14 patients with familial adenomatous polyposis have been published, showing that supplementation of the diet for 9 months with 100 g black raspberry powder significantly (P <0.05) reduced the polyp burden and decreased staining of the mucosal proliferation biomarker Ki-67, compared with seven patients given placebo[131].

Often the results of food studies on health produce disappointing results that cause confusion and dismay among the public, such as the effects of saturated fat and red meat on colorectal cancer and heart disease. Thus, it comes as a pleasant surprise to hear of the potential benefits of coffee and red wine on cancer and health. In a randomized crossover study on the effects of red wine (272 ml per day), nonalcoholic red wine (273 ml per day), or gin (100 ml per day) performed for 4 weeks, the microbiota, polyphenol and blood lipid responses in healthy volunteers were examined. Both red wine and nonalcoholic red wine significantly (P <0.05) increased the number of *Enterococcus, Prevotella, Bacteroides, Bifidobacterium, Bacteroides uniformis, Eggerthella lenta,* and *Blautia coccoides-Eubacterium rectale* groups, whereas gin had no effect[133]. Evidence that the changes were a consequence of increased polyphenol metabolism was provided by the observation that resveratrol metabolites derived from phase II metabolism were significantly (P <0.05) increased in 24 h urine collections after both red wines, compared with baseline and after gin intake. Furthermore, the concentration of dihydroresveratrol produced by the intestinal microbiota was also significantly (P <0.05) increased in the urine. Unfortunately, colonic saccharolytic fermentation products were not measured, nor were mucosal biomarkers of cancer risk. However, potential health benefits were shown as the microbiota changes were accompanied by reductions in levels of systolic and diastolic blood pressures, plasma triglyceride, total cholesterol, high-density lipoprotein cholesterol and C-reactive protein.

Although conflicting reports exist[134,135], studies report an inverse association between coffee consumption and colorectal cancer, with high coffee consumers, in particular, showing reduced risk. For example, in a study from Japan with 463 cases of colon cancer and 340 cases of rectal cancer, the multivariate-adjusted odds ratio of colon cancer was found to significantly (P trend=0.02) decrease with increasing intakes of coffee polyphenols[136]. The association with distal colon cancer was strongest, and no statistically significant association with tea consumption was seen. A further study from the Middle East in 5,145 colorectal cancer cases and 4,097 healthy controls also showed an inversely associated risk of colorectal cancer with coffee consumption in a dose-dependent manner[137]. Compared with <1 serving of coffee per day, an intake of 1 to >2.5 servings per day was associated with significantly (P trend <0.001) lower odds of colorectal cancer. The dose-response trend was significant for both colon and rectal cancers. In a case-controlled study of 251 colorectal cancer cases and 247 cancer-free matched individuals as controls from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, an untargeted metabolomic analysis of serum was performed and integrated with dietary analysis and colorectal cancer development[138]. The researchers identified three caffeine-related metabolites —

theophylline, caffeine and paraxanthine — that were significantly associated with the absence of colorectal cancer development. Evidence also suggests that colon cancer progression in humans can be retarded by high coffee consumption[135].

In summary, strong supportive evidence suggests that colorectal cancer suppression induced by a diet high in fruits, vegetables and grains is a consequence of, not only the high fibre content, but also the polyphenol content. This finding argues for the use of whole-foods rather than fibre supplements in the campaign to reduce the excessive rates of colorectal cancer in westernized societies.

Fat-stimulated metabolites

Epidemiological studies have shown that the relationship between dietary fat intake and colorectal cancer is not as strong as it is for meat and fibre. This finding might be explained by the weakness of questionnaires being able to segregate the effects of meat and energy from fat, as a high-meat diet is usually accompanied by a high intake of saturated fat. For example, in the Nurses' Health Study, animal fat intake was positively associated with colon cancer incidence in women, although fat intake was not independent of total energy intake in the study[139]. A further explanation might involve the modulating effects of luminal metabolites derived from calcium and fibre[140]. Further confusion has arisen from experimental findings showing that saturated fat protected against colon cancer development in the AOM mouse model, which was attributed to the ability of saturated fat to stimulate mucin synthesis, thereby increasing intestinal barrier integrity[141]. In an attempt to overcome the weaknesses of dietary recall, the relationship between colorectal cancer occurrence and blood biomarkers of fat intake, namely plasma phospholipid fatty acids, was examined in 4,205 high-risk Australian participants, which included 395 colorectal cancer cases[142]. The results showed no association between dietary fat intake by questionnaire and colorectal cancer, but there was a significant (P <0.005) association between saturated plasma phospholipid fatty acid measurements and a protective effect of n-3 plasma phospholipid fatty acids[142]. Mechanistically, a high-fat diet might increase colorectal cancer risk, both directly through its effect on inflammation[143], stem cell regulation[144] and prostanoid metabolism[145], and indirectly through its effects on the gut microbiota[43].

Another indirect effect on colorectal cancer comes from the stimulatory effect of hepatic bile acid synthesis by a high-fat diet. This process results in greater quantities of bile acids escaping the enterohepatic circulation and entering the colon, in which they expand the population of microorganisms responsible for their conversion to secondary bile acids, namely those containing the bile acid deconjugating enzyme, bile acid 7α-dehydratase (primarily Clostridia species)[146]. Strong experimental evidence suggests that the secondary bile acids lithocholic and deoxycholic acid are carcinogenic to the colon[147]. In a study of18 wild-type mice, the addition of 0.2% deoxycholic acid for 8–10 months to their diet induced colonic tumours in 17 mice, and cancer in 10. Furthermore, the addition of an antioxidant prevented tumour formation, again suggesting that a balanced diet containing fat and antioxidant phytochemicals from fruits and vegetables can prevent the inflammatory effects of procarcinogens[147]. Evidence also indicates that bile acids might be carcinogenic to the oesophagus in Barrett oesophagus[148], and to the gallbladder where chronic bacterial

infection might promote the formation of secondary salts[149]. In the same way, substantial experimental evidence suggests that the carcinogenic potential of meat and fat-stimulated metabolites are potentiated by colonic butyrate deficiency[115–119,147,150,151].

The evidence that fat directly affects gut microbiota composition and function in humans is fairly weak, and evidence that patients with steatorrhoea due to malabsorption or pancreatic insufficiency suffer an increased rate of colorectal cancer is lacking. A study in rats has shown that a change from a low-fat diet to a high-fat diet changes the microbiota composition (measured broadly by PCR) with an increase in the ratio of Firmicutes to Bacteriodetes[152], but this finding could equally have been a consequence of the associated reduction of carbohydrate intake to maintain isocaloric conditions. Similarly, another study showed that a high-fat diet substantially changed the microbiota measured by terminal restriction fragment-length polymorphism in mice, with a decrease in the order Lactobacillales and an increase in the *Clostridium* subcluster XIVa[153], but again this result could be a consequence of reduced carbohydrate intake. What is intriguing about these two studies is that in the first, supplementation of the high-fat diet with polyphenols from berries, and in the second, supplementation with a fibre source (agaro-oligosaccharides) suppressed the gut microbiota changes. Furthermore, fibre supplementation suppressed the development of AOM-generated aberrant crypt foci in the colon of mice treated with a high-fat diet, thereby inhibiting colon carcinogenesis[153]. These studies emphasize the fact that colorectal cancer risk is determined by dietary balance, and that the conclusions of dietary studies must take into consideration the modifying effects of luminal metabolism.

Finally, a study in a mouse model deficient in IL-10 showed that a diet high in saturated fat cultivated a blossom of *Bilophila wadsworthia* in the colon, which released hydrogen sulfide from taurine-containing bile acids, leading to acute inflammation that might predispose to neoplastic change[75]. Although hydrogen sulfide has been shown to be genotoxic in experimental cell lines[76], the study in IL-10 deficient mice, in which only saturated fat and not polyunsaturated fat stimulated the growth of *B. wadsworthia,* suggests that all fats might not be equal.

Good dietary oils

Notably, not all fats are the same: it is not only the quantity of fat that is important, but also its composition. For example, in a study in mice[154], a diet high in fish oil had no appreciable stimulatory effect on colonic 7α-dehydroxylating enzyme content, or the excretion of secondary bile acids. The balance between n-6 and n-3 fatty acid intake might be important, as exemplified by the Australian study that showed that plasma phospholipid saturated fatty acid concentrations were associated with high cancer risk, whereas high n-3 polyunsaturated fatty acid levels were associated with low risk[142]. Despite progressive westernization, n-3 fatty acid content in red blood cells continues to be considerably higher in Alaska Native people than in western societies, resulting in a ratio of n-6:n-3 fatty acids of approximately 1:1, compared with 10–30:1 in US white people[155,156]. Fish oils are a rich source of n-3 fatty acids, which have their own anti-inflammatory and antiproliferative properties[157–159], in contrast to the pro-inflammatory and tumorigenic n-6 fatty acids that predominate in the western diet[156]. Thus, Alaska Natives should be protected from colorectal cancer by their

high consumption of fish oil, and yet they have the highest reported rate in the world[2,160]. Interestingly, all Arctic native people are reported to have high rates of colorectal cancer[160]. The explanation for this finding is currently unknown, but might be related to their extremely low intakes of fibre and phytochemical-rich foods. This interpretation raises the intriguing possibility that dietary intervention in Alaska Native people aimed at increasing butyrogenesis and polyphenol intake will have profound antineoplastic effects and obliterate their excessive rate of colorectal cancer deaths. Indeed, experimental evidence shows that when provided together, butyrate and n-3 fatty acids have synergistic antineoplastic effects[161,162].

Obesity

Obesity (BMI >30 kg/m^2) is becoming increasingly prevalent in the USA; one survey found that more than one-third (34.9%) of adults were obese in 2011–2012 (REF 163). Obesity increases the risk of most cancers, including colorectal cancer, possibly through the generation of a chronic inflammatory state and the associated hormonal dysregulation, including hyperinsulinaemia, insulin resistance, and raised leptin and oestrogen levels, which all might contribute to a hyperproliferative state in the colonic mucosa[164,165]. Obesity is also commonly associated with changes in gut microbiota composition, but the reported changes are inconsistent and are probably more likely to reflect the composition of the diet[166]. In Gordon's studies on distal gut microbiota of genetically obese mice and their lean littermates, as well as those of obese and lean human volunteers, they showed that the microbiome associated with obesity had an increased capacity to harvest energy from the diet[167]. The obese microbiome was enriched with functional groups involved in starch degradation and butyrate production, and faecal content of fermentation products was increased. Remarkably, when they transplanted the microbiome from obese mice to germfree mice they found a significantly (P <0.05) greater increase in total body fat than colonization with the microbiota from lean mice despite isocaloric feeding. However, others have reported different changes in the ratios of Firmicutes to *Bacteriodes* in humans with obesity[168] and the dream of being able to change the risk of obesity by changing the gut microbiota has not yet been realized: the situation is clearly far more complex as reviewed elsewhere[168,169].

Evidence suggests that the high-fat content of the obesogenic diet drives carcinogenesis. For example, in a study comparing African Americans and rural South Africans, obesity in South Africans was associated with high intakes of carbohydrate and not fat, in tandem with low colonic bile acids, low biomarkers of colon cancer risk, and low colon cancer rates in the population[170]. Furthermore, a study in a mouse model genetically susceptible to intestinal tumorigenesis demonstrated that a high-fat diet promoted tumour progression independently of obesity[43].

How can colon cancer be prevented?

Fibre supplementation

Owing to the strong experimental evidence that butyrate suppresses colorectal neoplasia, multiple clinical trials have been conducted to determine whether fibre supplementation

could reduce the risk of colorectal polyp recurrences. Unfortunately, few studies were positive[171], most likely because the level of fibre supplementation achieved was insufficient. For example, the most-quoted Polyp Prevention Trial, in which 2,079 participants with a previous history of colorectal polyps were randomly assigned to an advised low-fat, high-fibre diet, found no overall difference in polyp recurrence after 4 years and 8 years compared with a control group given a brochure on healthy eating and assigned to follow their usual diet[172]. However, the high-fibre group were estimated to have consumed an average of only 32 g per day, which might have been further reduced as compliance was low, indicated by the change in biomarkers of fat and green vegetable intake (blood cholesterol and vitamin A, respectively).

What is the threshold intake of fibre?

We now have strong human and experimental evidence that there is a threshold level for fibre consumption, above which the level of butyrogenesis is sufficient to overcome the inflammatory, neoplastic and carcinogenic potential of nitrogenous microbial metabolites (such as ammonia and nitrosamines) and environmental carcinogens (such as polycyclic aromatic hydrocarbons and heterocyclic amines). First, the traditional African diet identified to be protective from colon cancer contained >50 g fibre per day[173]. Second, increasing the fibre consumption in African Americans at high risk of colorectal cancer to 55 g per day was associated with a substantial decrease in colonic mucosal biomarkers of cancer risk (epithelial proliferation) within 2 weeks of change (discussed in the next section)[50]. Third, in the Polyp Prevention Trial, a significantly reduced odds ratio for advanced (>1 cm, >25% villous, or high-grade dysplasia) adenomatous polyp recurrence was found in the subgroup consuming the highest quartile of high fibre beans[174]. These observations suggest that the required fibre consumption level should be >50 g per day and that the recommended intake level in the USA of 25 g per day for women and 38 g per day for men, which was based on the level required to maintain cardiovascular health, needs revising[175,176]. Fibre recommendations are similar in the UK, Europe, Australia and New Zealand[177–179].

Changing diet to change the microbiota

A series of studies have been performed over the past 20 years to identify the dramatic difference in colorectal cancer prevalence between African Americans (65 cases per 100,000) and native Africans (<5 cases per 100,000)[49–51,180–186]. One study provided evidence that the fibre and fat content of the diet were chiefly responsible, and that the effect of these diets on biomarkers of cancer risk were mediated by the microbial metabolites[50]. This study was unique in that it was tightly controlled and what the participants consumed and returned was examined under observation for the total duration of the 2-week in-house dietary intervention[50]. Diets were switched such that the healthy middle-aged African Americans increased their fibre intake to 55 g per day and reduced their fat intake to 51 g per day, while the rural Africans reduced their fibre intake to 7 g per day and increased fat intake to 134 g per day. Remarkably, epithelial proliferative rates in colonic mucosal biopsies taken from African Americans decreased to levels lower than those of rural Africans at baseline, whereas rates increased in rural Africans to levels greater than the African Americans at baseline[50]. Inflammatory biomarkers followed a similar pattern, with a decrease in $CD3^+$ intraepithelial lymphocytes and $CD68^+$ lamina propria macrophages in

African Americans and an increase in rural Africans. These changes were accompanied by substantial reciprocal changes in the gut microbiota and metabolome, and specifically in microbial butyrate and secondary bile acid production, supporting the hypothesis that mucosal biomarkers of cancer risk are modified by the diet through the microbiota. Reciprocal changes in urinary metabolites derived from microbial metabolism of green vegetables were also observed following the diet switch, suggesting that some of the reduction in mucosal proliferation in African Americans could also have been related to the effects of increased phytochemical consumption in the Africanized diets.

A further fascinating finding was that the mucosal biopsy samples from all the rural Africans at baseline showed intense lymphocytic infiltration consistent with lymphocytic colitis, (associated with parasitic infections in some) despite the absence of any gastrointestinal symptoms[50]. That this finding has not led to an increase in colon cancer within the community is surprising considering the strong link between chronic inflammation and neoplasia[187]. One can speculate that the high rate of butyrogenesis arrests neoplastic progression which, if true, implies that the inevitable progressive global westernization and associated reduced consumption of fibre-rich foods will lead to an explosion in colorectal cancer incidence.

Conclusions

Evidence based on controlled epidemiological, cell culture, animal and human studies has been presented to support the view that the high incidence of colorectal cancer in westernized countries is a consequence of dietary imbalance, principally a deficiency of high-fibre fruits, vegetables and whole grains. Evidence has been presented that the evolution of man has been accompanied by high complex carbohydrate feeding patterns, which have a profound effect on the colonic microbiota and their ability to produce metabolites that support colonic health and prevent carcinogenesis. Industrialization, westernization and food refinement has resulted in disturbance of the homeostatic role of our gut microbiota due to 'starvation' of the microbiota, impaired butyrogenesis and loss of host defense against environmental carcinogens. National guidelines for fibre requirements have not been based on robust scientific studies. Such studies are urgently needed to define the ideal metabolic requirements of the gut microbiota, which, when met, could result in a suppression of colorectal cancer risk in westernized communities to that of rural Africa.

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Key points

- Colorectal cancer is a so-called westernized disease and the second leading cause of cancer death worldwide; approximately half of those with the disease will die from it
- Geographical variation, migration studies and experimental studies provide compelling evidence that environmental factors, rather than genetic dysfunction, are responsible for the development of colorectal cancer
- Convincing evidence suggests that risk of colon cancer is increased by processed and unprocessed meat consumption but suppressed by fibre-rich foods
- Dietary risk is mediated by the colonic microbiota; carbohydrate residues stimulate production of metabolites that maintain mucosal health, proteinaceous residues and fat-stimulated bile acids might result in pro-inflammatory and carcinogenic metabolites
- A moderate intake of meat and fat is part of our omnivorous diet and the carcinogenic potential can be suppressed by the induction of butyrogenesis from fibre-rich foods
- Current dietary fibre recommendations need to be reviewed as they are based on the maintenance of cardiovascular health and are below the levels associated with low colon cancer risk

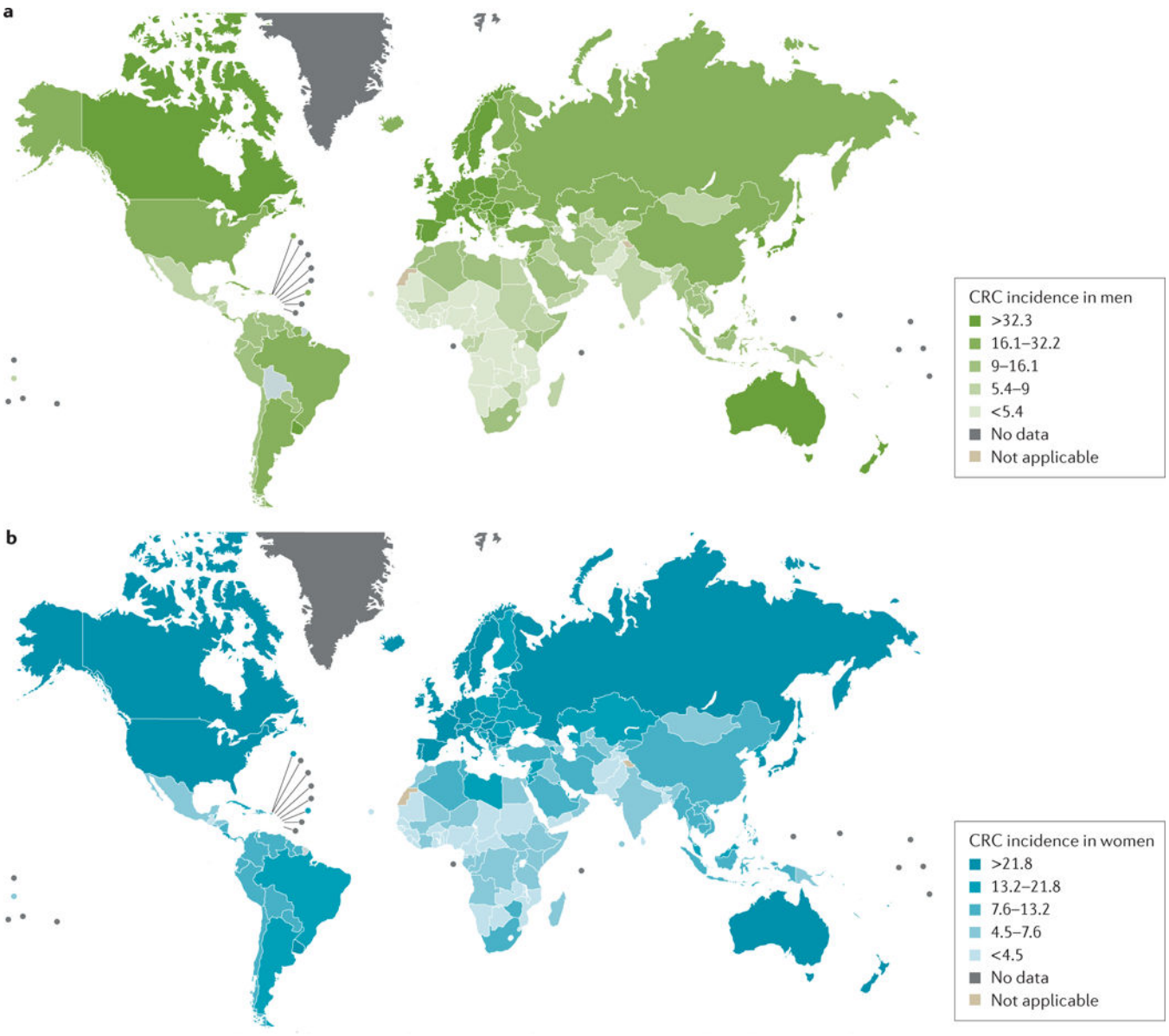

Figure 1 |. Geographical variation in colorectal cancer rates.

Data from the WHO[1] demonstrating the high incidence of colorectal cancer (CRC) in westernized countries in **a** | men and **b** | women. Reprinted from GLOBOCAN 2012, v1.0, Ferlay, J. et al., Cancer incidence and mortality worldwide: International Agency for Research on Cancer CancerBase No. 11 http://globocan.iarc.fr/Pages/factsheetscancer.aspx?cancer=colorectal (2013).

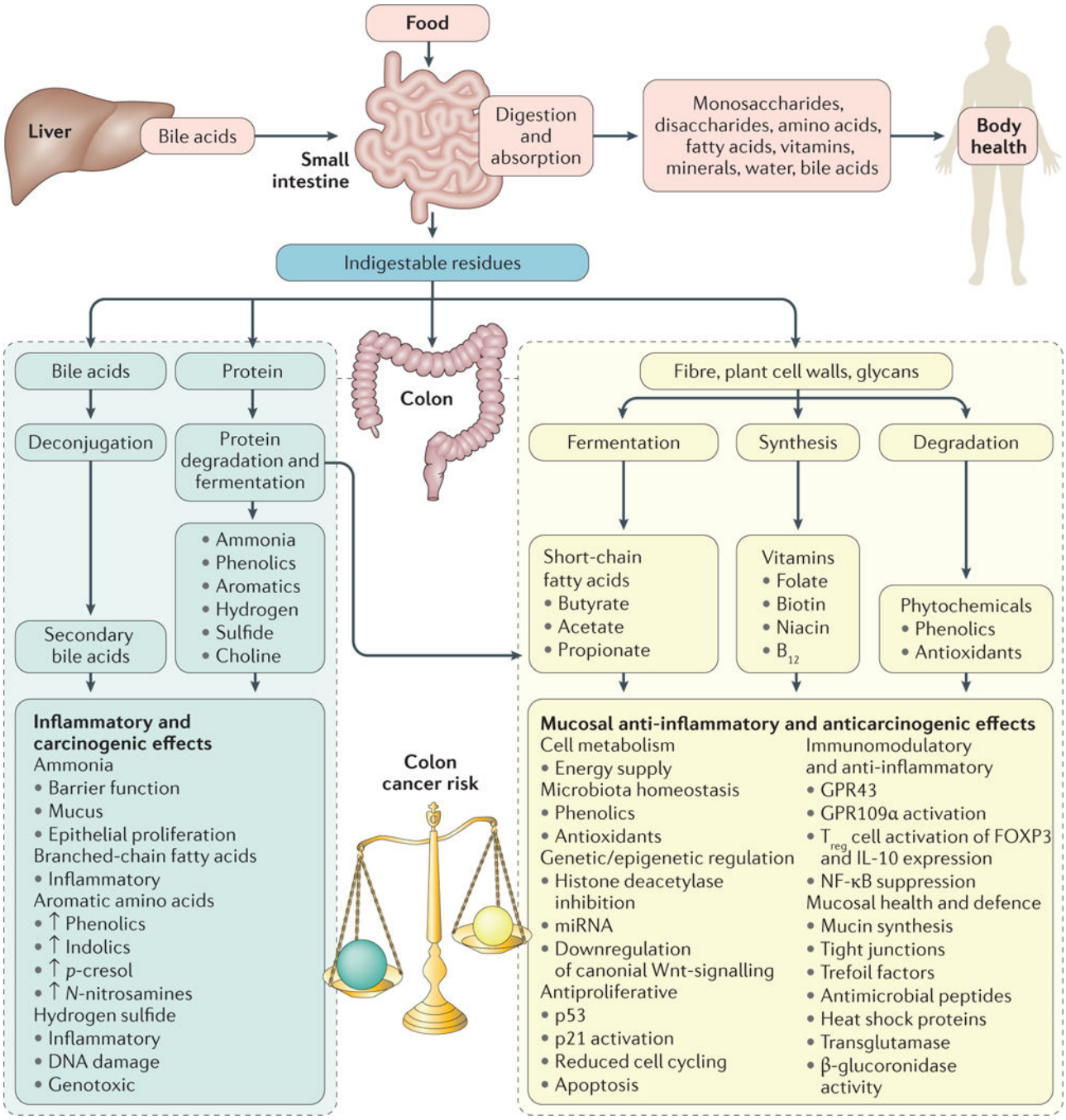

Figure 2 |. Importance of dietary residues and the colonic microbiota in determining colon cancer risk.

In health, >90% of a normal diet is absorbed in the small intestine and nutrients are distributed to maintain general body health. Residues entering the colon are chiefly complex carbohydrates (fibre), but also contain protein residues and primary bile acids secreted by the liver in response to fat ingestion. These residues have a critical role in the maintenance of colonic health as they determine the composition and metabolic activity of the colonic microbiota which, through fermentation, maintain mucosal and colonic health. With a balanced diet, saccharolytic fermentation of carbohydrates is dominant, producing short-

chain fatty acids, particularly butyrate, which is the preferred energy source for colonocytes and has anti-inflammatory and antineoplastic properties through actions shown. With an imbalanced high-fat, high-meat, low-fibre diet, the proinflammatory and proneoplastic properties of protein fermentation and bile acid deconjugated residues predominate, leading to increased colon cancer risk.

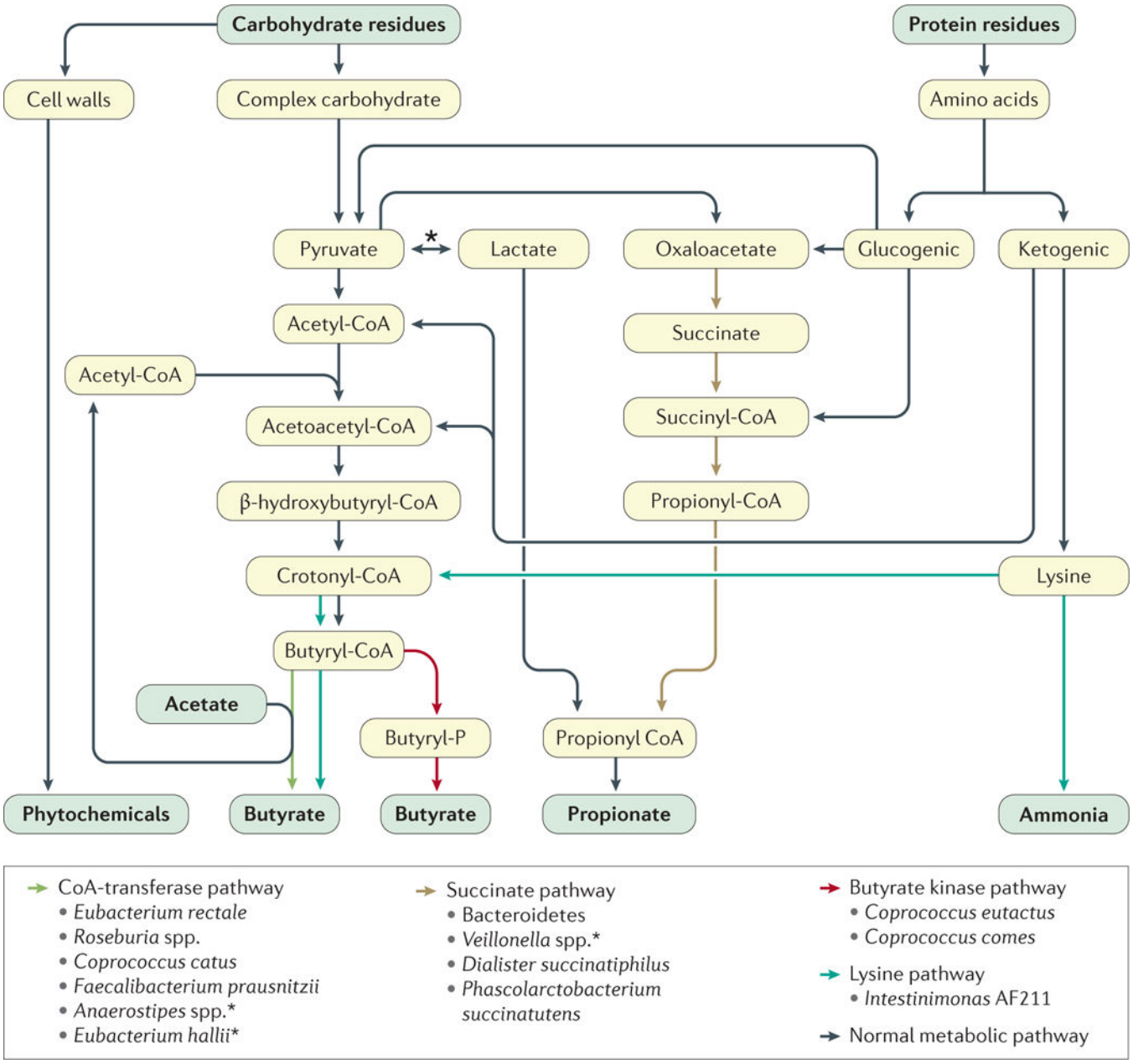

Figure 3 |. Key metabolic pathways of the colonic microbiota.

Through the process of fermentation, these metabolic pathways culminate with the production and release of the major short-chain fatty acids (acetate, propionate and butyrate) and phytochemicals from plant cell walls[56]. Note that there are two microbial enzymes that are responsible for the final synthesis of butyrate, namely butyryl-CoA transferase (dominant, formed by a variety of genera and species) and butyrate kinase (favoured in proteolytic fermentation). Also shown is the pathway for converting the amino acid lysine into butyrate, which also generates ammonia[69]. The main propionate production pathway is the succinate pathway. Bacterial species found to use certain pathways are shown but are not exhaustive. The asterix indicates species shown to use lactate to form butyrate[56].