

REVIEW

Three Measurable and Modifiable Enteric Microbial Biotransformations Relevant to Cancer Prevention and Treatment

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ABSTRACT

Interdisciplinary scientific evaluation of the human microbiota has identified three enteric microbial biotransformations of particular relevance for human health and well-being, especially cancer. Two biotransformations are counterproductive; one is productive. First, selective bacteria can reverse beneficial hepatic hydroxylation to produce toxic secondary bile acids, especially deoxycholic acid. Second, numerous bacterial species can reverse hepatic detoxification—in a sense, retoxify hormones and xenobiotics—by deglucuronidation. Third, numerous enteric bacteria can effect a very positive biotransformation through the production of butyrate, a small chain fatty acid with anti-cancer activity. Each biotransformation is addressed in sequence for its relevance in representative gastrointestinal and extra-intestinal cancers. This is not a complete review of their connection with every type of cancer. The intent is to introduce the reader to clinically relevant microbial biochemistry plus the emerging evidence that links these to both carcinogenesis and treatment. Included is the evidence base to guide counseling for potentially helpful dietary adjustments.

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Disclosure

The author completed the ICMJE Form for Disclosure of Potential Conflicts of Interest and had no conflicts to disclose.

INTRODUCTION

As far back as the 1890s, a connection between cancer and bacteria was noted with the successful treatment of inoperable sarcomas by injections of bacteria.¹ Since then, the most widely understood link between cancer and bacteria has been the induction of gastric MALT (mucosa-associated lymphoid tissue) lymphoma by the bacterial pathogen *Helicobacter pylori*. This microbe has been officially recognized as a carcinogen. And, surprisingly, successfully treating the gastric lymphoma means first successfully treating the bacteria.

But the relationship between gastric cancer and *H pylori* is much more complex than the easily understood 1:1 causality of a given carcinogen with a given illness or a given pathogen with a given illness. For example, we now know that *H pylori* alone is not enough to induce stomach cancer. Promotion requires the presence of a complex microbiota. Mice with just *H pylori* develop fewer tumors than regular mice.² Moreover, the presence of *H pylori* infection lowers the risk of esophageal cancer.^{3,4} These observations emphasize the need to move from a specific pathogen/infection model to an ecological model of the microbiota as a system.

The importance of an ecological approach has certainly been suggested by studies on germ-free mice that document the microbiota have tumor-promoting capacity in multiple carcinogenesis models both directly (eg, colon)⁵ and indirectly (eg, liver).⁶ And, likewise, in regular mice, treatment with antibiotics to eliminate bacteria can reduce the development of colon⁷ and liver cancers.⁸

In late 2013, the microbiota-cancer link was firmly established by several rigorous studies that addressed both prevention and treatment. First came documenta-

tion of a causal link between dysbiosis of the intestinal microbiota and colon tumorigenesis.⁹ Next came two reports that documented how the microbiota can alter a patient's response to chemotherapeutic agents.^{10,11}

For clinicians, translating these basic science insights and breakthroughs to everyday practice may seem impractical. After all, few have the technology to document the complexity of a given patient's intestinal microbiota. However, all clinicians and researchers do have access to three accessible, measurable, and modifiable products of the microbiota.

For this reason, this review focuses on the three microbial biotransformations readily measurable in stool samples: deoxycholic acid (DCA), beta-glucuronidase, and butyrate. Each is addressed in sequence for its relevance in selected gastrointestinal and extra-intestinal cancers. This is not a complete review of their connection with every type of cancer. The intent is to introduce the reader to clinically relevant microbial biochemistry plus the emerging evidence that links these to both carcinogenesis and treatment. Included is the evidence base to guide counseling for potentially helpful dietary adjustments.

1. FIRST MICROBIAL BIOTRANSFORMATION

Microbial Enzyme: 7- α -dehydroxylase

Microbial Biotransformation: Dehydroxylation

Functional Result: Production of toxic secondary bile acids

Background

Bile acids and bile salts are best known as the highly effective detergents necessary for the fat solubiliza-

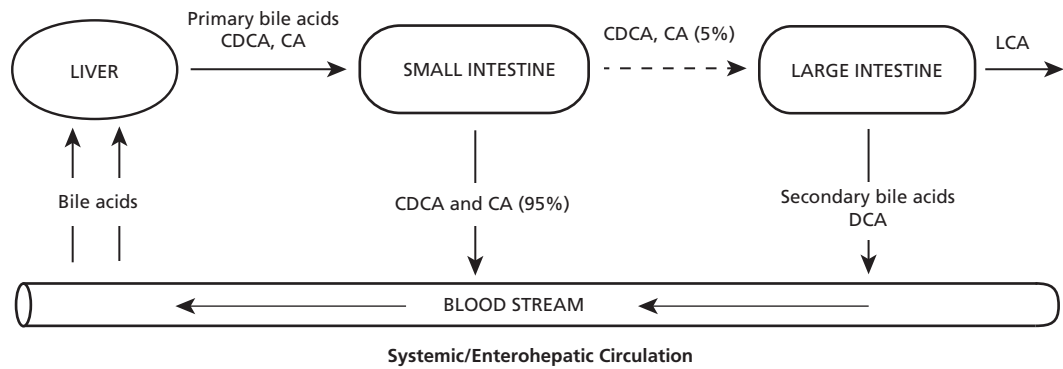


Figure 1 The primary bile acids chenodeoxycholic acid (CDCA) and cholic acid (CA) are produced in the liver, stored in the gallbladder, and, when prompted, discharged into the small intestine. These support the digestion of fats, and 95% are reabsorbed in the distal ileum and returned to the liver via the enterohepatic circulation. These can also circulate to the entire body. Approximately 5% pass on to the large intestine, where they may be transformed into the potential toxins deoxycholic acid (DCA) and lithocholic acid (LCA). DCA is subject to uptake, systemic circulation, and return to the liver where it can be concentrated and stored in the gall bladder.

tion and emulsification of dietary lipid and lipid-soluble vitamin absorption throughout the small intestine.

Each day, approximately 500 mg of cholesterol undergoes hydroxylation as well as oxidation of the sterol side chain to become a bile acid. The two primary bile acids produced in the liver are cholic acid (CA) and chenodeoxycholic acid (CDCA). These are conjugated to glycine or taurine, ionized into amphipathic salts, secreted actively, and carried in the bile to the gallbladder for concentration and storage (Figure 1).¹²

With meals, bile acids are released from the gallbladder into the duodenum and flow to the terminal ileum, where they are absorbed by passive diffusion and transported back to the liver via the portal vein. They are then taken up by the liver and re-exported into the bile. This enterohepatic circulation from liver to intestine and back occurs in 4 to 12 cycles per day for each bile acid molecule.¹³

A very small percentage of bile salts are not absorbed from the small intestine and instead enter the large intestine where approximately 0.0001% of all colonic bacteria have the capacity to reverse the hepatocyte synthesis that produced the bile acids.¹⁴ Enteric bacteria first deconjugate the bile salts and then, if the correct species and strains are present, dehydroxylate them. The result is not cholesterol but what are termed secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA). DCA, but not LCA, can be reabsorbed through passive non-ionic diffusion across the colonic epithelium. DCA, like CA and CDCA, participates in the enterohepatic circulation from intestines back to the liver. This accounts for the approximately 25-fold difference in concentration of DCA and LCA in the gallbladder.¹⁵

Surprisingly, bile acids are also more than detergents. High concentrations of the secondary bile acid DCA have been linked to several cancers. DCA does not appear to be directly toxic but instead is a promoter of carcinogenesis.^{16,17} This is likely due to its role as a signaling molecule related to the control of lipid, bile acid, and carbohydrate metabolism.¹⁸

DCA can activate multiple cell signaling pathways

related to carcinogenesis including protein kinase C, ERK1/2 via the epidermal growth factor receptor (EGFR), beta-catenin, Jun-N-terminal kinase 1 and 2 (JNK 1/2), and p38 MAPK.^{15,19} DCA is closely linked to inflammatory pathways because NF-kappa B DNA binding activity and subsequent pro-inflammatory cytokine transcription, occurs only in the presence of a dissociating agent such as DCA.²⁰

Bile acid activated receptors are found not only in epithelial cells in the enterohepatic system but also in multiple extra-intestinal sites including the breast, the adrenal glands, and immune cells. These receptors are comprised of the G-protein-coupled receptor TGR5 (GP-BAR1, G-protein-coupled bile acid receptor) as well as the superfamily of nuclear receptors including the farnesoid-X-receptor (FXR), the constitutive androstane receptor (CAR), the pregnane-x-receptor (PXR) and the vitamin D receptor (VDR). These nuclear receptors regulate the cell cycle, mitosis, proliferation, and apoptosis.

Of all these bile acid receptors, FXR is the ligand-activated transcription factor responsible for bile acid and triglyceride synthesis, bile acid uptake and export, plus bile acid conjugation and detoxification.²¹ Additionally, FXR appears to play a significant role in many cancers. It has high affinity for the primary bile acids CA and CDCA as well as the major secondary bile acids DCA and LCA.²² Four isoforms exist so tissue-to-tissue variability in function may exist for primary and secondary bile acids. FXR appears to be activated by unconjugated bile acids.²³

Pertinent Microbial Biotransformation

Bile salts are synthesized from cholesterol in hepatocytes via cholesterol-7 α -hydroxylase (CYP 7A1). The primary bile acids produced, CA and CDCA, after deconjugation are then 7 α -dehydroxylated in the large intestine by enteric bacteria to form the secondary bile acids DCA and LCA. This biotransformation occurs only with intestinal bacteria. This is inhibited at low colonic pH associated with the fermentation of resistant starches.²⁴⁻²⁶

Responsible Microbiota Bacteria

Secondary bile acids are produced by large intestine anaerobic bacteria from the genus *Clostridium*, specifically clostridial cluster XIVa. These are gram-positive, spore-forming anaerobes that are members of the phylum Firmicutes.²⁷ Only members of this cluster with the bai operon can produce these secondary bile acids.¹⁵

Laboratory Measurements

Fecal dexoycholic acid (DCA).

Representative Consequences for Cancer

Esophageal and Gastroesophageal Cancers

Despite the widespread use of proton pump inhibitors (PPIs), and despite the rapid decline in the prevalence of *Helicobacter pylori*, the incidence of both esophageal and gastroesophageal cancers has increased at a dramatically greater rate than for any other cancer.

One under-recognized factor in these cancers is the role of unconjugated bile acids including DCA from gastroduodenal reflux. Bile acids are not expected to be found at the gastro-esophageal junction, but their presence has been clearly documented and correlates with the degree of pathology seen in the progression from Barrett's esophagus (BE) to esophageal adenocarcinoma.²⁸ In patients with both GERD and BE, refluxed fluids show high concentrations of DCA.²⁹ Use of acid-suppressing medications, such as PPIs, ironically can result in bacterial overgrowth in the stomach and small intestine with increased production of unconjugated secondary bile acids, particularly DCA.^{30,31} This is important because in biopsies or cell lines derived from such patients, *ex vivo* and *in vitro* bile acid exposure induces expression of multiple inflammatory mediators, oxidative stress, and DNA damage.³² This is confirmed in *in vivo* animal models where mice fed a zinc-deficient diet supplemented with DCA demonstrate increased oxidative stress and development of BE-like pathologic changes.³³

The bile acid receptor FXR is over expressed in Barrett's esophagus and esophageal adenocarcinoma. FXR mediates multiple bile acid-induced alterations in gene expression relevant to cancer cell growth.³⁴ Overexpression is associated with higher tumor grade, larger tumor size, and lymph node metastasis. Inhibition of FXR (by guggulsterone) induced apoptosis *in vitro* and reduced tumor formation and growth in nude mouse xenografts. This reduced viability of esophageal and cancer cells occurred in a time-dependent and dose-dependent manner.³⁵

Breast Cancer

Although breast tissue is not considered to be a bile acid target, the intestinal microbiota and secondary bile acids were recognized as potential agents in breast cancer as far back as 1971.³⁶ The reasoning is as follows. Extra-intestinal effects are possible when secondary bile acids produced in the large intestine are passively absorbed and circulate via the blood stream to other tis-

sues. Most surprisingly, intestinal bile acids are found in breast cyst fluids in concentrations up to 50 times that of the serum.³⁷ Human studies using labeled chenodeoxycholate administration prior to breast cyst aspiration demonstrated rapid uptake and concentration of intestinal bile acids into benign breast cysts.³⁸ However, with an unsupplemented diet, day-to-day variation is minimal.³⁹

Additionally, the bile acid receptor FXR is also found in normal breast ductal epithelial cells as well as breast cancer cell lines and tissue specimens.⁴⁰ The FXR plays several roles in breast cancer. The primary bile acid CDCA activates FXR for beneficial purposes including growth inhibition of MCF-7, MDA-MB-468 and tamoxifen-resistant breast cancer cells (MCF-7 TR1). Specifically, CDCA *in vitro* treatment significantly reduced epidermal growth factor (EGF)-induced growth and blocked HER2/MAPK signaling.⁴¹ Additionally, in breast cancer cell lines MCF-7 and MDA-MB-468, the FXR CDCA-like ligand GW4064 induced SHP, the atypical nuclear receptor that down-regulates genes by interacting with other nuclear receptors including the estrogen receptor to prevent gene transcription. In this case, the FXR bile acid receptor activation results in inhibited induction of aromatase.⁴⁰

In contrast to CDCA, the secondary bile acids DCA and LCA activate FXR in non-beneficial ways. In this case, FXR activation results in multiple pro-cancer effects relevant to breast health including: (1) estrogen-receptor activation,^{42,43} (2) promotion of cancer cell survival,⁴⁴ (3) induced migration of metastatic human breast cancer MDA-MB-231 cells⁴⁵ and (4) expression of drug resistance proteins.⁴⁶ This appears to be quite important. Post-menopausal women with newly diagnosed breast cancers have demonstrated mean serum levels of DCA that were 52% higher ($P=0.12$) than those of controls.⁴⁷

Possible Therapeutic Interventions: 7- α -dehydroxylase

1. Low-animal fat, low meat, low processed food diet. Western or standard American diets are associated with elevated serum levels of bile acids³⁹ and elevated fecal levels of the potentially toxic secondary bile acids DCA and LCA.^{48,49} Persons on a low animal fat or vegetarian diet require less primary bile acid production and demonstrate reduced concentrations of 7- α -dehydroxylating bacteria.^{7,8,50} Omnivorous diets are associated with increased, and vegetarian diets are associated with reduced, concentrations of clostridial cluster XIVa bacteria.^{51,52}
2. Resistant starch diet. Natural sources of resistant starches include cereal starches, legume starches, green banana and potatoes, raw, cooked and cooled, or as unmodified potato starch. Resistant starches have multiple health benefits including reduced colonic pH and decreased DCA and LCA production.
3. Discontinuation of acid suppressing medications. Use of acid suppressing medications are associated with a high prevalence of bacterial overgrowth

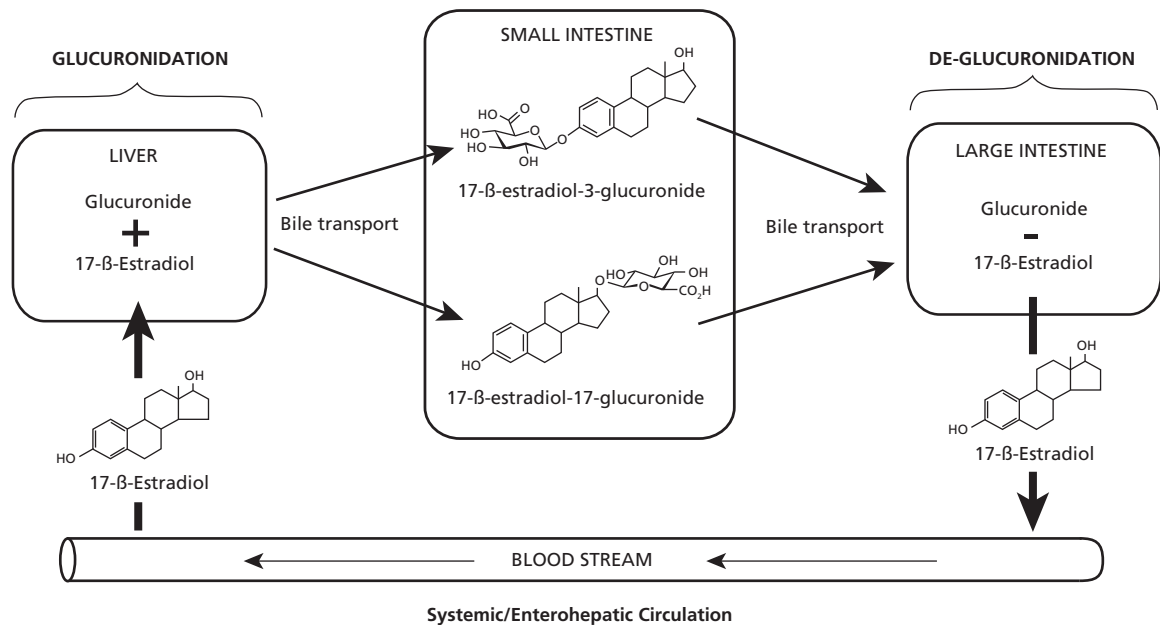


Figure 2 Hepatic glucuronidation of hormones and xenobiotics can be undone in the large intestine via bacterial beta-glucuronidase-mediated de-glucuronidation. The original products for disposal in the stool are then eligible for re-uptake and recirculation.

- and markedly increased amounts of unconjugated secondary bile acids.¹²
4. Curcumin/Turmeric in diet or by supplementation 500 mg per day. In one randomized study of 33 patients with Barrett's esophagitis, esophageal biopsies demonstrated *in vivo* increased apoptosis and reduced NF-kappa B activation. *In vitro* studies of tissues from these patients demonstrated that curcumin abrogated bile-driven effects.⁵³
 5. Z-guggulsterone (gugulipid). This is a plant sterol from the resin of *Cammiphora mukul* that is an effective antagonist of FXR that lowers cholesterol⁵⁴ and may have anti-esophageal cancer effects.^{13,14} and multiple anti-breast cancer effects including migration prevention and induced apoptosis^{55,56} The gugu plant has been used in the Ayurvedic healing tradition for states similar to metabolic syndrome as well as cancer.⁵⁷
 6. Ursodeoxycholic acid (UCDA) This secondary bile acid, sold as Ursodiol, may serve as an anti-dote to the toxic secondary bile acids DCA and LCA. UCDA prevents indomethacin-induced intestinal barrier dysfunction,⁵⁸ protects mitochondria against DCA-induced oxidative stress,⁵⁹ and attenuates chemically-induced colitis and colitis-associated adenocarcinoma and squamous cell carcinoma.⁶⁰
 7. Probiotic supplementation: Secondary bile acid production may be reduced by administration of lactobacilli and bifidobacteria probiotics. Specifically, several species can assimilate or accumulate the primary bile acid cholic acid.^{61,62} Less cholic acid can mean less deoxycholic acid. Likewise, increased cholic acid substrate supports increased population of clostridial cluster XIVa species and increased DCA production.^{63,64}

Precaution

Taurine supplementation: The amino acid taurine, after deconjugation from bile acids, is transformed in the colonic bacteria into hydrogen sulfide. This itself is a risk factor for both inflammatory bowel disease and colon cancer. Moreover, increased hydrogen sulfide production results in 7- α -dehydroxylation stimulation and increased DCA production.⁶⁵ No human taurine supplementation trial exists that assesses hydrogen sulfide and DCA production.

2. SECOND MICROBIAL BIOTRANSFORMATION

Microbial Enzyme: β -glucuronidase

Microbial Biotransformation: Deglucuronidation
Functional Result: Reversal of liver detoxification

Background

In the liver's phase II of detoxification, xenobiotic molecules such as drugs and pollutants as well as estrogens, androgens, bile acids, glucocorticoids, mineralocorticoids, retinoids, and fatty acid derivatives are made more hydrophilic by conjugation with glucuronic acid. This process, termed *glucuronidation*, allows excretion via the bile or urine (Figure 2). The class of responsible liver enzymes is termed *UDP-glucuronyl transferase*. The resulting molecules are termed *glucuronides*. Estrogens are metabolized primarily in the liver via conjugation, which includes glucuronidation.^{66,67} The resulting conjugated estrogens are not ligands for estrogen receptors and are excreted from the liver into the bile and later from the body in the stool.⁶⁸ However, in the large intestine, these conjugated estrogens can be subjected to deconjugation of the added glucuronic acid, a complete reversal by intestinal bacteria of the hepatic

detoxification.⁶⁹ These deconjugated estrogens can then be reabsorbed through the mucosa and re-enter the circulation via the portal vein.⁷⁰

Pertinent Microbial Biotransformation

Reversal or deconjugation of liver glucuronidation depends upon the presence of the bacterial enzyme beta-glucuronidase.⁷¹ This enzyme hydrolyzes β -D-glucuronides to glucuronic acid and an aglycone.

The intestinal microbiome can vary significantly in its capacity to deconjugate hormones such as estrogens and xenobiotics like chemotherapy drugs and heterocyclic amines. Altering the microbiome by administration of antibiotics results in significant increases in fecal progesterone and estriol metabolites.⁷²

Responsible Microbiota Bacteria

Numerous bacteria harbor genes for beta-glucuronidase activity including Firmicutes genera (*Lactobacillus*, *Streptococcus*, *Clostridium*, *Ruminococcus*, *Roseburia*, *Faecalibacterium*), the Proteobacteria genus *Escherichia* and in one species from the phyla Actinobacteria (*Bifidobacterium dentium*).⁷³ Many of these bacteria are found within the *Clostridium leptum* group (cluster IV) and Lachnospiraceas (cluster XIVa).⁷⁴

Laboratory Measurements

Fecal β -glucuronidase.

Representative Consequences for Cancer

Colon, Pancreatic, Ovarian, and Lung Cancers

The chemotherapeutic agents topotecan and irinotecan are pro-drugs that undergo transformation into the active drug SN-38 by hepatic carboxylesterases.⁷⁵ SN-38 is then metabolized into the inactive metabolite SN38G by the liver via glucuronidation and excreted in the bile. Reactivation of SN38 in the intestines can occur via bacteria-mediated removal of the glucuronide group.⁷⁶ SN38 does not appear to be subject to enterohepatic recirculation and remains active as a poison of human topoisomerase I. This means inhibition of both DNA replication and transcription with preferential activity in rapidly dividing cells, both malignant and normal.

With deconjugation and no enterohepatic recirculation, bacterial β -glucuronidase activity results in high concentrations of the activated forms of the chemotherapeutic pro-drugs irinotecan and topotecan in the intestinal tract. These drugs harm rapidly dividing intestinal epithelial cells and cause tight junction defects and mucosal barrier dysfunction.⁷⁷ The result can be dose-limiting, or even life-threatening, diarrhea. Inhibitors of bacterial β -glucuronidases protect mice from diarrhea without altering the microbiome or harming mammalian cells.⁷⁸

Gastrointestinal Cancers

Heterocyclic aromatic amines are genotoxic and carcinogenic compounds formed in meat and fish during cooking.^{79,80} These are metabolized in the liver by

UDP-glucuronosyl transferases to harmless glucuronidated derivatives that are excreted via the bile. However, the presence of β -glucuronidase will reverse this. For example, in the digestive lumen in one animal model, the presence of β -glucuronidase increased the genotoxicity of heterocyclic amines by 300%.⁸¹ This phenomenon may explain why prebiotics, such as inulin and non-digestible oligosaccharides, both reduce β -glucuronidase concentrations and protect against carcinogenesis in animal models.⁸²⁻⁸⁴

Breast Cancer

With deconjugation and enterohepatic recirculation, β -glucuronidase bacterial activity can result in sustained elevation of sex hormone levels including estrogens. This is concerning because breast cancer risk for postmenopausal women is associated with the concentration of serum estrogens and androgens^{85,86} and circulating sex hormone concentrations are strongly associated with severely established risk factors for breast cancer.⁸⁷

In a study of 51 male and female epidemiologists at the National Institutes of Health, fecal β -glucuronidase correlated inversely with fecal total estrogens, both conjugated and unconjugated, as well as serum estrone. The study documented that non-ovarian systemic estrogens were strongly and directly associated with all measures of fecal microbiome richness and Clostridia taxa. The authors concluded that intestinal microbial richness as well as β -glucuronidase influence the levels of non-ovarian estrogens via enterohepatic circulation.⁸⁸

Possible Therapeutic Interventions: β -glucuronidase

1. Adoption of a plant-based low meat or vegetarian diet. β -glucuronidase activity is markedly increased in human volunteers on a high meat diet compared to a vegetarian diet.⁸⁹ Omnivorous diets are associated with increased, and vegetarian diets are associated with reduced, concentrations of clostridial cluster XIVa bacteria.^{51,52}
2. Adoption of a raw vegan diet. This change from a standard diet rapidly and significantly reduces β -glucuronidase activity.⁹⁰
3. Avoidance of charred meat or fish (heterocyclic amines) plus ingestion of probiotics containing *L casei*,⁹¹ or *L helveticus* and *S thermophilus* groups or either *Bifidobacterium animalis*⁹² or *B longum*⁹³ to bind carcinogenic heterocyclic amines generated with grilling meats and fish.⁹⁴ Of note, heterocyclic amines are less genotoxic and carcinogenic in individuals who consume mainly plant-derived foods.⁹⁵
4. Ingestion of cultured or fermented dairy products⁹⁶ along with cruciferous vegetables and other prebiotics.⁹⁷
5. Ingestion of a multispecies probiotic (that includes *Lactobacillus GG*)⁹⁸ or *Lactobacillus* strain GG by itself.⁹⁹
6. The Kampo (traditional Japanese herbal medicine)

formula termed *Hangeshashinto* may alter intestinal ecology and reduce irinotecan and topotecan diarrhea.¹⁰⁰ This formula contains root of the herb *Scutellaria baicalensis* (Skullcap) that contains the beta-glucuronidase inhibitor baical.¹⁰¹

- Ingestion of *Kanjika*, a rice-based Ayurvedic fermented food or the probiotic *L. plantarum* along with prebiotic fructooligosaccharides (FOS).¹⁰²
- Ingestion of blackcurrant. Consumption of blackcurrant products (First Leaf, composed of blackcurrant extract powder, lactoferrin, and lutein, or Cassis Anthomix, blackcurrant extract powder) by healthy volunteers resulted in significant reduction in activity, significant reduction in pH, significant increases in lactobacilli and bifidobacteria, with significant reductions in *Clostridium* and *Bacteroides* species.¹⁰³

3. THIRD MICROBIAL BIOTRANSFORMATION

Microbial Enzymes: Butyrogenic Pathway (6 enzymes)

Microbial Biotransformation: Dietary Fiber to Acetyl-CoA to Butyrate

Functional Result: Colonocyte energy production

Background

Butyrate is a four-carbon short-chain fatty acid (SCFA) produced in the colon by bacterial fermentation (anaerobic respiration) of dietary fiber, complex carbohydrates that are unable to be either digested or absorbed in the small intestine. We depend upon saccharolytic anaerobic bacteria in our intestines for providing the enzymes necessary to break down such dietary fiber. Unlike the human genome, the intestinal microbiome is highly enriched with genes for digestion of dietary fiber.¹⁰⁴ Moreover, by comparative analysis of microbial genes via the COG (clusters of orthologous groups) database, the colon's microbiome is enriched with genes for the production of small chain fatty acids especially butyrate kinase,¹⁰⁵ the last of six enzymes in the production of butyrate from acetyl-CoA (Figure 3).¹⁰⁶

Butyrate is the preferred substrate and the major source of energy for human colonocytes.¹⁰⁷ We do not provide our colonocytes with this nourishment: our bacteria do. Butyrate is actively transported by two means (CMT1 and MCT1) into colonocytes¹⁰⁸ whose expression is reduced in colonic epithelial tumor cells.¹⁰⁹ Within colonocyte mitochondria, butyrate undergoes beta-oxidation into acetyl CoA and then enters the Krebs' cycle with subsequent oxidative phosphorylation for ATP production.¹¹⁰ Unless energy production from butyrate is maximized, very little accumulates in either the cytoplasm or the nucleus.¹¹¹ Butyrate produced in the gut can also be found in the systemic circulation.

Interest in butyrate follows from its capacity to effect histone acetylation, an epigenetic modification that is regulated by two classes of enzymes: histone deacetylases (HDACs) and acetylases (HATs). Control of acetylation

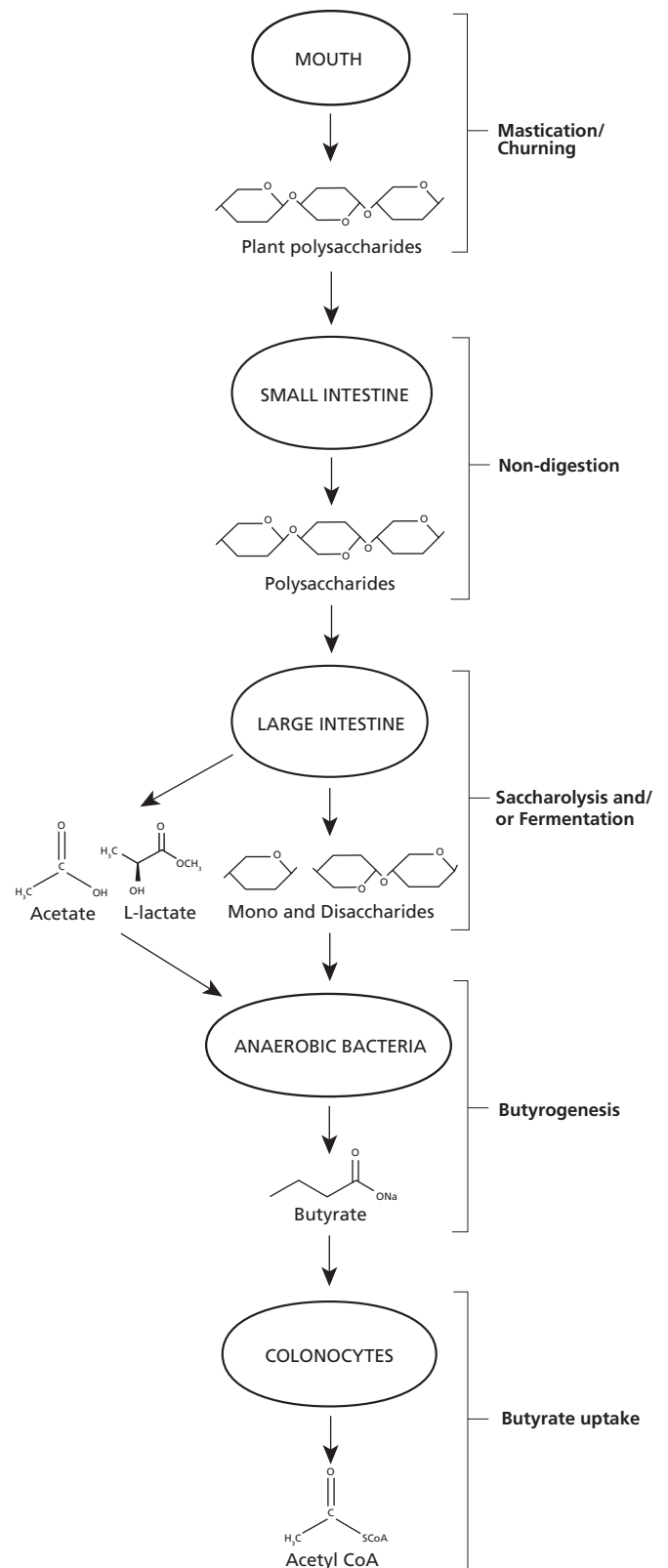


Figure 3 Ingested complex plant polysaccharides undergo significant digestion not in the small intestine, as expected, but instead undergo saccharolysis and fermentation in the large intestine. The mono- and disaccharides produced, as well as products of fermentation, are then transformed by subsets of anaerobic bacteria into butyrate, the predominant energy source for colonocytes. This is one example of cross-kingdom mutualism in the human intestinal tract.

means control of gene expression.¹¹² Butyrate was the first HDAC inhibitor to be discovered.¹¹³

Acetylation is important for regulation of chromatin, the tight complex of DNA and associated proteins that enable DNA to fit inside the nucleus. The fundamental units of chromatin are nucleosomes, 147 bp of DNA wrapped 1.65 times around an octamer of core histone proteins. Acetylation of these histones decreases chromatin's electrostatic interaction. This relaxes the tight structure of chromatin and allows transcription factors access to DNA to target gene promoters.¹¹⁴ In brief, acetylation induces transcription, and deacetylation represses transcription.

Butyrate effects acetylation by inhibiting HDAC deacetylation as well as supporting HAT acetylation. The latter is effected via butyrate undergoing beta-oxidation to acetyl CoA in the mitochondria and then combining with oxaloacetic acid in the first step of the Krebs's cycle to yield citrate. The citrate shuttle transports citrate out of the mitochondria where it is converted by ATP citrate lyase (ACL) back to acetyl CoA and oxaloacetate. ACL is found in the nucleus, produces acetyl CoA, and regulates histone acetylation.¹¹⁵ ACL is in turn upregulated by glucose-induced signal transduction, an important finding for cancerous cells.¹¹⁶

Butyrate paradoxically has different effects in normal and cancerous cells. In normal colonocytes, butyrate metabolism results in oxidative phosphorylation to produce ATP. In tumor cells, however, glucose rather than butyrate is the primary source of energy. This means that the active transport of butyrate results in levels that exceed metabolic capacity for utilization. This results in elevated butyrate levels in the nucleus where key genes are regulated via butyrate-mediated HDAC inhibition. Additionally, in cancer cells, upregulation of ACL by glucose means that butyrate functions as an acetyl-CoA donor and also stimulates HAT-mediated histone acetylation. The result is upregulated expression of downstream target genes including genes for cell cycle arrest/cellular proliferation, differentiation, and apoptosis.¹¹⁷

Specifically, butyrate's anti-cancer activities include cell cycle arrest via upregulation of p21¹¹⁸ and downregulation of cyclin D1 plus numerous pro-apoptotic mechanisms including WAF1, downregulation of apoptotic regulator Neuropilin-1 (NRP-1),¹¹⁹ upregulation of BAK,¹²⁰ downregulation of Bcl-xL and cyclin D1,¹²¹ activation of the JNK MAP kinase pathway,¹²² upregulation of membrane death receptors (DR4/5), higher-level and activation of Smad3 protein in TGF-beta-dependent apoptotic pathway, and activation of proapoptotic tBid protein, as well as lower levels of antiapoptotic proteins (cFLIP, XIAP).¹²³

Independently of histone acetylation, butyrate is also an agonist for G protein-coupled receptors including GPR109A. This receptor is silenced in colorectal, breast, and other cancers but in the presence of butyrate is re-expressed. Butyrate binding results in induction of apoptosis via downregulation of Bcl-2, Bcl-xL, and cyclin D1

and upregulation of the death receptor pathway as well as suppression of nuclear factor-kappaB activation.¹²⁴

Butyrate's anti-cancer effects include suppression of NF-kappaB activation and thus gene expression for pro-inflammatory cytokines, inflammation-inducing enzymes, adhesion molecules, growth factors, heat shock proteins, and immune receptors.^{125,126} Butyrate also induces the expression of adhesion molecules including ICAM-1, V-CAM, and E-selectin.^{127,128}

Moreover, butyrate may potentiate radiation therapy¹²⁹ and chemotherapy with cisplatin,^{130,131} ARA-C, vincristine and etoposide¹³² as well as celecoxib.¹³³

Pertinent Microbial Biotransformation

Complex carbohydrates are subjected in the colon to unique bacterial digestive enzymes not available from the human genome. Digestion results in glucose that can undergo anaerobic glycolysis to acetyl-CoA, the terminal oxidation product of glycolysis.

In obligate anaerobic bacterial cells, acetyl-CoA can then proceed through the butyrogenic pathway of six enzymes, resulting in butyrate production and ATP. For maintaining redox balance with ATP production, butyrate is the terminal electron acceptor.

In aerobic colonocytes, bacterially produced butyrate is subjected to beta-oxidation and returned to acetyl-CoA, which can then undergo oxidative phosphorylation with production of significant ATP.

Responsible Microbiota Bacteria

Gene-encoding enzymes for this pathway are widespread in genome-sequenced clostridia and related species.¹³⁴

Laboratory Measurement

Fecal N-butyrate.

Representative Consequences for Cancer Colorectal

Increased butyrate stool concentration may optimize cancerous colonocyte apoptosis. Numerous studies in multiple colorectal cell lines have demonstrated that butyrate inhibits cell proliferation and stimulates apoptosis.¹³⁵⁻¹³⁷ In addition to the means noted above, sodium butyrate upregulates expression of annexin A1 (ANXA1) in human colon adenocarcinoma cells. Annexins are proteins that are important as factors in the invasiveness and proliferation of cancer cells.¹³⁸ ANXA1 specifically is involved in both proliferation and apoptosis. Expression of ANXA1 appears relevant to outcomes in gastrointestinal cancers^{139,140} but not in other cancers, including breast cancer.¹⁴¹ At this time, the butyrate hypothesis has not been subject to any human trials.

Esophageal

Butyrate may augment the efficacy of fractionated ionizing radiation (IR) therapy. In KYSE-150R radio-resistant cells, butyrate increased radio-sensitivity, IR-induced ROS generation, and IR-induced G2/M arrest

and apoptosis. Butyrate also increased p21 and inhibited Bmi-1 expression.¹⁴² Bmi-1 plays a key role in the functioning of endogenous stem cells and cancer stem cells.

Breast

The colonic SCFA butyrate appears to inhibit breast tumorigenesis.¹⁴³ Multiple mechanisms appear relevant. In MCF-7 cells, butyrate induced P53-independent, Fas-mediated apoptosis.¹⁴⁴ The G Protein-coupled receptor GPR109A activation inhibits genes relevant to cell survival and pro-apoptotic signaling. Specifically, activation potentiates anti-inflammatory pathways, decreases cyclic AMP production, induces apoptosis, and blocks colony formation and breast tumor growth. This receptor is expressed in normal mammary tissue irrespective of hormone receptor status and silenced in multiple breast cancer cell lines. Evidence supports GPR109A as a tumor suppressor in breast tissue. As with colon cancer, its re-expression and butyrate binding may induce apoptosis. In the MMTV/neu mouse model of spontaneous breast cancer, deletion of GPR109A increased tumor incidence, triggered early onset of tumorigenesis, and increased lung metastasis.¹⁴⁵ In ER-positive breast cancer cell lines, butyrate was more potent than the steroidal anti-estrogen ICI in regulating expression of cell cycle proteins and cell growth. Specifically, butyrate-mediated transcriptional and post-transcriptional regulation and ER α phosphorylation resulted in marked depletion of ER α expression. Butyrate appears to antagonize E₂-dependent responses.¹⁴⁶ In HER2/neu over-expressing cell lines, butyrate functioned synergistically with trastuzumab.¹⁴⁷ In both differentiation-induced (HT-29) and cell death-induced (HeLa) cell lines, pretreatment of cells with butyrate followed by butyrate + paclitaxel resulted in increased therapeutic results in the HT-29 cells but proved detrimental in HeLa cells.¹⁴⁸

Possibly Therapeutic Interventions: Butyrate

1. Prebiotic diet
 - a. High plant-based diet for fiber from difficult-to-digest plant polysaccharides (cellulose, hemicellulose, and lignins).
 - b. Resistant starch (green bananas, raw potato, cooked then cooled potato)¹⁴⁹
 - c. Inulin-containing foods (wheat, onion, bananas, garlic, asparagus, and chicory).
 - d. Pectin-containing foods (dried citrus peels and apples, carrots, guavas, gooseberries, oranges, pears, plums, quince)
 - e. Oligosaccharides: Fructooligosaccharides (FOS) include asparagus, bananas, barley, chicory, Jerusalem artichoke, jicama, leeks, wheat, and yacón. Galactooligosaccharides (GOS) include soybeans and bovine lactose derivatives.
2. Citrus pectin as a supplement.
3. Italian pecorino, Greek feta and other sheep cheeses made from lamb rennet paste^{150,151} as well as butter¹⁵² are rich in butyrate or its prodrug form tributyrin.¹⁵³ Of note, the term *butyrate* comes from the

word butter, its best-known dietary source.

4. Butyrate supplementation as an enteric coated tablet such as ButyrEn (Allergy Research Group, Alameda, California). Oral butyrate has both a short half-life and is subject to first-pass hepatic clearance. Multigram doses are needed to achieve therapeutic concentrations *in vivo*.^{154,155} Side effects with oral use include headache, nausea, and anorexia.
5. Green tea ingestion.¹⁵⁶ EGCG, an active ingredient of green tea, appears to work synergistically with butyrate in promoting apoptosis including cell cycle arrest and DNA damage in colorectal cancer cells.
6. DHA supplementation.¹⁵⁷ DHA, one of the two long-chain fatty acids found in fish and krill oils, appears to work synergistically with butyrate to induce apoptosis.

Precaution

Glutamine supplementation: This is widely marketed for N-butyrate production support. However, many cancers actually depend upon glutamine for mitochondrial function, carbon and nitrogen donation, and NADH production for redox control and macromolecule synthesis.¹⁵⁸ Cancer cell lines can consume 10 times greater rates of glutamine than any other amino acid.¹⁵⁹ Many types of cancer cells are sensitive to glutamine deprivation. Pancreatic, lung, and glioma cancer cells, for example, cannot maintain viability, much less proliferate, in the absence of glutamine. Likewise, lymphoma cells can use glutamine for ATP production even in the absence of either oxygen or glucose.¹⁶⁰

CONCLUSION

This article's explicit intention was to describe three biotransformations by intestinal microbiota relevant to both gastrointestinal and non-gastrointestinal cancers. Unintentionally, this review strengthens the biological importance of a diet low in animal fat and high in vegetables. In all three microbial transformations discussed here, a low-animal fat/high-vegetable diet is associated with beneficial ecological profiles including decreased production of toxic bile acids, decreased de-glucuronidation, and increased butyrate production.

Laboratory technologies now allow both clinicians and researchers to measure bacterial factors very relevant to cancer prevention and treatment. Specifically, quantification of deoxycholic acid, β -glucuronidase, and butyrate provides clinically relevant data that can guide dietary interventions and monitor clinical progress toward goals. Increased testing of these bacterial factors could lead to improved dietary adherence and enhanced supplementation strategies that reduce costs and improve outcomes for many conditions, including cancer.

The benefits for researchers appear to be quite promising as well. First, quantification of these three factors may help researchers define "super donors" for fecal microbial transplant. Second, use of these measures will help define the functional consequences to

the intestinal ecology of antibiotics, surgery, and chemotherapy. Third, these functional measures may provide greater insight into the potential confounders of clinical trials including chemotherapy trials.

The rapid emergence of a robust basic science literature on the intestinal microbiota in cancer means that many exciting hypotheses are being generated that need to be tested. The existing evidence suggests that the microbiota might be managed via diet, prebiotics, probiotics and even targeted antibiotics to minimize risk or optimize therapies. Most importantly, these three biotransformations represent microbial factors that are easily accessible, measurable, and modifiable. As such, fecal testing to quantify these will likely play significant roles in future cancer prevention and treatment.

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