AIMS Microbiology, 1(1): 48-71.
DOI: 10.3934/microbiol.2015.1.48
Received date 15 September 2015,
Accepted date 18 November 2015,
Published date 23 November 2015

http://www.aimspress.com/

*Review*

# Healthy effects of prebiotics and their metabolites against intestinal diseases and colorectal cancer

**Javier Fernández [1], Sául Redondo-Blanco [1], Elisa M. Miguélez [1], Claudio J. Villar [1], Alfonso Clemente [2], and Felipe Lombó [1,*]**

[1] Research Unit "Nutraceuticals Biotechnology and Cotherapy". IUOPA (Instituto Universitario de Oncología del Principado de Asturias), Universidad de Oviedo, Oviedo ES33006, Spain
[2] Estación Experimental del Zaidín, Spanish Council of Scientific Research (CSIC), Granada, Spain

* **Correspondence:** Email: lombofelipe@uniovi.es; Tel: +34-985-10-3593.

**Abstract:** A specific group of plant and animal oligosaccharides does not suffer enzymatic digestion in the human upper intestinal tract, achieving the colon microbial ecosystem in intact form. The reason for that is their diverse glycosidic bond structure, in comparison with common energetic polysaccharides as starch or glycogen. In this complex ecosystem, these molecules serve as energy sources, via fermentation, of distinctive beneficial bacterial groups, mainly belonging to the *Anaerostipes, Bifidobacterium*, *Coprococcus, Faecalibacterium, Lactobacillus, Roseburia* and other genera. The main catabolic products of these fermentations are short-chain fatty acids (SCFA) as acetate, propionate and butyrate, which appear in high concentrations in the lumen around the colon mucosa. Acetate and propionate are associated to energetic purposes for enterocytes, hepatocytes and other cells. Butyrate is the preferred energy source for colonocytes where it controls their cell cycle; butyrate is able to induce cell cycle arrest and apoptosis in tumor colonocytes. These oligosaccharides that increase beneficial colon bacterial populations and induce SCFA production in this ecosystem are called prebiotics. Here, different sources and chemical structures for prebiotics are described, as well as their modulatory effect on the growth of specific probiotic bacterial groups in the colon, and how their fermentation renders diverse SCFA, with beneficial effects in gut health.

**Keywords:** prebiotics; short chain fatty acids; gut microbiota; acetate; propionate; butyrate; colorectal cancer

**Abbreviations**

AOS: arabino-oligosaccharides
BUK: butyrate kinase
BUT: butyryl-CoA:acetyl-CoA transferase
CDKI: cyclin dependent kinases
CRC: colorectal cancer
FAP: familial adenomatous polyposis
FOS: fructo-oligosaccharides
GlOS: gluco-oligosaccharides
GLP-1: glucagon-like peptide-1
GLUT: glucose transporter
GOS: galacto-oligosaccharides
HAT: histone acetyltransferases
HDAC: histone deacetylase
HMO: human milk oligosaccharides
IMO: isomalto-oligosaccharids
IBD: inflammatory bowel disease
IBS: irritable bowel syndrome
LPL: lipoprotein lipase
LPS: lipopolysaccharides
Neu5Ac: *N*-acetylneuraminic
Neu5Gc: *N*-glycolylneuraminic acid
OAA: oxaloacetic acid
OS: oligosaccharides
PTB: phosphotransbutyrylase
PYY: peptide YY
SCFA: short-chain fatty acids
TCA: tricarboxylic acids cycle
UC: ulcerative colitis
XOS: xylo-oligosaccharides

## 1. The Prebiotic Concept

The human gut tract is inhabited by a complex, diverse and highly metabolically active microbial community, called intestinal microbiota that comprises an extraordinary number (up to 100 trillion cells and each person having around 200 prevalent species) of resident commensal bacteria [1,2]. Their combined genomes, known as microbiome, contain more than 5 million genes, thus outnumbering the host´s genetic potential by two orders of magnitude [3]. The microbiota plays a critical role in health and well-being of their host. Gut microbes facilitate nutrients and energy to the host, fermenting and absorbing undigested dietary components but also releasing a variety of metabolites including essential vitamins and short-chain fatty acids (SCFA) that influence locally and/or systemically host physiology [4,5]. At the same time, some intestinal microorganisms have the capacity for harmful effects, via their metabolic outputs and gene products (as $H_2S$), or potential for pathogenicity [6,7].

Among other important functions, the gut community has a major role in control of epithelial cell proliferation/differentiation, confers protection against invasion by opportunistic pathogens and plays a key role in maturation of the immune system and host metabolism [8,9]. The overall balance of the gut microbial community in terms of its distribution, diversity, species composition and metabolic outputs is relevant in ensuring host homeostasis [5]. There is a large body of evidence linking alterations in the gut microbial composition, and the induced changes in interactions with the host, to several diseases [10], including obesity [11], inflammatory bowel disease (IBD) [12], cardiovascular disease [13], autoimmune arthritis [14] or colorectal cancer (CRC) [15,16]. Therefore, the extent to which the gut microbiota is clinically relevant to human diseases needs to be investigated more deeply.

Nutrient availability is critical in influencing the composition and metabolic activity of gut microbiota [17]. Many studies have shown an increased proportion of bifidobacteria in breast-fed

infants compared to formula-fed infants due mainly to the presence of human milk oligosaccharides. A study over a period of 2.5 years of the gut microbiome in a single infant showed a gradual increase in diversity over time alongside abrupt shifts in the abundance of major taxonomic groups associated to changes in diet or health [18]. The most significant change in microbiome composition and functionality occurs during weaning with introduction to solid foods resulting in a shift within the early 2–3 years of life towards an adult microbiota [19].

Dietary patterns have been demonstrated to affect the structure of the gut microbial community, having consequences in health and disease. Remarkably, some authors demonstrated that the composition of the intestinal microbiota differs significantly between children living in a rural African village and those living in Europe [20]. It has been shown that most diet-driven changes on microbiota occur rapidly and might reverse equally rapidly. Thus, short-term diets, such as those devoid of carbohydrates have been shown to have a pronounced effect on gut microbiome [21]. Shifting from a high-fat/low fiber diet to a low-fat/high-fiber diet caused significant changes in the gut microbiota within 24 h; however, the enterotype identity remained stable [22]. A diet rich in fruit and vegetable fiber has been associated with greater microbial diversity and might exert a positive influence in gut health [23]. In controlled human studies, variations in intake of resistant starch and non-starch carbohydrates induced significant changes in the gut microbiota and these seemed to be dependent of the initial composition of an individual´s gut microbiota [24]. Dietary supplementation with prebiotic fibers (as fructo-oligosaccharides and inulin) and dietary animal reduction can promote the growth of specific groups of bacteria, including bifidobacteria and *Bacteroides*, reducing simultaneously the relative amount of Firmicutes [6,25]. The presence of probiotics in milk products has been shown also to affect microbiota composition. Thus, higher consumption of fermented milk containing *Lactobacillus helveticus* in healthy subjects increased *Bifidobacterium* composition [26].

These relationships between diet and gut microbiomes are not only restricted to humans, as it has been shown that gut microbiome diversities from various vertebrates are adapted to the corresponding species diet (carnivorous, omnivorous, herbivorous). Thus, total protein intake in 33 vertebrate species was associated with more metabolic functions encoded by gut microbiome genes (as proteases), whereas total insoluble dietary fiber was associated with higher diversity in microbial taxons. So, for each giving species, gut microbiome function and structure are associated with dietary intake. As an example, carnivorous gut microbiomes are shifted towards degradation of proteins and amino acids as energy source (conversion of mitochondrial pyruvate into oxaloacetic acid (OAA) devoted for energy generation in TCA cycle); whereas herbivorous ones are shifted towards biosynthesis of amino acids building blocks (generation of more pyruvate and phosphoenolpyruvate from OAA as building block for essential amino acids) [27]. In humans, links have been shown between people consuming more diverse insoluble dietary fiber (that one achieving the colon ecosystem and able to be fermented by this microbial community in order to produce SCFAs) and a healthier status with respect to western lifestyle autoimmune (Crohn's disease, IBD) or inflammatory diseases (type 2 diabetes, insulin resistance, blood lipids concentrations, CRC, etc.) [28].

It is well known that non-digestible food ingredients or substances are capable of modulating composition and metabolic function of gut microbiota, therefore preventing diverse infectious, inflammatory and neoplastic intestinal diseases [29]. These compounds are called prebiotics and since introduction of this concept, its definition has evolved over the last twenty years. In early studies, prebiotics were defined as "non-digestible food ingredients that beneficially affect host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in

the colon" [17]. The major beneficial effects of prebiotics seem to occur in the large intestine due to the slow transit of the substrates to be fermented and their effects on microbial diversity, which plays an important role in host health [30].

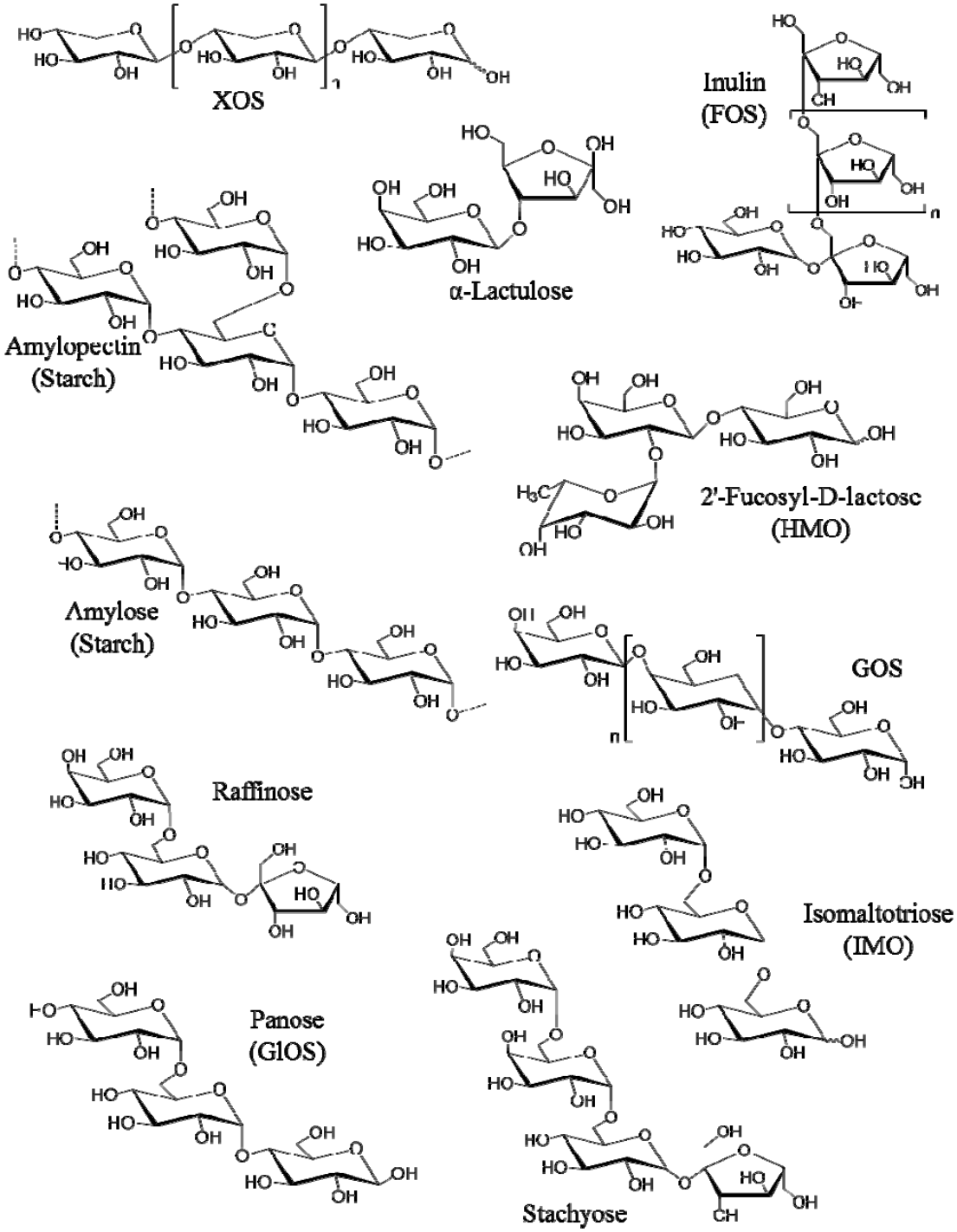


**Figure 1. Chemical structures of the different prebiotics families.** FOS: fructo-oligosaccharides, GlOS: gluco-oligosaccharides, GOS: galacto-oligosaccharides, HMO: human milk oligosaccharides, IMO: isomalto-oligosaccharids.

More recently, prebiotics have been redefined as "selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" [31]. The metabolic end-products (as SCFAs) that result from this fermentation have been shown to exert beneficial effects, not only at large intestine but also within the entire human body and/or contribute to the prevention/remission of intestinal or systemic pathologies [32,33]. Indeed, the prebiotic definition is still matter of discussion and recently the concept has been revisited in an effort to shift the focus towards ecological and functional features of the microbiota more likely to be relevant in host physiology [34].

Prebiotics are, therefore, non-digestible oligosaccharides with various origin and chemical properties, differing in chain length, monosaccharide composition, linkage type and branching degree. Although there are several carbohydrates marketed as prebiotics worldwide, there are only a few oligosaccharides for which prebiosis has been clearly demonstrated in human intervention trials. These are inulin, oligofructose (also called fructo-oligosaccharides, FOS), galacto-oligosaccharides (GOS), the synthetic disaccharide lactulose (4-*O*-*β*-D-galactopyranosyl-D-fructose) and human milk oligosaccharides (HMO) [34] (Figure 1). Other candidate prebiotics are under investigation; among them, isomalto-oligosaccharides (IMO), arabino-oligosaccharides (AOS), xylo-oligosaccharides (XOS), gluco-oligosaccharides (GlOS), soy oligosaccharides and resistant starch have been regarded as emerging prebiotics that may show similar or improved properties than the market well-established oligosaccharides (Figure 1) [29,39,123].

An aspect common to most of these prebiotic compounds is the fact that they mostly constitute carbohydrate energy reserves from diverse plant species, regardless of their accumulation in seed´s cotyledons (rye), leaves (agave), stems (artichoke), bulbs (garlic), roots (chicory) or tubers (yam) [63,123,161]. In contrast, with the most commonly widespread plant reserve carbohydrate, starch, which can also be found in seeds (maize), stems (sago), bulbs (onion), rhizomes (ginger), roots (cassava), tubers (potato) and fruits (banana) [36,56], prebiotics are not digested by mammalian enzymes responsible for starch degradation and absorption during intestinal digestion. Starch is usually composed of 30% amylose (long linear chains of glucose (linked through α-(1,4) bonds) and 70% amylopectin (tridimensional complex networks of linear glucoses linked through α-(1,4) bonds and branched through α-(1,6) bonds to other linear chains) [9]. Mammals as humans degrade starch into glucose disaccharides (maltose) using the amylases produced from salivary glands and pancreas [4]. Then, the maltases produced at the enterocyte lumen degrade these maltoses, rendering free glucoses which are absorbed at the small intestine [55].

To exert their potentially prebiotic properties, oligosaccharides have to resist the digestive enzymes and survive, at least to some extent, the acidic environment and enzymatic digestion by pancreatic and intestinal brush border enzymes in the upper digestive tract. The oligosaccharides resistance to digestion is associated with several factors, including the identity of their monomeric units (fructose, xylose, galactose, arabinose, rhamnose, etc.), linkages type (as fructose chains linked by β-(1,2) bonds in inulin), as well as their ring form and anomeric conformation [21,68]. Dietary oligosaccharides that escape digestion and absorption in small intestine arrive at the colon and there, they are selectively fermented by a number of different bacterial populations, via competitive and/or cooperative interactions, giving rise to healthy metabolites, mainly SCFA as acetate, propionate and butyrate. Such modulatory effect on gut microbiota has been associated with improvement in overall health, enhancement of host defense mechanisms to gut infections, accelerated recovery of gut disturbances and better absorption of minerals [62].

## 2. Structural Diversity of Prebiotics and Biological Activities

The most widespread prebiotic compounds in nature are formed by fructose chains, where the monomers are linked by glycosidic β-(1,2) bonds. These polymers are called fructans, and their fructose chains may contain or not in one of its ends a glucose molecule. Linear fructan molecules may possess from 2 to more than 60 fructose moieties, calling them fructo-oligosaccharides (FOS) (2 to 10 moieties) or inulin (more than 10 fructose residues, as in chicory (*Cichorium intybus*)) (Figure 1) [21,31]. In about 10% of vascular plant species (like banana, garlic, onion and leek) this is the main energy reserve carbohydrate [48]. Several inulin types occur in nature and they differ in polymerization degree and molecular weight, depending on the source plant, harvest time and processing conditions [39].

Fructo-oligosaccharides (FOS) can be produced either by chemical degradation or controlled enzymatic hydrolysis of inulin by endoglycosidases [49]. Furthermore, FOS can be produced from sucrose at commercial scale, using fungal enzymes [50]. Several studies have shown the bifidogenic effect of inulin and FOS favoring *Bifidobacterium* and *Lactobacillus* growth, and decreasing that of *Bacteroides* and *Clostridium* [51].

Some arabino-oligosaccharides (AOS) from sugar beet pectin [52] and lemon peel [53] promote *Bifidobacterium* growth to the same extent as fructo-oligosaccharides (FOS) and inulin, respectively [54–57], with the highest bifidogenic response obtained with those having higher molecular weight [58]. These compounds are chains of L-arabinose moieties linked by α-(1,5) bonds, branched via α-(1,2) or α-(1,3) bonds with other L-arabinose moieties (Figure 1) [59]. The bifidogenic activity of high molecular weight rhamnogalacturonan I from potato pulp has been reported to be superior to the FOS one [60]. AOS fermentation may diminish the inflammatory conditions in ulcerative colitis (UC) patients. An *in vitro* approach to this has been carried out by fermenting AOS with fecal samples from UC patients and healthy control people. These experiments showed that AOS stimulated bacteria genera as *Bifidobacterium* and *Lactobacillus*, and an increase in SCFA as acetate, which are known to elicit anti-inflammatory responses. AOS may therefore represent a new prebiotic candidate for reduction of flare-ups risk in UC patients in the near future, once *in vivo* tests will be carried out [61].

Xylo-oligosaccharides (XOS), are sugar oligomers made up of xylose units. They are naturally present in fruits, vegetables, bamboo, honey and milk and can be produced at industrial scale from xylan-rich materials (as straw, wood and some macroalgae, where they form the hemicellulose portion). These unusual oligosaccharides are composed by chains of xylose moieties linked by β-(1,4) bonds, with a polymerization degree ranging from two to ten monosaccharides (Figure 1). Several studies have shown the bifidogenic effect of XOS [62,63]. They are considered soluble dietary fibers with prebiotic activity, favoring the improvement of bowel functions and immune function and having antimicrobial and other health benefits as being able to decrease blood glucose levels, total cholesterol and LDL in patients with type 2 diabetes mellitus [64].

Raffinose and stachyose (Figure 1) are non-digestible oligosaccharides present in soybean seeds but also in other legumes like peas or lentils. Raffinose is a trisaccharide containing a galactose moiety linked α-(1,6) to the glucose unit of sucrose. Stachyose is a tetrasaccharide containing a galactose molecule linked α-(1,6) to the terminal galactose unit of raffinose [65]. Deshipu stachyose granules is a novel oligosaccharide preparation (55.3% stachyose, 25.8% raffinose and 9.7% verbascose) which promotes the growth of beneficial intestinal bacteria in mice gut, inhibiting

pathogenic bacteria and also facilitating intestinal peristalsis and fecal excretion, thereby enhancing intestinal health and relieving constipation [66].

Resistant starch is present in some plants, where the starch amylose/amylopectin (Figure 1) relation is much higher than the usual 30%/70% respectively. This abundance facilitates the amylose crystallization and compaction processes, and hindering its digestion by amylases along the intestinal tract [67].

Galacto-oligosaccharides (GOS) are naturally-occurring compounds present in mammals' milk. These prebiotics can be produced industrially from whey lactose by trans-galactosylation of lactose, carried out by β-galactosidases. This fact results in complex mixtures of oligosaccharides with different degrees of polymerization, ranging from two to eight moieties, and different glycosidic linkages: β-(1,1), β-(1,2), β-(1,3), β-(1,4) and β-(1,6) (Figure 1). Appropriate enzymes from different *Bifidobacterium* species can specifically hydrolyze these β-glycosidic linkages once they arrive at the colon [51]. Recently, the synthesis of lactulose-derived GOS has attracted the attention of the scientific community due to their prospective prebiotic applications, being recognized by their ability to promote the growth of bifidobacteria *in vitro* (human fecal slurries) [68] and *in vivo* (rat as a model) [46,69].

Human milk contains, apart from lactose (70 g/L) and GOS (5 g/L), other complex carbohydrates called HMO (human milk oligosaccharides, 10 g/L) that are important dietary factors with multiple functions during breast feeding ages [70,71]. HMO composition is very complex since at least may consist of more than 200 types of combinations of five monosaccharides: glucose, galactose, fucose, N-acetylglucosamine, and N-acetylneuraminic acid (Figure 1). Neutral HMO are composed by glucose and galactose but also contain multiple units of N-acetylglucosamine and fucose. Acid HMO contains, besides the above-mentioned carbohydrates, units of N-acetylneuraminic acid (also known as sialic acid). The presence of sialic acid and fucose in terminal positions render these polysaccharides non-digestible by human digestive enzymes. This facilitates its arrival to colon as unmodified carbohydrates, where they promote growth of diverse beneficial bacterial groups. In fact, HMO are responsible for the large numbers of *Bifidobacterium* present in breast-fed infants' feces, as they get regular amounts of these prebiotics from mother´s milk [72,73]. In goat´s milk, the presence of other oligosaccharides (OS) similar to those found in human milk has been reported. Among them, i) the existence of neutral oligosaccharides, whose structures are mainly based on lactose with the addition of neutral monosaccharides such as glucose or galactose (Hex), *N*-acetylglucosamine or *N*-acetylgalactosamine (Hex*N*Ac) and fucose or deoxyhexose (Fuc) and ii) acidic oligosaccharides, containing acidic components such as *N*-acetylneuraminic (Neu5Ac) or *N*-glycolylneuraminic acid (Neu5Gc) [74]. Some of these oligosaccharides, such as those containing fucosyl- or sialyl- groups have been described to have prebiotic and/or pathogen binding activities [75].

Lactulose (4-O-β-D-galactopyranosyl-D-fructofuranose) is a synthetic prebiotic disaccharide composed of galactose and fructose linked by a β-(1,4) glycosidic bond (Figure 1), which can be industrially obtained by chemical isomerization of lactose present in whey permeate [76]. This enzyme-catalyzed synthesis offers new potential for food technology since it can be carried out with crude lactose materials derived from the dairy industry [77]. Lactulose shows bifidogenic properties stimulating the growth of health-promoting bacteria such as *Bifidobacterium* and *Lactobacillus* [78]. Lactulose is used not only in nutrition, as prebiotic, but also for treatment of chronic constipation at doses of 10 g/day [79], to maintain blood glucose and insulin levels [80], and to prevent hepatic

encephalopathy in liver failure patients, as lactulose reduces the intestinal absorption of toxic ammonium from lumen, preventing it from reaching the brain [81,82].

Isomalto-oligosaccharides (IMO) are a mixture of short-chain carbohydrates obtained enzymatically from starch. They contain α-(1,6) and α-(1,4) linkages (Figure 1) [30]. While human intestinal enzymes can digest α-(1,4)-glycosidic bonds, α-(1,6)-linkages are not easily hydrolyzed, and therefore they can reach the colon, where they are partially fermented by the microbiota [83]. Different studies have shown that IMO show bifidogenic properties [84,85,86]. IMO can also diminish total cholesterol and triacylglycerides levels in patients treated with hemodialysis [87] and stimulate intestinal and systemic immunity via a shift in the Th1/Th2 balance towards Th1-dominant immunity. Such effects may be due, at least in partially, to the relative increase of *Lactobacillus* numbers in the gut microbiota of mice [88].

Finally, gluco-oligosaccharides (GlOS) are glucose oligomers with α-(1,6) linkages that can also branch via α-(1,2) and α-(1,3) (Figure 1). These glycosidic linkages are resistant to hydrolysis by mammals' digestive enzymes [89]. They can be produced from sucrose in the presence of maltose by enzymatic synthesis, or biosynthesized by *Leuconostoc mesenteroides*. *In vitro* fermentation studies have shown that they favor *Bifidobacterium* growth against harmful bacteria [90,91,92].

## 3. Modulation of Colon Microbiota by Dietary Prebiotics

Once ingested, prebiotics can carry out a selective action for health-promoting taxonomic groups with beneficial metabolic activities [29]. In this sense, prebiotics might favor the growth of *Bifidobacterium* and/or *Lactobacillus* species in the colon, considered a major shift in gut microbiota towards a healthier composition. This overgrowth of such beneficial taxons inhibits the uncontrolled growth of undesirable, potentially pathogenic bacteria including *Salmonella* sp., *Campylobacter jejuni*, *Helicobacter pylori* and *Escherichia coli*, among others [51]. In a pioneered human study to demonstrate their bifidogenic effects, eight healthy volunteers having a controlled diet were supplemented with 15 g/day of oligofructose or inulin, both of which resulted in almost a ten-fold increase in luminal bifidobacteria, which then returned to baseline following withdrawal of the prebiotic supplement [93]. Since then, both prebiotics, and also GOS, have been used in many healthy human trials, with a general significant increase in colon bifidobacteria [94–100]

Intestinal microbiota dysbiosis (an imbalance in body microbiota towards harmful species versus beneficial ones) scenarios are related to diseases as obesity, acting on the harvest and storage of dietary energy [101,102]. Dysbiosis is also a key factor in the development of inflammatory conditions as IBD [103,104] and Crohn's disease, one of the main forms of IBD, which also shows an increased risk of CRC compared with healthy people [105]. Prebiotics can help in these cases, as for example, daily consumption of 15 g inulin in patients with Crohn's disease improved disease symptoms and increased gut bifidobacteria [106].

Fecal microbiota diversity is reduced in cases of CRC, where is frequent the presence of opportunistic pro-inflammatory pathogens from *Fusobacterium, Campylobacter, Collinsella, Peptostreptococcus, Porphyromonas, Mogibacterium* and *Anaerotuncus g*enera, as well as from *Enterococcaceae* and *Erysipelotrichaceae* families. In these cases there is also a reduction of *Clostridium* cluster IV members (as *Faecalibacterium prausnitzii* and *Roseburia*), and *Bifidobacterium.* Some of these taxons have been defined as CRC driver bacteria: those giving an advantage to tumor progression, as production of superoxide radicals (*E. faecalis*), genotoxic (*E. coli*

strains), toxigenic (*B. fragilis*) or pro-inflammatory compounds (*Shigella, Citrobacter* or *Salmonella*). NF-kB transcription factor is one of the main connections between these pro-inflammatory factors and CRC during its early stages, when inflammation induces genetic mutations, inhibits apoptosis and stimulates proliferation and angiogenesis [107].

Also, there is a potential role of the gastrointestinal microbiota in pathogenesis of irritable bowel syndrome (IBS), and in particular in its diarrhea-predominant type, where low numbers of bifidobacteria are found. This fact has suggested that by adding prebiotics in higher amounts in the diet of these patients, their symptoms could remit due to an increase in bifidobacteria populations [108]. A clinical trial using two different doses of GOS patients with IBS found an increase in the number of bifidobacteria in both groups, compared with the placebo group [109].

Apart from bifidobacteria, beneficial attributes are constantly discovered for many other gut bacteria, and there is ongoing debate on extending the range of target beneficial gut microorganisms. Thus, abundant species in the healthy microbiota such as *Faecalibacterium prausnitzii*, *Eubacteriumrectale* and *Roseburia* spp. have been reported to produce relatively high amounts of butyrate from prebiotics fermentation, which plays different roles in important processes linked to colonic health, including protection against inflammation and CRC [110].

Furthermore, some studies have shown that CRC is related to variations in the amount of certain species of bacteria in the gut microbiota. For example, it has been reported a detectable increase in the diversity of the *Clostridium leptum* and *Cl. coccoides* subgroups in patients with CRC [111]. Differences in bacterial composition between healthy and tumor colon tissue have been described within cancer patients, with some genera of non-pathogenic bacteria, such as *Coriobacteria*, *Roseburia*, *Fusobacterium* and *Faecalibacterium* overrepresented in tumor tissue [112]. It has been observed that microbiota changes associated to CRC were not only limited to adenomas areas; as normal tissue beside tumors showed also increased levels of Proteobacteria and decreased levels of species in the Bacteroidetes phyla [113]. In other studies, the abundance of *Bacteroides* and *Prevotella* genera was higher in cancer tissue of patients than in adjacent normal tissue [114]. This later work is in accordance with a previous study where 15 colon microbial species were associated with an increased risk of cancer (including *Bacteroides vulgatus, B. stercoris, Bifidobacterium angulatum* and *B. longum*). In this previous study, higher levels or *Bacteroides* are expected in diets with high intake of red meat and fat, as both stimulates bile flow, which stimulates *Bacteroides* growth and are then converted by these bacteria into diverse colon mutagens. In this same study, *Lactobacillus acidophilus* and others, as well as *Eubacterium aerofaciens* were associated with a lower risk of the disease, likely due to their high production of SCFA as acetic acid. The presence of other bacteria, as *Collinsella aerofaciens*, has been also linked to a reduced risk of CRC [115]. In another study, patients with IBD showed lower levels of this species compared with normal subjects. The administration of inulin-type fructans increased levels of *Co. aerofaciens*, a considered beneficial effect associated with its fermentation [116].

## 4. Prebiotics Metabolic Effects and Their Catabolism by Gut Microbiome Species

Prebiotics pass along our digestive tract free of enzymatic transformations, but once they arrive at the colon, these oligosaccharides are recognized as a suitable carbon source for diverse probiotic colonic bacteria, such as lactic acid bacteria of the genera *Bifidobacterium* and *Lactobacillus*, which generate large amounts of SCFA after their fermentation [117,118], mainly lactate, pyruvate, acetate,

propionate and butyrate, together with significant amounts of carbon dioxide and molecular hydrogen; and at lesser extent, formate, valerate and caproate (the former three accounting for less than 5% total SCFA). As an example, with a daily prebiotic fiber intake of 10 g, about 100 mmol of SCFA can be generated in the colon [49,119].

Butyrate:propionate:acetate relative colon molar concentrations are 1:1:3. Butyrate is the most important SCFA in human health, as it is the preferred energy source of colonocytes, shows anti-inflammatory activity and regulates gene expression, differentiation and apoptosis in host colon cells. In contrast, acetate and propionate do not show a strong antitumor effect [120].

Propionate is readily absorbed by enterocytes, passing to the portal vein circulation, and being taken up by the liver for gluconeogenesis purposes. Acetate is also rapidly absorbed, and distributed by peripheral blood to all tissues, where it is metabolized [121].

Colon dysbiosis triggers alterations in the gut barrier function, allowing translocation to portal vein of bacterial components as lipopolysaccharides (LPS) and bacterial cells. Once distributed to other tissues, these pro-inflammatory factors promote local inflammation in liver and adipose tissue, giving rise to metabolic changes that may derive in obesity, metabolic syndrome and type 2 diabetes. These changes include higher circulating levels of triglycerides and free fatty acids, higher blood cholesterol levels, and insulin resistance in diverse tissues (muscle, liver, etc.). A first relationship between SCFA and metabolic syndrome derives from the observation that low SCFA blood concentrations have been associated to obesity status in laboratory animals and humans. SCFA can increase leptin levels in adipose tissue, a satiety hormone. At gut level, SCFA induce also production of GLP-1 hormone (glucagon-like peptide-1) after binding to GPR43 receptor. This mechanism increases insulin sensitivity. SCFA binding to gut GPR41 receptor induces PYY hormone (peptide YY) liberation. These two hormones act at the brain level by increasing satiety being at reduced level in obese individuals [119,122].

Butyrate induces lipolytic activity in human adipocytes and in animal adipose tissue, mainly through lipoprotein lipase (LPL) transcription activation. SCFA bind to GPR43 receptor also in adipocytes, suppressing insulin signaling and therefore inhibiting fat accumulation and obesity [119,122].

Acetate and propionate (the two main circulating SCFA) inhibit lipid accumulation in non-adipose tissues, as muscle, liver and pancreas. This inhibition helps in reducing side-effects (as local inflammation, insulin resistance and metabolic syndrome) caused by high levels of circulating fatty acids and triglycerides under metabolic syndrome circumstances. This effect seems to be associated with an increased fatty acid oxidation in these organs, and to the use of propionate for liver gluconeogenesis instead of using it for lipogenesis [119]. Acetate serum levels increase cholesterol biosynthesis, which is, however, inhibited by serum propionate [121].

SCFA increase the numbers of regulatory T cells, an anti-inflammatory cells with reduced numbers in obese individuals. Butyrate, for example, inhibits HDAC 6 and 9. This promotes histone H3 acetylation and expression of FoxP3 transcription factor, involved in development and function of these anti-inflammatory cells. This regulation on regulatory T cells may be of interest also in diseases as ulcerative colitis. Propionate regulates against allergic inflammations, hematopoiesis in the bone marrow and the proper function of dendritic cells. Also, SCFA improve epithelial barrier function and gut permeability, by modulating mucin and tight junction proteins expression [119,122,123].

Most butyrate producers are able to degrade complex polysaccharides towards monosaccharides

and glucose. For example, *Roseburia intestinalis* is a good colon xylan-degrading Firmicutes [124]. *Roseburia* is also a good producer of amylase for starch degradation, and some species can also degrade fructans as inulin, as in the case of *R. inulinivorans* and *F. prausnitzii* [125]. Fucose can be also a good metabolic substrate for fermentation by *R. inulinivorans*, in this case leading towards propionate formation instead of butyrate [126].

From these initial monomeric hexoses and pentoses, glycolysis pathway generates pyruvate, which is the substrate of pyruvate dehydrogenase decarboxylation, rendering acetyl-CoA [127]. Acetyl-CoA is then transformed into butyryl-CoA using diverse intermediates (acetoacetyl-CoA, 3-hydroxy-butyryl-CoA and crotonyl-CoA) [110,128]. Starting from this acetyl-CoA, two main catabolic pathways, with no phylogenetic relationship, are involved in butyrate generation from prebiotic compounds in colon bacterial species. The most common one is the route using butyryl-CoA:acetyl-CoA transferase (BUT) as the final enzyme, generating acetyl-CoA and butyrate from acetate and butyryl-CoA precursors. This enzyme is present in many families belonging to the phylum Firmicutes, as *Roseburia* spp., *Anaerostipes* spp. (family *Lachnospiraceae*), *Eubacterium.rectale, Eu. hallii* (family *Eubacteriaceae*) and *F. prausnitzii* (family *Ruminococcaceae*). Also, other phyla share this pathway for butyrate production, as Actinobacteria, Bacteroidetes, Fusobacteria, Proteobacteria, Spirochaetes and Thermotogae [129–133].

An alternative pathway uses as final step for production of butyrate the enzymes phosphotransbutyrylase (PTB) for converting butyryl-CoA in butyrylphosphate and butyrate kinase (BUK) for transforming this in butyrate. However, this second pathway is very uncommon in human colon microbiota species, and has been identified for example in the Firmicutes bacteria *Eu. ruminantium* (family *Eubacteriaceae*), *Coprococcus eutactus* (family *Lachnospiraceae*), *Cl. perfringens, Cl. beijerinckii, Cl. acetobutylicum, Cl. butyricum, Cl. eutactus* and *Cl. lituseburense* (family *Clostridiaceae*) [134]. Interestingly, BUT pathway is more common in gut microbiota from omnivores and herbivores, whereas BUK route is more frecuent in carnivores [135]. Also, BUT pathway seems to be more effective under colon conditions, where acetate concentrations are usually high (more than 30 mM) [127].

SCFA biosynthesis is usually a complex phenomenon, as these taxons usually collaborate to degrade insoluble prebiotics towards SCFAs after several rounds of enzymatic transformations, as colon syntrophic consortia. For example, inulin is fermented by *Bifidobacterium,* generating acetate. Then, this SCFA, together with the preexisting endogenous lactate, is used by *Clostridium* cluster XIVa and *Faecalibacterium praustnizii* to produce butyrate. Another example is *Roseburia inulinivorans* and *Faecalibacterium,* which can collaborate to generate butyrate from inulin. Starch is degraded by *Bacteroides* spp., producing acetate and succinate, which is useful for other species as propionate precursor. Xylane is degraded by *Roseburia intestinalis,* producing $H_2$ and $CO_2$, which are converted by *Ruminococcus hydrogenotrophicu*s into acetate [136].

## 5. Prebiotics as Effectors in CRC Prevention

CRC is the most common cancer (35 cases per 100,000 hab) in western populations, and an important cause of death, after coronary heart diseases and lung cancer, followed by breast cancer [137]. Human populations with lower consumption of saturated fat and red meat, and higher levels of fruits and vegetables (good sources of prebiotic fibers leading to SCFA) show lower CRC incidences [138–141]. This has been also proof in animal models for CRC [142], as in murine

models treated with colon mutagens, the number of tumor lesions in colon mucosa decreased in rats fed inulin, most probably due to the production of butyrate [143,144].

CRC initiates in the colonic mucosa crypts, which cover this surface completely. These crypts are tubular invaginations formed by structured cell layers [145]. In the bottom of the crypt, stem cells are in charge of carrying out the processes of continuous cell division. This is necessary to maintain the structure and function of intestinal epithelium. Division of these stem cells gives rise to more stem cells or to already differentiated progenitor cells of the different cell types present in the colon. These progenitor cells are located in the intermediate area of the mucosal crypts, and their division and differentiation gives rise to differentiated cells found at the top of each crypt and the entire surface of the colonic mucosa. If one of these stem cells at the bottom of a crypt suffers mutations towards uncontrolled growth, their descendants will expand throughout the crypt, replacing normal stem cells and the rest of normal progenitor cells. This will eventually lead to an aberrant crypt, then to a microadenoma and to a large adenoma (polyp); and finally to an adenocarcinoma with metastasis. The entire development process can take over 30 years [146,147].

The most frequent mutations associated with CRC are those affecting cytoplasmic levels of β-catenin, a protein that normally is bound to E-cadherin in complexes. Free cytosolic β-catenin gets bound to ubiquitin in the Wnt-APC complex and is degraded by the proteasome. Defects in APC (very common in CRC cells) cause an increase in free cytosolic β-catenin, which is translocated to the nucleus, where it binds to transcription factors, activating cell proliferation, maintenance the pluripotency of cancer stem cells, increased motility and metastatic capacity [102]. The *apc* gene is mutated in many familial versions of CRC, as in FAP (familial adenomatous polyposis), and also is an acquired mutation in advanced steps of CRC transformation. Mutations in Mismatch Repair System genes (MLH, MSH2, MSH6 and PMS2) are present in patients with Lynch Syndrome, another familial version of CRC [148].

A 33% of CRC cells possess mutations causing permanent KRAS activation. This membrane GTPase therefore get independent from upstream growth factors signaling pathways and activates constantly other downstream proteins involved in cell growth, cycle progression at the G1/S phase, metabolic changes towards anabolism, angiogenesis development, cell immortalization and metastasis [149,150].

50%–80% of CRC cells show overexpression in MET (the hepatocyte growth factor receptor), which then activates constitutively other cell signaling pathways involved in motility and metastasis (as MAPK and PI3K-AKT-mTOR pathways) [151]. Other membrane receptors, as the human epithelial growth factor receptors HER1, HER3 and HER4 are overexpressed in 35%–89% of CRC cells, giving rise to poor prognosis, lower survival and metastasis respectively. Their activation in CRC induces also MAPK and PI3K-AKT-mTOR cell multiplication pathways [152,153,154].

Several studies suggest that butyrate is able to modulate the activity of NFkB (and some cytokines as TNF-α), reducing the severity of colon inflammation associated to these transcription factor [155]. Butyrate inhibits specifically NFkB translocation to nucleus (which usually takes place once TNF-α activates it). This exerts a potent anti-inflammatory effect. A low inflammatory status has been also associated to lower risk of CRC in several human studies [156,157,158].

In CRC cells, several anti-apoptotic factors, as BCL2A1, block BAK, inhibiting the apoptosis cascade; butyrate is also an inhibitor of BCL2A1 and other relatives [159]. In addition, CRC cells treated *in vitro* with butyrate overexpressed enzymes involved in the defense against genotoxic and mutagenic agents, which involves a protective effect of butyrate also at the level of detoxifying

enzymes [160,161].

At the energetic metabolism level, butyrate is the main energy source for normal colonocytes, accounting for about 70% of all energy intake. At low concentrations and under reduced cytoplasmic levels of glucose and pyruvate (as in the crypts) butyrate acts as a normal growth and promoter for colonocytes [162,163]. In normal colonocytes, this intracellular butyrate concentration is maintained low because it is rapidly processed by β-oxidation in the mitochondria, as the preferred energy source.

However, in CRC cells, glucose is the main energy source. In these tumor cells, glucose uptake is increased about 10 times, due to overexpression of GLUT transporters and the Warburg effect taking place as in most of cancers. These cancer cells showing Warburg effect have increased levels of cytosol glycolysis, rendering huge amounts of cytosol pyruvate, which, instead of following the canonical aerobic oxidation process in mitochondria (Krebs cycle) is fermented anaerobically in cytosol to produce large amounts of lactate. This excess of glucose in CRC cells displaces butyrate as the main source of energy, and being butyrate accumulated in the CRC cell nucleus, causing histones hyperacetylation (as butyrate is a strong histone deacetylases (HDAC) inhibitor) and leading towards apoptosis induction, which proceeds *via* the intrinsic/mitochondrial pathway [163–167]. Histones acetylation is one of the main regulatory mechanisms to modulate genetic expression, not only in colonocytes but in other cell types. This acetylation alters the accessibility to DNA transcription, which is important in the processes of tumor formation [168]. Histone acetylation levels are dependent on histone acetyltransferases (HAT) and HDAC. Acetylation of histones H3 and H4 neutralizes the positive charges in their lysine residues and disrupts the nucleosome structure, enabling DNA unfolding and a more relaxed chromatin structure; allowing access of transcription factors and activation of pro-apoptotic genes [169,170]. Interestingly, HDAC inhibitors, as butyrate, appear to be selective within the regions in which gene expression is altered; as in CRC cells [171].

Histone acetylation by butyrate (due to this nuclear HDAC inhibition) causes an increase in p21 expression. p21 is an inhibitor of cyclin dependent kinases (CDKI) that plays a crucial role in cell cycle arrest at the G1/S phase in transformed cells and prevents tumor progression [172]. At the same time, the pro-apoptotic protein BAK is upregulated in the presence of high butyrate concentrations, and this protein is an essential issue for the apoptotic cascade in CRC cells. Both BAK and p21 upregulation is caused by a less efficient binding of the Sp1 transcription factor to their promoter regions, allowing binding by Sp3 (coupled to HDAC1 and HDAC2) [170]. Sp3 then activates transcription of genes involved in blocking cell cycle at G2/M interphase, and also lnvolved in apoptosis induction (cytochrome C release from mitochondria, formation of APAP-1 complex, caspase 9 activation, etc.) [173,174].

Based on this, a few studies have been carried out in order to demonstrate if prebiotic consumption is able to reduce surrogate markers for CRC in humans. One study analyzed if consumption of prebiotic inulin (6 g, twice per day) caused a reduction in pre-neoplasic lessions in colon mucosa (aberrant crypt foci), but failed demonstrating a statistical reduction in this [175]. However, the European SYNCAN study showed a reduction in DNA damage and in fecal water genotoxicity in patients (which were actually surgically operated of previous colon polyps) receiving a symbiotic (prebiotic inulin plus two probiotics, *B. lactis* Bb12 and *L. rhamnosus* GG) [176]. Also, a reduction in genotoxic bile acids was observed in another human trial using oligofructose during a period of three months [177].

## 6. Conclusions

Dietary intake of prebiotics has a major influence in the healthy status of the consumer. These nutraceuticals can be of plant origin (FOS, inulin, AOS, XOS, raffinose, stachyose, resistant starch), of animal milk origin (GOS, HMO), or generated by enzymatic industrial processes (lactulose, IMO, GlOS). Prebiotics are currently being extensively investigated by using a wide range of *in vitro* and *in vivo* approaches, not only as modulators of gut microbiota but also for their effects in a number of clinical conditions including CRC, intestinal disorders like ulcerative colitis, IBD and irritable bowel syndrome, prevention of obesity and constipation. The action mechanism/s by which these dietary oligosaccharides exert beneficial effects relays on their stimulation of the growth of colon probiotics and beneficial taxons with many anti-inflammatory capacities as well as in their catabolic products, especially butyrate, which is able to carry out a diverse and complex regulation of cell cycle in tumor colonocytes, leading towards their apoptosis.

## Acknowledgments

AC acknowledges support by Junta de Andalucía (AGR2011-7626) and CSIC (i-link0827). FL wishes to thank MINECO (Ministerio de Economía y Competitividad, Grant MINECO-14-RTC-2014-1525-2) and CDTI (Centro para el Desarrollo Tecnológico e Industrial, Grants IDI-20120896 and IDI-20120897) for financial support to JF. We also thank European Union H2020 Program (Grant UE-15-NOMORFILM-634588) for financial support to SRB.

## Conflict of Interest

Authors declare no conflict of interest in this paper.

## References

1. Eckburg PB, Bik EM, Bernstein CN, et al. (2005) Diversity of the human intestinal microbial flora. *Science* 308: 1635–1638.
2. Qin J, Li R, Raes J, et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464: 59–65.
3. Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* 486: 207–214.
4. Corthier G, Dore J (2010) A new era in gut research concerning interactions between microbiota in human health. *Gastroenterologie Clinique Biologique* 34: S1–S6.
5. Flint HJ, Scott KP, Louis O, et al. (2012) The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol* 9: 577–589.
6. Attene-Ramos MS, Wagner ED, Plewa MJ, et al. (2006) Evidence that hydrogen sulfide is a genotoxic agent. *Mol Cancer Res* 4: 9–14.
7. Nyangale EP, Mottram DS, Gibson GR (2012) Gut microbial activity, implications for health and disease: the potential role of metabolite analysis. *J Proteome Res* 11: 5573–5585.
8. Cerf-Bensussan N, Gaboriau-Routhiau V (2010) The immune system and the gut microbiota: friends or foes? *Nat Rev Immunol* 10: 735–744.

9. Sommer F, Backhed F (2013) The gut microbiota-masters of host development and physiology. *Nat Rev Microbiol* 11: 227–238
10. Clemente JC, Ursell LK, Parfrey LW, et al. (2012) The impact of the gut microbiota on human health: an integrative view. *Cell* 148: 1258–1270.
11. Aguirre M, Venema K (2015) The art of targeting gut microbiota for tackling human obesity. *Genes Nutr* 10: 20.
12. Hold GL, Smith M, Grange C, et al. (2014) Role of the gut microbiota in inflammatory bowel disease pathogenesis: What have we learnt in the past 10 years? *World J Gastroenterol* 20: 1192–1210.
13. Wang Z, Klipfell E, Bennett B, et al. (2011) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 472: 57–65.
14. Brusca SB, Abramson SB, Scher JU (2014) Microbiome and mucosal inflammation as extra-articular triggers for rheumatoid arthritis and autoimmunity. *Curr Opin Rheumatol* 26: 101–107.
15. Gao Z, Guo B, Gao R, et al. (2015) Microbiota dysbiosis is associated with colorectal cancer. *Front Microbiol* 6:20.
16. Mima K, Nishihara R, Qian ZR, et al. (2015) Fusobacterium nucleatum in colorectal carcinoma tissue and patient prognosis. *Gut*. pii: gutjnl-2015-310101. doi: 10.1136/gutjnl-2015-310101.
17. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J Nutr* 125: 1401–1412.
18. Koenig JE, Spor A, Scalfone N, et al. (2011) Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A* 108: 4578–4585.
19. Albenberg LG, Wu GD (2014) Diet and the intestinal microbiome: associations, functions, and implications for health and disease. *Gastroenterology* 146: 1564–1572.
20. De Filipo C, Cavalieri D, Di Paola M, et al. (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Nattl Acad Sci U S A* 107: 14691–14696.
21. David LA, Maurice CF, Carmody RN, et al. (2014) Diet rapidly and reproducibly alters the gut microbiome. *Nature* 505: 559–566.
22. Wu GD, Chen J, Hoffmann C, et al. (2011) Linking long-term dietary patterns with gut microbial enterotypes. *Science* 334: 105–108.
23. Simpson HL, Cambell BJ (2015) Review article: Dietary fibre-microbiota interactions. *Aliment Pharmocol Ther* 42: 158–179.
24. Walker AW, Ince J, Duncam SH, et al. (2011) Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J* 5: 220–230.
25. Bouhnik Y, Raskine L, Vicaut E, et al. (2004) The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans. A double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. *Am J Clin Nutr* 80: 1658–1664.
26. Saito Y, Hamanaka Y, Saito K, et al. (2002) Stability of species composition of fecal bifidobacteria in human subjects during fermented milk administration. *Curr Microbiol* 44:368–373.
27. Muegge BD, Kuczynski J, Knights D, et al. (2011) Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* 332: 970–974.
28. Sonnenburg ED, Sonnenburg JL (2014) Starving our microbial self: the deleterious consequences

of a diet deficient in microbiota-accessible carbohydrates. *Cell Metab* 20: 779–786.
29. Roberfroid M, Gibson GR, Hoyles L, et al. (2010) Prebiotic effects: metabolic and health benefits. *Br J Nutr* 104: S1–S63.
30. Gibson GR (2004) From probiotics to prebiotics and a healthy digestive system. *J Food Sci* 69: 141–143.
31. Gibson GR, Scott KP, Rastall RA, et al. (2010) Dietary prebiotics: current status and new definition. *Food Sci Technol Bull* 7: 1–19.
32. Bindels LB, Delzenne NM, Cani PD, et al. (2015) Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol* 12: 303–310.
33. Guarner F, Khan AG, Garish J, et al. (2012) World Gastroenterology Organization Global Guidelines Probiotics and prebiotics. *J Clin Gastroenterol* 46: 468–481.
34. Bindels LB, Porporato P, Dewulf EM, et al. (2012) Gut microbiota-derived propionate reduces cancer cell proliferation in the liver. *Br J Cancer* 107: 1337–1344.
35. Clemente A (2014) *In vivo* assessment of the bioactivity of food oligosaccharides. *In*: Moreno FJ, Sanz ML, editors. Wiley Blackwell, Food oligosaccharides: production, analysis and bioactivity; pp 238–254.
36. Charalampopoulos D, Rastall RA (2012) Prebiotics in foods. *Curr Opin Biotechnol* 23: 187–191.
37. Corzo N, Alonso JL, Azpiroz F, et al. (2015) Prebiotics: concept, properties and beneficial effects. *Nutr Hosp* 31: 99–118.
38. Rastall RA (2010) Functional oligosaccharides: application and manufacture. *Annu Rev Food Sci Technol* 1: 305–339.
39. Vijn I, Smeekens S (1999) Fructan: more than a reserve carbohydrate? *Plant Physiol* 120: 351–60.
40. Comparot-Moss S, Denyer K (2009) The evolution of the starch biosynthetic pathway in cereals and other grasses. *J Exp Bot* 60: 2481–2492.
41. Gallardo K, Thompson R, Burstin J (2008) Reserve accumulation in legume seeds. *C R Biol* 331: 755–762.
42. Bahaji A, Li J, Sánchez-López ÁM, et al. (2014) Starch biosynthesis, its regulation and biotechnological approaches to improve crop yields. *Biotechnol Adv* 32: 87–106.
43. Ao Z, Simsek S, Zhang G, et al. (2007) Starch with a slow digestion property produced by altering its chain length, branch density, and crystalline structure. *J Agric Food Chem* 55: 4540–45547.
44. Galand G (1989) Brush border membrane sucrase-isomaltase, maltase-glucoamylase and trehalase in mammals. Comparative development, effects of glucocorticoids, molecular mechanisms, and phylogenetic implications. *Comp Biochem Physiol B* 94: 1–11.
45. Cairns AJ (2003) Fructan biosynthesis in transgenic plants. *J Exp Bot* 54: 549–567.
46. Hernandez-Hernandez O, Marín-Manzano MC, Rubio L, et al. (2012). Monomer and linkage type of galacto-oligosaccharides affect their resistance to ileal digestion and prebiotic properties in rats. *J Nutr* 142: 1232–1239.
47. Chi ZM, Zhang T, Cao TS, et al. (2011). Biotechnological potential of inulin for bioprocesses. *Bioresour Technol* 102: 4295–4303.
48. Ernst MK, Chatterton NJ, Harrison PA, et al. (1998) Characterization of fructan oligomers from species of the genus *Allium* L. *J Plant Physiol* 153: 53–60.
49. Roberfroid MB, Van Loo JAE, Gibson GR (1998) The bifidogenic nature of chicory inulin and

its hydrolysis products. *J Nutrition* 128: 11–19.
50. Yun JW (1996) Fructooligosaccharides—occurrence, preparation, and application. *Enzyme Microbial Technol* 19: 107–117.
51. Macfarlane GT, Steed H, Macfarlane S (2008) Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *J Appl Microbiol* 104: 305–344.
52. Al-Tamimi MAHM, Palframan RJ, Cooper JM, et al. (2006) In vitro fermentation of sugar beet arabinan and arabinooligosaccharides by the human gut microflora. *J Appl Microbiol* 100: 407–414.
53. Hotchkiss AT, Nunez A, Rastall RA, et al. (2010) Growth promotion of beneficial bacteria in gut of human comprises administering composition comprising arabino oligosaccharide as prebiotic. U.S. patent 2010316766–A1.
54. Gómez B, Miguez B, Veiga A, et al. (2015) Production, purification and in vitro evaluation of the prebiotic potential of arabinoxylooligosaccharides from brewer´s spent grain. *J Agric Food Chem* [In press].
55. Palframan RJ, Gibson GR, Rastall RA (2002) Effect of pH and dose on the growth of gut bacteria on prebiotic carbohydrates in vitro. *Anaerobe* 8: 287–292.
56. Rycroft CE, Jones MR, Gibson GR, et al. (2001) A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *J Appl Microbiol* 91: 878–887.
57. Sanz ML, Gibson GR, Rastall RA (2005) Influence of disaccharide structure on prebiotic selectivity in vitro. *J Agric Food Chem* 53: 5192–5199.
58. Sulek K, Vigsnaes LK, Schmidt LR, et al. (2014) A combined metabolomic and phylogenetic study reveals putatively prebiotic effects of high molecular weight arabino-oligosaccharides when assessed by in vitro fermentation in bacterial communities derived from humans. *Anaerobe* 28: 68–77.
59. Westphal Y1, Kühnel S, de Waard P, et al. (2010) Branched arabino-oligosaccharides isolated from sugar beet arabinan. *Carbohydr Res* 345: 1180–1189.
60. Thomassen LV, Vigsnaes LK, Licht TR, et al. (2011) Maximal release of highly bifidogenic soluble dietary fibers from industrial potato pulp by minimal enzymatic treatment. *Appl Microbiol Biotechnol* 90: 873–884.
61. Vigsnæs LK, Holck J, Meyer AS, et al. (2011) In Vitro Fermentation of Sugar Beet Arabino-Oligosaccharides by Fecal Microbiota Obtained from Patients with Ulcerative Colitis To Selectively Stimulate the Growth of Bifidobacterium spp. and Lactobacillus spp. *Appl Env Microbiol* 77: 8336–8344.
62. Chung Y, Hsu C, Ko C, et al. (2007). Dietary intake of xylooligosaccharides improves the intestinal microbiota, fecal Moisture, and pH Value in the elderly. *Nutr Res* 27: 756–761.
63. Lecerf JM, Depeint F, Clerc E, et al. (2012) Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. *Br J Nutr* 108: 1847–1858.
64. Sheu WHH, Lee IT, Chen W, et al. (2008) Effects of xylooligosaccharides in type 2 diabetes mellitus. *J Nutr Sci Vitaminol* 54: 396–401.
65. Koga Y, Shibuta T, O'Brien R (1993) Soybean oligosaccharides. In Oligosaccharides. Production, properties and applications. Japan. *Technol. Rev. Section E*: Biotechnology vol. 3, No 2. Nakakuki T Ed; Gordon and Breach Science Publishers: Switzerland, pp. 175–203
66. Li T, Lu X, Yang X (2013) Stachyose-enriched α-galacto-oligosaccharides regulate gut microbiota

and relieve constipation in mice. *J Agric Food Chem* 61: 11825–11831.
67. Ao Z, Quezada-Calvillo R, Sim L, et al. (2007) Evidence of native starch degradation with human small intestinal maltase-glucoamylase (recombinant). *FEBS Lett* 581: 2381–2388.
68. Cardelle-Cobas A, Olano A, Corzo N, et al. (2012) *In vitro* fermentation of lactulose-derived oligosaccharides by mixed fecal microbiota. *J Agric Food Chem* 60: 20124–2032.
69. Marín-Manzano MC, Abecia L, Hernandez-Hernandez O, et al. (2013) Galacto-oligosaccharides derived from lactulose exert a selective stimulation on the growth of *Bifidobacterium animalis* in the large intestine of growing rats. *J Agric Food Chem* 61: 7560–7567.
70. Georgi G, Bartke N, Wiens F, et al. (2013) Functional glycans and glycoconjugates in human milk. *Am J Clin Nutr* 98: 578S–85S.
71. Li M, Bauer LL, Chen X, et al. (2012). Microbial composition and in vitro fermentation patterns of human milk oligosaccharides and prebiotics differ between formula-fed and sow-reared piglets. *J Nutr* 142: 681–689.
72. Barile D, Rastall RA (2013) Human milk and related oligosaccharidesas prebiotics. *Curr Opin Biotechnol* 24: 214–219.
73. Ruiz-Moyano S, Totten SM, Garrido DA, et al. (2013) Variation in consumption of human milk oligosaccharides by infant gut-associated strains of Bifidobacterium breve. *Appl Environ Microbiol* 79: 6040–6049.
74. Martin-Ortiz A, Barile D, Salcedo J, et al. (2015) Characterization of goat colostrum oligosaccharides by nano-liquid chromatography on chip quadrupole time of flight mass spectrometry and hydrophilic interaction liquid chromatography-quadrupole mass spectrometry. *J Chrom A* [in press].
75. Lara-Villoslada F, Debras E, Nieto A, et al. (2006) Oligosaccharides isolated from goat milk reduce intestinal inflammation in a rat model of dextran sodium sulfate-induced colitis. *Clin Nutr* 25: 477–488.
76. Padilla B, Frau F, Ruiz-Matute AI, et al. (2015). Production of lactulose oligosaccharides by isomerisation of transgalactosylated cheese whey permeate obtained by β-galactosidases from dairy Kluyveromyces. *J Dairy Res* 82: 356–364.
77. Panesar PS, Kumari S (2011) Lactulose: Production, purification and potential applications. *Biotechnol Adv* 29: 940–948.
78. Olano A, Corzo N (2009) Lactulose as a food ingredient. *J Sci Food Agric* 89: 1987–1990.
79. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2010) Scientific opinion on the substantiation of healthclaims related to lactulose and decreasing potentially pathogenicgastro-intestinal microorganisms (ID 806) and reductionin intestinal transit time (ID 807) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA J* 8: 1806–1821.
80. Schumann C (2002) Medical, nutritional and technological properties of lactulose. An update. *Eur J Nutr* 41:17–25.
81. Foster KJ, Lin S, Turck CJ (2010) Current and emerging strategies for treating hepatic encephalopathy. *Crit Care Nurs Clin North Am* 22: 341–350.
82. Sussman NL (2015) Treatment of Overt Hepatic Encephalopathy. *Clin Liver Dis* 19: 551–563.
83. Kaneko T, Yokoyama A, Suzuki M (1995) Digestibility characteristics of isomalto-oligosaccharides in comparison with several saccharides using the rat jejunum loop method. *Biosci Biotechnol Biochem* 9: 1190–1194.
84. Hu Y, Ketabi A, Buchko A, et al. (2013) Metabolism of isomalto-oligosaccharides by

Lactobacillus reuteri and bifidobacteria. *Lett Appl Microbiol* 57: 108–114.
85. Ketabi A, Dieleman LA, Gänzle MG (2011) Influence of isomalto-oligosaccharides on intestinal microbiota in rats. *J Appl Microbiol* 110: 1297–1306.
86. Yen CH, Tseng YH, Kuo YW, et al. (2011) Long-term supplementation of isomalto-oligosaccharides improved colonic microflora profile, bowel function, and blood cholesterol levels in constipated elderly people-a placebo-controlled, diet-controlled trial. *Nutrition* 17: 445–450.
87. Wang HF, Lim P-S, Kao MD, et al. (2011) Use of isomalto-oligosaccharide in the treatment of lipid profiles and constipation in hemodialysis patients. *J Renal Nutr* 11: 73–79.
88. Mizubuchi H, Yajima T, Aoi N, et al. (2005) Isomalto-Oligosaccharides Polarize Th1-Like Responses in Intestinal and Systemic Immunity in Mice. *J Nutr* 20: 2857–2861.
89. Valette P, Pelenc V, Djouzi Z, et al. (1993) Bioavailability of new synthesised glucooligosaccharides in the intestinal tract of gnotobiotic rats. *J Sci Food Agricult* 62:121–127.
90. Chung CH, Day DF (2002) Glucooligosaccharides from Leuconostoc mesenteroides B-742 (ATCC 13146): a potential prebiotic. *J Indus Microbiol Biotechnol* 29: 196–199.
91. Sarbini SR, Kolida S, Gibson GR, et al. (2013) In vitro fermentation of commercial α-gluco-oligosaccharide by faecal microbiota from lean and obese human subjects. *Bri J Nutr* 109: 1980–1989
92. Wichienchot S, Prasertsan P, Hongpattarakere T, et al. (2006) In vitro Fermentation of Mixed Linkage Glucooligosaccharides Produced by Gluconobacteroxydans NCIMB 4943 by the Human Colonic Microflora. *Curr Issues Intestinal Microbiol* 7: 7–12.
93. Gibson GR, Beatty ER, Wang X, et al. (1995) Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 108: 975–982.
94. Bouhnik Y, Achour L, Paineau D, et al. (2007) Four-week short chain fructo-oligosaccharides ingestion leads to increasing fecal bifidobacteria and cholesterol excretion in healthy elderly volunteers. *Nutrition* 6: 42–46.
95. Depeint F, Tzortzis G, Vulevic J, et al. (2008) Prebiotic evaluation of a novel galactooligosaccharide mixture produced by the enzymatic activity of Bifidobacterium bifidum NCIMB 41171, in healthy humans: a randomized, double-blind, crossover, placebo-controlled intervention study. *Am J Clin Nutr* 87: 785–791.
96. Kleessen B, Sykura B, Zunft HJ, et al. (1997) Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. *Am J Clin Nutr* 65: 1397–1402.
97. Kruse HP, Kleessen B, Blaut M (1999) Effects of inulin on faecal bifidobacteria in human subjects. *Br J Nutr* 82: 375–382.
98. Ladirat SE, Schoterman MH, Rahaoui H, et al. (2014) Exploring the effects of galacto-oligosaccharides on the gut microbiota of healthy adults receiving amoxicillin treatment. *Br J Nutr* 112: 536–546.
99. Petry N, Egli I, Chassard C, et al. (2012) Inulin modifies the bifidobacteria population, fecal lactate concentration, and fecal pH but does not influence iron absorption in women with low iron status. *Am J Clin Nutr* 96: 325–331.
100. Tuohy KM, Kolida S, Lustenberger AM, et al. (2001) The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides – a human volunteer study. *Br J Nutr* 86: 341–348.
101. Bäckhed F, Ding H, Wang T, et al. (2004) The gut microbiota as an environmental factor that

regulates fat storage. *Proc Natl Acad Sci U S A* 101: 15718–15723.
102. Tenbaum SP, Ordóñez-Morán P, Puig I, et al. (2012) β-catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. *Nat Med* 18: 892–901.
103. Leenen CH, Dieleman LA (2007) Inulin and oligofructose in chronic inflammatory bowel disease. *J Nutr* 137: 2572S–2575S.
104. Puertollano E, Kolida S, Yaqoob P (2014) Biological significance of short-chain fatty acid metabolism by the intestinal microbiome. *Curr Opin Clin Nutr Metab Care* 17: 139–144.
105. Canavan C, Abrams KR, Mayberry J (2006) Meta-analysis: colorectal and small bowel cancer risk in patients with Crohn's disease. *Aliment Pharmacol Ther* 23: 1097–1104.
106. Lindsay JO, Whelan K, Stagg AJ, et al. (2006) Clinical, microbiological and immunological effects of fructooligosaccharide in patients with Crohn's disease. *Gut* 55: 348–355.
107. Candela M, Turroni S, Biagi E, et al. (2014) Inflammation and colorectal cancer, when microbiota-host mutualism breaks. *World J Gastroenterol* 20: 908–922.
108. Silk DB, Davis A, Vulevic J, et al. (2009) Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharmacol Ther* 29: 508–518.
109. Whelan K (2011) Probiotics and prebiotics in the management of irritable bowel syndrome: a review of recent clinical trials and systematic reviews. *Curr Opin Clin Nutr Metab Care* 14: 581–587.
110. Louis P, Hold GL, Flint HJ (2014) The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol*12: 661–672.
111. Scanlan PD, Shanahan F, Clune Y, et al. (2008) Culture-independent analysis of the gut microbiota in colorectal cancer and polyposis. *Environ Microbiol* 10: 789–798.
112. Marchesi JR, Dutilh BE, Hall N, et al. (2011) Towards the human colorectal cancer microbiome. *PLoS One* 6: e20447. doi: 10.1371/journal.pone.0020447.
113. Shen XJ, Rawls JF, Randall T, et al. (2010) Molecular characterization of mucosal adherent bacteria and associations with colorectal adenomas. *Gut Microbes* 1: 138–147. doi: 10.4161/gmic.1.3.12360.
114. Sobhani I, Tap J, Roudot-Thoraval F, et al. (2011) Microbial dysbiosis in colorectal cancer (CRC) patients. *PLoS One* 6: e16393.
115. Moore WE, Moore LH (1995) Intestinal floras of populations that have a high risk of colorectal cancer. *Appl Environ Microbiol* 61: 3202–3207.
116. Dewulf EM, Cani PD, Claus SP, et al. (2013) Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* 62: 1112–1121.
117. Bosscher D, Breynaert A, Pieters L, et al. (2009). Food-based strategies to modulate the composition of the intestinal microbiota and their associated health effects. *J Physiol Pharmacol* 60(Suppl 6):5–11.
118. Roberfroid MB (2007) Inulin-type fructans: functional food ingredients. *J Nutr* 137: 2493S–2502S.
119. Canfora EE, Jocken JW, Blaak EE (2015) Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 11: 5775–5791.
120. Hamer HM, Jonkers D, Venema K, et al. (2008). Review article: the role of butyrate on colonic

function. *Aliment Pharmacol Ther* 27: 104–119.
121. Wong JM, de Souza R, Kendall CW, et al. (2006) Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* 40: 235–243.
122. Tilg H, Moschen AR (2014) Microbiota and diabetes: an evolving relationship. *Gut* 63: 1513–1521
123. Wagner NM, Brandhorst G, Czepluch F, et al. (2013) Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk**.** *Obesity Silver Spring* 21: 461–468.
124. Duncan SH, Aminov RI, Scott KP, et al. (2006) Proposal of Roseburia faecis sp. nov., Roseburia hominis sp. nov., and Roseburia inulinivorans sp. nov., based on isolates from human faeces. *Int J Syst Evol Micr* 56: 2437–2441.
125. Duncan SH, Hold GL, Harmsen HJM, et al. (2002) Growth requirements and fermentation products of Fusobacterium prausnitzii, and a proposal to reclassify it as Faecalibacterium prausnitzii gen nov., comb. nov. *Int J Syst Evol Micr* 52: 2141–2146.
126. Scott KP, Martin JC, Campbell G, et al. (2006) Whole-genome transcription profiling reveals genes upregulated by growth on fucose in the human gut bacterium Roseburia inulinivorans. *J Bacteriol* 188: 4340–4349.
127. Louis P, Flint HJ (2009) Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett* 294: 1–8.
128. Louis P, Flint HJ (2007) Development of a semi quantitative degenerate real-time PCR-based assay for estimation of numbers of butyryl-coenzyme A (CoA) CoA transferase genes in complex bacterial samples. *Appl Environ Microb* 73: 2009–2012.
129. Barcenilla A, Pryde SE, Martin JC, et al. (2000) Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol* 66: 1654–1661.
130. Duncan SH, Flint HJ (2008) Proposal of a neotype strain (A1-86) for Eubacterium rectale. Request for an opinion. *Int J Syst Evol Microbiol* 58:1735–1736.
131. Louis P, Young P, Holtrop G, et al. (2010) Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. *Environ Microbiol* 12: 304–314.
132. Vital M, Howe AC, Tiedje JM (2014) Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *MBio* 5: e00889.
133. Vital M, Gao J, Rizzo M, et al. (2015) Diet is a major factor governing the fecal butyrate-producing community structure across Mammalia, Aves and Reptilia. *ISME J* 9: 832–843.
134. Louis P, Duncan SH, McCrae SI, et al. (2004) Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. *J Bacteriol* 186: 2099–2106.
135. Vital M, Penton CR, Wang Q, et al. (2013) A gene-targeted approach to investigate the intestinal butyrate-producing bacterial community. *Microbiome* 1: 8.
136. Candela M, Maccaferri S, Turroni S, et al. (2010). Functional intestinal microbiome, new frontiers in prebiotic design. *Int J Food Microbiol* 140: 93–101.
137. Jemal A, Bray F, Center MM, et al. (2011) Global cancer statistics. *CA Cancer J Clin* 61: 69–90.
138. Hester CM, Jala VR, Langille MG, et al. (2015) Fecal microbes, short chain fatty acids, and colorectal cancer across racial/ethnic groups. *World J Gastroenterol* 21: 2759–2769.
139. Kim YS, Milner JA (2007) Dietary modulation of colon cancer risk. *J Nutr* 37: 2576S–2579S.

140. Le Leu RK, Winter JM, Christophersen CT, et al. (2015) Butyrylated starch intake can prevent red meat-induced O6-methyl-2-deoxyguanosine adductsin human rectal tissue: a randomised clinical trial. *Br J Nutr* 114: 220–230.
141. Winter J, Nyskohus L, Young GP, et al. (2011) Inhibition by resistant starch of red meat-induced promutagenic adducts in mouse colon. *Cancer Prev Res (Phila)* 4: 1920–1928.
142. Conlon MA, Kerr CA, McSweeney CS, et al. (2012) Resistant starches protect against colonic DNA damage and alter microbiota and geneexpression in rats fed a Western diet. *J Nutr* 142: 832–840.
143. Roller M, Femia AP, Caderni G, et al. (2004) Intestinal immunity of rats with colon cancer is modulated by oligofructose-enriched inulin combined with Lactobacillus rhamnosus and Bifidobacterium lactis. *Brit J Nutr* 92: 931–938.
144. Verghese M, Walker LT, Shackelford L, et al. (2005) Inhibitory effects of nondigestible carbohydrates of different chain lengths on azoxymethane-induced aberrant crypt foci in Fisher 344 rats. *Nutr Res* 25: 859–868.
145. Humphries A, Wright NA (2008) Colonic crypt organization and tumorigenesis. *Nat Rev Cancer* 8: 415–424.
146. Bruce WR, Corpet DE (1996) The colonic protein fermentation and insulin resistance hypotheses for colon cancer etiology: experimental tests using precursor lesions. *Eur J Cancer Prev* 2: 41–47.
147. Kinzler KW, Vogelstein B (1996) Lessons from hereditary colorectal cancer. *Cell* 87: 159–170.
148. Losi L, Di Gregorio C, Pedroni M, et al. (2005) Molecular genetic alterations and clinical features in early-onset colorectal carcinomas and their role for the recognition of hereditary cancer syndromes. *Am J Gastroenterol* 100: 2280–2287.
149. De Roock W, Claes B, Bernasconi D, et al. (2010) Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 11: 753–762.
150. Ying H, Kimmelman AC, Lyssiotis CA, et al. (2012) Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell* 149: 656–670.
151. Vermeulen L, De Sousa E, Melo F, et al. (2010) Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 12: 468–476.
152. Beji A, Horst D, Engel J, et al. (2012) Toward the prognostic significance and therapeutic potential of HER3 receptor tyrosine kinase in human colon cancer. *Clin Cancer Res* 18: 956–968.
153. Kountourakis P, Pavlakis K, Psyrri A, et al. (2006) Prognostic significance of HER3 and HER4 protein expression in colorectal adenocarcinomas. *BMC Cancer* 6: 46.
154. Montagut C, Dalmases A, Bellosillo B, et al. (2012) Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. *Nat Med* 18: 221–223.
155. Kanauchi O, Serizawa I, Araki Y, et al. (2003) Germinated barley foodstuff, a prebiotic product, ameliorates inflammation of colitis through modulation of the enteric environment. *J Gastroenterol* 38: 134–141.
156. Hofmanová J, Straková N, Vaculová AH, et al. (2014) Interaction of dietary fatty acids with tumour necrosis factor family cytokines during colon inflammation and cancer. *Mediators Inflamm* 2014: 848632.
157. Inan MS, Rasoulpour RJ, Yin L, et al. (2000). The luminal short-chain fatty acid butyrate modulates NF-kappaB activity in a human colonic epithelial cell line. *Gastroenterology* 118:

724–734
158. Izano M, Wei EK, Tai C, et al. (2015) Chronic inflammation and risk of colorectal and other obesity-related cancers: The health, aging and body composition study. *Int J Cancer*.
159. Campos-Vega R, Guevara-González RG, Guevara-Olvera BL, et al. (2010). Bean (Phaseolus vulgaris L.) polysaccharides modulate gene expression in human colon cancer cells (HT-29). *Food Research International* 43: 1057–1064.
160. Scharlau D, Borowicki A, Habermann N, et al. (2009) Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. *Mutat Res* 682: 39–53.
161. Stein K, Borowicki A, Scharlau D, et al. (2011) Effects of synbiotic fermentation products on primary chemoprevention in human colon cells. *J Nutr Biochem* 23: 777–784.
162. Cruz-Bravo RK, Guevara-González RG, Ramos-Gómez M, et al. (2014) The fermented non-digestible fraction of common bean (Phaseolus vulgaris L.) triggers cellcycle arrest and apoptosis in human colon adenocarcinoma cells. *Genes Nutr* 9: 359.
163. Zhang J, Wu G, Chapkin RS, et al. (1998) Energy metabolism of rat colonocytes changes during the tumorigenic process and is dependent on diet and carcinogen. *J Nutr* 128: 1262–1269.
164. Donohoe DR, Collins LB, Wali A, et al. (2012) The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. *Mol Cell* 48: 612–626.
165. Gatenby RA, Gillies RJ (2004) Why do cancers have high aerobic glycolysis? *Nat Rev Cancer* 4: 891–899.
166. Heerdt BG, Houston MA, Augenlicht LH (1997) Short-chain fatty acid-initiated cell cycle arrest and apoptosis of colonic epithelial cells is linked to mitochondrial function. *Cell Growth Differ* 8: 523–532.
167. Singh B, Halestrap AP, Paraskeva C (1997) Butyrate can act as a stimulator of growth or inducer of apoptosis in human colonic epithelial cell linesdepending on the presence of alternative energy sources. *Carcinogenesis* 18: 1265–1270.
168. Archerand SY, Hodint RA (1999) Histone acetylation and cancer. *Curr Opin Genet Dev* 9: 171–174.
169. Struhl K (1998) Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev* 12: 599–606.
170. Wilson AJ, Chueh AC, Tögel L, et al. (2010) Apoptotic sensitivity of colon cancer cells to histone deacetylase inhibitors is mediated by anSp1/Sp3-activated transcriptional program involving immediate-early gene induction. *Cancer Res* 70: 609–620.
171. Van Lint C, Emiliani S, Verdin E (1996) The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. *Gene Expr* 5: 245–253.
172. Chen YX, Fang JY, Zhu HY, et al. (2004) Histone acetylation regulates p21WAF1 expression in human colon cancer cell lines. *World J Gastroenterol* 10: 2643–2646.
173. Ruemmele FM, Schwartz S, Seidman EG, et al. (2003) Butyrate induced Caco-2 cell apoptosis is mediated via the mitochondrial pathway. *Gut* 52: 94–100.
174. Waby JS, Chirakkal H, Yu C, et al. (2010) Sp1 acetylation is associated with loss of DNA binding at promoters associated with cell cyclearrest and cell death in a colon cell line. *Mol Cancer* 9: 275.
175. Limburg PJ, Mahoney MR, Ziegler KL, et al. (2011) Randomized phase II trial of sulindac, atorvastatin, and prebiotic dietary fiber for colorectal cancer chemoprevention. *Cancer Prev Res* 4: 259–269.

176. Pool-Zobel BL, Sauer J (2007) Overview of experimental data on reduction of colorectal cancer risk by inulin-type fructans. *J Nutr* 137: 2580S–2584S.
177. Boutron-Ruault MC, Marteau P, Lavergne-Slove A, et al. (2005) Effects of a 3-mo consumption of short-chain fructo-oligosaccharides on parameters of colorectal carcinogenesis in patients with or without small or large colorectal adenomas. *Nutr Cancer* 53:160–168.


AIMS Press

©2015 Felipe Lombó, et al., licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0)