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The gut microbial metabolome: modulation of cancer risk in obese individuals

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Obesity is a critical health concern and although genetic factors may predispose an individual to become obese, changes in diet and lifestyle over the last few decades are likely to be significant contributors. Even so, it has been suggested that the causes of the current obesity crisis are not simply explained by changes in eating and exercise habits. Evidence suggests that the gut microbiota may play an important role in obesity and may be a factor in the development of associated disease including diabetes, CVD, non-alcoholic fatty liver disease and cancer. There have been tremendous advances in knowledge regarding the composition of human gut microbiota, but less is known about their function and role within the human host. It is becoming widely accepted that the products of microbial metabolism influence human health and disease, particularly with respect to immune response and inflammation. However, in most cases, the products of microbial metabolism are uncharacterised and their mechanism of action remains unknown. This review addresses the role of the metabolites produced by gut microbiota in cancer and obesity. It is clear that only if the link between microbial diversity and metabolic functionality is firmly established, will the mechanism by which gut microbiota maintains health or contributes to disease development be elucidated.

Gut microbiota: Obesity: Inflammation: Anti-inflammatory: Antioxidant

The colonic microbiota is likely to play an important role in health maintenance or progression to diseases such as colorectal cancer (CRC). Furthermore, the gut microbial community has been proposed by a number of studies to play a role in the development of obesity. Diet is a significant driver both for the composition of the gut microbiota[1–3] and for the development of obesity and cancer. This makes it challenging to untangle causative factors that may be due to dietary components, individual gut microbes and the metabolic products of microbial metabolism that are largely derived from dietary components. The diversity of bacterial species in the colon means that collectively they perform an impressive array of metabolic activities, as is evident from both metabolomic and metagenomic analyses[4–6]. There is an increasing awareness that in addition to understanding the composition of the microbiota, their metabolic output will have a profound impact in modulating both gut and systemic health[2,7,8]. Functional analysis of microbial metabolites will therefore be crucial to understanding the impact of diet and of gut micro-organisms on maintenance of health and prevention of disease. This review will focus on the importance of the microbial metabolome in the development of obesity-linked co-morbidities.

The gut microbiota

From birth, the gastrointestinal (GI) tract becomes colonised by a succession of bacterial species and the composition of the microbiota is similar to that of adults from around the time of weaning. In the days following birth, the baby GI tract is primarily colonised by

Abbreviations: CRC, colorectal cancer; GI, gastrointestinal; RS, resistant starch; TLR, Toll-like receptor.
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facultative anaerobes and microaerophiles, followed later by obligate anaerobes. In breast-fed infants, for example, *Bifidobacterium* species generally predominate while appreciable numbers of *Bacteroides* species are only detected after weaning. In early life *Bacteroides* species may persist in the gut largely through their ability to utilise host-derived substrates, followed after weaning by the utilisation of plant-derived polysaccharides from the diet[9].

The adult human large intestine usually contains more than 200 g of contents and is colonised by hundreds of bacterial species, reaching a total cell density of about 10^{11} bacterial cells/ml thereby outnumbering host cells about 10-fold. This complex microbial community also harbours about 100-fold more genes than the human genome[10]. This dense community of bacteria mainly comprise the Gram-negative Bacteroidetes and the Gram-positive Firmicutes and Actinobacteria[2] with the Bacteroidetes accounting for about one-quarter of the microbiota. Most of the remainder belong to the Firmicutes, which mainly comprise the *Lachnospiraceae* and *Ruminococcaceae* families. The next most abundant phylum of Gram-positive bacteria is Actinobacteria that includes the *Bifidobacterium* species[3]. Inter-individual variation is observed for the gut microbiota and may be greater in infants than in adults[11]. Nevertheless, a dominant group of bacterial species has been identified in faecal samples from healthy adult individuals[1,4,12]. The top ten most abundant phylotypes or species make up about 30 % of the faecal microbiota[1,13]. Two of the most dominant species are *Faecalibacterium prausnitzii* and *Eubacterium rectale*, which employ the butyryl CoA: acetate CoA transferase route for butyrate formation[14–16]. These species appear to be present in a majority of individuals although *F. prausnitzii* abundance is diminished in Crohn's sufferers[17,18] and in the elderly[19]. Two other dominant species, *Eubacterium hallii* and new *Anaerostipes* species are butyrate producers that can utilise lactate[15]. Microbiota variation may be a consequence of several factors including acquisition of bacteria at birth[20], host immune responses, antibiotic usage[21] and diet[1,2,22–24].

Methodologies used to study the microbial genome: metagenomics

Large genome programmes including the National Institutes of Health-funded Human Microbiome Project (nihroadmap.nih.gov/hmp/) and the EU funded MetaHIT project (www.metahit.eu/) have sequenced more than fifty *Bacteroides* and *Prevotella* species and many Gram-positive bacterial species. The increasing speed and decreasing cost of sequencing is now making metagenomic analysis of the whole microbial community a popular option[4,11]. A recent metagenomic analysis of faecal samples of volunteers from four countries suggested that the microbiota belonged to three distinct clusters or 'enterotypes'[25]. On the other hand, another study detected only two putative enterotypes among the ninety-eight adults examined, and presented evidence that these were in fact diet-driven[26]. While metagenomic analyses can provide an incredible amount of information, interpretation of the data is very much dependent on information gained from previously isolated and characterised bacteria. Functional screening of metagenomic libraries can be achieved using high-throughput screening assays, as have been employed in other ecosystems such as soil[27].

Methodologies used to study the microbial metabolome: metabolomics

Metabolomics is a comprehensive and non-selective analytical chemistry approach aimed at providing a global description of all the metabolites present in a given biological sample. Although metabolic profiling has been used for decades, modern instrumentation and statistical methodology has found recent application in predicting the outcome of dietary and clinical studies. In particular, metabolomics can prove valuable where the lack of effective biomarkers has made it difficult to establish the long-term implications of intervention, e.g. in outwardly healthy individuals. Human genotyping can give useful information regarding the predisposition to disease and proteomics can provide indicators of disease occurrence. However, metabolites have always been an excellent indicator of human disease, and for this reason they are also likely to be a useful predictor of human health. In addition, metabolomic data provide vital information on the overall function of the gut microbiota. Metabolomic analysis can rely on NMR spectroscopy which can be coupled to liquid chromatography, allowing separation of the metabolites prior to detection. The main advantages of MS over NMR are sensitivity and the ability to perform quantitative and targeted analysis. Recent advances in MS have resulted in robust and powerful methods to study the human metabolome[28,29] and the real potential of MS has been achieved through prefacing to GC–MS and liquid chromatography–MS. The plethora of ionisation techniques (e.g. electron ionisation and chemical ionisation) and wide availability of mass analysers make modern MS analysis an extremely versatile technique[28,29].

Influence of host and dietary factors on the gut metabolome

The composition of the gut microbiota may be is subject to selective pressure from the host and diet, which can alter the ecosystem homoeostasis affecting the abundance of specific groups. Many factors have the potential to influence the microbiome and these could include sex, age, BMI, gut physiology and immune/inflammatory status (Fig. 1). The colonic microbiota is found to be relatively stable in individuals consuming their normal diet but it is evident that dietary change can influence the abundance of specific bacterial groups[1]. There are major compositional differences in the gut microbiota between population groups depending upon staple food intake, as suggested by a study on Italian and African children[30]. The amount and type of the three main macronutrients, carbohydrate, protein and fat, consumed in our diets will impact on the composition of the gut microbiota. The ability of gut microbes to utilise and transform these macro- and

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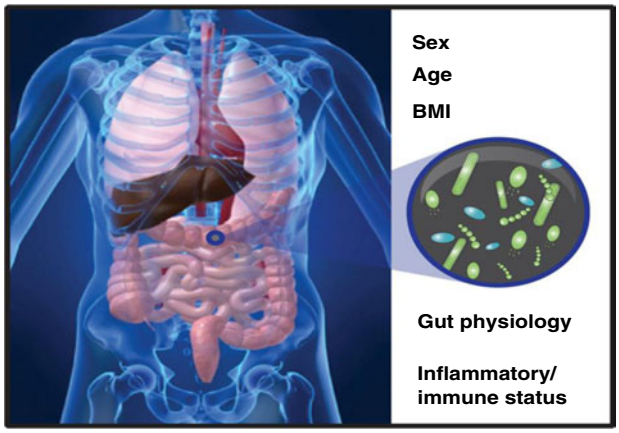

Fig. 1. Host factors with potential to impact on the gut microbiota.

micronutrients, however, has consequences for human health that may be more important than the detailed taxonomic changes in the microbiota.

Macronutrient influences on the gut microbiota

Carbohydrate is critical for the host and is the major energy source for the gut microbiota. The principal carbohydrate sources for individuals consuming a western-style diet are resistant starch (RS) with usually lower amounts of NSP, which are consumed mainly in cereals. In addition, oligosaccharides such as fructans are consumed in foods including onions and artichokes. The extent of dietary starch breakdown in the colon is dependent on a number of factors that include amylose content and state of gelatinisation which is mainly determined by the cooking process[31]. Starch is a complex polysaccharide consisting of a mixture of amylose (1,4-α-linked glucose residues) and amylopectin, a branched polymer composed of amylose chains linked to an amylose backbone by 1,6-α-linkages. The relative proportion of amylose and amylopectin has an effect on the availability of different types of starch for bacterial growth. In a model system, 80% of sequences recovered from starch particles belonged to *Ruminococcus bromii*, *E. rectale* and *Bifidobacterium* species[32].

Moreover, bacteria related to *R. bromii* increased almost two-fold in faecal samples when the volunteers switched from normal diets to starch enriched diets[22]. Faecal populations of the *Roseburia/E. rectale* group[33] have been shown to respond to controlled variations in dietary carbohydrate intake[2,8] that may be explained by a dependence on RS for growth. Another recent study compared the effects of diets supplemented either with a type three RS or a source of NSP (wheat bran) in obese subjects. The groups showing the most significant responses to RS on average were Firmicutes bacteria related to *R. bromii* and *E. rectale*, with the former group increasing 10-fold on average as a percentage of total bacteria. Two of the fourteen individuals showed no detectable ruminococci in faecal samples, and carbohydrate analysis of these samples revealed <40% fermentation of RS compared with >95% in the other subjects[1].

High fibre diets increase faecal bulking, SCFA production and transit rates along the large intestine[34,35]. Faecal butyrate concentrations have been shown to correlate with the abundance of the *Roseburia/E. rectale* group[2] which is likely to be a major contributor to butyrate formation from starch. This group is stimulated at mildly acidic pH *in vitro* largely because of the inhibition of competing *Bacteroides* species[36] suggesting that its preferred niche may be in the proximal colon where rapid fermentation creates mildly acidic conditions. Active fermentation of other fibre sources and prebiotics also tends to decrease pH in the proximal large intestine[37] and these shifts in pH can impact the gut microbial community structure[36,38,39].

There is much interest in modulating microbial metabolism in the intestine through dietary additives such as prebiotics that are defined as 'a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the GI microbiota that confers benefits upon host well being and health'[40]. Current prebiotics are mainly carbohydrates of low digestibility that are found naturally in foodstuffs. Candidate prebiotics include xylo-oligosaccharides and galacto-oligosaccharides[41,42] with most studies focusing on the use of inulin and fructo-ologosaccharides. The high numbers of *Bifidobacterium* species in the faeces of breast-fed babies is thought to result from their ability to utilise oligosaccharides in breast milk, including galacto-oligosaccharides while formula fed infants tend to harbour a greater diversity of organisms including a higher abundance of enterococci[43]. The metabolite profiles also differ with higher levels of NH_3, amines and phenols in bottle fed babies compared with breast-fed infants[44]. Not all oligosaccharides, however, are highly selective[3] and fructans, for example, have been shown to promote one or more groups of bacteria in addition to their effects on bifidobacteria[45–47]. As there is extensive inter-individual variation in composition of the gut microbiota there is likely to be inter-individual variation in the response of the microbial communities to prebiotics[46]. Recent evidence supports the view that there are likely to be detrimental health consequences of reduced carbohydrate intake especially when combined with an increase in the consumption of protein and/or fat, both of which have been potentially associated with an increased risk of colon cancer[8,48].

Many protein- and protein-derived metabolites are considered to be potentially mutagenic and genotoxic and these include heterocyclic amines and N-nitroso compounds[8]. Studies with germ-free animals implied that the gut microbiota was essential in the formation of N-nitroso compounds[49] and the DNA damage by heterocyclic amines was decreased compared with that of conventional animals[50]. Additional by-products of amino acid metabolism include polyamines (including putrescine, cadaverine, spermine, spermidine, pyrollidine and piperidine), indoles, NH_3, hydrogen sulphide, branched chain fatty acids and the aromatic amino acid metabolites. Various species have been shown to produce these products[51,52], but comprehensive screening of the predominant gut species has not been performed.

Significant changes in the gut metabolome were observed for volunteers consuming diets in which the

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protein and carbohydrate ratios were modulated[8]. Using liquid chromatography–MS, derivatives of a wide range of plant phenolics considered to be cancer-protective were reduced in diets high in protein (137 g/d) and low in carbohydrate (22 g/d). Phenylacetic acid, a major metabolite related to protein metabolism was found to have significantly increased. Increasing the carbohydrate content (181 g/d) resulted in significantly increasing some phenolic acids and their derivatives, principally fibre-related phenolics, namely, ferulic acid, 4-hydroxy-3-methoxyphenylpropionic acid and 3-hydroxyphenylpropionic acid.

Bile metabolism, along with lipase activity, are likely to be major factors affecting the delivery of lipids to the colon. Bile acids are predominantly deconjugated and dehydroxylated to secondary bile acids and *Bacteroides intestinalis*, *Bacteroides fragilis* and *Escherichia coli* have been shown to be involved in the production of deoxycholic and lithocholic acid[53]. Two major lipid metabolism pathways have been identified; hydration and reduction. The species responsible for the formation of hydrated lipids (i.e. hydroxy fatty acids) were predominantly *Clostridium perfringens*[54] and *Roseburia* species[55], although some other species could produce these metabolites to a lesser extent[55]. The species catalysing fatty acid reduction are less well characterised; only species responsible for the conversion of linoleic acid to vaccenic acid have been identified, namely *Butyrivibrio fibrisolvens* and *Roseburia* species[56].

Micronutrient influences on the gut microbiota

It has been shown that the microbiota can synthesise some vitamin B (B_3, B_5 and B_6) and related molecules including biotin, tetrahydrofolate, vitamin K and the corrinoids[57]. Microbes are considered to affect absorption of certain dietary minerals, with many studies demonstrating that carbohydrate and associated SCFA modulate uptake of Na, Ca and K. Many bacteria also actively accumulate Fe as a siderophore complex[58]. There is very little information regarding the direct effect of the gut microbiota on mineral uptake and the species associated have not been identified.

Non-nutrient/phytochemical influences on the gut microbiota

Epidemiological studies suggest that there is an inverse association between the intake of phytochemical-rich diets and the incidence of CVD, diabetes and cancer[59]. Edible plant material contains hundreds of compounds most of which play an important role for the plant including protection against pathogens. Biochemically, they can be broadly categorised according to their structure and biosynthetic pathways, but it should be appreciated that many secondary metabolites are derived by combining elements from all of these biosynthetic routes. Some of these compounds particularly if available as small molecules or as their aglycones, may be absorbed in the upper GI tract and directly enter systemic circulation. However, many and in particular those bound to other plant components such as carbohydrates will be available in the colon. Within the colon these phytochemicals are extensively metabolised.

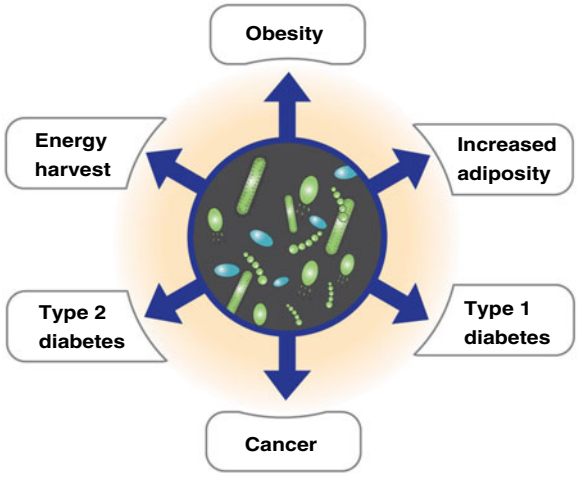

Fig. 2. Potential impact of the gut microbiota on the human host.

Gut microbiota are capable of performing many transformations including: hydrolysis, deamination, dehydrogenation, demethylation, decarboxylation, ring cleavage and chain shortening.

Impact of the gut metabolome on the human host

It is becoming increasingly important to determine which microbial-derived products are responsible for disease development and/or progression (Fig. 2). Once identified these molecules may be used as a potential diagnostic/prognostic tool for inflammatory diseases and related co-morbidities.

SCFA

Active fermentation of carbohydrates in the colon results in the formation of SCFA[60] together with gases, mainly H_2, CO_2 and methane. The SCFA detected in stool samples are a sub group of fatty acids with aliphatic tails with less than six carbons and include fornic, acetic, propionic, butyric and valeric acid. Branched-chain examples include isovaleric and isobutyric acid. There are also substituted short-chain carboxylic acids such as lactic acid. The total concentration of SCFA in the large intestine may reach upwards of 100 mM[61]. Dietary shifts can result in changes in SCFA production rates and in the molar proportions of different SCFA detected in faeces. Weight loss diets that are high in protein but low in carbohydrates, for example, were recently shown to reduce faecal butyrate up to fourfold[2] while higher proportions of propionate and butyrate and lower acetate have been reported to result from increasing prebiotic or fibre intake[62]. SCFA are likely to have several effects upon health. Butyrate is largely considered beneficial for gut health as it is the major energy source for the colonocytes and has a role in CRC prevention as discussed later[63–65]. Propionate is metabolised in the liver and is gluconeogenic. Activation of the gut receptors G protein-coupled receptor (GPR) 41 and GPR43 (also known as NEFA receptors NEFA2 and NEFA3) by SCFA influences gut motility as well as reducing

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inflammatory responses[66,67]. Acetate is metabolised in the peripheral tissues and is a precursor for cholesterol metabolism and lipid formation. A shift in fermentation products away from acetate (normally present at the highest concentration) towards propionate and butyrate may therefore be beneficial and explain the decrease in cholesterol levels in volunteers consuming fibre[68]. Increased SCFA concentrations may also increase the solubility of certain minerals such as Ca, and enhance absorption and expression of Ca-binding proteins[69]. Changes in intestinal microbial metabolism following the consumption of inulin fructans has also been shown to improve bone health by increasing Ca absorption while β-glucans may lower total cholesterol levels[70].

Phytochemicals

There is a vast amount of literature suggesting that plant secondary metabolites have properties beneficial to health[71] In particular, almost all plant foods considered to have cancer-preventative properties are rich in compounds derived from the phenylpropanoid pathway. Most of this information is obtained from *in vitro* data and there is very little evidence from both pre-clinical and human interventions to support this. Bioavailability may be defined as the fraction of an ingested nutrient or metabolite that reaches systemic circulation and specific sites where it can exert specific biological activity. The most abundant phytochemicals consumed may not, however, be the most bioavailable to the host. Bioavailability may be influenced by food composition as most of the phytochemicals are likely to be complexed with other plant components. Host factors will also influence bioavailability including enzyme activity and gut transit time. Following ingestion, the absorption of certain phytochemicals will occur in the small intestine where some glycosides will be hydrolysed. Rapidly absorbed plant metabolites will enter systemic circulation following methylation, sulphation and/or glucuronidation. However, many phytochemicals escape absorption early in the GI tract and in particular those bound to plant polymers reach the colon and are metabolised and released by the gut microbiota[72]. Data regarding the metabolism and bioavailability of these products are severely lacking, particularly with reference to the mechanisms of transformation and the species responsible. The involvement of the gut microbiota in conversion of these plant metabolites is demonstrated by the fact that their metabolites appear after 6–8 h in systemic circulation[8]. The majority of plant metabolites present in the form of glycosides will be converted to aglycones prior to further transformation. It is likely that the compounds that reach the functional sites such as cells and tissues will be chemically different from those consumed in the diet. To evaluate their potential role in cancer prevention, the structure, concentration and site of action must be established. In the colon, they can exert a direct action such as an anti-inflammatory effect on the gut mucosa or be absorbed from the colon via hepatic circulation and suppress low-level chronic systemic inflammation.

Both the parent compounds and the metabolites produced have the potential to influence specific microbial groups. Of the secondary metabolites, the group most widely studied are products of the phenylpropanoid pathway, as nearly all plant foods considered to have cancer-preventative properties are rich in these compounds[73] including cinnamic, phenylacetic, phenylpropionic, coumarins, flavonoids and anthocyanidins[72].

Although these phenylpropanoid derivatives are most widely studied, many other secondary metabolites and their derivatives are present in the human colon. Moreover, glucosinolates and their metabolites (isothiocyanates and indoles) have also been extensively studied in relation to protection against carcinogenesis and mutagenesis[74,75], while many other N- and S-related compounds have been generally overlooked in terms of metabolism. For some studies, the presence of specific gut metabolites was detected in plasma and urine by MS indicating that these compounds have entered systemic circulation via enterohepatic circulation. For example, urinary phenolic acid metabolites, measured by GC–MS, were significantly increased following consumption of red wine and red grape juice extracts[76]. The best markers of intake included syringic acid, 3- and 4-hydroxyhippuric acid and 4-hydroxymandelic acid. Reductions in p-cresol sulphate, 3-indoxylsulphuric acid and increases in indole-3-acetic acid and nicotinic acid were also observed in urine following consumption of red wine and grape extracts[77]. In addition, sesamin, a major bioactive lignin found in sesame seeds was metabolised to enterolactone by *in vitro* incubation with mixed faecal microbiota. Sesamin consumption also demonstrated that this compound was a precursor to enterolactone *in vivo*[78]. Ingestion of a range of ellagitannin-rich foods, demonstrated that the microbial metabolite 3,8-dihydroxy-6*H*-dibenzo[b,d]pyran-6-one (urolithin B) conjugated with glucuronic acid was detected in urine by liquid chromatography–MS[79]. These urolithin metabolites were also presently found to be present in human plasma[80].

Gut metabolites and human disease

Obesity and cancer are characterised by chronic low-grade inflammation and the products of microbial metabolism have the ability to modulate these effects (Fig. 3). Although the molecular mechanisms are still uncertain, particular receptors appear to have a clear role. These include Toll-like receptor (TLR) 4 and TLR5. The lipopolysaccharide of *B. fragilis* is unusual and likely to be at least 100–1000-times less toxic than that of *E. coli*. There is currently much interest in the role of bacterial lipopolysaccharide signalling via TLR4 and invoking a low grade inflammatory response which in turn may impact on metabolic health. Mice deficient in TLR5[81] showed increased adipose-tissue mass, reduced insulin sensitivity, increased blood lipids and higher blood pressure when compared with normal mice and a high-fat diet exacerbated these effects and demonstrated features of human metabolic syndrome. Interestingly, when germ-free normal mice were inoculated with the microbiota obtained from TLR5 deficient mice, these mice also developed symptoms

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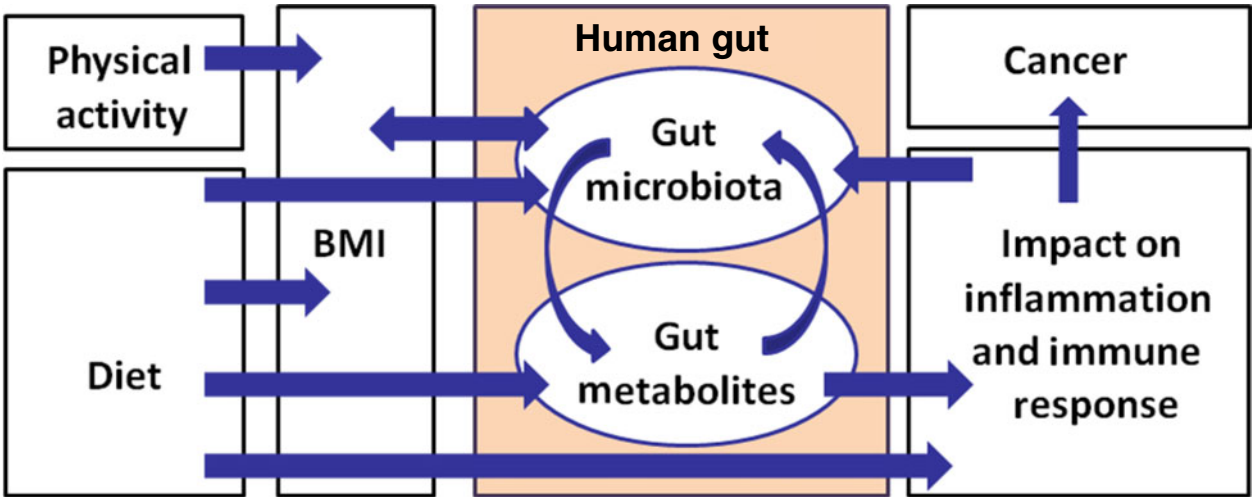

Fig. 3. Effect of the gut microbiota on the relationship between obesity and cancer.

of metabolic syndrome[81]. In this case, the ligands acting on these receptors are unknown.

Bacteria ferment dietary residues to SCFA and acetate, propionate and butyrate are the major acids detected. In addition to being the major energy source for the colonocytes[82], butyrate has a role in inhibition colonic inflammation and oxidative stress. At the molecular level, the anticarcinogenic effect of butyrate is a result of regulation of gene expression and the inhibition of histone deacetylease activity. Butyrate is transported across the colonic epithelium by two specific carrier-mediated transport systems both of which have been reported to function as tumour suppressors. SCFA generated by the microbiota modulate the immune response through GPR43[83] which are expressed in a wide range of host tissues. In particular, butyrate and propionate, have been identified as physiological ligands. Activation of GPR43 by SCFA contributes to the inhibition of lipolysis and to adipocyte differentiation which may be modulated by fructo-oligosaccharides[65,84,85]. Peptidoglycan released from the microbiota has also been shown to prime the innate immune system through NOD1[86].

In this context, the potential impact of dietary metabolites extends beyond gut health to include cardiovascular and metabolic health. This includes Type 2 diabetes mellitus which was found to be associated with changes in the gut microbiota regardless of BMI. Specifically, clostridial species were reduced and the ratio of Bacteroides to Firmicutes correlated positively with plasma glucose concentration, but not with BMI[87]. *Bacteroides vulgatus* and *Bifidobacterium* species were also lower in the diabetic group[88]. Subjects with Type 2 diabetes mellitus were also found to have reduced numbers of *F. prausnitzii*, which correlated with increased inflammatory markers[89]. Evidence also supports the hypothesis that host recognition of the gut microbiota is essential in preventing onset and progression of Type 1 diabetes. This is likely to involve the myeloid differentiation factor 88 signalling pathway, but the microbial product initiating the response is yet to be identified.

Obesity

Obesity is a major health problem both in developed and in developing nations and arises when energetic content of food ingested is in excess of energy expenditure. Excess body fat is associated with a number of metabolic diseases such as diabetes, CVD and cancer and can also have a major impact on longevity and quality of life. There has been much interest in the potential role of non-digestible dietary carbohydrates for body weight control and obesity[90]. Drastic reduction in total dietary carbohydrate intake in weight loss diets alters the composition of the colonic microbial community as well as faecal metabolite profiles[2,91]. Colonic fermentation provides an additional source of energy to the host via absorption of SCFA that is estimated to contribute about 10% of dietary energetic intake[92] and bacterial fermentative activity in the colon may contribute to fat deposition[93,94]. The energy recovered from ingested sugar by this route is, however, less than that for sugar directly absorbed in the small intestine[95]. The net effect must therefore depend largely on how alternative sources of dietary carbohydrate influence satiety. High intakes of monosaccharides such as glucose and fructose present in soft drinks appear to increase serum ghrelin, activating hunger signals and decreasing satiety[96,97]. It has been suggested that fructo-ologosaccharides intake, on the other hand, results in decreased ghrelin levels that may help in the control of food intake[98].

Obese human subjects on weight loss diets were shown to have altered microbial profiles[20,99]. Also, drastic reduction in total dietary carbohydrate intake in weight loss diets alters the composition of the colonic microbial community as well as faecal metabolite profiles[2,91]. Microbial metabolites associated with increased weight gain include increased excretion of hypoxanthine, hippurate, dimethylglycine and creatinine in the urine[100]. Studies where weight loss was achieved via gastric bypass surgery demonstrated that asparagine, lysophosphatidylcholine (C18:2), nervonic (C24:1) acid, p-cresol sulfate, lactate, lycopene, glucose and mannose were all significantly reduced[101].

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Cancer

CRC accounts for approximately 17 000 deaths in the UK annually and progression of the disease is likely to result from a combination of genetic and environmental factors. The majority of CRC is thought to be sporadic in origin with the most common form, adenocarcinoma, developing from glandular cells lining the colonic wall. There are several genetic events commonly occurring in CRC at the molecular level that include inactivation of the tumour suppressor genes such as *p53* along with activation of oncogenes including the *ras* family of genes. Not all colorectal polyps progress to cancer, suggesting that perhaps other factors can influence malignant transformation. There is increasing evidence supporting the role of inflammation in the pathogenesis of the gut, including diseases such as CRC. Compared with healthy subjects, patients suffering from inflammatory bowel disease are considerably more likely to develop CRC[102]. One of the similarities between inflammatory bowel disease-associated and sporadic CRC is the importance of cyclooxygenase-2 induction and this enzyme is induced in response to mediators of inflammation, cytokines and endotoxins. In the healthy colon, the thick mucin layer helps to protect the epithelial cells from direct contact with bacterial cells[103]. Moreover, tight junctions are located in the epithelium that create a barrier which regulates permeability of the epithelial layer in response to various signals including cytokines. Maintenance of an intact epithelial layer is one of the mechanisms that limit bacterial translocation. Bacterial cells may also provide a source of regulatory signals that, for example, direct the differentiation of T-helper cells producing IL-17 (T-helper 17 cells) and T-regulatory cell activity. These signals influence the maturation of the gut and the immune system. In the absence of bacteria these defence systems are likely to be weakened.

Most of the bacteria in the colon possess a number of microbial-associated molecular pattern moieties that are well recognised by cells of the innate immune system. These molecular motifs are recognised by TLR. For example, bacterial lipopolysaccharide, an endotoxin is recognised by TLR4, a recognition receptor of the innate immune system. Bacterial flagellin is recognised by TLR5 and lipoteichoic acid by TLR2. Deficiency in TLR2 has been reported to lead to both increased tumour burden and size in mice with dextran sodium sulphate-induced colitis. This has been related to increased cytokine levels of, for example, IL-6; TNFα that may drive inflammation and induce CRC. Separately, both TLR2 and TLR5 play a role in the suppression of tumour formation, via the activation of specific anti-tumour immunity. Mice deficient in TLR4 were protected against the development of neoplasia as TLR4 signalling can promote colon carcinogenesis by stimulating tumour infiltration of T-helper 17 cells. One of the key lipopolysaccharide producing species in the colon is *E. coli*[104] although the abundance of this species is likely to be a small proportion of the total bacterial load. There have been a number of bacterial species associated with CRC including the Gram-positive *Streptococcus* species and the Gram-negative *Helicobacter* species, *B. fragilis* and *E. coli*. It is not only the balance of bacterial species in the colon that is important for maintaining gut health; in addition the impact of changing bacterial composition in response to dietary intake also drives bacterial metabolite formation.

Microbial metabolites may be a key factor in regulating inflammatory and immunological responses in the colon. Metabolism of dietary carbohydrates will result in the formation of SCFA and as discussed earlier butyrate plays an important role in health maintenance. Increasing the protein content of the diet particularly by increasing red meat intake is likely to result in increased levels of toxic metabolites that include heterocyclic amines, fecapentaenes, nitrosamines, super oxide radicals and hydrogen sulphide[105]. Fecapentaenes are mutagens that are reportedly synthesised by *Bacteroides* species that may alkylate DNA to form mutagenic adducts. Hydrogen sulphide is also a toxic microbial metabolite formed by sulphate reducing bacteria, including *Desulfovibrio piger*, in the colon. *D. piger* can metabolise lactic acid that may accumulate in bowel disease[106,107] while reducing sulphate to sulphide[108] and meat is a major source of sulphur that promotes the growth of sulphate reducing bacteria[109]. The genotoxic potential of hydrogen sulphide is in part mediated by oxidative free radicals and cyclooxygenase-2 is up-regulated in epithelial cells following administration of hydrogen sulphide at physiological concentrations[110]. Colonic bacteria also play a role in the formation of N-nitroso compounds, the levels of which are elevated following intake of high protein diets, particularly meat[8]. Cooking meat generates heterocyclic amines that can be further transformed to genotoxic intermediates. The production of these products is likely to be linked to increased risk of CRC[111].

Secondary bile acids, mainly deoxycholic acid and chenodeoxycholic acid, are formed by microbial conversion of the primary acids that are formed in the liver and secreted into the duodenum. Approximately one litre of bile enters the duodenum each day; however, bile acid excretion is related to fat and red meat intake that are potential risk factors for CRC[112]. Epidemiological studies reported higher concentrations of secondary bile acids in CRC patients compared with healthy controls[105]. Secondary bile acids can cause DNA damage[105] by the production of oxygen radicals and reactive nitrogen species[113,114]. Bile acids enter enterohepatic circulation therefore the concentration decreases along the GI tract, however, elevated bile acid levels may modulate the abundance of certain bacterial species including *F. prausnitzii*[115] that has been reported to have potent anti-inflammatory activity[116].

Conclusions

The large intestine may appear to be a hostile environment for bacteria to inhabit, but nonetheless represents a densely populated microbial ecosystem. Moreover, on balance the host is likely to benefit from these multi-species communities in the gut when the balance of species provides mostly beneficial metabolites. Microbial imbalance however may well result in a less favourable metabolic output

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that will make an impact on inflammatory status and disease progression. As an example, carefully controlled dietary intervention studies have revealed that high protein diets (mainly meat-based) result in elevated levels of hazardous metabolites and a decrease in cancer protective metabolites. High protein diets are satiating and therefore, in the short term may be efficacious in achieving weight loss[117]. In addition, the health risks associated with consuming high meat protein diets may be partially ameliorated by including cereals in the diet and/or exchanging meat protein for plant protein. The phenolic content of the latter may afford some health benefits. Understanding of the microbial ecosystem of the human colon will continue to benefit from a range of molecular approaches which should be developed in parallel with metabolomic approaches. Our dietary intake clearly has an important influence on the species composition of gut microbiota, but this appears insufficient to explain the extent of variation that is seen between individuals. As MS methodologies continue to develop and its usage increases a clearer and much needed understanding of the complex interplay between diet, the gut microbiota and human health will be achieved. There is also a need to link detailed microbial diversity to metabolic functionality to ascertain if general dietary advice is sufficient or there may be a need, in certain circumstances, to provide personalised nutritional advice.

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