Metabolic phenotyping of the human microbiome [version 1; peer review: 2 approved]

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Abstract
The human microbiome has been identified as having a key role in health and numerous diseases. Trillions of microbial cells and viral particles comprise the microbiome, each representing modifiable working elements of an intricate bioactive ecosystem. The significance of the human microbiome as it relates to human biology has progressed through culture-dependent (for example, media-based methods) and, more recently, molecular (for example, genetic sequencing and metabolomic analysis) techniques. The latter have become increasingly popular and evolved from being used for taxonomic identification of microbiota to elucidation of functional capacity (sequencing) and metabolic activity (metabolomics). This review summarises key elements of the human microbiome and its metabolic capabilities within the context of health and disease.

Keywords
microbiome, metagenomics, metabolomics, short chain fatty acids, bile acids
Introduction

The human microbiome as it relates to metabolic function and health

It has been established that communities of microorganisms, *microbiota*, reside on or within nearly every physical substrate on our planet (and associated artificial satellites)\(^1\)\(^-\)\(^10\). Composed of organisms encompassing multiple divisions of the tree of life, such as protozoa\(^1\)\(^-\)\(^36\), fungi\(^37\)\(^-\)\(^39\), viruses\(^21\)\(^-\)\(^24\) and prokaryota\(^3\)\(^-\)\(^29\), these microbial communities are intricate ecological structures driven by the production and exchange of metabolic products\(^29\)\(^-\)\(^34\). Indeed, these communities can cause metabolic cascades that have measurable influences on their macroscopic hosts. Through recognition of these influences, the importance of the microbiome as an integral component of human biology has come to be appreciated, not only by microbiologists but by clinicians and the general public. This review describes essential background to the human microbiome, providing an overview of microbiomes delineated by human anatomy within the framework of microbe–host metabolic interaction before focusing on these interactions as they relate to the gut.

Womb to tomb

Present from birth to death, an individual’s microbiome maintains a constant presence as a chimeric organ\(^3\)\(^-\)\(^8\). Seeding of this microbial system occurs at the beginning of life via transmission of a mother’s microbiome to her infant during the birthing process\(^8\)\(^-\)\(^11\). Influenced by direct environmental transmission, a delivered infant will inherit either the mother’s vaginal and fecal microbiota as it passes through the birthing canal or the skin microbiota during caesarean delivery\(^11\)\(^-\)\(^14\). Either route of delivery imposes prolonged multifaceted effects on the infant\(^11\)\(^-\)\(^16\). Vaginal birth confers a microbiome of the mother’s urogenital system which has undergone specific alterations throughout the pregnancy which are conducive to the development of robust and functional immune and gastrointestinal (GI) systems of the infant\(^17\). Alternatively, numerous deleterious health effects for infants delivered by caesarean section have been identified. Immediate influences upon the infant include increased risk of exposure to antibiotic-resistant bacteria from the mother’s skin\(^18\). Long-term insults to health arising from caesarean delivery include greater risk of developing obesity, sensitivity to food and inhalant allergens, and asthma\(^18\)\(^-\)\(^20\). In light of increasing awareness of potential negative health effects associated with caesarean delivery, an experimental procedure of vaginal seeding has been developed to simulate the microbial exposures present in vaginal birth via administration of vaginal swabs to newly delivered infants\(^19\). However, implementing vaginal seeding is a contentious issue, and many clinical practitioners are wary of the intervention prior to extensive investigation of its effects\(^19\)\(^,\)\(^20\)\(^,\)\(^21\).

Throughout infancy, an individual’s core microbiome is continuously influenced by the mother and environment. Whether nourished by the mother’s natural breast milk or formula, the infant microbiome continues to be moulded through supplied nutrition. In this regard, a positive health bias towards biological ‘tradition’ persists, as both the process of breast feeding and breast milk itself, and potentially the microbes therein, convey health benefits superior to those of formula\(^22\)\(^-\)\(^25\).

Progressing through infancy, the microbiome goes through highly variable changes, beginning to stabilise at about 2 years of age. Flux of the microbiome during this period is attributed to numerous factors, including dietary variations (for example, milk versus solid food), immunological development, introduction to novel microbes, and antibiotic exposure\(^26\)\(^-\)\(^30\).

Through the transition from infancy to childhood and onto adulthood, the microbiome of an individual stabilises while still being influenced by drug exposure\(^30\)\(^,\)\(^31\), physical activity\(^30\)\(^,\)\(^32\), the environment\(^3\)\(^,\)\(^33\) (discussed more elaborately in proceeding sections)\(^34\)\(^-\)\(^37\). The microbiome changes again with old age\(^38\)\(^,\)\(^39\), and microbes ultimately contribute to decomposition after death\(^40\)\(^-\)\(^42\).

The human body: a microbiome perspective

Microbial communities take form within any accessible area of a host’s body. The defined niches with stable communities in humans and other mammals are currently generalised to the respiratory system\(^43\)\(^,\)\(^44\), nasal\(^45\)\(^,\)\(^46\) and oral\(^27\)\(^-\)\(^30\), cavi
dies, skin\(^21\)\(^,\)\(^23\)\(^-\)\(^25\), vagina and urinary tract\(^32\)\(^-\)\(^34\),\(^40\)\(^-\)\(^42\), and GI system\(^21\)\(^-\)\(^25\)\(^,\)\(^27\)\(^-\)\(^30\)\(^,\)\(^36\)\(^-\)\(^38\)\(^,\)\(^40\)\(^-\)\(^42\). For each of these unique communities, varied challenges are involved in their sampling and analysis and in interpreting their impact on health or disease.

The skin

Comprising a relatively large surface area (~1.8 m² for an adult human) and an array of subsystems defined by folds, crevices, pH, secretion profiles, and environmental exposures, the skin supports highly varied microbial communities functioning in diverse ecological constraints (Figure 1A)\(^49\)\(^,\)\(^50\). Ecological partitioning of the skin microbiome is further defined by elementary biological traits of the host. Microbial composition at specific anatomical locations coordinates with gender\(^50\)\(^,\)\(^100\)\(^,\)\(^101\). Indeed, topical sampling of hand palms demonstrates greater diversity of bacterial taxa in women than men, and specific taxa are differentially abundant between the two sexes\(^100\)\(^,\)\(^101\). Similar results have been presented for other body sites, such as the thigh and torso\(^99\)\(^,\)\(^100\). Expectedly, cohabitation of sexually active partners results in a shared skin microbiome that accurately matches couples 86% of the time\(^100\). Ancestral host genetics have also been demonstrated to influence the composition of the skin microbiome. Male participants of diverse ethnic backgrounds, all dwelling in a single geographic location, were shown to have microbial differences specific to ethnicity\(^102\). Furthermore, a study of both monozygotic and dizygotic twins described an association between *Corynebacterium jeikeium* and single-nucleotide polymorphisms of a host gene involved in epidermal barrier function\(^103\). This finding suggests that the establishment of specific skin microbes is dependent on heritable factors of the host. Despite such associations with the skin microbiome, ancestral genetics have been shown to exert a negligible influence on the gut microbiome, where instead other factors, such as environment, play a more profound role in the form and function of the microbial community\(^104\).

Continuous environmental interaction unsurprisingly results in the skin being our most exposed microbial ecosystem. Environmental factors shown to be influential include hygiene
Figure 1. Demonstration of key microbiota and metabolites of the human microbiome, delineated according to human physiology. (A) The skin, (B) oral cavity, (C) respiratory tract, (D) urogenital system and (E) gastrointestinal tract are each highlighted with examples of microbiota (Taxa) and relevant metabolic activity (Metab). Beneficial associations to host health are denoted as (+) and negative associations as (−).
routines, topical medication and cosmetic use, and residential environment (for example, rural versus urban). Despite its vulnerability to external perturbations, an individual’s skin microbiome maintains a consistent core structure. Though capable of opportunistic pathogenicity under certain conditions, constituents of this stable community perform homeostatic functions and act as a barrier against transient and potentially pathogenic species, subsequently maintaining a role in a variety of cutaneous conditions. Among these residential members are strains of Propionibacterium acnes, the fungal genus Malassezia, and Staphylococcus epidermidis. Lipophilic P. acnes and species of Malassezia proliferate in sebaceous gland-rich body sites, such as the face and back. The rich pool of triglycerides found in sebum are hydrolysed by microbes to produce fatty acids that assist in bacterial adherence and maintaining an acidic pH. Low pH environmental conditions select for lipophilic commensals while inhibiting colonisation by potentially pathogenic strains of Staphylococcus aureus and Staphylococcus pyogenes. P. acnes additionally contributes to suppression of metcillin-resistant S. aureus through glycerol fermentation to short-chain fatty acids (SCFAs) and in particular propionic acid, which also inhibits growth of Escherichia coli and Candida albicans.

Immunological training by microbiota seeded early in life enables the host to distinguish between the commensal core and transient pathogenic microbes, wherein selected commensals create biological barriers through biofilm formation, alter pH and oxygen levels, and produce antimicrobial molecules. Bacteriocins (that is, small peptide antimicrobials that include lantibiotics and microcins) are one such means of microbial-derived molecular regulation of community composition within the mouth (and other microbial systems). The underlying mechanisms coordinating this antagonistic inter-noc system unless challenged with disease. Now, however, it is clear that a respiratory microbiome exists. Periodontal disease is also caused by microorganisms. Prolonged biofilm formation at the interface of gingival tissue and the tooth surface leads to the accumulation of pathogenic bacteria that exacerbate inflammation through cytoxic compounds such as lipopolysaccharides. Resultant bleeding from inflammation provides a source of iron from heme, a molecule used by pathogenic microbes (for example, Porphyromonas gingivalis). Without disruption from inflammation provides a source of iron from heme, a molecule used by pathogenic microbes (for example, Porphyromonas gingivalis). Without disruption from inflammation, periodontitis-associated microbes thrive and, with continued immunological antagonism of the gingival tissue, contribute to induction of a dysregulated inflammatory response, permanently damaging connective tissue and bone.

The mouth

The oral cavity microbiome represents a reasonably well-defined ecosystem. Structure morphology and different tissue types within the human mouth offer a variety of microbrial habitats, further delineated by conditions of oxygenation, pH, and nutrient availability. Control of the oral microbiome is mediated in concert by factors produced by the host and the microbiota.

Within this environment, saliva moistens the mouth, aiding in the mastication, swallowing and digestion of food. Saliva also provides an essential nutrient source for microbes, containing complex molecules such as glycoproteins (for example, mucins). Similarly, saliva-derived proline-rich glycoproteins contribute to pellicle formation on mouth surfaces, immobilising microbes through their adherence to the structures. Bioactive compounds found within saliva also include potent factors that inhibit growth or otherwise modify the microbial complex’s activity within the mouth. For example, bacterial growth is curbed by lysozyme-mediated cell lysis and interference of glucose metabolism with lactoperoxidase-catalysed conversion of hydrogen peroxide and thiocyanate to hypohypochromate.

Sustaining a balanced oral microbiome is thought to confer numerous local and systemic health benefits. Nitric oxide (NO) is an important cellular signalling molecule, crucially involved with various physiological functions: metabolism, nerve function, and cardiovascular function. Key oral microbiome constituents have demonstrated the ability to reduce dietary nitrates to nitrite. Converted nitrite is deposited into saliva, which is ingested after oral cavity circulation, leading to NO conversion and the subsequent transmission to tissues across the body. Countering the potential health benefits of bacterial nitrite supplementation, the compound may stimulate cancer development through formation of carcinogenic N-nitrosamines. Posing a similar risk of carcinogenesis, acetaldehyde is produced from ethanol by oral bacteria.

Dysfunction of the oral microbiome contributes directly to dental diseases; the most widely recognised such condition is tooth decay or dental caries. Caries formation begins with bacterial fermentation of carbohydrates to organic acids, resulting in localised pH reduction and subsequent tooth demineralisation. Once the site has been acidified, the affected environment becomes increasingly selective for bacteria that are tolerant of low pH conditions, thus stimulating proliferation of destructive communities and worsening of the condition. Although Streptococcus mutans is implicated in tooth decay, it is evident that no single organism is the causative agent, and instead polymicrobial activity drives the condition with diverse actors from genera such as Actinomyces, Slackia, Propionibacterium and Lactobacillus.

Periodontal disease is also caused by microorganisms. Prolonged biofilm formation at the interface of gingival tissue and the tooth surface leads to the accumulation of pathogenic bacteria that exacerbate inflammation through cytoxic compounds such as lipopolysaccharides. Resultant bleeding from inflammation provides a source of iron from heme, a molecule used by pathogenic microbes (for example, Porphyromonas gingivalis). Without disruption from inflammation provides a source of iron from heme, a molecule used by pathogenic microbes (for example, Porphyromonas gingivalis). Without disruption from inflammation, periodontitis-associated microbes thrive and, with continued immunological antagonism of the gingival tissue, contribute to induction of a dysregulated inflammatory response, permanently damaging connective tissue and bone.

The nose and respiratory system

At one time, the human lung had been considered a sterile biological system unless challenged with disease. Now, however, it is clear that a respiratory microbiome exists. When healthy, the lung environment reflects many characteristics of the mouth and nose interiors, namely moderate thermal stability, high oxygen availability, mucosa-lined internal surfaces, and a continuous influx of environmental
microbes. Despite these similarities, modern investigation of respiratory-related microbes in the lungs projects a microbiome of low phylogenetic diversity\(^\text{124-126}\). The simplicity of the lung microbiome contrasts with that of the oral cavity, although the latter acts as a major channel for microbiota translocation, and microaspiration of aerosolised material from the upper respiratory tract and direct migration along the oropharynx mucosa occur\(^\text{126,127}\).

Whereas some human microbial communities exhibit high levels of diversity when healthy, presenting associations between disease and reduced diversity, the respiratory microbiome is thought to be more susceptible to malignancy when the complexity of its composition increases\(^\text{25,116,126,128,129}\). This is observed as far up in the respiratory system as the nasal cavity, and elevated diversity of the inner nostril is associated with a number of allergies\(^\text{10}\). Conversely, post-surgical outcome of sinus surgery is better with more diverse sinonasal microbial communities, suggesting an unpredictable complex relationship between upper respiratory tract microbial diversity and health\(^\text{130}\). Ultimately, caution needs to be used when considering diversity as a marker of health.

A clear association between the lung microbiota and compromised pulmonary health has been demonstrated with asthma, an inflammatory disease\(^\text{35,36,37,129,130}\). As is the case for many microbiome–health interactions, evidence supports early-life microbial exposures as being critically influential with respect to respiratory health. Strong epidemiological associations assert an increased risk of inflammatory respiratory disease with caesarean birth and reduced risk from diverse antigen presentation (such as rural and farm exposures)\(^\text{46,47,133-135}\). More specifically, bacterial species of *Lachnospira*, *Veillonella*, *Fae-*

nal delivery. The establishment of this microbiome can have lifelong influences on the health of the infant\(^\text{35,44,139-141}\).

Substantial effort has been put towards characterisation of vaginal microbial components and associated metabolic function (Figure 1D). The healthy vaginal microbiome is characterised as maintaining low microbial diversity, and *Lactobacillus* species typically dominate\(^\text{25,96,142}\). Disruptions to the healthy vaginal microbiome’s stable low complexity are linked to severity of cervical intra-epithelial neoplasia and bacterial vaginosis (BV), and the latter is also associated with an increased susceptibility to acquiring sexually transmitted infection, pelvic inflammatory disease, and preterm birth\(^\text{94,143-148}\).

*Lactobacillus* dominance of the vaginal microbiome appears to be specific to humans and contrasts greatly with levels found in other animals (>70% and ~1%, respectively)\(^\text{149}\). Several theories have been proposed for the *Lactobacillus*-centric human vaginal microbiome, including a suggestion of a conserved common function of vaginal microorganisms that in humans happens to be fulfilled by *Lactobacillus* species, and that these species are also adapted to the starch rich diets that are typical of humans\(^\text{149}\). Indeed, the diet hypothesis further suggests that the high glycogen concentrations found within the human vaginal tract reflect dietary carbohydrate catabolism which is facilitated by abundant salivary amylase levels.

Irrespective of its evolutionary basis, the growth of lactobacilli in the vaginal environment is supported by glycoprotein- and mucin-rich genital fluid and high levels of glycogen and α-amylase, and the latter increases the energy availability of glycogen through its by-products\(^\text{149-151}\). With *Lactobacillus* proliferation, the oestrogen-mediated low pH of the vagina is further acidified by microbial-derived lactic acid, which is metabolised from glycogen through anaerobic glycolysis\(^\text{152,153}\). Low pH (~3.5) and high lactic acid concentrations contribute in 

as a factor in atopic and seborrhoeic dermatitis, providing a further potential link between the deleterious translocation of skin microbiota and asthma\(^\text{10}\). It should be noted that these potential links need to be definitely established.
conjunction with cervicovaginal fluid, a highly effective antimicrobial and antiviral medium, to maintain a healthy vaginal environment\textsuperscript{15,157}. With BV, when the vaginal pH rises (>4.5) and microbial composition shifts away from being \textit{Lactobacillus}-dominant to allow other taxa (such as \textit{Gardnerella}) to proliferate, lactic acid levels drop and a more prominent SCFA profile develops\textsuperscript{15}. Although SCFAs are generally associated with health benefits, particularly in the gut, an undesirable pro-inflammatory response appears to be induced by acetate and butyrate within the vaginal tract\textsuperscript{13,107,113,155,158,159}.

The vaginal microbiome appears to considerably influence the efficacy of microbicide HIV prevention therapy\textsuperscript{64}. Tenofovir microbicidal gel was 59.2\% effective in HIV infection prevention for \textit{Lactobacillus}-dominant vaginal communities, but in individuals with a microbiome containing greater proportions of \textit{Gardnerella}, the prevention rate was only 18\%\textsuperscript{64}. Controlled doses of tenofovir administered to patients with either \textit{Gardnerella}- or \textit{Lactobacillus}-oriented microbiomes showed significantly lower concentrations of the drug in \textit{Gardnerella}-dominated vaginal communities; indeed, detected drug concentration negatively correlated with \textit{Gardnerella} abundance\textsuperscript{64}. \textit{In vitro} analysis demonstrated that \textit{Gardnerella} and other BV-associated microbes efficiently metabolised the drug through a cleavage of an oxy-methylphosphonic acid side chain of the compound\textsuperscript{64}.

The male urogenital tract microbiome has received less attention. However, emerging investigation of the subject suggests health-relevant microbial activity within this system. Circumcision significantly modifies microbial composition of the coronal sulci of the penis, decreasing the total microbial load, including anaerobic taxa putatively associated with BV\textsuperscript{160,161}. Reduced HIV infection rates have independently been associated with circumcision, but the underlying factors of this protective effect are unknown\textsuperscript{162}.

The gut

Of the microbial communities delineated by human physiology, those associated with the GI system have been investigated with the greatest intensity (Figure 1E)\textsuperscript{12,21,27,29}.

Microbes travel, generally in a uni-directional manner, through the GI tract within ingested material, and the associated communities follow a gradient of community complexity that peaks in the colon\textsuperscript{163–165}. Once established, the gut microbiome is subject to influence from a limited number of known factors. Perhaps the factor that most profoundly affects this community is host diet, supplying both microbes and nutrients to the host–microbiota process with enzymes that are absent from the human host, are one such important dietary factor\textsuperscript{163–165}. Through metabolism of these polysaccharides, microbial fermentation yields SCFAs, compounds with a broad range of purportedly profound effects on the host\textsuperscript{83,107,108}.

In addition to dietary constituents, host-derived metabolites can be used by the gut microbiome\textsuperscript{97,166–172}. Examples highlighting this host–microbe interaction include bile acids (BAs), which, once acted upon by bacteria, can trigger complex host–microbe signalling cascades, and intestinal mucins, compounds used by mucin specialists (for example, \textit{Akkermansia muciniphila}), providing protective properties to the host\textsuperscript{167,169–173}.

It is worth noting that, in addition to drugs explicitly affecting microorganisms (that is, antibiotics), the interaction between other medications and microorganisms can be key, affecting microbiome composition and function as well as the pharmacokinetics of the drugs\textsuperscript{171,174–177}. Indeed, an \textit{in vitro} screen of more than 1000 pharmaceutical compounds to assess their activity against core representative strains of gut bacteria demonstrated that growth of at least one strain was inhibited by 24\% of compounds intended to target human cells\textsuperscript{177}. Similarly, the type 2 diabetes drug metformin was shown to alter both the composition and function of the human intestinal microbiota, resulting in an enrichment of genes associated with SCFA metabolism and faecal concentrations of propionate and butyrate\textsuperscript{156}. However, the specifics of microbial metabolic interactions with metformin have yet to be elucidated.

It should also be noted that drugs of intoxication (for example, alcohol and cannabis) are indicated to interact with the microbiome, although studies in this field are somewhat rare and often limited to non-human animal models\textsuperscript{158–162}. An exception to the pattern, whereby the gut microbiome of chronic cannabis users was investigated\textsuperscript{161}, revealed that, in comparison with controls, chronic cannabis users had a 13-fold reduction in the ratio of \textit{Prevotella} to \textit{Bacteroides}. Lower \textit{Prevotella} abundance was further associated with poor cognition test performance and reduced mitochondrial ATP production\textsuperscript{163}.

Host behaviour, and more specifically physical exercise and fitness, are also recognised as potential modulators of microbial composition and function\textsuperscript{60–70}. Illustrating the potential influence of extremes of exercise, professional athletes have been shown to harbour a gut microbiome that exhibits a high compositional diversity of microbial taxa and contains a gene profile with robust potential for environmental energy capture\textsuperscript{60,61}. More specifically, the gut microbiome of a cohort of professional rugby players, in comparison with age-matched controls with similar body mass index to represent the range of body composition in the athletes, contained greater proportions of metabolic pathways associated with potential health benefits. These pathways ranged from those associated with organic cofactor and antibiotic biosynthesis to degradation and biosynthesis of carbohydrates. Such biosynthetic pathways could result in an increased capacity for energy utilisation by the microbiome\textsuperscript{60}. Metabolomic profiling of the athlete gut microbiome revealed elevated levels of SCFAs, which (as noted above) are metabolites with wide health-associated attributes (detailed further below) and are associated with a lean body composition\textsuperscript{83}. The faecal metabolome of these athletes also exhibited elevated levels of trimethylamine-N-oxide (TMAO), a compound that has been associated with cardiovascular disease and atherosclerosis, although these negative associations have been disputed because of the occurrence of high levels of TMAO in populations with a low occurrence of
cardiovascular disease\textsuperscript{164}, and thus the significance of these findings with respect to athletes has yet to be determined. From another study (in this instance, of the microbiome of high-performance cyclists), it was shown that the genus \textit{Prevotella} was significantly associated with reported time of exercising\textsuperscript{68}. The study further revealed higher transcriptional activity of \textit{Methanobrevibacter smithii} genes, particularly those related to methanogenesis, in professional cyclists when compared with amateurs. Investigation of amateur half-marathon runners demonstrated that, through the course of high-intensity running, significant changes occurred in certain taxa (for example, \textit{Coriobacteriaceae}) and metabolites within the gut environment\textsuperscript{69}. Intriguingly, the introduction of exercise as a novel stimulus appears to elicit more subtle changes in the gut microbiome. After undergoing a short period (8 weeks) of moderate-intensity exercise, healthy but inactive adults were shown to exhibit only minor changes in the composition of their gut microbiome\textsuperscript{69}. A separate analysis of a combination of lean and obese individuals undergoing a period of structured exercise conversely asserted that concentrations of faecal SCFAs increased in lean participants following exercise while an obesity-dependent shift in microbial diversity was present after exercise and dissipated after a washout period\textsuperscript{185}. In sum, it is apparent that there remains much to be done to completely understand the mechanisms underlying the interaction of exercise and the gut microbiome.

Gut microbiome analysis is carried out predominantly on the terminal end of the GI tract because of the relative ease with which samples can be non-invasively acquired as stool. These samples provide insight into the intestinal microbiome as excreted samples retain microbial cells and metabolites from the lumen and mucosa, although it is important to note that stool does not provide an exact recapitulation of the intestine’s various subsites\textsuperscript{183,184,185}.

**Systemic implication of the gut microbiome in health and disease**

The GI system acts as the primary site for the uptake and metabolic processing of nutrients. The gut accordingly contributes substantially to health regulation. As extensive evidence now indicates, intestinal microbes have similar significance in health maintenance and modulation of various disease states via interaction with the host’s biology and intestinal environment. Microbial contributions to this health dynamic are mediated by numerous metabolic modalities. The most prominent such metabolic circuit is between the microbiome and ingested nutrients, whereby microbes use dietary nutrients to proliferate and produce metabolites, such as SCFAs, that are involved in cross-talk with the host (Figure 2)\textsuperscript{20,37,32,166,167,187,188}.

**Short-chain fatty acids**

SCFAs act locally within the intestinal system but also impact on hepatic, neurological and immunological function\textsuperscript{186,188-192}. As previously noted, microbial SCFA generation results primarily from polysaccharide utilisation, although it has also been demonstrated that some gut microbes have the capacity to produce butyrate from the metabolism of protein\textsuperscript{18,193-195}. Upon excretion from microbial cells, SCFAs entering the intestinal environment are used by colonocytes as an energy source or pass into broader circulation via the portal vein\textsuperscript{159,188}. Acting locally on colonocytes, butyrate is incorporated into luminal cells through diffusion or direct transport mediated by the Na\textsuperscript{+}-coupled transporter SLC5A8\textsuperscript{159,159}. Butyrate within colonocytes contributes to energy production through conversion to acetyl-CoA or alternatively inhibits histone deacetylase (HDAC) activity\textsuperscript{159,196,197}. HDAC inhibition occurs within colorectal cancer cells, wherein glucose is preferentially used as an energy source, leading to butyrate accumulation and the subsequent action upon HDAC which results in a cascade of effects on cell proliferation, differentiation and apoptosis\textsuperscript{159,196,197}.

Propionate enters systemic circulation through the portal vein, where it is metabolised primarily in the liver while acetate is more broadly circulated, for example, crossing the blood–brain barrier, where it may influence satiety through action on the hypothalamus\textsuperscript{186}. On the basis of murine studies, gut-derived acetate and propionate have separately been suggested to influence asthma\textsuperscript{170,170,191}. While regulatory T–cell activity is enhanced by acetate-mediated inhibition of histone deacetylase 9 (HDAC9), resulting in suppression of environmental allergen hypersensitivity, propionate affects lung dendritic cells, dampening promotion of T helper type 2 cell–driven inflammation while leaving the cells’ phagocytic ability intact\textsuperscript{138,198-200}.

**Bile acids**

BAs have been shown to be at the centre of a metabolic interplay between the host and microbes\textsuperscript{172,169,170,174,176,201-203}. Following post-meal metabolic cues, bile released from the canalicular membrane of hepatocytes enters the intestinal system. Primary BAs, cholic acid and chenodeoxycholic acid are converted from cholesterol and conjugated with taurine or glycine and, within the context of host physiology, are used as detergents to allow intestinal absorption of dietary lipids and fat-soluble vitamins\textsuperscript{202,203,204}. Microbial bile salt hydrolases (BSHs) facilitate the hydrolysis of conjugated BAs (CBAs), converting the compounds back to BAs, which permits small intestine reabsorption or additional metabolic processing\textsuperscript{203,204}. Unconjugated and glycine-CBA absorption by passive diffusion and active transport creates a circulating pool of BAs, establishing continuous bioavailability of the compounds\textsuperscript{204,204}. As detergents, BAs have the capacity to disrupt the lipid membrane of bacterial cells, subsequently exerting considerable influence on the microbiome. Microbes accordingly employ myriad strategies to circumvent the antimicrobial action of BAs, such as outer membrane lipid and protein modifications\textsuperscript{205,204}. In conjunction with BA resistance, microbial alterations to BAs, affecting the hydrophobicity of the compounds, also enable some microbes to evade lipid membrane degradation while creating an inhospitable environment for competing organisms\textsuperscript{203,204}. Microbial BSH-driven hydrolysis of CBAs to unconjugated primary BAs enables subsequent conversion to secondary BAs deoxycholic acid (DCA) and lithocholic acid\textsuperscript{203,204}. DCA, in particular, accumulates in the enterohepatic BA pool. Relatively high concentrations of DCA result from intestinal diffusion and hepatic...
reuptake that is facilitated by the compound’s hydrophobicity and the human liver’s inability to rehydroxylate DCA.\(^{203}\)

Notably, the fat- and protein-enriched ‘Western’ diet that contributes to obesity development modifies not only gut microbiome composition but also microbial BA pool contributions.\(^{72,167,202,205,206}\) Indeed, the negative consequences of dietary insult have been shown to be ameliorated through intervention with BA-binding resins.\(^{207}\) Roux-en-Y gastric bypass surgery has intriguingly been shown to also have an effect on BAs, and serum concentrations are raised in individuals who have undergone the procedure when compared with obese and severely

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**Figure 2. Host–microbe metabolic interaction.** A simplified demonstration of the metabolic interactions between host and microbiome. The cross-section of the small intestine illustrates the metabolic exchange between the intestine and two taxonomic representatives (**Prevotella** spp. and **Faecalibacterium prausnitzii**). Polysaccharides act as an example of dietary substrate used by the microbiota for the production of short-chain fatty acid (butyrate and acetate). Similarly, host-derived substrate in the form of lactate presented with excretion of mucin from the intestine can be used by the microbiota. Within the example, acetate can be either absorbed by the intestine and subsequently the bloodstream where systematic influences take place or converted to butyrate, exerting a localised effect on intestinal epithelial cells. NO, nitric oxide.
obese controls, suggesting that anatomical manipulation of the procedure modifies the dynamics of the BA pool\(^{[18,29]}\).

Among the numerous detrimental effects of obesity, evidence supports a role for microbial-derived DCA as a potent tumour promoter, contributing to the development of hepatocellular carcinoma and the colorectal cancer precursor colorectal adenoma\(^{[23,20,218–212]}\). Although the associated mechanisms involved have not been studied in the human gut, DCA-driven hepatocellular carcinoma in mice is suggested to result from the compound’s provocation of the senescence-associated secretory phenotype (SASP) in hepatic stellate cells\(^{[21]}\). SASP is characterised by broad alterations in gene expression and secretory profile, which affect neighbouring cells through numerous factors, namely the release of cytokines (for example, interleukin-1α and -1β), insulin-like growth factor–binding proteins, NO and reactive oxygen species and potentially the glycoprotein fibronectin\(^{[11,211]}\). The influence of DCA on colorectal tumorigenesis is proposed to mediate derangement of epidermal growth factor receptor–mitogen-activated protein kinase (EGFR-MAPK) regulation, specifically with DCA pre-ectal tumorigenesis is proposed to mediate derangement of mitogen-activated protein kinase; NO, nitric oxide; SASP, senescence-associated secretory phenotype; SCFA, short-chain fatty acid; T2-high, type 2-high; TMAO, trimethylamine-N-oxide.

Conclusions and Outlook

Examination of microbiome–host interaction has revealed the integral role of microbiota in health and disease. Extensive characterisation of the microbiome’s taxonomic structure and associations between states of microbial composition and aspects of health have established the groundwork for recognition of the microbiome as a component of human biology. However, the challenge now lies in elucidating the mechanisms underlying the associations between our microbes and health. Metabolic phenotyping and identification of the microbial metabolites interacting with the host will be pivotal to this challenge. With such knowledge, progress can be made in the development of defined microbial cultures (for example, probiotics) and substrates conducive to selective growth or function of microbes (for example, prebiotics) for health enhancement. In short, there is need and opportunity for the innovative deployment of metabolic phenotyping of the human microbiome to develop a new generation of interventions to improve health.

Abbreviations

BA, bile acid; BSH, bile salt hydrolase; BV, bacterial vaginosis; CBA, conjugated bile acid; DCA, deoxycholic acid; EGFR, epidermal growth factor receptor; GI, gastrointestinal; HDAC, histone deacetylase; MAPK, mitogen-activated protein kinase; NO, nitric oxide; SASP, senescence-associated secretory phenotype; SCFA, short-chain fatty acid; T2-high, type 2-high; TMAO, trimethylamine-N-oxide.

References

1. Xie W, Wang F, Guo L, et al.: Comparative metagenomics of microbial communities inhabiting deep-sea hydrothermal vent chimaeras with contrasting chemistries. ISME J. 2011; 5(3): 414–26. Published Abstract | Publisher Full Text | Free Full Text

2. Alshinnakeo E, Meydan C, Chowdhury S, et al.: Geospatial Resolution of Human and Bacterial Diversity with City-Scale Metagenomics. Cell Syst. 2015; 1(1): 72–87. Published Abstract | Publisher Full Text | Free Full Text

3. Ruiz-Calderon JF, Cavallin H, Song SJ, et al.: Walls talk: Microbial biogeography of homes spanning urbanization. Sci Adv. 2016; 2(2): e1501061. Published Abstract | Publisher Full Text | Free Full Text

4. Coughlan LM, Cotter PD, Hill C, et al.: New Weapons to Fight Old Enemies: Novel Strategies for the (Bio)control of Bacterial Biofilms in the Food Industry. Front Microbiol. 2016; 7: 1641. Published Abstract | Publisher Full Text | Free Full Text

5. Bourie BC, Wiling BP, Cotter PD: The Microbiota and Health Promoting Characteristics of the Fermented Beverage Kefir. Front Microbiol. 2016; 7: 647. Published Abstract | Publisher Full Text | Free Full Text

6. Doyle CJ, Gleeson D, O’Tosca PW, et al.: High-throughput metatxinomic characterization of the raw milk microbiota identifies changes reflecting lactation stage and storage conditions. Int J Food Microbiol. 2017; 255: 1–6. Published Abstract | Publisher Full Text

7. Walsh AH, Crispe F, Dasari K, et al.: Strain-Level Metagenomic Analysis of the Fermented Dairy Beverage Nunu Highlights Potential Food Safety Risks. Appl Environ Microbiol. 2017; 83(16): pii: e01144–17. Published Abstract | Publisher Full Text | Free Full Text

8. McHugh AJ, Feshley C, Hill C, et al.: Detection and Enumeration of Spore-Forming Bacteria in Powdered Dairy Products. Front Microbiol. 2017; 8: 109. Published Abstract | Publisher Full Text | Free Full Text

9. Verkeswattewaran K, Vaishampayan P, Cisneros J, et al.: International Space Station environmental microbiome - microbial inventories of ISS filter debris. Appl Microbiol Biotechnol. 2014; 98(14): 6453–66. Published Abstract | Publisher Full Text

10. Be NA, Avila-Herreras A, Allen JE, et al.: Whole metagenome profiles of particulates collected from the International Space Station. Microbiome. 2017; 5(1): 81. Published Abstract | Publisher Full Text | Free Full Text

11. Scanlan PD: Blastocystis: past pitfalls and future perspectives. Trends Parasitol. 2012; 28(8): 327–34. Published Abstract | Publisher Full Text | Free Full Text

12. Scanlan PD, Knight R, Song SJ, et al.: Prevalence and genetic diversity of Blastocystis in family units living in the United States. Infect Genet Evol. 2016; 45: 95–7. Published Abstract | Publisher Full Text

13. Scanlan PD, Stensvold CR, Rajlič-Stojanović M, et al.: The microbial eukaryote Blastocystis is a prevalent and diverse member of the healthy human gut microbiota. FEMS Microbiol Ecol. 2014; 90(1): 326–30. Published Abstract | Publisher Full Text

14. Burgess SL, Gilchrist CA, Lynn TC, et al.: Parasitic Protozoa and Interactions with the Host Intestinal Microbiota. Infect Immun. 2017; 85(8): pii: e00101–17. Published Abstract | Publisher Full Text | Free Full Text

15. Chudnovskiy A, Mortha A, Kana V, et al.: Host-Protozoan Interactions Protect from Mucosal Infections through Activation of the Inflammasome. Cell. 2016; 167(2): 444–456.e14. Published Abstract | Publisher Full Text | Free Full Text

16. Hannafin K, Doozd V, Langland N, et al.: Development of functional gastrointestinal disorders after Giardia lamblia infection. BMC Gastroenterol. 2009; 9: 27. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

17. Ghannoum MA, Jurcovic RJ, Mukherjee PK, et al.: Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog. 2010; 6(1): e1000713. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

18. challnagie GB, Noverr MC: The emerging world of the fungal microbiome. Trends Microbiol. 2013; 21(7): 334–41. Published Abstract | Publisher Full Text | Free Full Text

F1000Research 2019, 8(F1000 Faculty Rev):1956 Last updated: 31 MAR 2022

Page 10 of 16
202. Ridlon JM, Kang DJ, Hylemon PB, et al.: Bile acids and the gut microbiome. Curr Opin Gastroenterol. 2014; 30: 332–6. PubMed Abstract | Publisher Full Text | Free Full Text

203. Lefebvre P, Cario B, Lien F, et al.: Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev. 2009; 89(1): 147–91. PubMed Abstract | Publisher Full Text

204. Ridlon JM, Harris SC, Bhowmik S, et al.: Consequences of bile salt biotransformations by intestinal bacteria. Gut Microbes. 2016; 7(1): 22–39. PubMed Abstract | Publisher Full Text | Free Full Text

205. Hartstra AV, Bouter KE, Bäckhed F, et al.: Insights into the role of the microbiome in obesity and type 2 diabetes. Diabetes Care. 2015; 38(1): 159–65. PubMed Abstract | Publisher Full Text

206. Turnbaugh PJ, Ley RE, Mahowald MA, et al.: An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444(7122): 1027–31. PubMed Abstract | Publisher Full Text | F1000 Recommendation

207. Kobayashi M, Ikegami H, Fujisawa T, et al.: Prevention and treatment of obesity, insulin resistance, and diabetes by bile acid-binding resin. Diabetes. 2007; 56(1): 239–47. PubMed Abstract | Publisher Full Text

208. Penney NC, Kinross J, Newton RC, et al.: The role of bile acids in reducing the metabolic complications of obesity after bariatric surgery: A systematic review. Int J Obes (Lond). 2015; 39(1): 1565–74. PubMed Abstract | Publisher Full Text

209. Patti ME, Houten SM, Bianco AC, et al.: Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. Obesity (Silver Spring). 2009; 17(9): 1671–7. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

210. Centuori SM, Gomes CJ, Trujilo J, et al.: Deoxycholic acid mediates non-canonical EGFR-MAPK activation through the induction of calcium signaling in colon cancer cells. Biochim Biophys Acta. 2016; 1861(7): 663–70. PubMed Abstract | Publisher Full Text | Free Full Text

211. Yoshimoto S, Loo TM, Atarashi K, et al.: Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013; 499(7456): 97–101. PubMed Abstract | Publisher Full Text | F1000 Recommendation

212. Bayerdörffer E, Mannes GA, Ochsenkühn T, et al.: Unconjugated secondary bile acids in the serum of patients with colorectal adenomas. Gut. 1995; 36(2): 268–73. PubMed Abstract | Publisher Full Text | Free Full Text

213. Coppé JP, Desprez PY, Krtolica A, et al.: The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol. 2010; 5: 99–118. PubMed Abstract | Publisher Full Text | Free Full Text
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