Gut microbial metabolites as multi-kingdom intermediates

Kimberly A. Krautkramer[1], *Jing Fan*[2] *and Fredrik Bäckhed*[1,3,4]✉

Abstract | The gut microbiota contributes to host physiology through the production of a myriad of metabolites. These metabolites exert their effects within the host as signalling molecules and substrates for metabolic reactions. Although the study of host–microbiota interactions remains challenging due to the high degree of crosstalk both within and between kingdoms, metabolite-focused research has identified multiple actionable microbial targets that are relevant for host health. Metabolites, as the functional output of combined host and microorganism interactions, provide a snapshot in time of an extraordinarily complex multi-organism system. Although substantial work remains towards understanding host–microbiota interactions and the underlying mechanisms, we review the current state of knowledge for each of the major classes of microbial metabolites with emphasis on clinical and translational research implications. We provide an overview of methodologies available for measurement of microbial metabolites, and in addition to discussion of key challenges, we provide a potential framework for integration of discovery-based metabolite studies with mechanistic work. Finally, we highlight examples in the literature where this approach has led to substantial progress in understanding host–microbiota interactions.

The mammalian gut microbiota is a vast collection of trillions of microorganisms (viruses, bacteria, archaea and eukaryotes) found within and on the body, including the skin, saliva, oral mucosa, vaginal mucosa and conjunctiva, although the vast majority reside in the gastrointestinal tract. Estimates place the ratio of human to bacterial cells at roughly 1:1–1.3, depending on age, gender and body habitus[1], and the gut microbiome exceeds the human genome by a factor of nearly 1,000 (>22 million genes identified in the gut microbiome versus 23,000 genes in the human genome)[2]. In rodents and humans, the caecum and proximal colon are the areas of highest microbial biomass, whereas the small intestine makes a lesser, but still substantial, contribution. In this Review, we focus on the bacteria residing in the gut. A key contribution of the gut microbiota to host physiology is the production of a diverse array of metabolites and other small molecules. These metabolites are absorbed across the host gut and are measurable in host circulation, often at concentrations equal to or in excess of those achieved by typical pharmaceutical agents[3–6].

Notably, microbial compounds can be both health-promoting and toxic, even within a single metabolite, and the effects depend upon factors such as the type and metabolic status of affected tissues[7–9], dietary context[10] and circulating levels of the metabolite[11]. Furthermore, production of microbial metabolites is driven by a combination of dietary substrate availability and interindividual variability both within populations and across geographic or ethnic groups[12–18]. The relationship between microbial metabolite production and the luminal microenvironment is also cyclical. Changes in dietary substrate availability impact microbial fermentation, which in turn impacts the luminal pH as a function of short-chain fatty acid (SCFA) production from the fermentation of complex carbohydrates, leading to changes in microbial community structure and function[19–22]. Therefore, the interaction between microbial metabolites and host physiology is complex, with variation arising due to environmental, microbial and host sources.

The reported effects of gut microbial metabolites on host health are myriad. Yet, despite a wealth of high-quality associative studies, there remains a need for more mechanistic insight into host–microbiota interactions. In the setting of extensive crosstalk between gut microorganisms and host–microbiota co-metabolism of compounds, measurement of metabolites provides a direct read-out of the host–microbiota system as a whole. Thus, metabolite-centric study design is a reasonable approach to population-based research in the pursuit of microbial mediators of host health. In this Review, we cover the major microbial metabolite classes and the relevant biochemistry and regulation of these pathways. Additionally, we highlight instances in the literature where microbial metabolites affect host health, we provide a survey of available methodologies for exploration

[1]*Wallenberg Laboratory, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.*

[2]*Morgridge Institute for Research, Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, WI, USA.*

[3]*Department of Clinical Physiology, Region Västra Götaland, Sahlgrenska University Hospital, Gothenburg, Sweden.*

[4]*Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.*

✉*e-mail: Fredrik.Backhed@wlab.gu.se*

https://doi.org/10.1038/s41579-020-0438-4

Box 1 | **Metabolomics in host–microbiome studies**

Quantitative measurement of microbiome-derived or microbiome-modified metabolites provides a functional read-out of the metabolic activities of microbiota and host–microbiome interactions. This rapidly developing area has substantially contributed to our understanding of host–microbiota interactions. Metabolomics analysis coupled with in vitro culture and bioreactor techniques has proven to be a powerful strategy to screen for and validate both microbial community-based and species-specific enzymatic activities on dietary substrates, host metabolites and drugs[4,107]. Metabolite profiling from various host compartments is increasingly used to investigate the effects of specific gut microbial modifiers and the impacts of microbiome-derived or host–microbiota co-substrates on host health.

Mass spectrometry is becoming more widespread in host–microbiota studies due to its high sensitivity, capacity for unbiased and high-throughput discovery, and applicability to a wide variety of metabolite classes. Owing to the high complexity of biological samples, mass spectrometry analysis is usually preceded by chromatographic separation to improve sample resolution and subsequent metabolite identification and quantification. Gas chromatography–mass spectrometry (GC-MS) is particularly suitable for volatile metabolites such as short-chain fatty acids, but can also be used for non-volatile metabolites such as sugar metabolites, amino acids and amino acid derivatives when combined with specific pre-analytical chemical derivatization steps[175]. Liquid chromatography–mass spectrometry (LC-MS) is widely used for the analysis of both non-polar metabolites (bile acids and lipids) and polar metabolites (purines, amino acids, vitamins and their derivatives). LC-MS also uses softer ionization and lower temperature than GC-MS, making it more suitable for larger, non-volatile and less stable metabolites.

Nuclear magnetic resonance (NMR) spectroscopy is also used for metabolomics, although it generally has lower sensitivity than mass spectrometry-based methods. NMR spectroscopy allows for quantification of abundant metabolites in biological samples with relatively simple sample preparation. It also provides valuable structural information, particularly when sample complexity can be mitigated, which is beneficial for identifying novel microbiota-derived compounds. Although both mass spectrometry and NMR spectroscopy allow for untargeted metabolite analysis, identifying the structure of spectral hits remains challenging due to the high diversity of microbial products, many of which have not been previously characterized. Both NMR-based and mass spectrometry-based metabolomics allow for the investigation of nutrient assimilation and metabolic activity in the microbiota through isotope tracing[176–178]. However, full characterization of microbiota-specific metabolic flux using isotopic labelling remains challenging, as many metabolites are shared and exchanged among the host and various microorganisms in the microenvironment, making it difficult to determine the origin of specific metabolites. Imaging mass spectrometry (desorption electrospray ionization mass spectrometry or matrix-assisted laser desorption/ionization imaging mass spectrometry[179] and nanoscale secondary ion mass spectrometry[176,180]) and biological spectroscopy methods (Raman spectroscopy[176]) can provide high-throughput spatial information as well, and can be combined with fluorescent probes and/or stable isotope tracing to gain single-cell resolution within host and microbial cells[176].

Additional analytical methods available for metabolite measurements include immunochemistry-based methods and enzymatic assays. These methods are low throughput and targeted in nature but offer unique advantages. Antibodies against some small molecules are available, which enables ELISA-based or imaging-based measurement of these metabolites[59]. Imaging-based methods can provide not only high sensitivity but also spatial information that is otherwise challenging to obtain.

Method	Key features	Metabolites and references
Mass spectrometry-based methods	High sensitivity, high throughput, capable of rapidly measuring a large volume of samples, coupling with online chromatography separation methods reduces complexity and improves resolution, specificity and quantification; allows quantification of isotopic labelling, provides structural information, may provide high-resolution, high-sensitivity spatial information, can be performed under ambient environmental conditions without sample preparation with preservation of tissue morphology (DESI-MS)	GC-MS: SCFAs and ketones[181–183], sugars and sugar metabolites[184], amino acids[183,184] LC-MS: bile acids[140,183,184], lipids and fatty acids[140,183–185], sugar metabolites[185], vitamins and related compounds[158,177], amino acids and amino acid derivatives[4] Untargeted analysis[184–186] Imaging mass spectrometry: MALDI/DESI-IMS[179] and nanoSIMS[176]
Nuclear magnetic resonance (NMR) spectroscopy	Provides structural information, lower sensitivity than mass spectrometry, high throughput, allows quantification of isotopic labelling, provides spatial information (NMR imaging or MRI)	Sugar metabolites[187,188], amino acids and amino acid derivatives[187,189], SCFAs[190], vitamins[177] Untargeted analysis and metabolome finger printing[191]
Raman microspectroscopy	Spatial information, high throughput, structural information, measurement is non-destructive enabling other downstream methodologies, lower sensitivity versus mass spectrometry and NMR	Can be combined with fluorescent probes and isotopic labelling for single cell-resolved assessment of nutrient assimilation[176]
Immunochemistry and enzymatic assays	Low throughput, high specificity, may provide spatial information (immunohistochemistry or immunofluorescence)	Eicosanoids, uric acid, serotonin and other neurotransmitters[59], lipopolysaccharide, some vitamins, sugar metabolites[192]

DESI-MS, desorption electrospray ionization mass spectrometry; GC-MS, gas chromatography–mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; MALDI, matrix-assisted laser desorption; nanoSIMS, nanoscale secondary ion mass spectrometry; SCFA, short-chain fatty acid.

of microbial metabolites and we conclude with a discussion of both challenges and opportunities, along with a possible framework for metabolite-centred studies in clinical and translational research. Given the essential nature of metabolite measurement to the exploration of host–microbiota interactions and the growing role of the epigenome as a complex signal integrator, we also provide coverage of these topics in BOXES 1 and 2, respectively.

Fermentable substrates

Bacterial metabolism of dietary macronutrients and micronutrients in the distal gut results in the production of many compounds. SCFAs are perhaps the most commonly studied class of small-molecule metabolites that are produced by gut microbial fermentation of dietary fibre and, to a lesser degree, other substrates depending on availability of fermentable carbohydrates.

Mucosa
An epithelial layer comprising epithelial cells and mucus-secreting cells, among other specialized cell types, that lines multiple body surfaces (gastrointestinal tract, oropharynx, airways and vaginal tract) and functions as an innate barrier.

Fermentation
The chemical breakdown of organic substrates (for example, carbohydrates and amino acids) by various enzymes in the absence of molecular oxygen.

Gas chromatography–mass spectrometry
(GC-MS). An analytical method that couples chromatographic separation of complex biological samples in the gas phase to mass spectrometry for the identification and quantification of the compounds that comprise the sample.

Liquid chromatography–mass spectrometry
(LC-MS). An analytical method that couples chromatographic separation of complex biological samples in the liquid phase to mass spectrometry for the identification and quantification of the compounds that comprise the sample.

Desorption electrospray ionization mass spectrometry
A soft electrospray ionization technique that relies on solvent extraction directly on the sample under ambient conditions that is primarily used on tissues for imaging mass spectrometry.

Raman spectroscopy
A vibrational spectroscopy technique wherein a biological sample is subjected to a beam of light and differences in photon scatter (based on the molecular composition of the sample) are used to produce a unique chemical fingerprint.

Carbohydrate-active enzymes
(CAZymes). A collective term for enzymes that can synthesize or break down saccharides.

Dietary fibre, which impacts the gut microbiota in host health and disease (reviewed in REF.[22]), is a broad term that encompasses polysaccharides, oligosaccharides and resistant starches. These complex dietary carbohydrates evade breakdown by a limited repertoire of host enzymes in the small intestine and pass to the distal gut, where they serve as substrates for a multitude of microbial carbohydrate-active enzymes (CAZymes) that vastly expand the host's metabolic capacity[23,24]. Dietary fibre is estimated to constitute 5–10% of the energy intake in western society, but may be higher in communities with higher fibre intake[25]. Acetate, propionate and butyrate comprise ≥95% of the total SCFA pool and are present at a molar ratio of approximately 60:20:20 in the gut of mice and humans[26,27]. Although far lower in abundance, the branched-chain fatty acids (BCFAs) isobutyrate, 2-methylbutyrate and isovalerate, and the propionate intermediates lactate and succinate, are also produced and can exert biological effects[20,28,29]. There is a wealth of literature concerning microbial SCFAs in host physiology, including recent reviews dedicated entirely to this subject[20,30]. Our goal here is to discuss key fermentative pathways, the microorganisms that utilize them and regulation of these pathways, and to highlight both salient and recent examples in the literature of SCFAs impacting host health.

Although the majority of the SCFAs are derived from dietary fibre, another source of microbial accessible carbohydrates (MACs) is the colonic mucus layer. The inner mucus layer is impermeable under normal physiological conditions, but the outer layer functions as a microbial habitat where bacteria can utilize mucus as an energy source[31]. Mucus is secreted by goblet cells in both the small and large intestine, and comprises large proteins (>5,000 amino acids long) called mucins that are heavily *O*-glycosylated[31,32]. In fact, >80% of the molecular weight of mucins is due to these complex carbohydrate post-translational modifications. Thus, host mucins can serve as a major source of both carbohydrate and protein under conditions where dietary MACs are limiting[19,33,34]. Complex glycans are released from mucins via bacterial exoglycosidases one monosaccharide at a time[34], and if enough glycan removal occurs to expose

Box 2 | **The epigenome as a signal integrator of supra-organismal metabolic status**

Modulation of chromatin by endogenous metabolites has been an area of intense research for over two decades[193], but it has only recently become clear that microbial metabolites can also exert control over host chromatin modifications[194,195]. Numerous small-molecule metabolites, including the methyl donor *S*-adenosyl methionine (SAM) and the central carbon rheostat acetyl-CoA, regulate the activity of enzymes that add and remove chromatin modifications[193,196]. Chromatin comprises genomic double-stranded DNA that is wrapped around an octamer of histones, which are small, highly basic, globular proteins with flexible amino-terminal tails that are subject to an extensive array of post-translational modifications (PTMs). Acetylation, methylation and phosphorylation are the most commonly studied, but the list of histone PTMs is steadily growing[197]. Histone PTM states collectively form what has been termed the 'histone code', a vastly complex and combinatorial set of histone modifications that regulate processes requiring physical access to genomic DNA, including transcription, DNA replication and DNA repair[198]. Chromatin has also been hypothesized to function as a signal integrator within cells, taking in environmental cues in the form of small-molecule metabolites from both endogenous and exogenous sources to elicit new gene expression programmes in response to various stimuli, thus allowing a static genome to adapt to a dynamic environment[199]. Although genomic DNA undergoes a less diverse, but also expanding, set of chemical modifications than histone proteins, metabolite availability impacts DNA methylation as well[200].

In addition to the small list of known host chromatin–microbial metabolite relationships, there are numerous putative gut microbial metabolites that may be chromatin modifiers[194]. Although butyrate has been known to inhibit histone deacetylases (HDACs) since the late 1970s (REF.[201]), several decades passed before microbial butyrate was directly linked to histone acetylation[8,65]. Microbial-derived butyrate was shown to acetylate histones through two distinct mechanisms in normal colonic epithelium versus highly glycolytic, Warburg-like cancerous colonocytes, resulting in opposing gene expression programmes[8]. Further, dietary fibre-driven butyrate production and subsequent histone acetylation was shown to be protective against colonic adenocarcinoma in mice and humans[64]. However, the protective role of butyrate remains controversial, as there is also evidence that this metabolite promotes cancer[7], suggesting specific and/or local effects.

In the setting of diet-induced obesity, we demonstrated that global histone modification states (acetylation and methylation) are regulated by the gut microbiota in multiple host tissues in a diet-dependent manner, and that short-chain fatty acid supplementation in germ-free mice was sufficient to recapitulate a significant fraction of the microbiota-induced chromatin signature and hepatic gene expression[62]. Gut microbial control of host histone PTMs is also circadian. Diurnal changes in microbial proximity to the intestinal mucosa and metabolite production have been shown to alter histone acetylation and methylation in intestinal epithelial cells[202]. Additional microbiota-driven histone PTMs have also been recently reported, including ethylation[177] and lactylation[203].

Finally, although members of the prokaryotic gut microbiota possess neither a nucleus nor chromatin, there is evidence for epigenetic regulation through DNA methylation[204] and supercoiling[205] in microorganisms. Host microRNAs can also impact the microbiota[206]. Thus, epigenetic regulation has an important role in both the host and the microbiota. Spanning multiple kingdoms, chromatin may serve as an intracellular integrator of both endogenous and microbial signals to elicit appropriate cellular and tissue responses to environmental stimuli. There is also evidence that early life exposures alter both the gut microbiome[207,208] and the host epigenome[209]. Combined, this evidence suggests that early life may be a key developmental window for both the microbiota and host epigenome, and that some of these early life events may not only be imprinted at the level of chromatin but also inherited across generations. In support of this, two recent studies have linked early-life antibiotic exposure to altered gut histone PTM states in type 1 diabetes mellitus[183] and maternal host–microbiota choline competition with alterations in both maternal and offspring DNA methylation[210].

Microbial accessible carbohydrates
(MACs). Complex polysaccharides and oligosaccharides that are available to the gut microbiome's vast repertoire of carbohydrate-active enzymes.

Mucus
A gel-like layer(s) secreted by and resting on top of the mucosa comprising mucins and functions as an essential barrier between the environment and the mucosal layer.

Mucins
Large, heavily decorated proteins characterized by proline-rich, serine-rich and threonine-rich tandem repeats (PTS domains) that are modified by complex *O*-glycans and form large polymeric protein networks that function as the building blocks of mucus in the intestinal tract.

Exoglycosidases
Enzymes that hydrolyse the glycosidic bond at the terminal monosaccharide in a polysaccharide or oligosaccharide.

Polysaccharidases
Enzymes that hydrolyse polysaccharides to form smaller saccharide chains.

Glycosidases
A general term for enzymes that hydrolyse glycosidic bonds in polysaccharides and oligosaccharides.

the mucin protein core, bacterial proteases then dissolve the mucus gel[31].

The colonic microbiota can also ferment protein of dietary, host and microbial origin, which contributes a small amount to the total SCFA pool, including acetate, propionate, butyrate and the BCFAs isobutyrate, 2-methylbutyrate and isovalerate from the branched-chain amino acids (BCAA) valine, leucine and isoleucine[28]. In vitro batch culture studies from human gut contents suggest that protein fermentation accounts for ~17% of caecal and 38% of distal gut (sigmoid colon or rectum) SCFA pools[29]. Based on human ileostomy[35] and pancreatic juice collection[36] studies, the major source of substrate for protein fermentation in the colon appears to be dietary; however, pancreatic hydrolases secreted into the gut may account for up to an estimated 19% of nitrogenous compounds available to the colonic microbiota (3.5 g pancreatic enzymes secreted into the gut out of an estimated total 18 g nitrogenous compounds available to the gut microbiota per day)[35]. Thus, protein fermentation is a small, but substantial, contributor to microbial organic acid production.

Fermentation of dietary substrates also liberates and/or modifies bioactive polyphenols[22,37,38]. Dietary polyphenols are a large class of plant-derived compounds found in common foods such as fruits, vegetables, cereals, tea, coffee and wine[39]. These phytochemicals possess immense structural diversity (for example, >9,000 structurally diverse, naturally occurring flavonoids have been identified)[38] and are grouped into families by structural similarity: phenolic acids, flavonoids, lignans, lignins, coumarins and stilbenes[37]. They are generally glycosylated in their native forms, but esterification, acylation and polymerization also occur, resulting in complex and very high molecular weight compounds up to nearly 40,000 Da[38,40]. Given their high structural complexity, only ~10% of dietary polyphenols are metabolized and absorbed in the small intestine. The remaining ~90% pass to the distal gut where they may undergo extensive modification and degradation by the microbiota, which increases their absorption and bioavailability[38,41]. Owing to their structural diversity and limited bioavailability, polyphenols have been difficult to study, but there is evidence that these compounds impact gut microbial community composition and function[38]. Dietary polyphenols exert their effects through anti-inflammatory, antioxidant and antimicrobial properties, and have been associated with beneficial effects in the setting of cardiovascular disease (CVD), cancer, metabolic disease, Alzheimer disease and inflammatory bowel disease[22,38,41]. An encouraging hypothesis is that these compounds exert measurable physiologic effects despite low bioavailability owing to substantial metabolism of parent compounds by the gut microbiota. Indeed, a purified flavone-rich extract from citrus fruits was recently shown to be protective against high-fat feeding in a microbiota-dependent manner, suggesting that this extract may be a therapeutic prebiotic agent in the treatment of metabolic disease[42]. Nonetheless, additional research is necessary to better understand the implications of this complex group of compounds on host–microbiota interactions.

Microbial fermentation pathways for carbohydrates and protein. Fermentative pathways of dietary carbohydrates and proteins by the colonic microbiota are depicted in FIG. 1. Broadly, complex carbohydrates from either the diet or mucins are hydrolysed by microbial polysaccharidases and glycosidases to either five-carbon or six-carbon monosaccharides that undergo further catabolism to pyruvate via either the classical pentose phosphate (five-carbon) pathway or the Embden–Meyerhof–Parnas (six-carbon) pathway. Pyruvate (or its precursor phosphenolpyruvate) then proceeds through numerous biochemical pathways that ultimately produce SCFAs. In the case of protein fermentation, the fraction of dietary and host proteins that escape host digestion in the proximal gut are first hydrolysed to amino acids through a combination of host endopeptidases and microbial proteases[35]. The resulting amino acids are then subject to various microbial fermentative reactions, depending on the substrate, that yield not only SCFAs and BCFAs but also numerous other compounds, including ammonia, and phenol and indole compounds[28,35].

Regulation by environmental, host and biochemical factors. The production of SCFAs is a function of diet, microbial community composition and host factors. Dietary macronutrient composition (that is, the carbohydrate to protein to fat ratio), which dictates the amount and source of fermentable substrate for the microbiota, is a major driver of microbial structure and function. Microbial responses to dietary fibre are highly individualized[14,18] and may depend on the presence of certain 'keystone' species[43]. There are also a multitude of data showing differences in SCFA production as a function of dietary fibre type[27,30], and a recent small randomized controlled trial showed that, despite individualized responses, the effects of treatment with three distinctly structured resistant starches elicited consistent effects on microbiome composition and SCFA production in humans[44]. Members of Firmicutes and Actinobacteria phyla are the primary responders to changes in availability of MACs and, generally, occupy more specialized roles in fibre degradation[22]. By contrast, bacteria such as *Bacteroides thetaiotaomicron* can alter their transcriptome to digest mucus rather than dietary MACs[34]. Furthermore, *B. thetaiotaomicron* can also induce the host to produce fucose-containing glycans that can be used as fermentable substrates[45]. TABLE 1 presents a list of microbial fermentation products, relevant pathways and microbial producers.

High-MAC diets are generally associated with increased gut microbial diversity and SCFA production. When dietary fibre is limiting, the microbiota shifts towards fermentation of less favourable substrates, namely dietary and endogenous proteins and dietary fats, resulting in lower amounts of SCFA and poor health outcomes[19–22]. Changes in microbial community composition have been observed, within as little as 24 h of a macronutrient shift, that reflect trade-offs between primary utilization of carbohydrate versus protein[46–48]. In mice, feeding a western diet low in MACs induced changes in microbial community structure that were

largely reversible within a single generation, but continuation of this diet over multiple generations resulted in extinction of several microbial species that worsened over progressive generations (that is, more species were lost) and were not recoverable upon resumption of a higher MAC diet[49]. Similar dietary responses have also been observed in human populations, particularly in response to industrialization and/or immigration[13,15,17,50], including one study linking immigration from a non-western country to loss of microbial diversity and function that was compounded by the length of time spent in the USA and increasing generation[16].

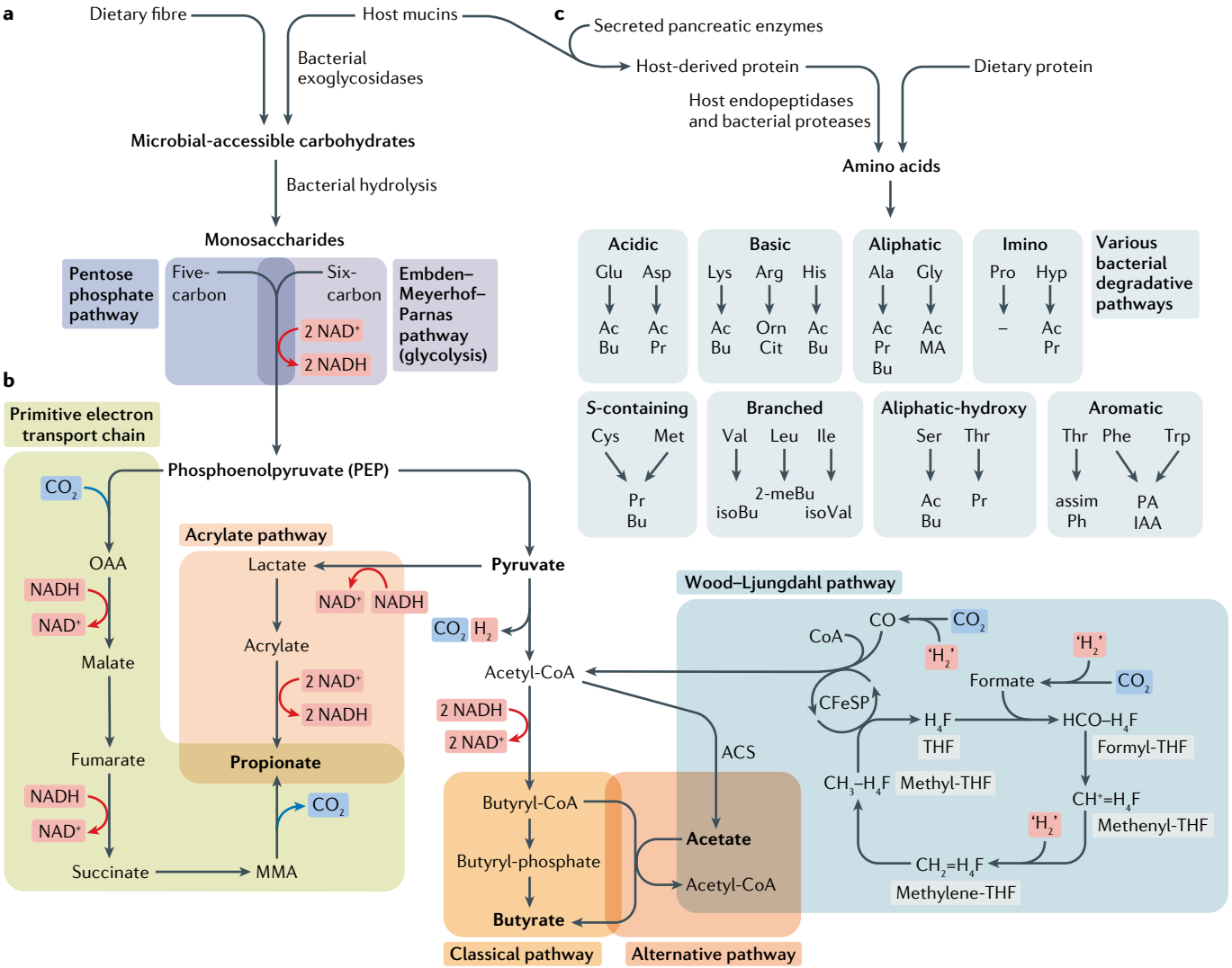

Fig. 1 | **Fermentation of microbial-accessible carbohydrates and proteins by the colonic gut microbiota. a** | Dietary fibre and carbohydrates liberated from host mucins comprise microbial-accessible carbohydrates, which are hydrolysed to monosaccharides by a myriad of bacterial polysaccharidases and glycosidases. These monosaccharides are catabolized via either the pentose phosphate pathway or the Embden–Meyerhof–Parnas pathway (for five-carbon and six-carbon substrates, respectively) to first form phosphoenolpyruvate (PEP) and, ultimately, pyruvate, generating 2 NADH in the process. **b** | The short-chain fatty acids acetate, propionate and butyrate can be derived from PEP or pyruvate via several pathways driven by redox equivalents (NADH and molecular H_2) and the partial pressures of H_2 and CO_2. Acetate is formed either through decarboxylation of pyruvate yielding acetyl-CoA followed by hydrolysis to acetate by acetyl-coenzyme A synthetase (ACS) or from CO_2 via the stepwise Wood–Ljungdahl pathway[172] that utilizes formate and several one-carbon cycle intermediates. Propionate is generated either from PEP via a primitive anaerobic electron transport chain comprising NADH dehydrogenase and fumarate reductase or through reduction of lactate via the acrylate pathway[20,52]. Butyrate is formed either by condensation of two moieties of acetyl-CoA to butyryl-CoA, which is then converted to butyrate via the classical pathway consisting of phosphotransbutyrylase and butyrate kinase, or a more recently discovered alternative pathway that uses exogenously derived acetate to generate butyrate and acetyl-CoA, which actually dominates over the classical pathway in the human gut microbiota[173]. **c** | Fermentation of proteins (grey insets), from both host (mucins and gut-secreted enzymes) and dietary sources, leads to production of acetate (Ac), propionate (Pr) and butyrate (Bu), in addition to smaller amounts of ornithine (Orn), citrulline (Cit), methylamine (MA), phenolic compounds (Ph), phenylacetate (PA) and indoleacetate (IAA). The branched-chain amino acids valine, leucine and isoleucine form the branched-chain fatty acids isobutyrate (isoBu), 2-methylbutyrate (2-meBu) and isovalerate (isoVal). The majority of these compounds are produced by dissimilatory amino acid catabolic pathways, but tyrosine also undergoes rapid assimilatory metabolism (assim) and proline appears to be a poorly used substrate for fermentation (denoted '–')[28]. CFeSP, corrinoid iron–sulfur protein; 'H_2' designates the requirement for two electrons and two protons in the reaction; MMA, methylmalonate; OAA, oxaloacetate; THF, tetrahydrofolate.

Table 1 | **Products of gut microbial fermentation of carbohydrates, protein and dietary polyphenols**

Metabolite	Pathway	Genera or species
Acetate	Pyruvate decarboxylation to acetyl-CoA	*Akkermansia muciniphila*, *Bacteroides* spp., *Bifidobacterium* spp., *Prevotella* spp., *Ruminococcus* spp.[21,26–28]
	Wood–Ljungdahl pathway	*Blautia hydrogenotropphica*, *Clostridium* spp., *Streptococcus* spp.[21,26–28]
Propionate	Acrylate pathway	*Coprococcus catus*, *Eubacterium hallii*, *Megasphaera elsdenii*, *Veillonella* spp.[21,26–28]
	Succinate pathway	*Bacteroides* spp., *Dialister* spp., *Phascolarctobacterium succinatutens*, *Veillonella* spp.[21,26–28]
	Propanediol pathway	*Roseburia inulinivorans*, *Ruminococcus obeum*, *Salmonella enterica*[21,26–28]
Butyrate	Classical pathway via butyrate kinase	*Coprococcus comes*, *Coprococcus eutactus*[21,26–28]
	Alternate pathway using exogenous acetate	*Anaerostipes* spp., *C. catus*, *E. hallii*, *Eubacterium rectale*, *Faecalibacteerium prausnitzii*, *Roseburia* spp.[21,27,28,35]
Short-chain fatty acids and branched-chain fatty acids	Amino acid fermentation through various dissimilatory proteolytic reactions	*Acidaminococcus* spp., *Acidaminobacter* spp., *Campylobacter* spp., Clostridia spp., *Eubacterium* spp., *Fusobacterium* spp., *Peptostreptococcus* spp.[21,26–28,44]
'Kynurenines' (kynurenine and its derivatives)	Various bacterial enzymes homologous to mammalian enzymes of the kynurenine pathway	*Lactobacillus* spp., *Pseudomonas aeruginosa*[89], *Pseudomonas fluorescens*[95] Putative: *Pseudomonas* spp., *Xanthomonas* spp., *Burkholderia* spp., *Stenotrophomonas* spp., *Shewanella* spp., *Bacillus* spp., members of Rhodobacteraceae, Micrococcaceae and Halomonadaceae families[95]
Indole	Hydrolytic β-elimination of tryptophan to indole (tryptophanase)	*Achromobacter liquefaciens*, *Bacteroides ovatus*, *Bacteroides thetaiotamicron*, *Escherichia coli*, *Paracolobactrum coliforme*, *Proteus vulgaris*[11,89]
Indole derivatives	Multiple	*Bacteroides* spp., *Clostridium* spp. (*Clostridium sporogenes*, *Clostridium cadaveris*, *Clostridium bartlettii*), *E. coli*, *Lactobacillus* spp., *E. halli*, *Parabacteroides distasonis*, *Peptostreptococcus* spp. (*Peptostreptococcus anaerobius*)[4,11,89,167]
Tryptamine	Decarboxylation of tryptophan	*C. sporogenes*, *Ruminococcus gnavus*[168]
Serotonin	Induction of host synthesis[a]	Indigenous spore-forming bacteria, dominated by *Clostridium* spp.[59] and *Turicibacter* spp.[103]
Histamine	Decarboxylation of histidine (histidine decarboxylase (HDC))	*E. coli*, *Morganella morganii*, *Lactobacillus vaginalis*[105] Putative: *Fusobacterium* spp.[101]
Imidazole propionate (ImP)	Non-oxidative deamination of histidine to urocanate followed by reduction of urocanate to ImP by urocanate reductase (UrdA)	*Aerococcus urinae*, *Adlercreutziae equolifaciens*, *Anaerococcus prevotii*, *Brevibacillus laterosporus*, *Eggerthella lenta*, *Lactobacillus paraplantarum*, *Shewanella oneidensis*, *Streptococcus mutans*[106]
Dopamine	Decarboxylation of levodopa (L-DOPA) via tyrosine decarboxylase (TyrDC)	*Enterococcus* spp. (*Enterococcus faecalis*, *Enterococcus faecium*, 77 human isolates of *Enterococcus* spp.), *Lactobacillus brevis*, *Helicobacter pylori*[107,108]
p-Cresol	From tyrosine or phenylalanine via two pathways: direct cleavage of the Cα–Cβ bond in tyrosine to yield *p*-cresol by tyrosine lyase; and a series of reactions involving transamination, deamination and decarboxylation of tyrosine or phenylalanine via formation of the cresol precursor phenylacetic acid[4,169]	Assay proven: *Blautia hydrogenotrophica*, *Clostridioides difficile*, *Olsenella uli*, *Romboutsia lituseburensis*[169] Predicted: *Acidaminococcus fermentans*, *Anaerococcus vaginalis*, *Anaerostipes* spp., *Bacteroides* spp., *Bifidobacterium infantis*, *Blautia* spp., *Citrobacter koseri*, *Clostridium* spp., *Eubacterium siraeum*, *Fusobacterium* spp., *Klebsiella pneumoniae*, *Lactobacillus* spp., *M. elsdenii*, *Roseburia* spp., *Ruminococcus* spp., *Veillonella parvula*[169]
Phenylacetylglutamine (PAGln) and phenylacetylglycine (PAGly)	Synthesized during host hepatic phase II metabolism via conjugation of either glutamine or glycine to phenylacetic acid, an intermediate in microbial fermentation of phenylalanine[4,117]	Conjugation of phenylacetic acid to glutamine or glycine occurs in the host liver; see *p*-cresol (above) for information about its precursor, phenylacetic acid

[a]Despite studies identifying genomic potential within the microbiota for serotonin production[101], there are no reports to our knowledge of validated serotonin synthesis by the mammalian gut microbiota.

Of note, computational modelling of empirical data from 858 mice fed 25 unique diets revealed that, in addition to the gross energy density of the food, dietary nitrogen availability and its impact on competition for carbohydrates, rather than the carbohydrate content itself, was the key determinant of microbial community assembly and host–microbiome interactions[51].

In addition to dietary and host factors, there are also biochemical drivers of microbial fermentation. Two NADH molecules are produced each time a monosaccharide is catabolized to pyruvate, and these excess reducing equivalents are used to drive additional biochemistry (FIG. 1) or are sunk into molecular H_2 via ferredoxin-dependent reactions[52]. Reduction of

Chromatin
A highly structured nucleoprotein complex in eukaryotes that consists of the nucleic acids and histone proteins around which double-stranded genomic DNA winds to ultimately form chromosomes.

ferredoxin is the driving force behind numerous reactions that are essential to microbial SCFA production, including carboxylation of acetyl-CoA to pyruvate and acetogenesis via the Wood–Ljungdahl pathway, that would otherwise be thermodynamically prohibitive[53]. Fermentation also results in the production of both CO_2 and H_2 (FIG. 1), both of which have regulatory roles in SCFA production. Removal of these products by acetogens and methanogens may further increase the fermentative capacity of the microbiota[52,54].

SCFAs in mammalian host physiology. The roles of SCFAs in host health and disease are myriad[20,30]. SCFAs regulate a growing list of host physiological and biochemical functions, including maintenance of innate gut barrier function at the level of the colonic epithelium[55,56] and mucus[31,32], gut motility[57,58], secretion of the gut hormones (Peptide YY (PYY), serotonin/5-hydroxytryptamine (5-HT), cholecystokinin (CCK), gastric inhibitory peptide (GIP) and glucagon-like peptide 1 (GLP-1))[59–61], chromatin regulation[62–65], the gut–brain axis[30,66], immunological function[67,68] and more. These small organic acids have also been associated with a growing list of host health and disease states. Although this list is not exhaustive, it includes an array of cardiometabolic diseases, including atherosclerosis[69], obesity[70,71], metabolic syndrome and type 2 diabetes mellitus (T2DM)[72], and non-alcoholic fatty liver disease[72]; neurological and neuropsychiatric disorders, including Parkinson disease, Alzheimer disease, autism spectrum disorder, anxiety and depression[30]; and mixed effects in the setting of tumorigenesis[8,64,73]. The specific effects of SCFAs are largely mediated by selective activation of G-protein-coupled receptors (GPCRs) or free fatty acid receptors (FFARs), including GPR41 (FFAR3), GPR42, GPR43 (FFAR2), GPR81 (HCA1), GPR91 (SUCNR1), GPR109A (HCA2), GPR164 (ORE51E1) and OR51E2 (Olfr78)[20,30,74], although they also have direct roles as substrates for additional catabolic and anabolic pathways. Although coverage of GPCRs and their role in gut microorganism–host signalling is outside the scope of this Review, this vast topic has recently been expertly reviewed[74].

Less is known about the role of BCFAs in host physiology, but the BCAAs they originate from have recently emerged for their role in obesity, insulin resistance and T2DM. Elevated plasma BCAAs have been associated with insulin resistance across multiple ethnic groups and geographic locations, and generally covary as part of a BCAA-related metabolite cluster that includes aromatic amino acids (phenylalanine and tyrosine) and acylcarnitines, both of which are reflective of BCAA overload through separate mechanisms[75]. BCAAs may also predict the efficacy of bariatric surgery and thiazolidenediols in improving glucose homeostasis[76]. In a separate study, elevated fasting serum BCAAs associated with insulin resistance and a gut microbiome that was enriched for BCAA synthesis but depleted for uptake of these compounds[77]. BCAA catabolism is also lost in multiple cancers and dietary intake of BCAAs correlates with cancer risk in mice and humans[78]. Despite these associations with metabolic disease and cancer, BCAAs are an increasingly popular additive in sports supplements due to theoretical attenuation of the effects of strenuous resistance training[79]. Therefore, although the extent to which microbial fermentation of BCAAs impacts metabolic disease remains to be determined, alterations in microbial community composition and function that alter BCAA pools may be important etiologic factors.

Amino acids and their derivatives

An estimated 5–12 g per day of proteinaceous material is available to human colonic bacteria[35]. Despite efficient assimilation of protein by the host in the small intestine, studies in healthy volunteers using breath testing or direct sampling by ileostomy or nasogastric tube reveal that anywhere from 5 to 10% of dietary protein is not absorbed by the end of transit through the ileum, and thus passes into the colon as protein and peptides[80,81]. Once in the distal gut, protein and peptides have three possible fates: assimilation by the microbiota; serving as the substrate for microbial dissimilatory metabolism, the products of which either enter the host portal circulation or function as intermediates in extensive microbial crosstalk; and excretion in faeces. The degree to which the gut microbiota metabolizes amino acids is largely dictated by substrate availability and the luminal environment. Higher rates of bacterial fermentation of protein (versus carbohydrate) have been reported in the setting of higher colonic pH and low carbohydrate availability[82,83]. Degradation of protein by the microbiota results in considerably less SCFA production than that from carbohydrates. Furthermore, decreased organic acid production leads to a higher luminal pH, which in turn alters the structure and function of the microbiota[83,84]. By contrast, low luminal pH from SCFA production is thought to inhibit bacterial protease activity[84], and fermentable carbohydrates drive bacterial growth that, subsequently, increases bacterial protein assimilation at the expense of fermentation[85,86].

The study of protein degradation by the gut microbiota, to date, has been somewhat limited owing to the complexity of luminal contents, intricate interdependencies between a multitude of host and microbial pathways for metabolism of these substrates and technical limitations in classifying metabolite origins (host versus microbiota)[87]. Nonetheless, it has become clear in recent decades that the gut microbiota salvages substantial energy from proteins and peptides that escape host digestion to generate various bioactive compounds, some of which are potentially toxic, including SCFAs, BCFAs, ammonia, phenols, indoles, amines, sulfides and *N*-nitroso compounds[84]. Although not the focus of this section, fermentation of the canonical amino acids and the major products formed are depicted in FIG. 1, and genera with known proteolytic activity are presented in TABLE 1. Given the degree of substrate diversity and the complex crosstalk involved in degradative pathways, discussion of specific mechanisms for each nitrogenous compound is outside the scope of this Review and, in many cases, remains unknown. Therefore, the remainder of this section covers a focused list of bioactive amino acid derivatives with known effects on the host. These, and additional compounds, are presented in TABLE 1.

Tryptophan metabolism in host–microbiota crosstalk. Tryptophan is an essential amino acid that is found in common foods such as milk, cheese, fish, bananas, oats, poultry, chocolate and wine. This amino acid is particularly interesting in that despite being the largest canonical amino acid by molecular weight, it is the least abundant both in terms of its presence within proteins but also as a free amino acid within cells[88]. It is also the most chemically complex amino acid and can undergo biochemical transformation at nearly every atom within its structure, making it an optimal substrate for an extensive variety of transformations[88]. These characteristics make it an ideal molecule for inter-kingdom communication. In support of this, several signalling molecules in humans are derived from tryptophan, including serotonin and tryptamine. Dietary tryptophan has several possible fates within mammalian hosts (FIG. 2): the kynurenine pathway, which produces several intermediates and, ultimately, NAD^+; the serotonin pathway in enterochromaffin cells; protein synthesis; and direct transformation by the resident microbiota into one of many derivative compounds, including indoles, several of which are ligands for the aryl hydrocarbon receptor (AhR)[89].

The kynurenine pathway results in numerous distinct chemical intermediates, collectively termed 'kynurenines', and the end product NAD^+[90,91]. The rate-limiting step in the conversion of tryptophan to kynurenine is either indoleamine 2,3-dioxygenase 1 (IDO1; immune and gut epithelial cells) or tryptophan 2,3-dioxygenase (TDO; hepatocytes)[91]. The gut microbiota is a known driver of IDO1 expression[92,93], and IDO1 activity has also been shown to regulate microbial community composition[94]. These enzymes are known to be highly upregulated in various cancers, and it is thought that some of the kynurenine derivatives synthesized as a result of this upregulation act as AhR ligands to promote cell migration and immune tolerance, thus driving cancer progression[90,91]. In addition to host production of kynurenines, several members of the mammalian gut microbiota also have the genomic capacity to produce various intermediates in the pathway, and *Lactobacillus* spp., the pathogen *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* have been shown to synthesize several of these intermediates[89,95] (TABLE 1).

The physiological effects of kynurenines can be either protective or detrimental to host health, depending on the specific compound, target tissue and signalling pathways involved[89,95,96] (FIG. 2). In the central nervous system (CNS), levels of quinolinic acid and kynurenic acid, which exert excitotoxic and neuroprotective effects, respectively, have been found to be dysregulated in depression and schizophrenia, and mouse models of both Alzheimer disease and Huntington disease have also been associated with defects in the kynurenine pathway[89,90]. By contrast, regulation of the kynurenine pathway during exercise may be responsible for the beneficial mental effects of exercise. Exercise training in mice and human subjects caused a PGC-1α-mediated increase in skeletal muscle expression of several isoforms of kynurenine aminotransferase (KAT), which converts kynurenine to kynurenic acid, leading to a decrease in circulating levels of kynurenine and protection against stress-induced depression[96]. In the gut, kynurenine pathway metabolites are thought to function as a GPR35 (kynurenic acid receptor) agonist to mediate mucosal homeostasis and host–microbiota immune tolerance[89,90]. Kynurenine has also been shown to be an AhR ligand in the human hepatoma HepG2 cell line, although only at supraphysiologic concentrations[97]. Finally, multiple kynurenine pathway intermediates have also been shown to inhibit insulin synthesis, secretion and signalling in rats, and increased levels of kynurenic acid and xanthurenic acid have been found in the urine of individuals with T2DM[90].

Serotonin (5-HT) is another substantial product of tryptophan metabolism. 5-HT is synthesized from tryptophan via a two-step pathway, wherein the rate-limiting enzyme is tryptophan hydroxylase (TPH). There are two isoforms of this enzyme, with TPH1 expressed in enterochromaffin cells within the intestinal mucosa and TPH2 in neurons of the CNS and the enteric nervous system[59,98]. Approximately 90% of total body 5-HT is synthesized by enterochromaffin cells and, under physiological conditions, does not cross the blood–brain barrier. Binding of 5-HT to specific 5-HT receptors elicits various responses. In the CNS, 5-HT is important in regulation of mood, sleep, appetite and behaviour, whereas in peripheral tissues it regulates a diverse set of processes, including gut peristalsis and secretion, inflammation, platelet function, vascular tone, bone development and the development and maintenance of neurons and interstitial cells of Cajal within the gut myenteric plexus[98,99].

The gut microbiota has been shown to induce transcription of *Tph1* and subsequent serotonin production in the gut[59,100] (TABLE 1). There is also limited evidence that SCFAs induce *Tph1* expression in endocrine tumour BON and RIN14B cells. Faecal levels of the secondary bile acid deoxycholate (DCA) were increased in response to spore-forming microorganisms, which are dominated by Clostridia species, and correlated positively with 5-HT production. Some Clostridia species are known to produce DCA, and intrarectal injection of DCA rescued 5-HT levels in germ-free mice[59]. Although there is no evidence to date for gut microbial synthesis of 5-HT, one study profiled microbial pathways for metabolism of neuroactive compounds using a module-based framework and found that nearly 20% of microbial genomes tested have the potential for 5-HT synthesis[101]. Links between the microbiota and selective serotonin reuptake inhibitors, which are the first-line treatment for depression and anxiety, have also been identified. Treatment with various selective serotonin reuptake inhibitors altered the microbial community composition in mice, and *Ruminococcus flavefaciens* and *Adlercreutzia equolifaciens* attenuated the therapeutic effects of duloxetine[102]. Treatment with the common selective serotonin reuptake inhibitor fluoxetine was also shown to inhibit the *Turicibacter sanguinis* 5-HT transporter (homologous to the human protein), impacting its growth dynamics and resulting in elevated 5-HT concentrations and an increase in the abundance of spore-forming bacteria[103].

Indole and its derivatives modulate various processes involved in host–microbiota homeostasis through

Germ-free mice
Mice born and raised in the complete absence of any microorganisms, frequently in a laminar flow glovebox isolator or IsoCage setting

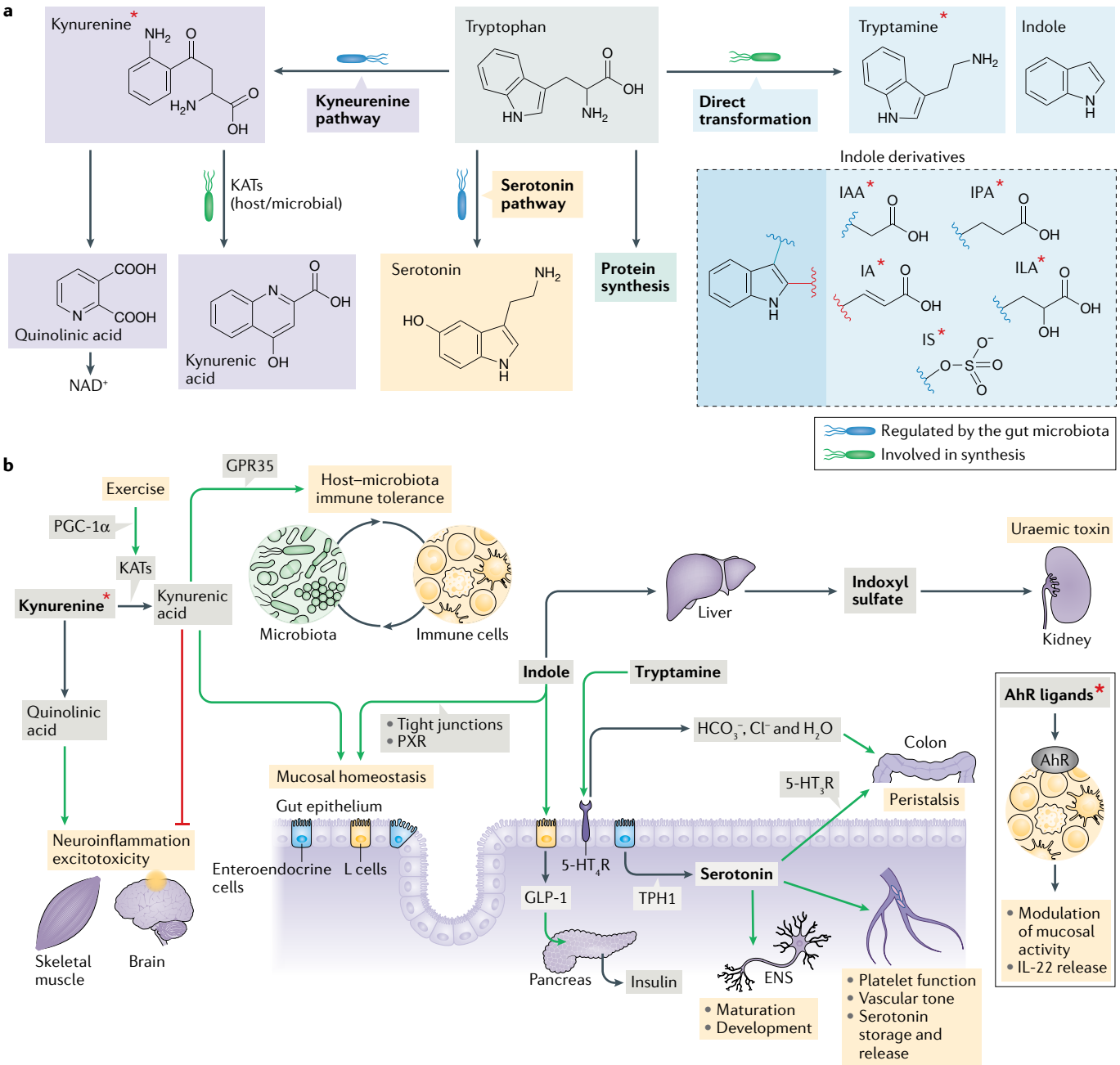

Fig. 2 | **Host–microbiota interactions during tryptophan metabolism.** **a** | Tryptophan is metabolized via one of four pathways: the kynurenine pathway, the serotonin pathway, protein synthesis or direct transformation to various compounds. Pathways regulated by the gut microbiota are denoted with a blue bacterium, whereas those in which the microbiota is involved in synthesis are denoted by a green bacterium. Side chains of indole derivatives attach at either the 2 (red) or 3 (blue) position on the indole ring. **b** | Tryptophan metabolites regulate various host processes through their functions as signalling molecules and toxins. Kynurenine and its derivatives exert effects in the central nervous system and the gut, with roles in neurotoxicity, mucosal homeostasis and host–microbiota immune tolerance. The effects of kynurenine metabolites are mediated through signalling at GPR35 and the aryl hydrocarbon receptor (AhR) (putative; only at supraphysiologic concentrations of kynurenine[89,90,96,97]). Indole-containing compounds have diverse roles in multiple host tissues, including regulation of insulin secretion, modulation of mucosal homeostasis and immunity, and kidney toxicity[11,89,110]. The tryptophan-derived monoamines, tryptamine and serotonin/5-hydroxytryptamine (5-HT), stimulate gut peristalsis by signalling through the serotonin receptors, 5-HT_4R and 5-HT_3R, respectively, in the gut[99,104]. Enteroendocrine cells in the gut produce serotonin through the enzyme tryptophan hydroxylase 1 (TPH1), whose expression is induced by the gut microbiota[59]. This serotonin is rapidly taken up by the gut epithelium through the serotonin-selective reuptake transporter (SERT) and impacts enteric nervous system (ENS) development and signalling[59,99]. In peripheral circulation, serotonin modulates vascular tone and is taken up by platelets through SERT[99]. Serotonin mediates platelet function and is released upon platelet activation[59,174]. Green arrows indicate activation of a process, red arrows indicate inhibition and black arrows represent all other relationships. AhR ligands are denoted with a red asterisk. Neuroendocrine cells within the intestinal epithelium are coloured blue (enteroendocrine cells) and orange (L cells). GLP-1, glucagon-like peptide-1; 5-HT_3R, serotonin type 3 (5-HT_3) receptor; 5-HT_4R, serotonin type 4 (5-HT_4) receptor; IA, indoleacrylic acid; IAA, indoleacetic acid; ILA, indolelactic acid; IPA, indole propionic acid; IS, indoxyl sulfate; KAT, kynurenine aminotransferase; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PXR, pregnane X receptor.

Conventionally raised mice
Mice born and raised in a normal ('conventional') mouse colony setting with exposure to normal environmental microorganisms from birth onwards.

control of both microbial and host physiology (FIG. 2). Many of these compounds are ligands of AhR, a cytosolic ligand-activated transcription factor that has a role in regulation of immune responses[89]. Although the effects of many indole derivatives remain somewhat uncharacterized, indoxyl sulfate has been implicated in the pathogenesis of chronic kidney disease (CKD) and may also mediate known co-morbidities of the disease[3,11]. Gut microbial tryptophanase converts tryptophan into indole, which then enters the host portal circulation and is converted into indoxyl sulfate in the liver. Indoxyl sulfate is excreted by the kidneys and is renal-toxic when present at high levels. Genetic manipulation of *Bacteroides* sp. tryptophanase has been shown to modulate levels of indoxyl sulfate in a gnotobiotic mouse model, suggesting a role for targeted manipulated of the gut microbiota in treatment of renal disease[11]. The gut microbiota carries out numerous other direct transformations of tryptophan. This includes the production of multiple indole derivatives and tryptamine, which stimulates colonic motility through activation of the serotonin type 4 receptor (5-HT_4R), and secretion of anions and fluid in the colonic mucosa[104]. Examples of these compounds and their physiological effects are depicted in FIG. 2.

In addition to tryptophan, microbial metabolism of histidine, phenylalanine and tyrosine by the gut microbiota results in numerous compounds associated with disease (TABLE 1). Histidine can be decarboxylated in both mammalian and gut bacterial cells to form histamine, which has a major regulatory role in the immune system[105]. Histidine can also be metabolized to imidazole propionate (ImP), which was found to be elevated in individuals with T2DM and impairs insulin signalling through activation of the p38γ–p62–mTORC1 pathway[106]. ImP is produced from histidine through non-oxidative deamination of histidine to ammonia and urocanate followed by reduction of urocanate by urocanate reductase (UrdA). Twenty-eight strains possessing authentic UrdA activity were elevated in individuals with treatment-naive T2DM, and many of these strains, including *Streptococcus mutans* and *Eggerthella lenta*, were verified as ImP producers[106].

Recent evidence points towards a role for microbial derivatives of phenylalanine and tyrosine in neurological disease, kidney disease and CVD. Multiple strains of *Enterococcus* and *Lactobacillus brevis* can produce the phenylalanine or tyrosine derivative dopamine in the gut through decarboxylation of levodopa by a conserved tyrosine decarboxylase (TyrDC)[107,108]. Dopamine is produced in mammalian hosts from phenylalanine via a pathway that includes tyrosine, which is converted to levodopa (L-DOPA) by the rate-limiting enzyme tyrosine hydroxylase. Dopamine is a neurotransmitter that has an important role in the control of movement and mood, and Parkinson disease is caused by deficient dopamine production due to loss of dopamine-producing neurons in the substantia nigra of the brain. L-DOPA can be converted to dopamine both in the CNS and peripherally by host aromatic amino acid decarboxylase (AADC). Peripheral dopamine, however, cannot cross the blood–brain barrier and can cause numerous negative effects related to movement, which is why L-DOPA is typically given with the AADC inhibitor carbidopa during treatment of Parkinson disease. Despite co-administration of L-DOPA with carbidopa, there is substantial heterogeneity in the efficacy and associated negative side effects of this drug combination that are not fully explained by differences in drug metabolism alone. However, recent evidence that the gut microbiota can decarboxylate L-DOPA to dopamine and that the abundance of *Enterococcus faecalis* and/or enrichment of the microbiome for *tyrDC* correlate with dopamine metabolism by complex microbiota from individuals with Parkinson disease offers a possible explanation for this heterogeneity and opens new avenues for treatment of the disease[107,108].

CKD, which can progress to end-stage renal disease (renal failure), is associated with shifts in microbiota function and accumulation of microbiota-derived renal toxins, as reviewed in REFS[109,110]. Metabolism and excretion of cellular wastes, including nitrogenous compounds, is a major physiological function of the kidney. As kidney function declines during disease, urea and other waste products accumulate in the blood, thereby promoting transfer of these compounds from the blood into the gut. Thus, the distal gut becomes the primary site of urea excretion as CKD progresses[111], which alters the intraluminal environment (pH and substrate ratio of carbohydrate to protein to fat) and microbial ecology.

Several early mouse studies point towards a role for microbial metabolites in CKD. Germ-free anephric mice survive significantly longer than their conventionally raised mice counterparts[112], and two unique mouse models of CKD have attenuated phenotypes when reared germ-free[113,114]. Further, an early plasma metabolome study revealed the presence of several microbiota-dependent uraemic toxins in mice, including the tryptophan derivative indoxyl sulfate (discussed above) and the tyrosine or phenylalanine derivatives *p*-cresol sulfate (pCS) and phenylacetylglutamine (PAGln)[3]. All three of these microbiota-dependent metabolites are known to accumulate in CKD and contribute to pathogenesis and disease progression by inducing renal damage, inflammation and fibrosis[109,110]. The synthesis of these compounds and the microbial taxa involved are detailed in TABLE 1. Notably, individuals with CKD have a significantly increased risk of adverse cardiovascular events that is only partly explained by an increase in traditional CVD risk factors such as hypertension, diabetes and metabolic syndrome, which suggests a contribution from renal-toxic microbiota-derived metabolites[115]. Notably, indoxyl sulfate, pCS and PAGln have all been associated with overall mortality and CVD in individuals with CKD[110,116], and PAGln was also recently identified as an independent risk factor for major adverse cardiovascular events (myocardial infarction, stroke or death)[117]. One study showed that PAGln contributes to platelet activation and enhanced thrombosis by signalling through multiple α-adrenergic and β-adrenergic GPCRs, and that treatment of platelet precursor MEG01 cells with the non-selective β-blocker propranolol significantly attenuated the response to PAGln[117]. Furthermore, haemodialysis and transplantation remain the only two

Bile salt hydrolases
Microbial enzymes that hydrolyse the amide bond in taurine and glycine-conjugated primary bile acids to yield a deconjugated bile acid.

effective treatments for end-stage renal disease, yet many microbiota-derived uraemic toxins are not efficiently removed by haemodialysis. Thus, microbiome-based therapeutics are an attractive target to augment current treatment modalities available for individuals with CKD and end-stage renal disease.

Bile acids as gut microbial messengers

Primary bile acids are synthesized from cholesterol in the liver. Prior to secretion from hepatocytes into bile canaliculi, bile acids are conjugated to taurine or glycine and then passed into the gall bladder, where they are concentrated together with phospholipids, cholesterol, electrolytes, minerals, bilirubin, biliverdin and small amounts of protein to form bile[118]. Following ingestion of a meal, bile is secreted into the duodenum, where bile salts emulsify dietary lipids and fat-soluble vitamins to aid in their absorption. More than 95% of the bile acid pool is reabsorbed in the ileum and circulates back to the liver several times a day via the hepatic portal vein in what is known as enterohepatic circulation. Upon reaching the colon, bile acids are subject to extensive metabolism by the gut microbiota and/or are excreted. Thus, bile acid pools are subject to a myriad of chemical transformations by both the host and gut microorganisms, as detailed further below. In addition to direct metabolism of primary bile acids, the microbiota also regulates bile acid synthesis and uptake[9,119].

The synthesis of bile acids in the liver requires at least 17 different enzymes and is carried out by two pathways, as thoroughly reviewed in REF.[118]. The rate-limiting step in the classic (or neutral) bile acid pathway, which contributes an estimated 75% of total bile acid synthesis, is cholesterol 7α-hydroxylase (CYP7A1), whereas the alternative (or acidic) bile acid pathway is regulated by sterol-27-hydroxylase (CYP27A1). The classic pathway produces either chenodeoxycholic acid (CDCA) or cholic acid, depending on the activity of sterol 12α-hydroxylase (CYP8B1), whereas the alternative pathway produces mainly CDCA[118]. The expression of both CYP7A1 and CYP27A1, but not CYP8B1, is regulated by the gut microbiota[119]. Once conjugated to glycine or taurine, bile salts can then be secreted from hepatocytes and stored in the gall bladder until release following ingestion of food. Of note, synthesis of taurine is also regulated by the gut microbiota[119]. Conjugation of cholic acid to phenylalanine, tyrosine and leucine has also been recently demonstrated in both mice and humans, although the role of these newly identified secondary bile acid conjugates in host physiology remains relatively unknown[120]. In the distal small intestine and colon, bile acids are subject to deconjugation by microbial bile salt hydrolases (removal of glycine and taurine), preventing their active reuptake during enterohepatic circulation. Deconjugated bile acids then undergo various microbial biotransformations, leading to a diverse array of secondary bile acids through dehydroxylation, epimerization and oxidation of hydroxyl groups, as summarized in TABLE 2. Mechanistic understanding of these microbial biotransformations remains an active area of research, and the complete enzymatic pathway for formation of two highly abundant secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA), was only very recently elucidated[121]. Notably, there are key differences (denoted in TABLE 2) in both bile acid pools and tissue-resident immune cell populations between mice and humans that may substantially impact the translation of murine bile acid studies to humans[122].

Table 2 | **Host and microbial chemical transformations of bile acids**

Class	Chemistry	Bile acids formed	Genera
Primary bile acids	Synthesized in the liver from cholesterol via the classical or alternative pathways and conjugated to taurine or glycine	Human: CA, CDCA Mouse: CA, CDCA, UDCA, α-MCA, β-MCA	Host (mouse and human)[9,123]
Secondary bile acids	Gut microbial deconjugation of primary and secondary bile acids through bile salt hydrolases	Unconjugated free forms of primary and secondary bile acids	*Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Lactobacillus*[9,123]
	Microbiota-mediated conjugation to phenylalanine, tyrosine or leucine	Phenylalanocholic acid, tyrosocholic acid, leucholic acid	*Clostridium boltae*[120]
	Gut microbial 7α/β-dehydroxylation of primary bile acids	DCA, LCA MDCA (mice)	*Bacteroides*, *Clostridium*, *Escherichia*, *Eubacterium*, *Lactobacillus*[9,121,123]
	Gut microbial 3α/β-epimerization of primary or secondary bile acids	Iso-bile acids	*Eubacterium lentum*, *Clostridium perfringens*, *Ruminococcus gnavus*[9,123]
	Gut microbial 5β/α-epimerization of primary or secondary bile acids	Allo-bile acids	*Eubacterium*[9]
	Gut microbial 6β-epimerization of β-MCA	ω-MCA (mice and rats)	*Eubacterium*, *Fusobacterium*[9,170]
	Gut microbial 7α/β-epimerization of CDCA	UDCA (human)	*Clostridium*[9]
	Gut microbial 6β-epimerization and 7β-dehydroxylation of β-MCA	Hyodeoxycholic acid	Unidentified Gram-positive rod[170,171]
	Gut microbial oxidation of primary or secondary bile acids at C3, C7 and C12	Oxo-bile acids or keto-bile acids	*Bacteroides*, *Clostridium*, *Eggerthella*, *Escherichia*, *Eubacterium*, *Peptostreptococcus*, *Ruminococcus*[9,123]

α-MCA, α-muricholic acid; β-MCA, β-muricholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; MDCA, murideoxycholic acid; UDCA, ursodeoxycholic acid.

Bile acid signalling through FXR and TGR5. Bile acids exert their effects in various host tissues, primarily through two receptors: the farnesoid X receptor (FXR) and G-protein-coupled bile acid receptor 1 (GPBAR1; also known as TGR5) — although unconjugated bile acids have also been shown to signal through pregnane X receptor (PXR), constitutive androstane receptor (CAR) and vitamin D receptor (VDR)[123]. Although there is some discrepancy as to which conjugated bile acids are more potent activators of TGR5 in vitro versus in vivo, it is well accepted that unconjugated bile acids are more potent activators than their conjugated counterparts[123]. TGR5 is a ubiquitously expressed transmembrane receptor that regulates energy balance by promoting intracellular thyroid hormone activity in brown adipose tissue, increasing energy expenditure in brown adipose tissue and muscle, and inducing release of the insulin secretagogue GLP-1 from intestinal L cells[9,123]. TGR5 activation also induces enteroendocrine cell differentiation to L cells[124]. FXR is a cytosolic ligand-activated transcription factor that translocates to the nucleus to induce transcription of target genes. In addition to its role as a regulator of bile acid synthesis[125] and transport, FXR also has a major role in regulation of inflammation and immunity and in liver regeneration, and induces protective cellular responses in hepatocytes and the gastrointestinal tract[9,123]. The role of FXR is quite complex and depends upon the tissue type and contextual factors such as diet and surgical anatomy following gastric bypass. Indeed, health benefits have been reported as a result of both FXR agonism[126] and antagonism[127] in separate contexts, and whereas FXR activation in liver is protective against steatosis[128], intestinal FXR facilitates diet-induced obesity and steatosis[129,130], highlighting the need for further study of FXR and its regulation by the gut microbiota in host health.

Microbial mediation of bile acid profiles in host disease. Bile acids have been linked to metabolic disease and malignancy, among other conditions. In particular, bile acids have received a lot of attention in the setting of bariatric surgery, which is currently the most effective treatment for long-term management of morbid obesity and also reduces cancer incidence in women[131]. Improvements in glucose control have been reported just a few days postoperatively, long preceding any weight loss, suggesting that the metabolic benefits of bariatric surgery are due, at least in part, to factors independent of weight loss[132]. In support of this, postoperative alterations in bile acid pools were observed in patients who underwent gastric bypass surgery via Roux-en-Y gastric bypass or biliopancreatic diversion but not via vertical sleeve gastrectomy and gastric banding[9]. It is important to note that both Roux-en-Y gastric bypass and biliopancreatic diversion reroute biliopancreatic juice to a more distal segment of the gut, altering both absorption and interaction of the gut microbiota with nutrients. Indeed, the microbiota is known to be altered following Roux-en-Y gastric bypass[133]. These observations suggest a strong role for bile acids and their interactions with the gut microbiota as mediators of the positive metabolic effects of bariatric surgery[134]. As bariatric surgery is the only treatment for morbid obesity resulting in sustained weight loss, understanding the underlying mechanisms is exceedingly important. Furthermore, new findings may lead to the development of microbiota-based or metabolite-based therapies to augment lifestyle-mediated or surgical weight loss methods.

Bile acid–microbiome crosstalk has also been indicated in both hepatocellular carcinoma (HCC) and colorectal cancer (CRC)[123]. In the setting of obesity-induced HCC, increased microbial production of the secondary bile acid DCA induces secretion of inflammatory and tumour-promoting factors from hepatic stellate cells, which then promotes HCC development[135]. Inhibition of DCA production or reducing the microbial biomass with a broad-spectrum antibiotic cocktail, however, was protective against obesity-induced HCC. FXR activation by secondary bile acids in the gut may also be a key event in the pathogenesis of both CRC and HCC. Activation of this nuclear receptor leads to host–microbiota homeostasis, gut barrier maintenance and control of immunity and inflammation, all of which are important in the setting of tumorigenesis[123]. Further, chronic inflammation, a hallmark of inflammatory bowel disease and multiple liver diseases, increases the risk of progression to either CRC or HCC, respectively. Treatment with the potent semi-synthetic FXR ligand obeticholic acid (OCA) in two mouse models of colitis resulted in an attenuation of disease, including improved mucosal homeostasis and decreased inflammation[136]. FXR expression has also been found to be decreased in human colonic polyps (~5-fold) and colonic adenocarcinoma (~10-fold)[134], and loss of FXR expression has been identified in numerous human colonic neoplasia studies[123,137]. Further underscoring the importance of FXR signalling in the development of gastroenterological cancers, whole-body FXR-deficient mice are known to develop spontaneous liver tumours, a phenotype that can be rescued with restoration of FXR expression and subsequent bile acid homeostasis[138,139]. More recently, a study also showed that natural killer T cell accumulation in response to production of secondary bile acids by Clostridiales is protective against HCC and liver metastases, which are commonly derived from CRC[140]. Finally, the large number of clinical trials in the setting of gastrointestinal diseases, all of which share the common feature of chronic inflammation promoting carcinogenesis, underscores the potential of harnessing bile acid signalling in the treatment of gastroenterological disease and cancer[118,123].

Vitamins and one-carbon metabolites

Although metabolism of the major macronutrients (carbohydrates, fats and protein) has been the focus of the majority of host–microbiota research, the gut microbiota also has a substantial impact on one-carbon metabolism and vitamin availability, particularly B vitamins (FIG. 3). The one-carbon cycle is a universal metabolic process that relies on the folate cycle to support a series of methyl (one-carbon) transfers. One-carbon intermediates facilitate numerous biosynthetic processes, including purine synthesis, methyl donor availability

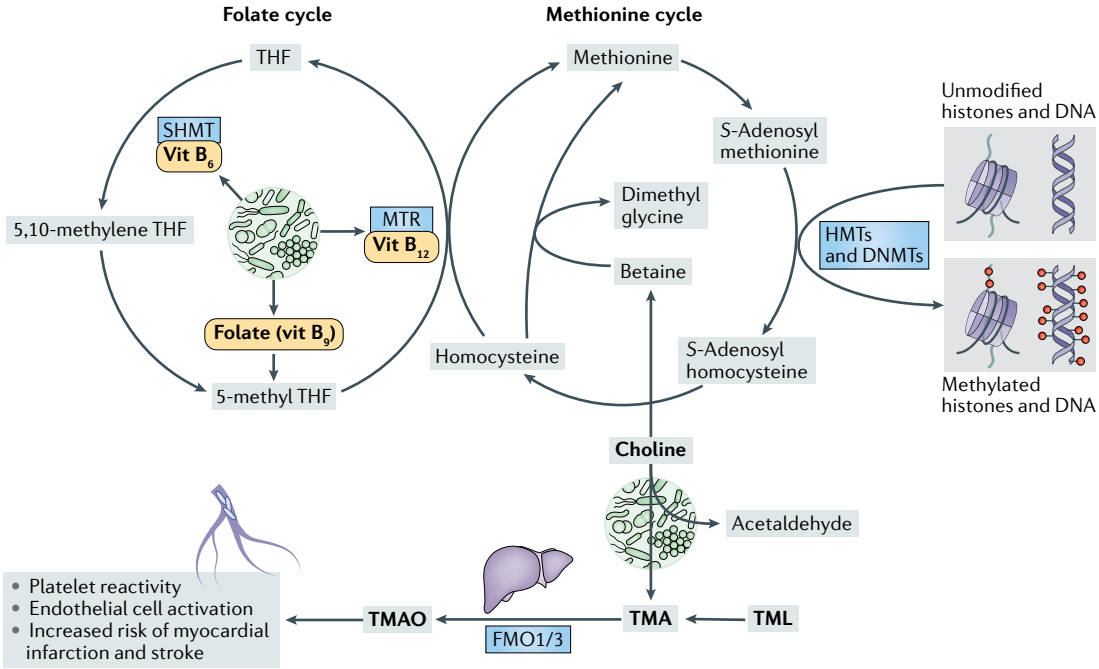

Fig. 3 | **Gut microbiota–host interactions in one-carbon metabolism.** Gut microbial production of B vitamins and competition with the host for choline intersects with both the folate cycle and the methionine cycle, which together impact availability of the methyl donor S-adenosyl methionine. Histone and DNA methyltransferases (HMTs and DNMTs, respectively) are regulated by availability of S-adenosyl methionine. The host and its gut microbiota compete for dietary choline. Gut microbial synthesis of trimethylamine (TMA) from choline is converted in the host liver to form trimethylamine-*N*-oxide (TMAO), which has several roles in cardiovascular disease. Trimethyllysine (TML) is a dietary nutrient precursor to TMA that has also been associated with adverse cardiovascular events. Microbial produced vitamins are highlighted in yellow and key host–microbiota co-metabolites are depicted in bold text. Enzymes are depicted in blue boxes. FMO1/3, flavin-containing monooxygenase 1/3; MTR, methionine synthase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; vit, vitamin.

and redox balance through the transsulfuration pathway. Additionally, one-carbon metabolism has an important role in embryogenesis, stem cell maintenance and haematopoiesis, methylation of DNA and histones, and immune cell function; and dysregulation of one-carbon metabolism has been associated with multiple cancers, liver disease and CVD[141]. Given the universal nature of one-carbon metabolism, which spans all kingdoms, crosstalk between mammalian hosts and resident gut microorganisms occurs through one-carbon intermediates as well. Therefore, this section will be focused on select cases of host–microbiota crosstalk involving one-carbon metabolites or cofactors.

Choline is a water-soluble compound that is an essential nutrient for humans. It contributes to cell membrane function, neurotransmission and methyl donor availability for numerous biosynthetic reactions[142]. Choline is both diet-derived and synthesized endogenously, and is used by anaerobic gut microorganisms to generate trimethylamine (TMA) and acetaldehyde[143] (FIG. 3). Once TMA is absorbed across the host gut, it is metabolized to trimethylamine-*N*-oxide (TMAO) in the liver by flavin-containing monooxygenases 1 and 3 (FMO1 and FMO3). This gut microbial–host co-metabolite was identified nearly a decade ago in a metabolomics study intended to identify small-molecule metabolites in serum that predict risk of major cardiovascular events, including both stroke and myocardial infarction in multiple cohorts and clinical trials[144–147]. Mechanistic work has since revealed that TMAO promotes atherosclerosis by inducing multiple macrophage receptors[144] and the hallmarks of thrombosis: arterial endothelial cell activation[148] and enhanced platelet reactivity[145]. Isotopic labelling studies have also shown that conversion of dietary L-carnitine, an abundant amino acid derivative in red meat, into TMA occurs via a 2-step microbiota-dependent transformation and results in a >20-fold increase in labelled atherogenic TMAO in omnivores versus vegans and vegetarians[149]. More recently, trimethyllysine (TML), a precursor to TMAO, has also been identified as a predictor of major adverse cardiac events. When combined with TMAO levels, TML improved risk stratification for individuals presenting with acute coronary syndrome (a term that encompasses myocardial infarction and unstable angina) and was able to predict the risk of a future major adverse cardiac event, even in individuals whose initial levels of troponin, a commonly used clinical marker of myocardial infarction, were negative[150].

Availability of the B vitamins pyridoxine (vitaminB_6), folic acid (vitaminB_9) and cobalamin (vitaminB_{12}) is essential to functional one-carbon metabolism (FIG. 3). These vitamins are necessary substrates or cofactors in the folate and one-carbon cycles. Host synthesis of B vitamins is not sufficient for optimal health, and thus B vitamins are also obtained from dietary sources and

Nuclear magnetic resonance spectroscopy
An analytical method frequently used in structural, quantitative and imaging applications wherein unique spectra are obtained for biomolecules based on nuclear resonance transitions that occur when atomic nuclei are immersed in a magnetic field and then subjected to specific magnetic energy levels.

Faecal microbiota transplantation
Delivery of processed stool from a donor into the intestinal tract of a recipient with the goal of stable engraftment.

are de novo synthesized by the gut microbiota[151,152]. In the case of folate, production by the colonic microbiota actually exceeds dietary intake[153]. Systematic assessment of the genomes of 256 common human gut bacteria for biosynthetic capacity of 8 B vitamins (vitamins B_1, B_2, B_3, B_5, B_6, B_7, B_9 and B_{12}) revealed that 40–65% of human gut bacteria have the genomic potential to produce some or all of the vitamins, and 88% of those predictions were validated by published data[152]. There are also age-dependent differences in gut microbial metabolism of B vitamins. Infant gut microbiomes have been shown to be enriched for genes involved in de novo biosynthesis of folate, whereas adult microbiomes are enriched for those involved in metabolism of folate and its reduced form tetrahydrofolate[154]. Despite dietary and gut microbial sources of these water-soluble vitamins, B vitamin deficiencies are very common and can result from poor dietary intake, malabsorption, certain medications that interfere with folate metabolism (methotrexate and sulfasalazine) and genetic disorders. Deficiency can cause numerous diseases that are treated with supplementation and addressing the cause where possible: pellagra (vitamin B_3), anaemias (vitamins B_9 and B_{12}), cerebellar ataxia (vitamin B_{12}) and cognitive impairment (vitamins B_9 and B_{12}). Thus, the gut microbiota is an important source of essential vitamins and may offer new strategies for treatment of vitamin deficiencies, particularly in cases where deficiency is due to intake-independent causes.

Perspectives

Massive complexity is perhaps the single most defining characteristic of the multi-kingdom supra-organism that is the host and its microbiota. Yet, despite this complexity, the need to understand these multifaceted interactions in the context of host health and ever-changing environments remains. Some key challenges facing the field include tracing the origin of metabolites to host versus microbiota, which is often challenging due to extensive co-metabolism; grasping the full range of context-specific and dose-specific effects of target metabolites, both of which have been demonstrated to exert sometimes opposing effects from the same compound[7,8,126–130]; and continued development of analytical and statistical frameworks for the acquisition and integration of multi-omics data types that are required for a systematic approach to this extensively complex system.

The combination of these factors makes it challenging to study host–microbiota interactions in a mechanistic way, which is required to progress beyond associations towards actionable microbiota-driven targets. Nonetheless, there are several examples within the literature where metabolite-centered approaches in large discovery cohorts guided a series of reductionist experiments that yielded mechanistic understanding of how microbial metabolites impact host health. Notable examples include TMAO[155], indoxyl sulfate[11] and ImP[106], which have roles in adverse cardiovascular events, CKD and T2DM, respectively. In each of these cases, there were commonalities in the experimental approach. First, the target metabolite was identified in human discovery cohorts using a combination of mass spectrometry and/or nuclear magnetic resonance spectroscopy-based methods. This enabled subsequent reductionist work involving a combination of computational exploration of the functional capacity within the microbiome, in vitro microbiology and biochemistry to validate identified metabolic pathways, and in vivo testing using cultured cells and gnotobiotic mouse models to understand how specific microbial metabolites signal to host cells and tissues, dose effects[11] and the impact of environmental factors, such as diet[10], disease status[7,8] and exposure to pharmacologic agents[107,156]. This approach necessitates combination of multiple high-throughput, discovery techniques with reductionist microbiological and biochemical techniques, thus requiring teams with diverse expertise. Although this approach may seem simple at the outset, it is noteworthy that, in all cases, discovery of a specific metabolite and the mechanisms underlying its synthesis and physiological effects occurred in multiple stages over the span years. For example, the wealth of information surrounding TMAO synthesis and its impact on host physiology available today was generated by multiple groups beginning as early as 1910, when the molecule was first reported[157]. More than a century later, TMAO was first linked to CVD in a human cohort study in 2011 (REF.[144]), and anaerobic conversion of choline to TMA was mechanistically detailed in 2012 (REF.[143]). Subsequent exploration of the relationship between microbial community composition and TMAO accumulation[158] and extensive characterization of TMAO in CVD by several teams then led to a multitude of mechanistic insights into the synthesis, physiological effects and potential clinical utility of TMAO as a biomarker (a non-exhaustive list of examples[145–148,155,159]). There are also examples where an informatics-driven approach has led to significant discovery. One study combined metagenome-mining with synthetic biology and genetic manipulation of common laboratory bacteria to discover microorganism-derived metabolites that inhibit specific host proteases[160]. A frequent feature of both metabolome-mining and metagenome-mining approaches is multi-omics integration, which yields a comprehensive view of the multi-organism system as a whole. Although not a focus of this Review, massive multi-omics integration that focused on inflammatory bowel diseases in the Integrative Human Microbiome Project is an excellent example of data integration and sharing through a publicly available database, both of which are necessary for synthesis and dispersal of information[161]. However, the authors appropriately caution that although the results identify host and microbial targets for follow-up characterization, substantial work remains to determine whether multi-omics features can predict disease events, and disease-relevant timescales for these molecular events have not yet been established. Ultimately, the common goal within these various approaches is identification and validation of candidate host or microbial targets within common clinical samples, such as peripheral blood or urine, that drive development of novel disease biomarkers and therapeutic agents.

Faecal microbiota transplantation is now used at several institutions for recurrent *Clostridioides difficile* (formerly

Enterotypes
Variants in human microbial community composition based on empirical population measurements that are dominated by a single genus (for example, *Bacteroides*, *Ruminococcus* or *Prevotella*).

Clostridium difficile) infection[162,163]; however, apart from faecal microbiota transplantation, microbiome-based therapies are not yet ready for clinical use, and even with faecal microbiota transplantation there are still lessons to be learned[164]. Although the use of microbiome-based therapies in medicine is currently in its infancy, there is considerable potential to change the practice of medicine, including the possibility of curbing antibiotic use but also augmenting or replacing current therapies in fields ranging from cardiovascular medicine[155] to neurology[107] and psychiatry[102,103]. As we move closer to using microbiome-based therapies in medicine, several factors, in addition to safety, must be taken into consideration. Given the vast complexity of host–microbiota interactions, is it necessary or even feasible to understand the mechanism of microbiome-based therapies prior to clinical use? Do additional biomarkers need to be developed to monitor stable engraftment of the donor microbiota in recipients and subsequent clinical efficacy? What measures must be taken to ensure scalable manufacturing and shelf stability, particularly in the context of anaerobic microorganism exposure to ambient oxygen?

Recognition of interindividual[14,18,43] and geographic[12,13,15,16] variation in host–microbiota responses points increasingly towards personalized nutrition[165,166] and medicine. In light of this known variation, metabolites may be a more robust clinical end point than microbial taxa, as they are the output of all combined microbial functions and, in some cases, even host–microbiota co-metabolism. Thus, quantitative measurement of microbiota-associated metabolites may supersede some of the known variance within and between populations. Regardless, multi-institutional strategies and support will undoubtedly be required to move forward successfully and efficiently, both in terms of designing and implementing sufficiently powered population-based studies but also in capturing the full variance in host–microbiota ecology that exists within diverse populations. An additional challenge will be incorporating these findings into medical practice, particularly as many are likely to apply to only subsets of individuals. However, consistent and standardized identification of enterotypes[48] and metabotypes[6] in the clinic has yet to be defined and implemented. As healthcare costs continue to rise across the world, in part owing to increasing recognition and use of personalized medicine, continued development of methods and policy will be required to allow for reliable, timely and sustainable use of these findings in the clinic.

Published online: 23 September 2020

1. Sender, R., Fuchs, S. & Milo, R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* **164**, 337–340 (2016).
2. Tierney, B. T. et al. The landscape of genetic content in the gut and oral human microbiome. *Cell Host Microbe* **26**, 283–295.e8 (2019).
3. Wikoff, W. R. et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc. Natl Acad. Sci. USA* **106**, 3698–3703 (2009).
4. Dodd, D. et al. A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. *Nature* **551**, 648–652 (2017).
5. Martin, F.-P. J. et al. Panorganismal gut microbiome–host metabolic crosstalk. *J. Proteome Res.* **8**, 2090–2105 (2009).
6. Claus, S. P. et al. Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. *Mol. Syst. Biol.* **4**, 219 (2008).
7. Lupton, J. R. Microbial degradation products influence colon cancer risk: the butyrate controversy. *J. Nutr.* **134**, 479–482 (2004).
8. Donohoe, D. R. et al. The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. *Mol. Cell* **48**, 612–626 (2012).
9. Wahlström, A., Sayin, S. I., Marschall, H.-U. & Bäckhed, F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metab.* **24**, 41–50 (2016).
10. Singh, V. et al. Dysregulated microbial fermentation of soluble fiber induces cholestatic liver cancer. *Cell* **175**, 679–694.e22 (2018).
11. Devlin, A. S. et al. Modulation of a circulating uremic solute via rational genetic manipulation of the gut microbiota. *Cell Host Microbe* **20**, 709–715 (2016).
12. Deschasaux, M. et al. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nat. Med.* **24**, 1526–1531 (2018).
13. Martínez, I. et al. The gut microbiota of rural Papua New Guineans: composition, diversity patterns, and ecological processes. *Cell Rep.* **11**, 527–538 (2015).
14. Martínez, I., Kim, J., Duffy, P. R., Schlegel, V. L. & Walter, J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS ONE* **5**, e15046 (2010).
15. Jha, A. R. et al. Gut microbiome transition across a lifestyle gradient in Himalaya. *PLoS Biol.* **16**, e2005396 (2018).
16. Vangay, P. et al. US immigration westernizes the human gut microbiome. *Cell* **175**, 962–972.e10 (2018).
17. Smits, S. A. et al. Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science* **357**, 802–806 (2017).
18. Davis, L. M. G., Martínez, I., Walter, J., Goin, C. & Hutkins, R. W. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS ONE* **6**, e25200 (2011).
19. Desai, M. S. et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* **167**, 1339–1353.e21 (2016).
20. Koh, A., De Vadder, F., Kovatcheva-Datchary, P. & Bäckhed, F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* **165**, 1332–1345 (2016).
21. Macfarlane, S. & Macfarlane, G. T. Regulation of short-chain fatty acid production. *Proc. Nutr. Soc.* **62**, 67–72 (2007).
22. Makki, K., Deehan, E. C., Walter, J. & Bäckhed, F. The impact of dietary fiber on gut microbiota in host health and disease. *Cell Host Microbe* **23**, 705–715 (2018).
23. Kaoutari, A. E., Armougom, F., Gordon, J. I., Raoult, D. & Henrissat, B. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat. Rev. Microbiol.* **11**, 497–504 (2013).
24. Bhattacharya, T., Ghosh, T. S. & Mande, S. S. Global profiling of carbohydrate active enzymes in human gut microbiome. *PLoS ONE* **10**, e0142038 (2015).
25. McNeil, N. I. The contribution of the large intestine to energy supplies in man. *Am. J. Clin. Nutr.* **39**, 338–342 (1984).
26. Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P. & Macfarlane, G. T. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **28**, 1221–1227 (1987).
27. Besten, G. den et al. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* **54**, 2325–2340 (2013).
28. Smith, E. A. & Macfarlane, G. T. Dissimilatory amino acid metabolism in human colonic bacteria. *Anaerobe* **3**, 327–337 (1997).
29. Macfarlane, G. T., Gibson, G. R., Beatty, E. & Cummings, J. H. Estimation of short-chain fatty acid production from protein by human intestinal bacteria based on branched-chain fatty acid measurements. *FEMS Microbiol. Lett.* **101**, 81–88 (1992).
30. Dalile, B., Oudenhove, L. V., Vervliet, B. & Verbeke, K. The role of short-chain fatty acids in microbiota–gut–brain communication. *Nat. Rev. Gastroenterol.* **12**, 453 (2019).
31. Johansson, M. E. V., Sjövall, H. & Hansson, G. C. The gastrointestinal mucus system in health and disease. *Nat. Rev. Gastroenterol.* **10**, 352–361 (2013).
32. Schroeder, B. O. Fight them or feed them: how the intestinal mucus layer manages the gut microbiota. *Gastroenterol. Rep.* **7**, 3–12 (2019).
33. Martens, E. C., Chiang, H. C. & Gordon, J. I. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. *Cell Host Microbe* **4**, 447–457 (2008).
34. Sonnenburg, J. L. et al. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science* **307**, 1955–1959 (2005).
35. Cummings, J. H. & Macfarlane, G. T. The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol.* **70**, 443–459 (1991).
36. Kukral, J. C., Adams, A. P. & Preston, F. W. Protein producing capacity of the human exocrine pancreas. *Ann. Surg.* **162**, 63–73 (1965).
37. Silberberg, M. et al. The bioavailability of polyphenols is highly governed by the capacity of the intestine and of the liver to secrete conjugated metabolites. *Eur. J. Nutr.* **45**, 88–96 (2006).
38. Cardona, F., Andrés-Lacueva, C., Tulipani, S., Tinahones, F. J. & Queipo-Ortuño, M. I. Benefits of polyphenols on gut microbiota and implications in human health. *J. Nutr.Biochem.* **24**, 1415–1422 (2013).
39. Puupponen-Pimiä, R. et al. Development of functional ingredients for gut health. *Trends Food Sci. Tech.* **13**, 3–11 (2002).
40. Marín, L., Miguélez, E. M., Villar, C. J. & Lombó, F. Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. *Biomed. Res. Int.* **2015**, 1–18 (2015).
41. Roopchand, D. E. et al. Dietary polyphenols promote growth of the gut bacterium *Akkermansia muciniphila* and attenuate high-fat diet-induced metabolic syndrome. *Diabetes* **64**, 2847–2858 (2015).
42. Zeng, S.-L. et al. Citrus polymethoxyflavones attenuate metabolic syndrome by regulating gut microbiome and amino acid metabolism. *Sci. Adv.* **6**, eaax6208 (2020).

43. Ze, X., Duncan, S. H., Louis, P. & Flint, H. J. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J.* **6**, 1535–1543 (2012).
44. Deehan, E. C. et al. Precision microbiome modulation with discrete dietary fiber structures directs short-chain fatty acid production. *Cell Host Microbe* **27**, 389–404.e6 (2020).
45. Bry, L., Falk, P. G., Midtvedt, T. & Gordon, J. I. A model of host–microbial interactions in an open mammalian ecosystem. *Science* **273**, 1380–1383 (1996).
46. David, L. A. et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2013).
47. Turnbaugh, P. J. et al. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci. Transl. Med.* **1**, 6ra14 (2009).
48. Wu, G. D. et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**, 105–108 (2011).
49. Sonnenburg, E. D. et al. Diet-induced extinctions in the gut microbiota compound over generations. *Nature* **529**, 212–215 (2016).
50. Clemente, J. C. et al. The microbiome of uncontacted Amerindians. *Sci. Adv.* **1**, e1500183 (2015).
51. Holmes, A. J. et al. Diet–microbiome interactions in health are controlled by intestinal nitrogen source constraints. *Cell Metab.* **25**, 140–151 (2017).
52. Besten, G. den et al. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am. J. Physiol. Gastrointest. Liver Physiol.* **305**, G900–G910 (2013).
53. Reniere, M. L. Reduce, induce, thrive: bacterial redox sensing during pathogenesis. *J. Bacteriol.* **200**, e00128-18 (2018).
54. Hylemon, P. B., Harris, S. C. & Ridlon, J. M. Metabolism of hydrogen gases and bile acids in the gut microbiome. *FEBS Lett.* **592**, 2070–2082 (2018).
55. Peng, L., Li, Z.-R., Green, R. S., Holzman, I. R. & Lin, J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J. Nutr.* **139**, 1619–1625 (2009).
56. Lewis, K. et al. Enhanced translocation of bacteria across metabolically stressed epithelia is reduced by butyrate. *Inflamm. Bowel Dis.* **16**, 1138–1148 (2010).
57. Cherbut, C. et al. Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat. *Am. J. Physiol. Gastrointest. Liver Physiol.* **275**, G1415–G1422 (1998).
58. Soret, R. et al. Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in rats. *Gastroenterology* **138**, 1772–1782.e4 (2010).
59. Yano, J. M. et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **161**, 264–276 (2015).
60. Martin, A. M., Sun, E. W., Rogers, G. B. & Keating, D. J. The influence of the gut microbiome on host metabolism through the regulation of gut hormone release. *Front. Physiol.* **10**, 428 (2019).
61. Fukumoto, S. et al. Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **284**, R1269–R1276 (2003).
62. Krautkramer, K. A. et al. Diet–microbiota interactions mediate global epigenetic programming in multiple host tissues. *Mol. Cell* **64**, 982–992 (2016).
63. Kaiko, G. E. et al. The colonic crypt protects stem cells from microbiota-derived metabolites. *Cell* **165**, 1708–1720 (2016).
64. Donohoe, D. R. et al. A gnotobiotic mouse model demonstrates that dietary fiber protects against colorectal tumorigenesis in a microbiota- and butyrate-dependent manner. *Cancer Discov.* **4**, 1387–1397 (2014).
65. Donohoe, D. R. et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab.* **13**, 517–526 (2011).
66. Vadder, F. D. et al. Microbiota-generated metabolites promote metabolic benefits via gut–brain neural circuits. *Cell* **156**, 84–96 (2014).
67. Smith, P. M. et al. The microbial metabolites, short-chain fatty acids, regulate colonic T_{reg} cell homeostasis. *Science* **341**, 569–573 (2013).
68. Bachem, A. et al. Microbiota-derived short-chain fatty acids promote the memory potential of antigen-activated $CD8^+$ T cells. *Immunity* **51**, 285–297.e5 (2019).
69. Kasahara, K. et al. Interactions between *Roseburia intestinalis* and diet modulate atherogenesis in a murine model. *Nat. Microbiol.* **474**, 327 (2018).
70. Samuel, B. S. et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc. Natl Acad. Sci. USA* **105**, 16767–16772 (2008).
71. Ridaura, V. K. et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* **341**, 1241214 (2013).
72. Canfora, E. E., Meex, R. C. R., Venema, K. & Blaak, E. E. Gut microbial metabolites in obesity, NAFLD and T2DM. *Nat. Rev. Endocrinol.* **15**, 261–273 (2019).
73. Louis, P., Hold, G. L. & Flint, H. J. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat. Rev. Microbiol.* **12**, 661–672 (2014).
74. Husted, A. S., Trauelsen, M., Rudenko, O., Hjorth, S. A. & Schwartz, T. W. GPCR-mediated signaling of metabolites. *Cell Metab.* **25**, 777–796 (2017).
75. Newgard, C. B. et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab.* **9**, 311–326 (2009).
76. Newgard, C. B. Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab.* **15**, 606–614 (2012).
77. Pedersen, H. K. et al. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* **535**, 376–381 (2016).
78. Ericksen, R. E. et al. Loss of BCAA catabolism during carcinogenesis enhances mTORC1 activity and promotes tumor development and progression. *Cell Metab.* **29**, 1151–1165.e6 (2019).
79. VanDusseldorp, T. A. et al. Effect of branched-chain amino acid supplementation on recovery following acute eccentric exercise. *Nutrients* **10**, 1389 (2018).
80. Evenepoel, P. et al. Amount and fate of egg protein escaping assimilation in the small intestine of humans. *Am. J. Physiol. Gastrointest. Liver Physiol.* **277**, G935–G943 (1999).
81. Mahé, S., Huneau, J. F., Marteau, P., Thuillier, F. & Tomé, D. Gastroileal nitrogen and electrolyte movements after bovine milk ingestion in humans. *Am. J. Clin. Nutr.* **56**, 410–416 (1992).
82. Neis, E. P. J. G., Dejong, C. H. C. & Rensen, S. S. The role of microbial amino acid metabolism in host metabolism. *Nutrients* **7**, 2930–2946 (2015).
83. Ratzke, C. & Gore, J. Modifying and reacting to the environmental pH can drive bacterial interactions. *PLoS Biol.* **16**, e2004248 (2018).
84. Smith, E. A. & Macfarlane, G. T. Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. *J. Appl. Bacteriol.* **81**, 288–302 (1996).
85. Birkett, A., Muir, J., Phillips, J., Jones, G. & O'Dea, K. Resistant starch lowers fecal concentrations of ammonia and phenols in humans. *Am. J. Clin. Nutr.* **63**, 766–772 (1996).
86. Stephen, A. M. & Cummings, J. H. Mechanism of action of dietary fibre in the human colon. *Nature* **284**, 283–284 (1980).
87. Sridharan, G. V. et al. Prediction and quantification of bioactive microbiota metabolites in the mouse gut. *Nat. Commun.* **5**, 5492 (2014).
88. Alkhalaf, L. M. & Ryan, K. S. Biosynthetic manipulation of tryptophan in bacteria: pathways and mechanisms. *Chem. Biol.* **22**, 317–328 (2015).
89. Agus, A., Planchais, J. & Sokol, H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe* **23**, 716–724 (2018).
90. Cervenka, I., Agudelo, L. Z. & Ruas, J. L. Kynurenines: tryptophan's metabolites in exercise, inflammation, and mental health. *Science* **357**, eaaf9794 (2017).
91. Houtkooper, R. H., Cantó, C., Wanders, R. J. & Auwerx, J. The secret life of NAD^+: an old metabolite controlling new metabolic signaling pathways. *Endocr. Rev.* **31**, 194–223 (2010).
92. Atarashi, K. et al. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* **331**, 337–341 (2010).
93. Rhee, S. J., Walker, W. A. & Cherayil, B. J. Developmentally regulated intestinal expression of IFN-γ and its target genes and the age-specific response to enteric *Salmonella* infection. *J. Immunol.* **175**, 1127–1136 (2005).
94. Zelante, T. et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* **39**, 372–385 (2013).
95. Vujkovic-Cvijin, I. et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Sci. Transl. Med.* **5**, 193ra91–193ra91 (2013).
96. Agudelo, L. Z. et al. Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. *Cell* **159**, 33–45 (2014).
97. Hubbard, T. D. et al. Adaptation of the human aryl hydrocarbon receptor to sense microbiota-derived indoles. *Sci. Rep.* **5**, 12689 (2015).
98. Walther, D. J. et al. Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* **299**, 76–76 (2003).
99. Mawe, G. M. & Hoffman, J. M. Serotonin signalling in the gut — functions, dysfunctions and therapeutic targets. *Nat. Rev. Gastroenterol.* **10**, 473–486 (2013).
100. Reigstad, C. S. et al. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J.* **29**, 1395–1403 (2014).
101. Valles-Colomer, M. et al. The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat. Microbiol.* **4**, 623–632 (2019).
102. Lukić, I. et al. Antidepressants affect gut microbiota and *Ruminococcus flavefaciens* is able to abolish their effects on depressive-like behavior. *Transl. Psychiat* **9**, 133 (2019).
103. Fung, T. C. et al. Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut. *Nat. Microbiol.* **4**, 2064–2073 (2019).
104. Bhattarai, Y. et al. Gut microbiota-produced tryptamine activates an epithelial G-protein-coupled receptor to increase colonic secretion. *Cell Host Microbe* **23**, 775–785.e5 (2018).
105. Barcik, W., Wawrzyniak, M., Akdis, C. A. & O'Mahony, L. Immune regulation by histamine and histamine-secreting bacteria. *Curr. Opin. Immunol.* **48**, 108–113 (2017).
106. Koh, A. et al. Microbially produced imidazole propionate impairs insulin signaling through mTORC1. *Cell* **175**, 947–961.e17 (2018).
107. Rekdal, V. M., Bess, E. N., Bisanz, J. E., Turnbaugh, P. J. & Balskus, E. P. Discovery and inhibition of an interspecies gut bacterial pathway for levodopa metabolism. *Science* **364**, eaau6323 (2019).
108. Kessel, S. P. van et al. Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. *Nat. Commun.* **10**, 310 (2019).
109. Nallu, A., Sharma, S., Ramezani, A., Muralidharan, J. & Raj, D. Gut microbiome in chronic kidney disease: challenges and opportunities. *Transl. Res.* **179**, 24–37 (2017).
110. Vanholder, R., Schepers, E., Pletinck, A., Nagler, E. V. & Glorieux, G. The uremic toxicity of indoxyl sulfate and *p*-cresyl sulfate: a systematic review. *J. Am. Soc. Nephrol.* **25**, 1897–1907 (2014).
111. Hatch, M. & Vaziri, N. D. Enhanced enteric excretion of urate in rats with chronic renal failure. *Clin. Sci.* **86**, 511–516 (1994).
112. Einheber, A. & Carter, D. The role of the microbial flora in uremia. *J. Exp. Med.* **123**, 239–250 (1966).
113. Werder, A. A., Amos, M. A., Nielsen, A. H. & Wolfe, G. H. Comparative effects of germfree and ambient environments on the development of cystic kidney disease in CFWwd mice. *J. Lab. Clin. Med.* **103**, 399–407 (1984).
114. Hallman, T. M. et al. The mitochondrial and kidney disease phenotypes of kd/kd mice under germfree conditions. *J. Autoimmun.* **26**, 1–6 (2006).
115. Consortium, C. K. D. P. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet* **375**, 2073–2081 (2010).
116. Poesen, R. et al. Microbiota-derived phenylacetylglutamine associates with overall mortality and cardiovascular disease in patients with CKD. *J. Am. Soc. Nephrol.* **27**, 3479–3487 (2016).
117. Nemet, I. et al. A cardiovascular disease-linked gut microbial metabolite acts via adrenergic receptors. *Cell* **180**, 862–877.e22 (2020).
118. de Aguiar Vallim, T. Q., Tarling, E. J. & Edwards, P. A. Pleiotropic roles of bile acids in metabolism. *Cell Metab.* **17**, 657–669 (2013).
119. Sayin, S. I. et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-β-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab.* **17**, 225–235 (2013).
120. Quinn, R. A. et al. Global chemical effects of the microbiome include new bile-acid conjugations. *Nature* **579**, 123–129 (2020).

121. Funabashi, M. et al. A metabolic pathway for bile acid dehydroxylation by the gut microbiome. *Nature* **582**, 566–570 (2020).
122. Schramm, C. Bile acids, the microbiome, immunity, and liver tumors. *N. Engl. J. Med.* **379**, 888–890 (2018).
123. Jia, W., Xie, G. & Jia, W. Bile acid–microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nat. Rev. Gastroenterol. Hepatol.* **15**, 111–128 (2017).
124. Lund, M. L. et al. L-cell differentiation is induced by bile acids through GPBAR1 and paracrine GLP-1 and serotonin signaling. *Diabetes* **69**, 614–623 (2020).
125. Inagaki, T. et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab.* **2**, 217–225 (2005).
126. Ryan, K. K. et al. FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature* **509**, 183–188 (2014).
127. Flynn, C. R. et al. Bile diversion to the distal small intestine has comparable metabolic benefits to bariatric surgery. *Nat. Commun.* **6**, 7715 (2015).
128. Schmitt, J. et al. Protective effects of farnesoid X receptor (FXR) on hepatic lipid accumulation are mediated by hepatic FXR and independent of intestinal FGF15 signal. *Liver Int.* **35**, 1133–1144 (2014).
129. Jiang, C. et al. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. *Nat. Commun.* **6**, 10166 (2015).
130. Li, F. et al. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. *Nat. Commun.* **4**, 2384 (2013).
131. Sjöström, L. et al. Effects of bariatric surgery on cancer incidence in obese patients in Sweden (Swedish Obese Subjects Study): a prospective, controlled intervention trial. *Lancet Oncol.* **10**, 653–662 (2009).
132. Sjöström, L. et al. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N. Engl. J. Med.* **351**, 2683–2693 (2004).
133. Tremaroli, V., Karlsson, F. & Werling, M. et al. Roux-en-Y gastric bypass and vertical banded gastroplasty induce long-term changes on the human gut microbiome contributing to fat mass regulation. *Cell Metab.* **22**, 228–238 (2015).
134. Gottardi, A. D. et al. The bile acid nuclear receptor FXR and the bile acid binding protein IBABP are differently expressed in colon cancer. *Dig. Dis. Sci.* **49**, 982–989 (2004).
135. Yoshimoto, S. et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* **499**, 97–101 (2013).
136. Gadaleta, R. M. et al. Activation of bile salt nuclear receptor FXR is repressed by pro-inflammatory cytokines activating NF-κB signaling in the intestine. *Biochim. Biophys. Acta* **1812**, 851–858 (2011).
137. Bailey, A. M. et al. FXR silencing in human colon cancer by DNA methylation and KRAS signaling. *Am. J. Physiol. Gastrointest. Liver Physiol.* **306**, G48–G58 (2014).
138. Degirolamo, C. et al. Prevention of spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice by intestinal-specific farnesoid X receptor reactivation. *Hepatology* **61**, 161–170 (2014).
139. Yang, F. et al. Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid X receptor. *Cancer Res.* **67**, 863–867 (2007).
140. Ma, C. et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science* **360**, eaan5931 (2018).
141. Ducker, G. S. & Rabinowitz, J. D. One-carbon metabolism in health and disease. *Cell Metab.* **25**, 27–42 (2016).
142. Zeisel, S. H. & Costa, K. D. Choline: an essential nutrient for public health. *Nutr. Rev.* **67**, 615–623 (2009).
143. Craciun, S. & Balskus, E. P. Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. *Proc. Natl Acad. Sci. USA* **109**, 21307–21312 (2012).
144. Wang, Z. et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472**, 57–63 (2011).
145. Zhu, W. et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell* **165**, 111–124 (2016).
146. Tang, W. H. W. et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N. Engl. J. Med.* **368**, 1575–1584 (2013).
147. Li, X. S. et al. Gut microbiota-dependent trimethylamine *N*-oxide in acute coronary syndromes: a prognostic marker for incident cardiovascular events beyond traditional risk factors. *Eur. Heart J.* **38**, 814–824 (2017).
148. Seldin, M. M. et al. Trimethylamine *N*-oxide promotes vascular inflammation through signaling of mitogen-activated protein kinase and nuclear factor-κB. *J. Am. Heart Assoc.* **5**, e002767 (2016).
149. Koeth, R. A. et al. l-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *J. Clin. Invest.* **129**, 373–387 (2018).
150. Li, X. S. et al. Trimethyllysine, a trimethylamine *N*-oxide precursor, provides near- and long-term prognostic value in patients presenting with acute coronary syndromes. *Eur. Heart J.* **40**, 2700–2709 (2019).
151. Hill, M. J. Intestinal flora and endogenous vitamin synthesis. *Eur. J. Cancer Prev.* **6** (Suppl 1), S43–S45 (1997).
152. Magnúsdóttir, S., Ravcheev, D., Crécy-Lagard, V. de & Thiele, I. Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. *Front. Genet.* **6**, 1–18 (2015).
153. Aufreiter, S. et al. Folate is absorbed across the colon of adults: evidence from cecal infusion of ^{13}C-labeled [6*S*]-5-formyltetrahydrofolic acid. *Am. J. Clin. Nutr.* **90**, 116–123 (2009).
154. Yatsunenko, T. et al. Human gut microbiome viewed across age and geography. *Nature* **486**, 222–227 (2012).
155. Roberts, A. B. et al. Development of a gut microbe-targeted nonlethal therapeutic to inhibit thrombosis potential. *Nat. Med.* **24**, 1407–1417 (2018).
156. Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R. & Goodman, A. L. Separating host and microbiome contributions to drug pharmacokinetics and toxicity. *Science* **363**, eaat9931 (2019).
157. Ackerman, D. & Schutze, H. The formation of trimethylamine by *Bacterium prodigiosum*. *Zentralb. Physiol.* **24**, 210–211 (1910).
158. Romano, K. A., Vivas, E. I., Amador-Noguez, D. & Rey, F. E. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-*N*-oxide. *Mbio* **6**, e02481 (2015).
159. Gregory, J. C. et al. Transmission of atherosclerosis susceptibility with gut microbial transplantation. *J. Biol. Chem.* **290**, 5647–5660 (2015).
160. Guo, C.-J. et al. Discovery of reactive microbiota-derived metabolites that inhibit host proteases. *Cell* **168**, 517–526.e18 (2017).
161. Lloyd-Price, J. et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* **569**, 655–662 (2019).
162. McDonald, L. C. et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin. Infect. Dis.* **66**, e1–e48 (2018).
163. Mullish, B. H. et al. The use of faecal microbiota transplant as treatment for recurrent or refractory *Clostridium difficile* infection and other potential indications: joint British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. *Gut* **67**, 1920 (2018).
164. DeFilipp, Z. et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. *New Engl. J. Med.* **381**, 2043–2050 (2019).
165. Korem, T. et al. Bread affects clinical parameters and induces gut microbiome-associated personal glycemic responses. *Cell Metab.* **25**, 1243–1253.e5 (2017).
166. Zeevi, D. et al. Personalized nutrition by prediction of glycemic responses. *Cell* **163**, 1079–1094 (2015).
167. Zhang, L. S. & Davies, S. S. Microbial metabolism of dietary components to bioactive metabolites: opportunities for new therapeutic interventions. *Genome Med.* **8**, 46 (2016).
168. Williams, B. B. et al. Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. *Cell Host Microbe* **16**, 495–503 (2014).
169. Saito, Y., Sato, T., Nomoto, K. & Tsuji, H. Identification of phenol- and *p*-cresol-producing intestinal bacteria by using media supplemented with tyrosine and its metabolites. *Fems. Microbiol. Ecol.* **94**, fiy125 (2018).
170. Eyssen, H., Pauw, G. D., Stragier, J. & Verhulst, A. Cooperative formation of ω-muricholic acid by intestinal microorganisms. *Appl. Env. Microb.* **45**, 141–147 (1983).
171. Madsen, D., Beaver, M., Chang, L., Bruckner-Kardoss, E. & Wostmann, B. Analysis of bile acids in conventional and germfree rats. *J. Lipid Res.* **17**, 107–111 (1976).
172. Ragsdale, S. W. & Pierce, E. Acetogenesis and the Wood–Ljungdahl pathway of CO_2 fixation. *Biochim. Biophys. Acta* **1784**, 1873–1898 (2008).
173. Duncan, S. H., Barcenilla, A., Stewart, C. S., Pryde, S. E. & Flint, H. J. Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Appl. Env. Microb.* **68**, 5186–5190 (2002).
174. Cloutier, N. et al. Platelets release pathogenic serotonin and return to circulation after immune complex-mediated sequestration. *Proc. Natl Acad. Sci. USA* **115**, 201720553 (2018).
175. Fiehn, O. Metabolomics by gas chromatography–mass spectrometry: combined targeted and untargeted profiling. *Curr. Protoc. Mol. Biol.* **114**, 30.4.1–30.4.32 (2016).
176. Berry, D. & Loy, A. Stable-isotope probing of human and animal microbiome function. *Trends Microbiol.* **26**, 999–1007 (2018).
177. Röth, D. et al. Two-carbon folate cycle of commensal *Lactobacillus reuteri* 6475 gives rise to immunomodulatory ethionine, a source for histone ethylation. *FASEB J.* **33**, 3536–3548 (2019).
178. Jang, C. et al. The small intestine converts dietary fructose into glucose and organic acids. *Cell Metab.* **27**, 351–361.e3 (2018).
179. Rath, C. M. et al. Molecular analysis of model gut microbiotas by imaging mass spectrometry and nanodesorption electrospray ionization reveals dietary metabolite transformations. *Anal. Chem.* **84**, 9259–9267 (2012).
180. Wagner, M. Single-cell ecophysiology of microbes as revealed by Raman microspectroscopy or secondary ion mass spectrometry imaging. *Annu. Rev. Microbiol.* **63**, 411–429 (2009).
181. Lourenço, C. et al. Monitoring type 2 diabetes from volatile faecal metabolome in Cushing's syndrome and single Afmid mouse models via a longitudinal study. *Sci. Rep.* **9**, 18779 (2019).
182. Hoving, L. R., Heijink, M., Harmelen, V., Dijk, K. W. van & Giera, M. Methods in molecular biology. *Meth. Mol. Biol.* **1730**, 247–256 (2018).
183. Zhang, X.-S. et al. Antibiotic-induced acceleration of type 1 diabetes alters maturation of innate intestinal immunity. *eLife* **7**, e1002358 (2018).
184. Robinson, J. I. et al. Metabolomic networks connect host–microbiome processes to human *Clostridioides difficile* infections. *J. Clin. Invest.* **129**, 3792–3806 (2019).
185. Fujisaka, S. et al. Diet, genetics, and the gut microbiome drive dynamic changes in plasma metabolites. *Cell Rep.* **22**, 3072–3086 (2018).
186. Zierer, J. et al. The fecal metabolome as a functional readout of the gut microbiome. *Nat. Genet.* **50**, 790–795 (2018).
187. Paul, H. A., Bomhof, M. R., Vogel, H. J. & Reimer, R. A. Diet-induced changes in maternal gut microbiota and metabolomic profiles influence programming of offspring obesity risk in rats. *Sci. Rep.* **6**, 20683 (2016).
188. Lamichhane, S. et al. Strategy for nuclear-magnetic-resonance-based metabolomics of human feces. *Anal. Chem.* **87**, 5930–5937 (2015).
189. Zhang, C. et al. Dietary modulation of gut microbiota contributes to alleviation of both genetic and simple obesity in children. *Ebiomedicine* **2**, 968–984 (2015).
190. Bui, T. P. N. et al. Production of butyrate from lysine and the Amadori product fructoselysine by a human gut commensal. *Nat. Commun.* **6**, 10062 (2015).
191. Lin, Y. et al. NMR-based fecal metabolomics fingerprinting as predictors of earlier diagnosis in patients with colorectal cancer. *Oncotarget* **7**, 29454–29464 (2015).
192. Kim, M., Qie, Y., Park, J. & Kim, C. H. Gut microbial metabolites fuel host antibody responses. *Cell Host Microbe* **20**, 202–214 (2016).
193. Etchegaray, J.-P. & Mostoslavsky, R. Interplay between metabolism and epigenetics: a nuclear adaptation to environmental changes. *Mol. Cell* **62**, 695–711 (2016).
194. Krautkramer, K. A., Rey, F. E. & Denu, J. M. Chemical signaling between gut microbiota and host chromatin: what is your gut really saying? *J. Biol. Chem.* **292**, 8582–8593 (2017).
195. Allen, J. & Sears, C. L. Impact of the gut microbiome on the genome and epigenome of colon epithelial cells: contributions to colorectal cancer development. *Genome Med.* **11**, 11 (2019).
196. Fan, J., Krautkramer, K. A., Feldman, J. L. & Denu, J. M. Metabolic regulation of histone post-translational modifications. *ACS Chem. Biol.* **10**, 95–108 (2015).

197. Zhao, Y. & Garcia, B. A. Comprehensive catalog of currently documented histone modifications. *Cold Spring Harb. Perspect. Biol.* **7**, a025064 (2015).
198. Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293**, 1074–1080 (2001).
199. Badeaux, A. I. & Shi, Y. Emerging roles for chromatin as a signal integration and storage platform. *Nat. Rev. Mol. Cell Bio.* **14**, 211 (2013).
200. Zhu, Q., Stöger, R. & Alberio, R. A lexicon of DNA modifications: their roles in embryo development and the germline. *Front. Cell Dev. Biol.* **6**, 24 (2018).
201. Riggs, M. G., Whittaker, R. G., Neumann, J. R. & Ingram, V. M. *n*-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. *Nature* **268**, 462–464 (1977).
202. Thaiss, C. A. et al. Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* **167**, 1495–1510.e12 (2016).
203. Zhang, D. et al. Metabolic regulation of gene expression by histone lactylation. *Nature* **574**, 575–580 (2019).
204. Sánchez-Romero, M. A. & Casadesús, J. The bacterial epigenome. *Nat. Rev. Microbiol.* **18**, 7–20 (2020).
205. Dorman, C. J. & Dorman, M. J. DNA supercoiling is a fundamental regulatory principle in the control of bacterial gene expression. *Biophys. Rev.* **8**, 89–100 (2016).
206. Liu, S. et al. The host shapes the gut microbiota via fecal microRNA. *Cell Host Microbe* **19**, 32–43 (2016).
207. Yassour, M. et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci. Transl. Med.* **8**, 343ra81–343ra81 (2016).
208. Kimura, I. et al. Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. *Science* **367**, eaaw8429 (2020).
209. Waterland, R. A. et al. Season of conception in rural gambia affects DNA methylation at putative human metastable epialleles. *PLoS Genet.* **6**, e1001252 (2010).
210. Romano, K. A. et al. Metabolic, epigenetic, and transgenerational effects of gut bacterial choline consumption. *Cell Host Microbe* **22**, 279–290.e7 (2017).

Acknowledgements
K.A.K acknowledges support from the Fulbright United States Scholar Program (US Fulbright Scholar Award) and the Human Frontier Science Program (HFSP; Long-term Fellowship LT000195/2018-L). Work in the Bäckhed laboratory is supported by Transatlantic Networks of Excellence Award from the Leducq Foundation (17CVD01), JPI (A healthy diet for a healthy life; 2017-01996_3), AFA insurances, the Swedish Research Council (2019-01599), the Swedish Heart Lung Foundation (20180600), the Knut and Alice Wallenberg Foundation (2017.0026), the Novo Nordisk Foundation (NNF19OC0057271, NNF17OC0028232 and NNF15OC0016798), grants from the Swedish state under the agreement between the Swedish government and the county councils, and the ALF-agreement (ALFGBG-718101). F.B. is Torsten Söderberg Professor in Medicine and recipient of an ERC Consolidator Grant (European Research Council; Consolidator grant 615362-METABASE).

Author contributions
All authors contributed substantially to the conceptualization and writing of the manuscript, focusing on the following sections: Introduction (K.A.K and F.B.), Fermentable substrates (K.A.K. and F.B.), Amino acids and their derivatives (K.A.K. and F.B.), Bile acids as gut microbial messengers (K.A.K. and F.B), Vitamins and one-carbon metabolites (K.A.K. and F.B.), Perspectives (K.A.K., J.F., and F.B.), Box 1 (K.A.K., J.F., and F.B.) and Box 2 (K.A.K. and F.B.)

Competing interests
F.B. receives research support from Biogaia AB and is founder and shareholder in Implexion pharma AB. All other authors declare no competing interests.

Peer review information
Nature Reviews Microbiology thanks P. Dorrestein, E. Gentry and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.