TOPIC HIGHLIGHT

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Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health

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Abstract

Gut microbes comprise a high density, biologically active community that lies at the interface of an animal with its nutritional environment. Consequently their activity profoundly influences many aspects of the physiology and metabolism of the host animal. A range of microbial structural components and metabolites directly interact with host intestinal cells and tissues to influence nutrient uptake and epithelial health. Endocrine, neuronal and lymphoid cells in the gut also integrate signals from these microbial factors to influence systemic responses. Dysregulation of these host-microbe interactions is now recognised as a major risk factor in the development of metabolic dysfunction. This is a two-way process and understanding the factors that tip host-microbiome homeostasis over to dysbiosis requires greater appreciation of the host feedbacks that contribute to regulation of microbial community composition. To date, numerous studies have employed taxonomic profiling approaches to explore the links between microbial composition and host outcomes (especially obesity and its comorbidities), but inconsistent host-microbe associations have been reported. Available data indicates multiple factors have contributed to discrepancies between studies. These include the high level of functional redundancy in host-microbiome interactions combined with individual variation in microbiome composition; differences in study design, diet composition and host system between studies; and inherent limitations to the resolution of rRNA-based community profiling. Accounting for these factors allows for recognition of the common microbial and host factors driving community composition and development of dysbiosis on high fat diets. New therapeutic intervention options are now emerging.

Core tip: The development of dysbiosis is driven by multiple factors. These include selective pressures imposed on the microbial community by the diet composition and feedback effects that involve either diet-host interaction or diet-microbiome-host interaction. The role of microbial signals in dysbiosis is well established but the involvement of host feedback mechanisms in aberrant host-microbial interactions is an under-appreciated part of disease progression. New opportunities to intervene in diseases of dysbiosis can result from targeting these distinct processes. These include stimulation of the host ability to self-regulate and blocking of deleterious host responses.

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INTRODUCTION

The gastrointestinal tract of animals typically harbours a large resident community of microorganisms that we will term the microbiome. The main function of the gut is to enable harvesting of nutrients from the external environment, however, animals live in a dynamic environment where their energy demands, exposure to foreign microorganisms and their access to nutrients are continually changing. Consequently gut functions also include containment of microbial activity to the intestinal lumen and integration of sensory perception of the intestinal environment with behavioural and physiological responses. Put simply, the gut is a major site for endocrine, immune and neural signalling in addition to digestion and nutrient absorption.

Many aspects of host physiology are strongly shaped by the presence and activities of the gut microbiome. The primary axis of host-microbiome interaction is in the intestinal tissues where microbial growth in the lumen contributes to the digestion of ingested food and directly shapes the chemical milieu of the gut. Host cells in the intestines are highly exposed to microbial activity, and microbial influence ranges from stimulation of receptors on those cells, to supply of energy sources to epithelial cells and triggering of developmental pathways in intestinal tissues[1,2] (Figure 1). Although the primary interaction with microbes is at the intestinal epithelium, their influence is projected beyond the gut through secondary host-microbiome interactions, which occur externally to the epithelium. Some of these influences such as nutrient uptake and systemic inflammation, result from translocation of or "escape" of microbial products[3,4]. Others such as appetite regulation, gut motility, energy balance and immune tone, result from the integration of multiple signals from the gut environment and bidirectional communication along the gut-brain axis[5,6]. Accordingly, it is now widely recognised that differences in microbial composition and activity result in effects of fundamental importance to health.

The breadth of potential influence of the microbiome means mechanisms that serve to regulate the microbial interface with host systems are critical for health. This view gives rise to the concept of dysbiosis: Disease states that result from dysregulated host-microbe interactions. Dysbiosis contributes to the underlying pathophysiology of a wide range of diseases, including obesity[7], diabetes[4,8], inflammatory bowel diseases[9], non-alcoholic fatty liver diseases[10,11] and cardiovascular diseases[12,13]. With awareness of the importance of dysbiosis in multiple diseases, attention has focused on how to define the microbe involvement in different diseases. The objectives here encompass the following: Identification of microbiota signatures (or biomarkers) that help define different dysbiosis states, ideally at the pre-clinical stage. Identifica-

tion of the triggers of dysregulated host-microbe interactions that ultimately lead to disease. Development of intervention strategies based around restoration of normal host-microbiome interactions. Underpinning all these objectives is the need to understand the dynamics of gut microbial community composition. This review focuses on mechanisms that drive the changes in microbial community composition that ultimately lead to shifts in host-microbiome interactions.

EVIDENCE FOR, AND LIMITS OF, MICROBIOME INFLUENCE ON HEALTH

Comparative studies on germ-free (GF) and conventionally raised (CONV) animals have been instrumental in establishing that the gut microbiome has influence on the physiological, immunological and nutritional state of its host. Such studies have consistently shown that GF animals are characterised by reduced intestinal vasculature[1], undeveloped gut-associated lymphoid tissue[14] and alterations in nutrition and energy metabolism[15], all of which are largely restored by reintroduction of gut bacteria. Collectively there is compelling evidence that the gut microbiome can influence postnatal development of gut tissues and the physiological state of animals.

The effects of microbes are interdependent with effects of diet or the host genotype. For instance, GF and CONV comparisons are not precisely recapitulated in different animal models[16], and there are also characteristic variations in microbiome composition between species[17]. Some of these variations almost certainly reflect genetically encoded differences in life history (carnivores *vs* herbivores) or gut structure (ruminants *vs* monogastrics). Others will reflect more subtle tissue specific differences, for example, the organisation of gut-associated lymphoid tissue in dogs and rodents are distinct[18]. Collectively these points serve to illustrate a broader issue. Host-microbiome interaction involves effects of the microbiome on the host, as well as effects of the host on the microbiome and these both occur within the context of environmental effects on the system (especially the nutritional environment). Studies that have addressed the influence of microbiome on differences between GF and CONV against defined genetic and diet differences in animals highlight the importance of this tripartite interaction[9,19].

The importance of variation in host diet and genotype has been observed through GF-CONV comparisons across different strains and species of inbred rodents. In a seminal paper Bäckhed, Gordon *et al*[15] raised the prospect that gut microbiota represent an environmental factor in obesity. They showed that GF C57BL/6 mice had less fat deposition than CONV counterparts despite higher food consumption. Moreover, the faecal caloric content of GF mice was significantly higher than that of CONV counterparts. These findings led to the conclusion that gut microbiota promote energy harvesting and fat storage, and the hypothesis that GF
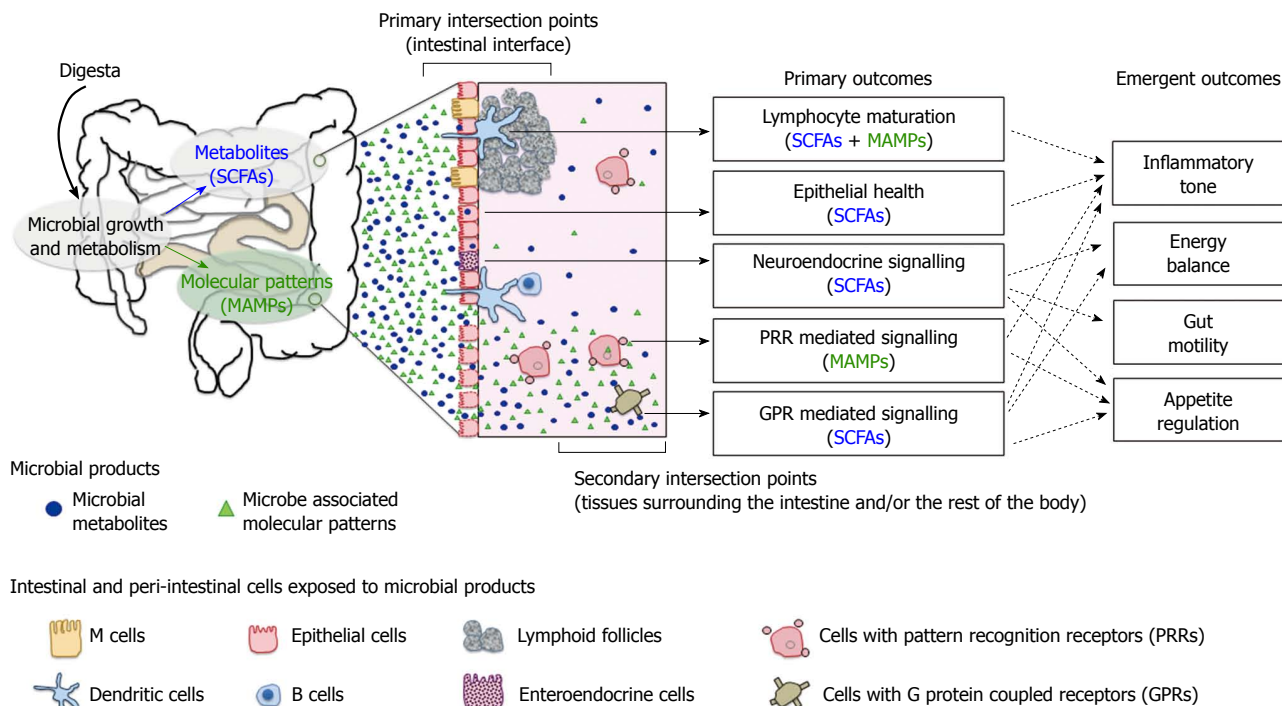
Figure 1 Axes of host-microbial interaction that influence health. Short chain fatty acids (SCFAs) and microbe-associated molecular patterns (MAMPs) are the key microbial signals detected by the host. Outcomes of host-microbiome interactions are contingent on the microbial product involved, the type of host cells exposed to microbial signals and the location of contact. The primary intersection points occur at the intestinal epithelial interface. Sampling of luminal MAMPs and uptake of SCFAs have a direct impact on gut epithelium, lymphoid and neuroendocrine systems. The secondary intersection points occur externally to the intestinal tissues. Translocated or "escaped" microbial products can activate pattern recognition receptors (PRRs) and specific G protein coupled receptors (GPRs) on a wide range of host cells beyond the epithelium. A compromised gut barrier amplifies host-microbiome interactions in the secondary intersection points and the downstream effects of PRR and GPR signalling cascades. Host outcome is an emergent property of all axes of interactions.

animals are protected from obesity[15,20]. In contrast to this mouse model, GF Fischer 344 rats displayed similar body weight and adiposity relative to CONV in two out of three experimental cohorts, and differences in daily food intake between the GF and CONV groups were insignificant[21]. Although this suggests different animal species may respond differently, it is important to note that these studies used standard rodent chow from different suppliers and almost certainly the diets were compositionally distinct[15,21].

Intersection between diet and genotype can also influence the phenotype of GF and CONV animals. The significance of this issue is highlighted in a report comparing the effect of three different diets on GF and CONV C3H mice[22]. There was no difference in weight gain between GF and CONV groups under low fat diet, but GF C3H mice actually showed significantly higher weight gain on a high fat diet (HFD) compared to CONV. Previous reports of obesity resistance on HFD in GF C57BL/6 mice had used a formulation with similar macronutrient balance but distinct sources of carbohydrates and fat[20]. When the two versions of high fat formulation were directly compared, GF and CONV C3H had comparable body fat content on the HFD with low sugar formulation but GF C3H mice was obesity resistant on the HFD with high sugar[22]. In summary, GF-CONV comparisons in different animal/diet models consistently show differences in energy harvest (faecal

caloric content), energy storage (weight and body fat) and energy expenditure. Typically the effect of microbial presence is to increase adiposity, however, this does vary between experimental models and even between cohorts in the same model system. The major identifiable variables are animal species/strain and diet composition which differ between experimental cohorts.

Further exploration of the importance of microbiome composition has provided robust evidence supporting a causal link between gut microbiome composition and host outcomes. Specifically, some phenotypic traits of CONV animals can be recapitulated by conventionalisation of GF animals through microbiome transplantation[11,23-25]. When GF mouse models are conventionalised with gut microbiota from either obese or lean mice, metabolic profiles and physiological attributes of the recipients reflect their donors[23,24]. Evidently emergent properties of the total microbial community can drive differences in metabolic and physiological phenotypes. Precisely which microbes or how many are needed is unclear. For example, monocolonisation of GF mice with *Enterobacter cloacae* (a member of *Proteobacteria* isolated from an obese human) induced obesity and systemic insulin resistance in mice on HFD, while GF mice on HFD did not exhibit the same disease phenotypes[26].

In conclusion, host metabolic health is strongly influenced by the gut microbiome. The influence of gut microbes is dependent on microbiome composition and

is interactive with the effects of diet and host genotype. The mechanisms of microbial influence stem from microbial activity in the intestinal tract, but are projected to the body system *via* multiple integrated pathways. The complexities of these interactions mean that although variations in microbial community composition can lead to different outcomes, associations may be diet or system-specific.

IDENTIFYING MICROBIAL MARKERS FOR METABOLIC DISEASES

Gut microbial community in health and disease-taxonomic insights

Broadly speaking microbiome association studies have two objectives: (1) To identify links with specific disease states[27]; and (2) To identify features of a healthy microbiome that may be a target in the restoration of health[28]. Although there have been many reports of microbiome associations with obesity or metabolic health indicators in cross-sectional studies[29,30], experimentally controlled treatments in humans[31,32] and animal models (Table 1), consistent patterns across studies are hard to discern. As discussed above the influence of the microbiome on host health is interdependent with diet and the host system. As such the apparent lack of consistent associations is likely to reflect the confounding effects of diet, host genotype and host epigenetic state. Since HFDs in Table 1 are not of the same formulation, some of the discrepancies observed almost certainly reflect variations in diet. Differences will also reflect some inherent limitations of taxon-based description of the gut microbial community.

Community profiling has two key requirements. These are the ability to recognise biologically distinct units and the capacity to effectively sample all such units in a community. The size and diversity of microbial communities mean that it is essential to meet these requirements with high throughput approaches. The limitations of the species concept in bacteriology, combined with poor cultivability of bacteria meant that historically this has been impossible. Advances in sequencing technologies and analysis programs over the past decade have made effective sampling possible for the first time. However, recognition of biologically meaningful taxonomic units is still limited.

The most widely used marker for community profiling is the 16S ribosomal RNA (rRNA) gene. Sample sizes of thousands to even millions of sequence reads are now readily obtained. A feature of the 16S rRNA is that it is a very flexible phylogenetic marker and taxonomic units can be readily made at a variety of scales. Generally defining taxonomic units at coarse scale (*e.g.*, phylum; about 80% 16S rRNA identity) simplifies the analytical task of comparing units but at the expense of explanatory power. Variation in the gut microbiome is readily observable at this scale[48]. Many studies have reported an association between the ratio of the two dominant gut phyla, *Bacteroidetes* and *Firmicutes*, with obesity in cross-sectional studies and in experimental treatments[24,29,49]. However

numerous exceptions have also been reported[50-52], and a recent exhaustive meta-analysis of human microbiome project data found no consistent relationship between the *Bacteroidetes*:*Firmicutes* ratio and obesity[53]. An almost certain contributing factor is that such coarse taxonomic units are less biologically meaningful than fine scale units.

There are some attributes of the gut microbiome that one can reasonably predict from the taxonomic profiles at phylum scale. For instance, *Firmicutes* and *Bacteroidetes* have fundamental differences in cell envelope composition, and polysaccharide foraging strategy[54]. However, detailed predictions of microbial functions and/or properties based on phylum classification alone are unrealistic. At finer scales of classification the biological homogeneity of taxa increases and more consistent patterns are observable. For example, it has been proposed that human gut microbiome variation occurs in three predominant variants termed enterotypes, which are recognisable through co-occurrence patterns defined by the genera *Bacteroides*, *Prevotella* and *Ruminococcus*[52]. Recently this concept has been intensively explored, highlighting that observation of specific patterns of association is subject to analytical and classification approaches[55], particularly how sequences are clustered into operational taxonomic units (OTUs) and how OTU-based distances between communities are calculated. This effect of analytical approach is likely to exist wherever community profiling does not (or cannot) classify into ecologically homogeneous units (ecotypes).

The inability to recognise ecotypes is an inherent limitation of 16S rRNA sequencing based approaches. Closely related species can have differential responses to specific nutrient sources and have divergent ecological roles[42,56,57]. Perhaps the most striking illustration of this issue derives from a study conducted by Li *et al*[58], where they used community fingerprinting and metabolomics to test for associations between *Clostridia* and urinary metabolites in humans. Distinct populations in the fingerprinting analysis that had mutually exclusive associations to different sets of urinary metabolites were classified to *Faecalibacterium prausnitzii* (*F. prausnitzii*). This indicates that strains of *F. prausnitzii* inseparable by rRNA-based classification had distinct metabolic impacts in the gut system. Hence, it is not surprising that even microbiome associations reported at the finest scales possible with rRNA-based classification are often contradictory between different studies. For instance, *F. prausnitzii* was found to be over-represented in obese subjects in comparison to the lean counterparts[59], which suggests high proportion of *F. prausnitzii* within the gut community is an indicator of poor health outcomes. Yet, other investigations have reported that healthy individuals carry more *F. prausnitzii* than patients with type 2 diabetes[30] or chronic inflammation[60]. Another example is the association of *Akkermansia muciniphilia* (*A. muciniphilia*) with health in some animal studies[61], other studies have noted an increased proportion of *A. muciniphilia* in obesity[33] and type 2 diabetes[30], or a role in

Table 1 Murine gut microbiome and host outcomes after exposure to high fat diets

Type of high fat diet and duration	Detection method	Key microbial features[1]						Observation and proposed mechanism for microbial outcomes	Reported host phenotype	Observation and proposed mechanism for host outcomes	Ref.
		F:B	Firmicutes	Bacteroidetes	Proteobacteria	Actinobacteria	Other				
HF/HS[2] for 8 wk	Fecal 454 [V4]	↑F:B	↑unclassified Lachnospiraceae, unclassified Ruminococcaceae, Turicibacter, Dorea, Roseburia ↓Oscillibacter	↓Barnesiella, unclassified Porphyromonadaceae		↑Bifidobacterium	↑Akkermansia	Host genotype influences gut microbiota plasticity in response to diet.	↑Body fat percent		[33]
HF/HS[3] for 8 wk	Cecal full length 16S sequencing, shotgun sequencing and transcriptomics	↑F:B	↑Mollicutes/ Erysipelotrichaceae				↓Microbial diversity ↑Genes for PTS system ↑SCFAs concentration	Altered substrate availability ↑ microbes with the capacity to import and degrade sugars found in diet and/or host mucosa.	Weight gain, ↑Body fat percent	Increased energy harvest. Specific microbes facilitate the transfer of calories from the diet to the host in the form of SCFAs.	[24]
HF/HS[4] for 12 wk	Colonic tissue 454 [V1-2], qPCR and DGGE [V3-5]	↓F:B	↑mucin-degrading Ruminococcus torques	↑Bacteroides-Prevotella spp	↑Proteobacteria		↓16S rRNA gene copies	Diet type and host genotype↑ bacteria with the ability to bind to glycosylated proteins and colonise mucosal surfaces.	Leaky gut	Diet-induced microbial changes at gut mucosa may aggravate inflammation in genetically susceptible host.	[34]
HFD[5] for 8 wk	Fecal 454 [V4] at baseline, Week 4 and Week 8	Progressive ↑F:B			Progressive ↓Proteobacteria		Fecal energy and SCFAs fluctuate overtime, varied patterns in cecum and stool	Dietary factors determine microbial composition. Microbial community may adapt to HFD overtime	Weight gain and ↑ fat mass	Microbes may promote obesity *via* LPS or SCFA modulation of host gene expression rather than energy harvesting,	[35]
HFD[5] for 20 wk	Fecal 454 [V4] and qPCR	↑F:B	↑Lactobacillus	↓Bacteroides					Weight gain, IR, fatty liver, adipose, and systemic inflammation	Antibiotic improves metabolic abnormalities. Gut microbiota modulates inflammatory responses.	[36]
HFD[5] for 21 wk	Fecal 454 [V1-2] and shotgun sequencing	↑F:B	↑Clostridiaceae	↓Bacteroidaceae, Prevotellaceae and Rickenellaceae	↑Desulfovibrionaceae		↑genes for ABC transporters, two-component system and cell motility ↓metabolic genes	Altered substrate availability ↑ microbes with the capacity to enhance nutrient uptake in an environment of limiting substrates	Weight gain		[37]
HFD[6] for 8 to 12 wk	Fecal 454 [V6-8]	↑F:B	↑Oscillibacter, Blautia ↓Lactobacillus	↓Barnesiella, Parabacteroides					Weight gain, leaky gut, IR, adipose, gut and liver inflammation	Gut bacteria modulate gut barrier integrity. Leaky gut coupled with aberrant microbiota drive metabolic dysfunction.	[38]

Model	Methods	F:B	Firmicutes changes	Bacteroidetes changes	Proteobacteria/other changes	Actinobacteria/other	Diversity & function	Mechanism	Host effects	Outcome	Ref
HFD[7] for 12 wk	Cecal MiSeq [V4], metaproteome, metabolomics		↓ Ruminococcaceae	↑ Rikenellaceae			↑ proteins for amino acid metabolism and transport and cell motility; No difference in microbial richness	Altered substrate availability shifts the composition and/or activity of microbiota, which favours amino acid metabolism	Weight gain, hyperglycemia		[39]
HFD[8] for 8 wk	Fecal 454 [V1-3], culture	↑ F:B	↑ Erysipelotrichales; ↑ Ruminococcaceae ↓ Clostridiales	↑ Rikenellaceae ↓ Bacteroidaceae	↑ Enterobacteriaceae	↓ Bifidobacterium	↑ LPS; No difference microbial diversity		Weight gain, hyperglycemia, adipose, systemic and gut inflammation	Leaky gut and LPS induce pro-inflammatory cascade and accelerate obesity development	[40]
HFD[8] for 12 wk	Fecal 454 [V3] at every 2-4 wk	Progressive ↑ F:B	↑ Lachnospiraceae, Ruminococcaceae, Lactococcus	↑ selected OTUs in Bacteroides, Alistipes ↓ Barnesiella	Progressive ↑ Desulfovibrionaceae	↓ Bifidobacterium	↓ microbial diversity; ↑ LPS binding protein	Age-related effects and/or altered substrate availability	Weight gain, ↑ fat mass, IGT	↑ antigen load (LPS) and H_2S production may lead to chronic inflammation and leaky gut	[41]
HFD[8] for 25 wk	Fecal 454 [V3], DGGE [V3] and T-RFLP		Lineages in Mollicutes/ Erysipelotrichaceae responded differentially		↑ Desulfovibrionaceae ↓ Bifidobacteriaceae			Altered substrate availability and host genetics have differential impact on gut microbial profile	Weight gain, IGT	↓ gut barrier protecting members, ↑ LPS and H_2S production promote leaky gut and trigger inflammation	[42]
HFD[8] for more than 35 wk	Cecal 454 [V1-2]	↓ F:B	↑ unclassified Lachnospiraceae, Lactococcus, Unclassified Ruminococcaceae, Roseburia ↓ Allobaculum	↑ Bacteroides	↑ Mucispirillum			Leptin may affect microbial composition by modulating mucin production in the intestine	Weight gain, ↑ leptin, adipose inflammation	Association between gut bacteria and body fat may be mediated by adipokines and inflammation	[43]
HFD[8] for life, gut microbiota at week 62 is described here	Fecal 454 [V3]		↑ selected OTUs in Allobaculum, Ruminococcaceae, Papillibacter, Lactococcus ↓ selected OTUs in Allobaculum	↑ selected OTUs in Rikenella, Alistipes; ↓ selected OTUs in Bacteroidiales, Prophyromonadaceae.	↑ Bilophila; ↓ Akkermansia ↑ Mucispirillum		↑ LPS binding protein	Altered substrate availability. Low plant polysaccharides may alter the balance of gut barrier protecting bacteria, butyrate producers and pathobionts	Weight gain, IGT, fatty liver, ↓ liver function,	↑ antigen load (mainly LPS) may contribute to metabolic abnormalities	[44,45]
HFD[9] for 4 wk	Cecal FISH		↓ Eubacterium rectale/ Clostridium coccoides	↓ Bacteroides-like species		↓ Bifidobacterium	↑ LPS		Weight gain, IR, fatty liver, systemic and adipose inflammation	Dietary fat modulates LPS level in plasma and ↓ gut barrier protecting bacteria, which trigger inflammation and the onset of diabetes and obesity	[4]
HFDs[10] with different sources of fat (safflower oil, milk fat or lard) for 24 d	Cecal 454 [V2-4]	↑ F:B in lard HFD ↓ F:B in other HFDs			↑ Bilophila in milk fat HFD		↓ microbial diversity in milk fat and safflower oil HFDs	Altered substrate availability. Milk-derived saturated fat ↑ the pool of sulphated bile acid, an antimicrobial but a growth substrate for Bilophila	Gut inflammation in genetically susceptible host	H_2S or secondary bile acids from pathobiont may damage gut barrier and drive pro-inflammatory responses	[9]

				↑Proteobacteria in milk fat and safflower oil HFDs	↑Actinobacteria	↑Tenericutes in lard HFD	Altered substrate availability. Dietary fat source modulates gut microbial profile	Weight gain (highest in milk fat), adipose inflammation (highest in safflower)	Diet induced alterations in the gut microbiota influence localised inflammation	[46]
HFDs[10] with different sources of fat (safflower oil, milk fat or lard) for 4 wk	Fecal Illumina [V3-4]	↑F:B in all HFDs								
HFDs[11] with different sources of fat (palm, olive or safflower oil) for 8 wk	Fecal MITChip (microarray)	↑F:B in palm oil HFD only	↑Bacilli, Clostridium cluster XI, XVII, and XV III in palm oil HFD only			↓microbial diversity in palm oil HFD only	Saturated fat diet leads to an overflow of dietary fat in the gut which may have an antimicrobial effect on microbiota	Weight gain (highest in palm oil), IR, fatty liver		[47]

[1]This table features the microbial shifts in wild type mice after dietary interventions, patterns for knockout models are excluded. Sampling site and technique used to monitor the hypervariable region of 16S rDNA are noted; [2]31.8% and 51.4% calories from fat (corn oil and butter fat) and carbohydrates, respectively, Research Diets, New Brunswick; [3]40.6% and 40.7% calories from fat (beef tallow, vegetable shortening) and carbohydrates, respectively, Research Diets, Harlan-Teklad, United States; [4]60.6% and 26.3% calories from fat (lard) and carbohydrates, respectively, SAFE, France; [5]45% and 35% calories from fat (lard and soybean oil) and carbohydrates, respectively, Research Diets, New Brunswick; [6]60% and 20% calories from fat (lard and sunflower oil) and carbohydrates, respectively, in house; [7]60% and 21% calories from fat (beef tallow and soybean oil) and carbohydrates, respectively, Sniff GmbH, Germany; [8]60% and 20% calories from fat (lard and soybean oil) and carbohydrates, respectively, Research Diets, New Brunswick; [9]72% and < 1% calories from fat (corn oil and lard) and carbohydrates, respectively, SAFE, France; [10]37.5% and 47% calories from fat and carbohydrates, respectively, Harlan-Teklad, United States; [11]45% and 35% calories from fat and carbohydrates, respectively, Research Diet Services, The Netherlands. F:B: Firmicutes to Bacteroidetes ratio; HF/HS: High fat and high sugar diet; HFD: High fat diet; PTS: Phosphotransferase system; SCFAs: Short chain fatty acids; ABC transporters: ATP-binding cassette transporters; DGGE: Denaturing gradient gel electrophoresis; T-RFLP: Terminal restriction fragment length polymorphism; IR: Insulin resistance; IGT: Impaired glucose tolerance; LPS: Lipopolysaccharides; OTUs: Operational taxonomic units; H2S: Hydrogen sulphide; FISH: Fluorescent in situ hybridisation.

exacerbating gut inflammation[62].

In summary, consideration of diet, host system and great care in methodological approaches to community profiling is necessary to identify consistent associations between microbes and metabolic health. The main limitation from a methodological perspective is linkage of relevant ecological properties of the microbial group to the taxonomic marker. An alternate approach to this is to profile the gut system and its resident bacteria from a functional perspective.

Gut microbial community in health and disease-functional insights

In effect functional profiling is delineation of taxonomic units based on a biochemical property. It is generally accepted that there is a high level of functional redundancy in the gut microbiome. This means bacteria from different taxonomic groups may contribute to the same ecological process (belong to the same guild) and they can substitute for one another. For instance, many gut bacteria can produce butyrate, a short chain fatty acid (SCFA) with widespread health implications, but the bacteria that carry out this function are phylogenetically diverse[63]. Associations between rRNA-based taxa and host outcomes that are critically dependent on butyrate availability are likely to be inconsistent because different members of the butyrate-producer guild may be dominant under different diets or host systems. Thus functional redundancy is almost certainly a contributor to the wide variation in associations of microbiome response and host outcomes to HFDs summarised in Table 1.

If the diet-microbiome-host outcomes listed in Table 1 are cross-examined from the perspective of microbial metabolites or microbe-associated molecular patterns (MAMPs) that are likely to be common features of ecological guilds, a more encouraging picture of associations between microbiome and metabolic health starts to emerge. Inferred or measured changes of microbial metabolite such as elevated total SCFA, elevated serum lipopolysaccharides (LPS) production are recurrently observed. In the case of LPS and H2S these are also associated to taxa that are recognisable by rRNA-based classification, such as *Enterobacteriaceae* and *Desulfovibrionaceae* from the phylum *Proteobacteria*.

Metagenomic analysis provides a global dataset for functional profiling whereby multiple guilds can be looked at simultaneously. Such analyses have reported differences in the total level of carbohydrate degradation genes in the metagenomes of obese *vs* lean microbiomes raising the prospect that energy harvesting may be predictable from
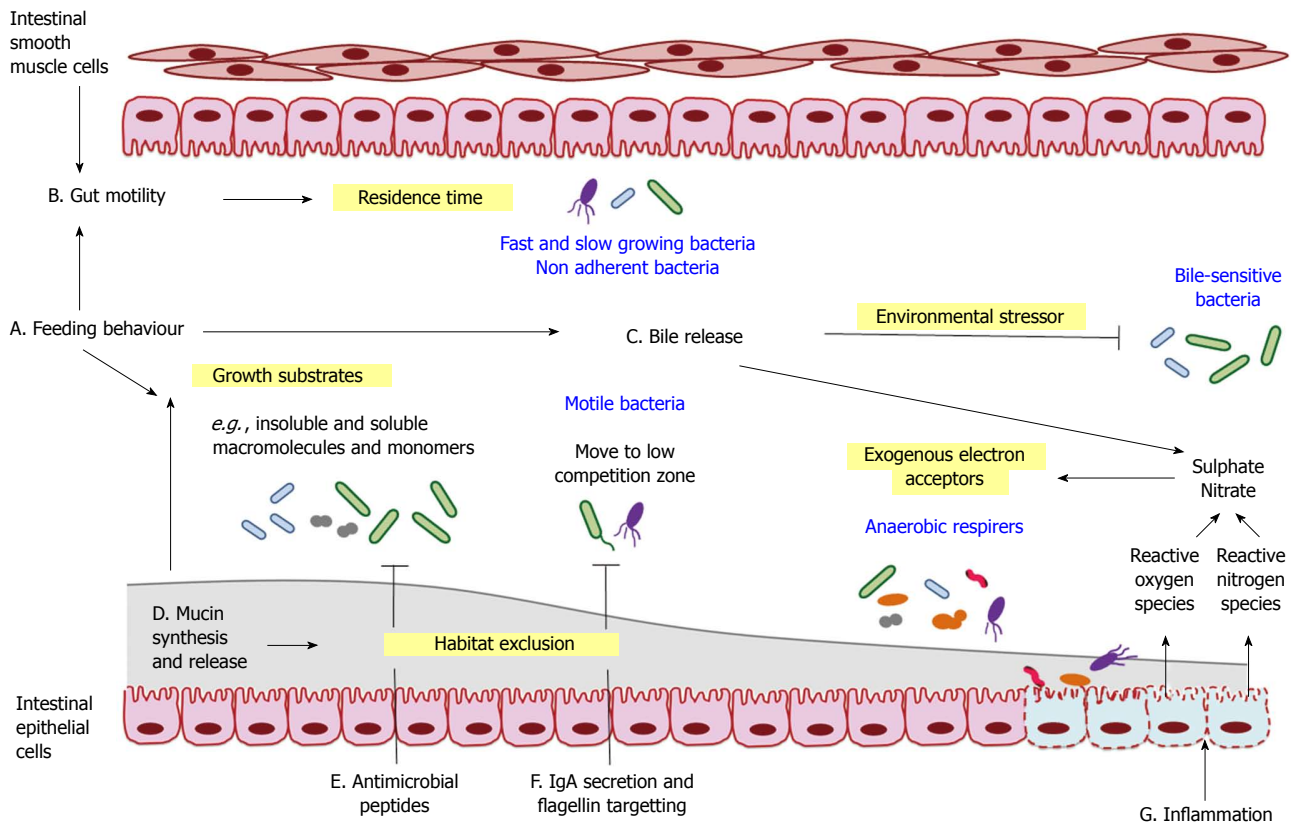
Figure 2 Multiple host-mediated mechanisms regulate bacterial growth and their activities. These pathways may act against the microbiota in a generalised manner or influence bacteria with distinct properties (blue). A: Substrates from diet are key energy sources for bacterial growth. Changes in feeding pattern will shape the microbiome structure and associated products; B: Ingestion of dietary fibre and osmotically active compounds promotes gut motility. Faster transit rate flushes out slow growing organisms and those without the ability to adhere to the intestines; C: Release of bile in response to dietary fat selects against bile-sensitive bacteria but promotes those with the capacity to obtain energy *via* anaerobic respiration; D: Mucin secreted by goblet cells physically prevents the penetration of bacteria into gut epithelium, and it also promotes bacteria that utilise mucin as growth substrates; E: Paneth cells in the gut epithelium secrete effector molecules with broad-spectrum antimicrobial activity, *e.g.* defensins, lysozyme and RegIIIγ, which contribute to the innate barrier against microbial colonisation; F: Migration of flagellated bacteria is inhibited by secretory immunoglobulin A (IgA), which facilitates the exclusion of bacteria at the epithelium; G: When mucin synthesis and release is impaired, pathobionts may penetrate the mucosal epithelium and trigger the inflammatory cascade. Byproducts of inflammation confer a growth advantage for organisms that obtain energy through anaerobic respiration.

metagenome signatures[64], but more specific signatures have also been reported. Aside from microbial metabolites, MAMPs also stimulate host responses. Consistent with this, metagenome studies have found enrichment of microbial genes that encode cell motility[37] as well as an increase in flagellin proteins[65] associated with the obese state.

In summary, small scale single-cohort, rRNA-based studies of diet-microbiome-host interactions in response to HFD typically identify associations. Cursory comparisons of such studies reveal a confusing picture, however more detailed consideration of common ecological or physiological features reveals common patterns. Microbial structural motifs and metabolites with robust associations to HFD formulations and disease states have been seen and are regarded as the mechanistic links between gut microbiome and systemic complications. It is noteworthy that these MAMPs and microbial metabolites are present in the intestinal lumen but their systemic loads are known to increase during a HFD challenge[4,66-68] and in various aspects of metabolic disorders[4,51,69]. This raises the question of feedback processes that may further shape

microbial community structure and the progression into dysbiosis.

FACTORS THAT SHAPE GUT COMMUNITY DYNAMICS AND FUNCTION

Intrinsic factors

Multiple host mechanisms are involved in restricting microbial growth and activity to the intestinal lumen (Figure 2). These processes may act against the gut microbiome in a generalised manner or target specific bacteria with distinct properties. Host secretions in the gut can function as environmental stressors that regulate bacterial growth. The primary role of bile acids is to facilitate dietary fat absorption but their amphipathic properties also disrupt bacterial membrane integrity and result in antibacterial activity[70]. When rats are fed with diet supplemented with bile acids, their gut communities are characterised by a reduction in *Bacteroidetes* and enrichment in *Clostridia* and *Erysipelotrichi*[71]. Intriguingly, this

compositional change mirrors the patterns reported in HFD studies[24,37,38]. Higher amounts of bile acids are also linked to lower caecal concentrations of butyrate[71], a metabolite produced by subsets of gut bacteria. This finding suggests bile acids either select against the proliferation of butyrate producing bacteria or inhibit the metabolic pathways leading to butyrate synthesis. Collectively, bile acids have a contributing role in determining microbial composition and the products released by the gut microbiome.

At the intestinal interface, host-derived molecules work in synergy to exclude microbial colonisation along the gut epithelium and modulate the microbial composition in the vicinity. Secretory immunoglobulin A (IgA) is known to control bacterial migration patterns by sequestering the movement of motile organisms, thereby preventing their penetration across the gut epithelium[72]. Antimicrobial peptides such as defensins and RegⅢγ also influence microbial composition[73,74]. Mice expressing human α-defensin genes had marked depletion of segmented filamentous bacteria and less interleukin 17-producing T cells in the lamina propria than those with α-defensin deficiency[75]. RegⅢγ, on the other hand, generally selects against Gram positive bacteria, as LPS on Gram negative bacteria inhibit RegⅢγ activity[74,76]. Host secretions can also shape the gut microbiome by providing an ecological niche for specific bacteria. For instance, mucin, a glycosylated protein covering the intestinal epithelium, is a specific growth substrate for many commensal gut microbes, including *Ruminococcus*[77], *Bacteroides*[78] and *Akkermansia*[79]. In the event of gut inflammation, byproducts of immune responses may alter the gut microbiome by favouring the growth of selected organisms. For instance, host cells release reactive oxygen and nitrogen species into the lumen, which react to form nitrate[80-82]. It has been shown that *Escherichia coli* uses exogenous nitrate as electron acceptors for anaerobic respiration, giving it a competitive advantage over fermentative organisms[83].

Host feeding behaviour

While host secretions play an important role in determining the gut community structure, external factors such as host feeding behaviour are equally influential (Figure 2). A main driver of microbial change is the macronutrient intake of the host, in particular the type of carbohydrate ingested[57,84]. Changes in intake are likely to influence the gut microbiota composition or their nutrient acquisition strategies[85]. For instance, experiments in monocolonised mice have found that *Bacteroides thetaiotaomicron* responded to depletion of dietary polysaccharides by upregulating a set of genes adapted to degradation of host mucus glycans[78]. Similarly, *Ruminccoccus gnavus* switches on different sets of carbohydrate-utilising enzymes in response to the availability of carbon sources (monosaccharides *vs* mucin) in the environment[86]. *Escherichia coli* can also adapt to nutrient changes in the environment by altering porin-mediated outer membrane permeability, broadening nutritional acquisition capacity[87], but at the expense of

reduced resistance against bile[88]. Increase in the amount of fermentable polysaccharides changes intestinal transit rate, which modulates the membership of the gut community[89]. Faster transit rate may flush out slow growing organisms and those without the ability to adhere to the mucosal lining of epithelial cells. Altered microbial composition and associated metabolites, in turn, feedback to gut motility[89,90], which strongly influences nutrient absorption in the gut[91,92]. Additionally, high consumption of dietary saturated fat enhances the secretion and taurine conjugation of bile acids[9,93,94], which provides a strong selection pressure on the gut commensals due to its antibacterial activity. However, influx of taurocholic acid presents an additional source of sulphated compounds for bile tolerant, sulphate/sulphite-reducing bacteria (SRBs) to utilise in anaerobic respiration[9], thereby promoting their expansion in the gut community. Changes in diet can alter microbial composition in the matter of days[95,96]. If the altered state persists over time, it will result in a different repertoire of microbial products accumulating in the gut system[97].

HOST-MICROBIOME FEEDBACKS IN METABOLIC DYSFUNCTION AND INFLAMMATION

MAMPs as mechanistic links between gut community and host outcome

A number of pattern recognition receptors (PRRs) on host cells, such as toll-like receptors (TLR4 and TLR5) and nucleotide-binding oligomerisation domain receptors (NOD1 and NOD2) are specialised for detection of MAMPs such as LPS, peptidoglycan (PGN) and flagellin. The structure and/or the extent to which MAMPs are released from bacterial cells can vary between species. Thus modification in community composition, or MAMPs expression, can promote changes in the host system. However MAMPs profile alone cannot determine host outcomes, specific host receptors and loss of gut barrier function are required to potentiate metabolic dysfunction. Localisation and expression of PRRs differ between cell types[98], this may explain the divergent outcomes of each MAMP/PRR interaction.

Flagellin

A wide range of gut bacteria have the capacity to produce flagella, including members of the phyla *Firmicutes*[99] and *Proteobacteria*[72]. Flagellin proteins derived from motile organisms are detected by TLR5, which is selectively expressed at a higher level in the cecum and proximal colon[100]. TLR5 are present on the basolateral surface of intestinal epithelial cells, apical surface of epithelial cells associated lymphoid follicles and mucosal dendritic cells[98,100]. TLR5 detection of flagellin is known to induce the secretion of anti-flagellin IgA, which quenches the motility of various *Proteobacteria* and *Firmicutes* species[72]. This restriction of microbial migration is a normal host

response. When flagellin gains access into the intestinal mucosa, it triggers pro-inflammatory responses and increases the risk of chronic inflammation[101].

Aside from localised responses in the gut, flagellin activation is linked to regulation of physiological processes beyond the gut system. Mice lacking TLR5 had higher food consumption, and developed obesity, dyslipidemia, insulin resistance and hypertension in comparison to wild type (WT)[102]. While some of these phenotypes can be explained by increased dietary intake, food restriction in TLR5 knockout (KO) mice was only effective in preventing obesity but not insulin resistance. Remarkably, antibiotic treatment of TLR5 KO mice normalised food intake and ameliorated metabolic defects, while transplantation of TLR5 KO gut microbiota into WT recipients recapitulated metabolic dysfunction[102]. These results suggest that appropriate flagellin/TLR5 signalling cascade have a beneficial role in host feeding behaviour and thus, promote metabolic health.

Lipopolysaccharides

LPS is a component of the outer membrane of most Gram negative bacteria, including *Bacteroidetes* and *Proteobacteria*. Chemical properties of LPS vary between species, which lead to differential capacity in activating the TLR4 signalling cascade[103]. It is thought that species from *Proteobacteria* exert a stronger immunostimulatory effect than *Bacteroides*[104]. In comparison to TLR5, TLR4 expression in intestinal epithelial cells is relatively low[105] and they are localised in the basolateral compartment[98]. Under normal circumstances, only small amounts of LPS pass through the gut epithelium and reach the bloodstream[4]. Consumption of HFD, however, is associated with reduced expression of tight junction proteins in the gut epithelium[106]. Loss of tight junction integrity increases the paracellular space in the epithelium and facilitates the leakage of luminal contents, including LPS, into adjacent tissues and the circulatory system[106]. Dietary fat is also believed to enhance chylomicron absorption of LPS from the intestinal lumen or enterocytes, which are then exported into the circulatory system[107,108]. Once LPS escapes from the intestinal lumen it can be recognised by cells with TLR4 in the peri-intestinal region or in insulin-targeting tissues, such as adipose tissue, liver, skeletal muscle and pancreas[109]. Activation of TLR4 induces the release of pro-inflammatory cytokines, which drives helper T cell (T$_{Helper}$) expansion and impairs insulin signalling[109,110]. In summary, LPS is an immunostimulatory agent but its exposure to TLR4 expressing cells and the capacity to drive dysbiosis is dependent on physiological properties of the host system such as intestinal permeability.

Physiological consequences of LPS/TLR4 signalling are demonstrated in mice with CD14 or TLR4 deficiencies. During HFD treatment or LPS infusion, both KO mouse models are protected from the hallmark features of metabolic dysfunction observed in the WT counterparts, including obesity, insulin resistance and inflamma-

tion[4,111]. These results indicate that TLR4 agonists, such as LPS, can influence health. Yet, TLR4 is also stimulated by non microbial structures, such as saturated fatty acids[112]. Systemic lipid infusion can trigger the TLR4 inflammatory cascade in adipose tissue and give rise to insulin resistance[113]. One might argue the activation of TLR4 cascade and associated metabolic defects is due to an excess of dietary lipid from HFD, rather than a consequence driven by a microbiota-derived compound. However, detoxification of LPS by intestinal alkaline phosphatase[114], reduced microbial load after antibiotic administration[106,115] or altered microbial profile after prebiotics treatment[61,116] can all lower plasma LPS. All these are thought to be concomitant with improved gut barrier function and/or restoration of metabolic health[106,114-116]. Since broad (antibiotics) and selective (prebiotics) alterations in the gut microbiota lead to improvements of metabolic parameters during HFD, these findings are in agreement that the availability of LPS has a fundamental role in driving metabolic outcomes.

Peptidoglycan

NOD1 and NOD2 are sensors of PGN, but each receptor has a different substrate preference. NOD1 preferentially binds to a structural variant commonly found in Gram negative bacteria[117], while NOD2 detects a common motif of gram positive and gram negative organisms[118]. Similar to TLR4, NOD1 activation is implicated in the development of insulin resistance. Administration of NOD1 agonist to adipocytes upregulates the expression of pro-inflammatory cytokine TNF-α and chemokine MCP-1 in a dose dependent manner, which affects insulin signalling and decreases insulin-mediated glucose uptake[119]. Mice lacking NOD1 are protected from HFD-induced glucose intolerance and translocation of intact Gram negative bacteria from the gut lumen to mesenteric adipose tissue (MAT) and blood, compared to the WT[120]. The authors also demonstrated that bacterial translocation to MAT and the associated inflammation preceded glucose intolerance, suggesting NOD1 interaction with Gram negative gut bacteria drives the pathophysiology associated with HFD.

Apart from NOD1 signalling, NOD2 activation in the skeletal muscle also influences insulin action and glucose homeostasis. Tamrakar *et al*[121] have shown that a NOD2 agonist significantly reduced insulin-stimulated glucose uptake in rat skeletal muscle cell line, whereas NOD1 activation had minimal effect. However, interference with the NOD2 cascade does not necessarily protect the host from dysbiosis. Malfunctions in NOD2 signalling in patients with Crohn's disease or in NOD2 KO mice, are linked to dysregulation of microbial containment, resulting in bacterial translocation to intestinal surface and aberrant stimulation of mucosal immune system[122,123]. Taken together, these findings demonstrate the diverse outcomes of host-microbial immune signalling. The net response is strongly dependent on the target site and is possibly linked to the ratio of Gram negative to

Gram positive organisms as different PGN ligands lead to divergent downstream response.

SCFAs as mechanistic links between gut community and host outcome

SCFAs, such as acetate, propionate and butyrate, are arguably the most influential microbial metabolites in the context of health and disease. Both community composition and the available fermentable substrates influence the net SCFA profile[54,124,125]. As a consequence SCFA profile is an emergent property of the community and it is difficult to predict from taxon-based analysis. The majority of SCFA production is utilised locally by the gut epithelial cells but significant amounts are also transported across the epithelium to distant tissues *via* the circulatory system. Butyrate is metabolised in the gut epithelium and is the key energy source for colonocytes[126]. Propionate and acetate are metabolised as substrates for energy metabolism and lipid synthesis in the liver and other peripheral tissues[127]. Absorption of SCFAs accounts for 6%-9% of the total energy intake for humans and can contribute up to 44% in other animals[128,129]. In addition to their role as an energy substrate, SCFAs are signalling molecules in modulating neuroendocrine and anti-inflammatory responses at various sites.

SCFA signalling: neuroendocrine function and energy regulation

G protein coupled receptors, GPR41 and GPR43, are the primary mediators of SCFA signalling. Butyrate and propionate have high stimulatory effect towards GPR41, while butyrate, propionate and acetate all show similar activity towards GPR43[130]. Evidence from KO models has led to the proposal that SCFA signalling *via* GPRs modulates energy balance, with WT mice having higher fat deposition than GPR41 KO[131]. The GPR41 KO is also characterised by a reduced expression of intestinal peptide YY (PYY), an enteroendocrine L cell hormone that in WT animals inhibits gut motility, potentially increasing the time for energy harvest and absorption[131]. Similarly, GPR43 KO mice are resistant to HFD-induced obesity, insulin insensitivity, and dyslipidemia[132], and there is supporting evidence that acetate and propionate promote adipogenesis through GPR43[133].

Other gut hormones are also influenced by SCFA signals. Glucagon-like peptide 1 (GLP-1) secreted by enteroendocrine cells has a range of effects that encompass promotion of satiety and glucose homeostasis[134], and its release can be stimulated by oral administration of butyrate[135]. Supplementation of butyrate to HFD fed mice reduced food intake and improved glucose control compared to HFD mice without the treatment[135], these phenotypic differences might be driven by differential secretion of GLP-1. Consistent with this observation, mice with impaired GPR43 signalling had reduced GLP-1 secretion, concomitant with glucose intolerance[136]. In adipocytes, SCFA activation of GPR41 induce the expression and production of leptin[137], a hormone that regulates feeding behaviour, metabolic rate and immune response.

Interactions *via* the gut-brain axis are also involved in the coordination of metabolic homeostasis. Propionate produced in the gut can activate GPR41 in the nerve fibres of the portal vein, which resulted in upregulation of genes required in intestinal synthesis of glucose, or intestinal gluconeogenesis (IGN)[138]. The IGN-derived glucose contributes to reduced appetite, improved glucose control and decreased hepatic glucose production, concomitant with lower body weight[138,139]. These emergent outcomes of propionate-induced IGN are mediated by the portal nervous system as denervation can abolish these effects[138,139].

It is evident that SCFA interactions with GPRs and subsequent neuroendocrine signalling affect a wide range of physiological functions, and the emergent outcomes are contingent on the type and location of the receptors as well as the agonists. As a consequence variation in microbial community composition that alters the SCFA profile can drive host responses *via* signalling pathways. The range of pathways triggered is influenced by other factors such as gut barrier function and SCFA translocation that impact which tissues are exposed to SCFA. The host responses, including appetite and intestinal motility, have potential to feedback to gut community composition.

SCFAs and immune regulation

The actions of SCFAs extend beyond energy balance and endocrine function, they are also involved in shaping immune regulation and possibly the progression of autoimmune diseases. In models of colitis, arthritis and asthma, GF mice and CONV GPR43 KO mice showed increased production of inflammatory mediators and enhanced recruitment of immune cells. Notably, exacerbated inflammation in GF mice was attenuated by acetate supplementation, supporting SCFA/GPR43 signalling resolves inflammatory responses[140]. However, other studies have proposed that SCFA mediated GPR43 signalling also has a role in potentiating tissue destruction[141,142].

Despite the competing views on the role of SCFAs/GPR signalling in inflammatory outcomes, SCFAs have emerged as the key microbial signal in modulating the balance of pro-inflammatory T_{Helper} and anti-inflammatory T regulatory cells (T_{Reg}). Atarashi *et al*[143] have shown that SCFA-producing species from *Clostridium* clusters IV and XIVa had greater capacity in expanding the population of colonic T_{Reg} than *Bacteroides fragilis,* which releases polysaccharide A (PSA) to promote immune homeostasis. More importantly, SCFAs on their own can modulate T_{Reg} responses and increase the expression of anti-inflammatory cytokine interleukin-10, which dampens pro-inflammatory responses and reduces the proliferation of effector CD4+ T cells[144]. Diets which promote SCFA production or administration of butyrate alone are able to recapitulate these effects[145,146]. Butyrate can also down regulate the expression of pro-inflammatory mediators in

intestinal macrophages, such as nitric oxide, interleukin-6, and interleukin-12 by histone deacetylase inhibition, a mechanism independent of GPR activation[147].

These host-microbial immune feedbacks in the gut are proposed to have a role in the pathophysiology of autoimmune diseases in genetically susceptible individuals, such as type 1 diabetes (T1D). T1D is characterised by T cell mediated destruction of pancreatic β cells and deficiencies in T$_{Reg}$ numbers or function[148,149]. Given the link between butyrate and T cell homeostasis, gut microbiota might be an environmental risk factor in T1D. High throughput sequencing studies have shown that the T1D gut is depleted in butyrate producing bacteria and a key gene involved in butyrate synthesis[8]. Butyrate depletion is linked to increased intestinal permeability, which precedes the clinical onset of T1D[150,151]. In individuals who are genetically susceptible to T1D, an aberrant gut microbiota with reduced butyrate production is predicted to increase the risk of the following events: increased intestinal permeability, leakage of MAMPs, subclinical intestinal inflammation, homeostatic imbalance of T cells and ultimately autoimmunity in pancreas[152,153].

In conclusion the widespread effects of SCFAs mean that factors altering their concentration and profile have multiple interacting consequences for the host and microbiome. SCFA are primary metabolites of microbial growth. Consequently the SCFA profile of the gut will be especially responsive to diet as changes in microbial nutrient supply can alter both community composition and their metabolic activity. These SCFA changes can lead to changes in gut barrier integrity, energy metabolism and inflammatory responses. All these may impact on host health, but also can feedback to impact microbial community structure. SCFAs are key factors in the interaction between gut microbiome and the host.

Hydrogen sulphide and gut epithelial function

While butyrate fortifies the structural integrity of gut epithelium, other microbial metabolites, such as H$_2$S, are implicated in impaired epithelial function. H$_2$S is produced when sulphated compounds are utilised as terminal electron acceptor in anaerobic respiration. Most gut bacteria with this capability belong to the *Desulfovibrionaceae* family[154]. H$_2$S is known to interfere with energy metabolism in the gut epithelium[155], ultimately leading to cell death, concomitant with gut inflammation[156]. *In vitro* studies of intestinal epithelial cells have demonstrated that H$_2$S influences the expression of genes linked to cell cycle progression and stimulates both inflammatory and DNA repair responses[157,158]. Collectively, there is robust evidence that H$_2$S has deleterious effects on the gut epithelium. A recurrent feature of HFD studies, especially those in which diet formulations have a high proportion of saturated fat, is an increase in *Desulfovibrionaceae* and gut inflammation (Table 1). Again the inferred loss of gut barrier function and associated changes in host-microbiome interaction have the potential to drive feedback responses in the microbial community.

## DIET, PATHOBIONT EXPANSION AND DYSBIOSIS-A MODEL REVISITED

The interplay between diet, gut microbiome and host health has been the subject of numerous studies, and mechanisms that tip homeostasis to dysbiosis are starting to emerge. Nutrient competition is a major driver of community dynamics. Available evidence indicates that access to inorganic electron acceptors such as nitrate and sulphate occupies a special place in determining the outcome of nutrient competition between pathobionts and commensals at the epithelial interface[9,82]. The availability of these is tightly linked to inflammation and cell damage[9,82]. We postulate that microbes whose competitive advantage is dependent on anaerobic respiration adopt a pro-inflammatory life history strategy (which results in increased nitrate) and that their competitors promote mucosal homeostasis (which limits nitrate). Obesity and diet can skew the outcome of these opposing strategies by altering the "tipping point" at which inflammatory processes lead to elevated gut nitrate (Figure 3).

The effect of obesity, or more specifically MAT, is due to their potential to amplify the host response to metabolites that escape the intestine. Adipose tissue macrophages stimulated by MAMPs such as LPS switch to a pro-inflammatory state and increase the production of pro-inflammatory cytokines[159]. Pro-inflammatory cytokines can "escape" from the adipose tissue and promote inflammation and insulin resistance in other tissues[160].

The effects of diet are multiple but can be summarised as driving microbial changes that alter gut barrier function and immune tone. Diets that are depleted in fermentable polysaccharides are associated with lower levels of SCFA production. This state increases the risk of epithelial cell starvation (due to low butyrate levels) and reduces the numbers of T$_{Reg}$ cells. Both host responses have the effect of increasing the potential for inflammation. Epithelial cell starvation and/or inflammation can both increase the availability of inorganic electron acceptors in the lumen that supports expansion of pro-inflammatory pathobionts, many of which are *Proteobacteria*. At this point the potential for positive feedback exists since the LPS of *Proteobacteria* is strongly pro-inflammatory. Diets that are also high in saturated fat exacerbate this basic model. Dietary fat results in increased bile secretion which has been observed to select against key groups of fermentative bacteria. Fat types that specifically promote taurocholate may exacerbate the inflammatory processes since they are strongly linked to expansion of SRBs and production of H$_2$S. Collectively these two aspects of diet composition, levels of fermentable polysaccharide and saturated fat, can operate in synergy to reduce the fitness of bacteria that promote mucosal function *via* butyrate production and enhance the competitiveness of bacteria that drive inflammation *via* LPS.

In this conceptual framework there are two independent host feedback pathways, bile secretion and nitrate production, that facilitate the enrichment of pathobionts
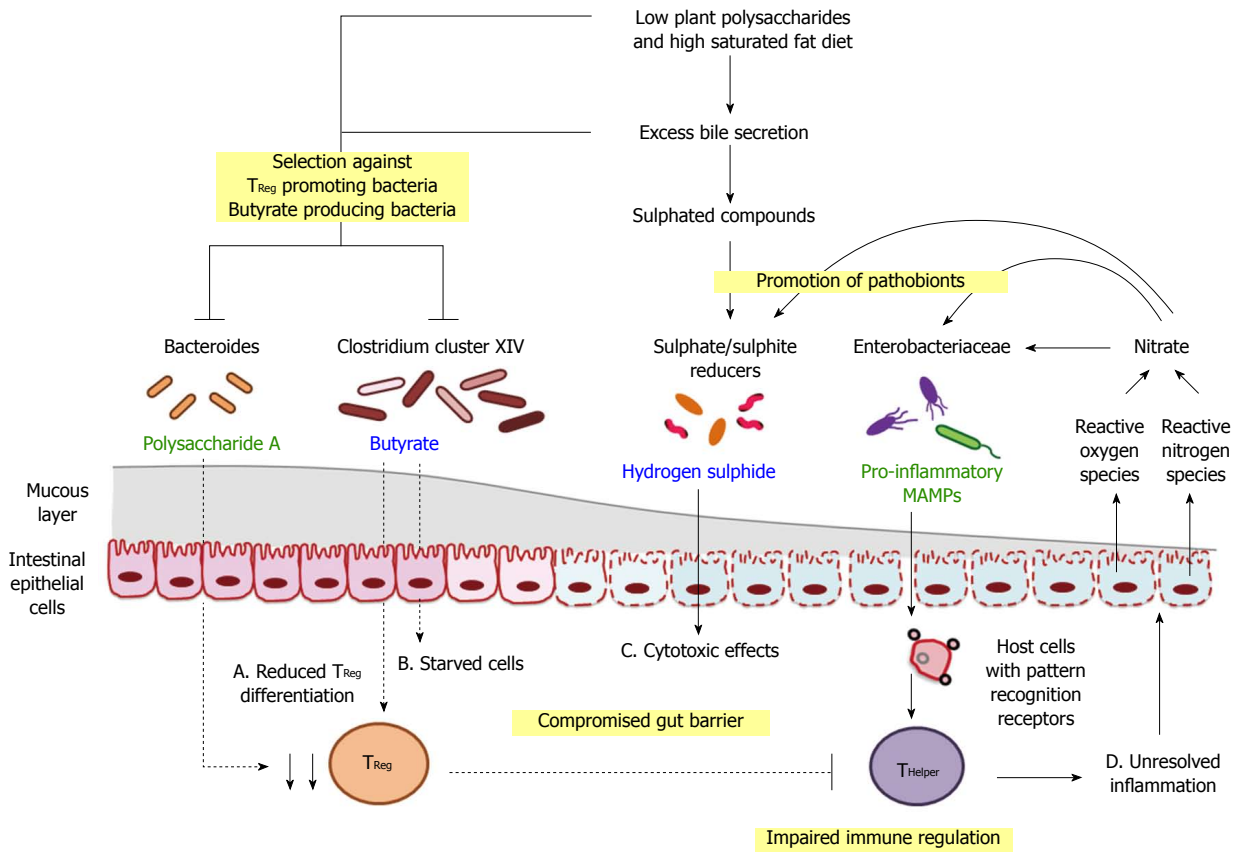
Figure 3 Hypothesised triggers and drivers in diet-induced dysbiosis. Progression from homeostasis to clinical manifestation of metabolic dysfunction may emerge from shifts in microbe-associated molecular patterns (MAMPs; green) and metabolites (blue), initiated by long-term consumption of diets with reduced amount of dietary fibre but high saturated fat. A: Reduction in the availability of fermenting substrates in conjunction with excess secretion of anti-bacterial bile acids can alter the competition dynamics of commensal organisms and pathobionts. Consequent depletion of polysaccharide A and butyrate promotes immune dysfunction by altering the balance of regulatory T cells (T_{Reg}) and helper T cells (T_{Helper}); B and C: Shifts in microbial products contribute to the impairment of gut barrier function and the leakage of MAMPs; D: Dietary factors, microbial signals and host responses act in concert to drive inflammation, which provides a positive feedback pathway in favour of chronic disease development.

and drive pro-inflammatory responses. Host feedbacks to the gut microbiome may be an important determinant in disease progression, which warrants further investigation. Furthermore, there may be more than one type of commensal or pathobiont that influence disease states, especially when alternate microbial groups fulfil similar ecological functions within the gut community. Although *Bilophila* was the leading SRB pathobiont in the initial saturated fat/taurocholic acid/inflammation model[9], the above mechanism is applicable to other SRBs that produce H_2S, such as *Desulfovibrio* in the *Desulfovibrionaceae* family and other representatives within the *Clostridia* class[154,161]. Similarly, several SRBs in the *Desulfovibrionaceae* family and other *Proteobacteria* have the capacity to utilise nitrate[162] and thus, *Enterobacteriaceae* such as *E. coli* may not be the only organisms with increased fitness during inflammation.

FUTURE DIRECTIONS AND CONCLUSION

With many mechanistic links between gut community dynamics and host health are now established, microbiome-based applications for preventing and attenuating the progression of gut-related diseases are emerging. Poten-

tial therapeutic strategies may be in the form of restoring function or blocking feedback at specific nodes of the host-microbial network. If pro-inflammatory tone at the intestinal interface is the predominant driver of disease states, improving T_{Reg} ability to suppress T_{Helper} actions may ameliorate local and systemic complications associated with aberrant immune responses. Prebiotics with fermentable dietary carbohydrates are known to promote the proliferation of organisms that produce butyrate and PSA[163,164]. Stimulation of T_{Reg} differentiation by these beneficial microbial signals may help resolve inflammation.

Aside from rational modifications in diet composition, a change in feeding cycle, *e.g.*, intermittent fasting, has been shown to have metabolic benefits[165]. Since periodic fasting will change nutrient availability to gut microbes and potentially interrupt host feedbacks to the gut microbiome, this may also help reverse dysbiosis. However, these postulated links require further investigations for validation. In conclusion, integration of metagenomics, metabolomics and taxonomic profiling has provided important insights into the functions of gut microbiome and the role of host-microbial crosstalk in dysbiosis. Our emerging understanding of interplay between nutrition,

gut microbial dynamics and host responses will further the development of effective interventions on pathophysiology of lifestyle diseases.

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