The intestinal microbiota fuelling metabolic inflammation

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Abstract | Low-grade inflammation is the hallmark of metabolic disorders such as obesity, type 2 diabetes and nonalcoholic fatty liver disease. Emerging evidence indicates that these disorders are characterized by alterations in the intestinal microbiota composition and its metabolites, which translocate from the gut across a disrupted intestinal barrier to affect various metabolic organs, such as the liver and adipose tissue, thereby contributing to metabolic inflammation. Here, we discuss some of the recently identified mechanisms that showcase the role of the intestinal microbiota and barrier dysfunction in metabolic inflammation. We propose a concept by which the gut microbiota fuels metabolic inflammation and dysregulation.

Chronic low-grade inflammation is a hallmark of many metabolic disorders, such as obesity and its related comorbidities, type 2 diabetes (T2D) and nonalcoholic fatty liver disease (NAFLD), which are rapidly increasing in prevalence worldwide[1–7]. Atherosclerosis is exacerbated in these disorders and is a leading cause of cardiovascular morbidity and mortality. In comparison with acute inflammation, which is usually a physiological response to injury or infection, chronic inflammation in metabolic disorders, termed 'metabolic inflammation', is characterized by low-level local or systemic inflammatory responses. As such, several studies have associated these conditions with increased circulating levels of acute phase proteins (such as C-reactive protein (CRP), fibrinogen, haptoglobin, serum amyloid A, cytokines and chemokines)[2,3,8–11].

NAFLD has recently emerged as the most prevalent chronic liver disorder in developed countries and comprises a broad spectrum of manifestations, ranging from steatosis and its ensuing inflammatory condition termed nonalcoholic steatohepatitis (NASH), to liver cirrhosis and hepatocellular carcinoma. T2D and NAFLD often co-occur, as patients with T2D tend to develop NAFLD and patients with NAFLD are at risk for developing T2D[12]. Similar to T2D, NAFLD is characterized by circulating proinflammatory mediators such as cytokines, acute phase proteins and adhesion molecules, which are present at high levels in patients with NASH[13–18].

Metabolic inflammation is considered sterile, as several non-infectious factors, such as nutrients and especially excess dietary lipid species (BOX 1), emerged as causative factors in experimental and clinical settings[19–21]. This so-called lipotoxicity occurs in organs that are involved in lipid metabolism such as the liver, muscle or adipose tissue. As such, it may not be surprising that key regulators of lipid metabolism and oxidative processes impact metabolic inflammation. For example, unresolved endoplasmic reticulum stress and oxidative stress are key drivers of an inflammatory response in metabolic dysregulation[22]. Similarly, hypoxia in expanding adipose tissue may trigger inflammatory responses[23,24] and suppress the synthesis of anti-inflammatory adipokines (such as adiponectin)[25,26]. It is well established that adipose tissue is an important cytokine sink in human obesity and related disorders[27–30]. Estimates suggest that human visceral and subcutaneous adipose tissues contribute to 15–35% of the body's total circulating IL-6 (REF.[31]). Notably, the degree of adipose tissue inflammation is correlated with the severity of liver inflammation in NAFLD[32]. These studies indicate that metabolic inflammation is a sterile process, fuelled by adipose and liver tissues[4,5].

In contrast to this notion, the gastrointestinal tract harbours a plethora of microorganisms, and specifically bacteria, that are a source of pathogen-associated molecular patterns (PAMPs) and metabolites. PAMPs communicate with the host by eliciting responses involving membrane-bound pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), or cytoplasmic PRRs such as NOD-like receptors or RIG-I-like receptors[33–35]. Healthy subjects are characterized by a diverse composition of the intestinal microbiota and an intact intestinal barrier, which prevents penetration and systemic dissemination of bacteria and their mediators[36–38]. By contrast, obesity and related metabolic disorders, such as T2D and NAFLD, exhibit profound functional and compositional alterations in the gut microbiota, collectively referred to as dysbiosis[39]. Dysbiosis per se does not necessarily result in systemic inflammation; however, it is frequently associated with a bloom of commensals that may become detrimental (so-called pathobionts)[40]. Additionally, obesity, T2D and NAFLD are characterized

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Box 1 | **Strategies to combat metabolic inflammation**

Interventions designed to control metabolic inflammation pose a compelling means to reduce the risk for developing metabolic syndrome. Dietary habits (for instance, composition, quantity and timing) may impact metabolic inflammation. For instance, a high-fat diet promotes metabolic dysfunction in various organs, including the central nervous system, pancreas, liver, muscle, adipose tissue and vasculature[233], and dietary lipids drive a low-grade inflammatory state through upregulation of Toll-like receptor 4-mediated inflammation in human peripheral blood monocytes[19]. Studies in mice have shown that dietary lipids can also drive adipose tissue inflammation and insulin resistance in a microbiome-dependent manner[234]. In contrast, non-digestible carbohydrates (for example, dietary fibre) improve glucose homeostasis by enrichment of short-chain fatty acid-producing bacteria in the gut[63], although a study in humans found that weight reduction and anti-inflammatory effects mediated by dietary fibre was not associated with alterations in the gut microbiome[235]. Similarly, diets restricted in carbohydrates reduced circulating proinflammatory cytokines and improved fat metabolism in humans with obesity and nonalcoholic fatty liver disease [236]; omega-3 fatty acids and nicotinamide supplementation improved metabolic dysregulation in mice[237,238]. Kynurenic acid reduced weight gain and promoted the expression of genes involved in lipid metabolism, thermogenesis and anti-inflammatory immune responses in adipose tissue[239] and palmitic acid hydroxy stearic acids improved insulin sensitivity and reduced adipose tissue inflammation in mice[240].

Experimental evidence also suggests that dietary timing impacts susceptibility to metabolic dysregulation. For example, time-restricted feeding ameliorated obesity and metabolic inflammation in mice[241]. Additionally, exercise was associated with decreased levels of circulating proinflammatory cytokines and a greater gut microbiota diversity in humans[242] and ameliorated metabolic inflammation in mice[243]. Finally, not only lifestyle modification but also bariatric surgery is considered an effective intervention to trigger weight loss and treat metabolic disorders in severely obese patients. The effect achieved by surgery is partially mediated by reduction in calorie intake but may also be modulated by the intestinal microbiome[244], bile acid metabolism[245] and metabolic inflammation[246,247]. A causal link between post-bariatric surgery microbiome alterations and attenuation of metabolic inflammation has been recently drawn in humans. Obese male human recipients with insulin resistance transplanted with faecal microbiota from human donors who underwent Roux-en-Y gastric bypass showed a decrease in insulin sensitivity and adipose inflammatory markers compared with recipients transplanted with microbiota from individuals with the metabolic syndrome[248].

by an impaired and defective intestinal barrier[41–43]. As a result of dysbiosis and intestinal barrier breach, it is perceived that the microbiota or its components (such as endotoxins) translocate into the circulation and instigate low-grade inflammation. In this Review, we examine the impact of the intestinal microbiota and its metabolites on intestinal permeability, metabolic inflammation and dysfunction and discuss how these insights can improve our understanding of human metabolic diseases.

The microbiota in metabolic disease

Dysbiosis in obesity. The advent of multi-omic sequencing in the past decade has allowed researchers to investigate the complexity of the intestinal microbiota in various human disorders[44–46]. Evidence for a role of the intestinal microbiota on host metabolism came from studies in germ-free mice. Conventionally raised mice had significantly more total body fat than mice raised under germ-free conditions[47]. Conventionalization of these germ-free mice with a caecum-derived microbial community resulted in a marked increase in total body fat content[47]. Earlier observations had shown that colonization of germ-free mice profoundly affected the transcription of various factors in the intestinal tract regulating nutrient absorption, mucosal barrier function and metabolic functions[48]. Pioneering studies in humans described a distinct gut microbiome signature associated with obese states[49]. Other studies found that obesity, the propensity to gain more weight, dyslipidaemia, insulin resistance and low-grade inflammation were more prevalent in subjects exhibiting low gut bacterial richness[50,51]. Additionally, it was suggested that certain 'proinflammatory' bacterial strains, such as *Ruminococcus gnavus* or *Bacteroides* species, might dominate in obesity and 'anti-inflammatory' strains, such as *Faecalibacterium prausnitzii*, are less prevalent[52]. A certain intestinal microbiome signature may also contribute to weight regain in obese mice after successful dieting[53]. Collectively, these studies suggest the existence of a gut microbial signature in obesity.

Dysbiosis in type 2 diabetes. Several landmark studies from the past several years have reported a microbiome signature in T2D and NAFLD[54–56] (TABLE 1). Intestinal dysbiosis in T2D was characterized by a decrease in *Roseburia intestinalis* and *F. prausnitzii*[54]. The altered microbiome was enriched in membrane transport of sugars, branched-chain amino acid transport and decreased butyrate biosynthesis. Enriched microbial genes mapped to oxidative stress signalling, suggesting a direct link between an altered microbiota composition and the inflammatory state in patients with T2D. European women with T2D demonstrated increased abundance of Lactobacillales (including *Lactobacillus gasseri* and *Streptococcus mutans*) and alterations in Clostridiales abundance (including *Clostridium clostridioforme* and other *Clostridium* species). Moreover, *R. intestinalis* and *F. prausnitzii*, both typical butyrate producers, were highly discriminant for T2D[55]. Indeed, transfer of the intestinal microbiota (and its metabolites) from lean donors to patients with metabolic syndrome ameliorated insulin resistance[57]. A recent report also showed that individuals with prediabetes exhibited an aberrant intestinal microbiota composition accompanied by decreased abundance of *Akkermansia muciniphila*[58]. Several other studies have provided evidence for a gut microbiome signature in T2D or linked microbial functional alterations with features of T2D. For example, Pedersen et al. found increased levels of branched-chain amino acids in insulin resistance[59]. Notably, *Prevotella copri* and *Bacteroides vulgatus*, two bacterial strains that are enriched in subjects with T2D, were able to promote insulin resistance and increased levels of branched-chain amino acids in rodents[59]. Mice with diet-induced obesity and treated with *Ralstonia pickettii* exhibited increased insulin resistance compared with control animals, suggesting a role for *R. pickettii* in T2D[60]. A potentially detrimental (proinflammatory) bacterial strain is *Bilophila wadsworthia*, which worsened high-fat diet (HFD)-induced metabolic dysfunction and hepatic steatosis in mice[40,61]. Both pharmacological suppression of *B. wadsworthia* and treatment with *Lactobacillus rhamnosus* CNCM I-3690 improved metabolic inflammation, glycaemic control and intestinal barrier integrity. Similarly, certain bacterial strains, such as *Eubacterium hallii*, can ameliorate insulin resistance in rodents[62]. *E. hallii* treatment improved energy expenditure, increased faecal concentrations of butyrate and affected bile acid metabolism. Zhao et al. recently

Table 1 | **Intestinal dysbiosis in type 2 diabetes and nonalcoholic fatty liver disease**

Change	Type 2 diabetes[a]	Nonalcoholic fatty liver disease[a]	Refs
Dysbiosis			
Bacteroidales	↑ *Bacteroides* spp.	↑ *Bacteroides vulgatus*[b]	54,56
	↑ *Alistipes*	↓ *Alistipes putredinis*[b]	54,56
	↑ *Parabacteroides*	–	54
Firmicutes	↓↑ Clostridiales	–	54,55
	↑ *Clostridium* spp.	↓ *Ruminococcus obeum*[b]	54,56
	↓ *Eubacterium rectale*	↑↓ *Eubacterium rectale*[b]	54,56
	↓ *Faecalibacterium prausnitzii*	–	54
	↓ *Roseburia* spp.	–	54
	↑ *Lactobacillus gasseri*	–	55
	↑ *Streptococcus mutans*	–	55
Proteobacteria	↑ *Escherichia coli*	↑ *Escherichia coli*[b]	54,56
Verrucomicrobia	↓↑ *Akkermansia muciniphila*	–	54,264
Associated altered microbial functions			
	Sugar membrane transport, branched-chain amino acid transport, butyrate production	Amino acid metabolism, butyrate metabolism	54,56
Impact on experimental metabolic disease			
Detrimental	*Bacteroides vulgatus*, *Prevotella copri*, *Bilophila wadsworthia*, *Ralstonia pickettii*	–	59–61
Beneficial	*Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Eubacterium hallii*, *Akkermansia muciniphila*	VSL#3 (*Lactobacilli*, *Streptococcus thermophilus*, *Bifidobacterium*)	61,62,76,145,264

[a]For gut microbiome changes in obesity, we refer to recent reviews[265,266]. [b]Examples of a consortium comprising 37 species.

observed that certain dietary fibres promoted the synthesis of short-chain fatty acids (SCFAs) by manipulating the gut microbiota[63]. Overgrowth of SCFA-producing bacteria directly correlated with improvements in glycaemic control (mirrored by decreased glycated haemoglobin levels), partly through upregulation of glucagon-like peptide 1 (GLP1). Interestingly, levels of potentially detrimental metabolites, such as indole or hydrogen sulfide, were reduced by dietary fibres. An analysis of eight studies supported the notion that dietary interventions in patients with T2D altered the gut microbiota and ameliorated metabolic dysfunction[64]. Notably, dietary factors that drive metabolic dysregulation may also have a strong effect on the gut microbiota[65], a topic that is beyond the scope of this Review.

Dysbiosis in NAFLD. Increasing evidence indicates that the gut microbiota is involved in the pathogenesis of NAFLD[66–69]. Recent human studies suggest an intestinal microbiome signature in NAFLD, with the dominance of certain phyla such as Proteobacteria[70]. Furthermore, the faecal microbiome composition reflects the degree of fibrosis in patients with NAFLD[56]. Advanced fibrosis was associated with an increased abundance of Proteobacteria and *E. coli* and a decrease in Firmicutes, allowing microbiome profiling to accurately diagnose advanced fibrosis in NAFLD. Moreover, the faecal microbiome of 2-week-old infants born to obese mothers promoted the development of experimental NAFLD when transplanted in germ-free mice[71]. These data indicate that the intestinal microbiota impacts the susceptibility for NAFLD. Interestingly, in more advanced liver diseases, more than 50% of the species detected in gut microbiome samples were of buccal origin, suggesting that the oral microbiota could contribute to disease progression when present in the lower gastrointestinal tract[72]. Concordantly, ectopic colonization of oral bacteria in the gut has been shown to drive T helper 1 (T_H1) cell induction and inflammation in *Il10*$^{-/-}$ mice[73].

Similar to these important observations in human NAFLD, microbial alterations have been linked to experimental NAFLD[47,74]; more than 20 years ago, dietary prebiotics were shown to improve experimental hepatic steatosis[75]. Furthermore, a 4-week therapy with VSL#3, a probiotic mixture comprising *Streptococcus thermophilus* and several strains within the *Lactobacillus* and *Bifidobacterium* genera, was as effective as therapy targeting tumour necrosis factor (TNF) in decreasing liver steatosis in *ob/ob* mice[76].

Collectively, these studies support the notion that obesity, T2D and NAFLD exhibit distinct gut microbiome signatures, characterized by a bloom of potentially harmful taxa and suppression of beneficial taxa. However, it must be acknowledged that the behaviour of certain bacteria may be context specific and determined by the integrated signature of host-related and microbiome-related factors that define their composite role as pathobionts or commensals[77].

Barrier dysfunction in metabolic disease

The interface partitioning the gastrointestinal microbiome from the underlying host tissue is a pivotal signalling hub between these two compartments, and breach of this interface has been linked to detrimental metabolic consequences. Pioneering work demonstrated that feeding mice a HFD modified the gut microbiome and triggered an influx of bacteria-derived lipopolysaccharides (LPS) into the systemic circulation (a state termed 'metabolic endotoxaemia'), which may contribute to an increased inflammatory tone, obesity and diabetes[78]. This observation has been replicated in work by others showing increased permeability across the gut barrier in the jejunum and colon of a mouse model for obesity[79] and in the small intestine of a rat model for NASH[79,80]. In humans with T2D, increased intestinal permeability was suggested more than three decades ago[43] and ever since has been described along with leakage of bacteria or bacterial products across the intestinal barrier during consumption of a HFD[81]. However, gut barrier dysfunction has been inconsistently described in other manifestations of the metabolic syndrome such as obesity[82–85] and NASH[86–91]. These discrepancies may derive from methodological issues, such as the diversity of means to assess gut permeability in humans, ranging from morphological analyses using histology

ob/ob mice
A mouse model of metabolic dysregulation and obesity that arises from increased appetite due to a leptin mutation (that renders these mice functionally leptin deficient).

Metabolic endotoxaemia
A state that favours the translocation of microbial components (such as lipopolysaccharide) to the bloodstream, which promotes metabolic disease.

and electron microscopy, to functional analyses such as enteral administration of non-digestible markers or measurement of translocated microbial PAMPs. Results may be altered based on the part of the gastrointestinal tract investigated and the molecular size of the administered marker. Unlike animal models, human studies are particularly prone to environmental and host bias; individuals may differ in the capacity of endotoxin production by their gut microbiome consortia, or in factors affecting absorption (such as dietary intake, gut motility, distribution of the markers in the body, use of interacting drugs and kidney function), which may reduce or confound statistical trends and complicate their interpretation (see below).

Over the years, research has elucidated the role of the host–microbiome interface and identified that intestinal epithelial cells constitute not only a physical barrier but also harbour immunological properties[92]. In this section, we describe mucosal mechanisms that orchestrate the host–microorganism crosstalk, disruption of which can instigate metabolic derangements. We discuss interventions that restore intestinal barrier integrity as novel therapeutic approaches against metabolic syndrome.

Molecular immune mechanisms of intestinal barrier disruption. Segregation between the host gut epithelium and the microbiome at homeostasis is maintained by various mechanisms that favour a tolerogenic immune response. In the small intestine, this is achieved primarily by PRRs, antimicrobial peptides and secreted IgA, and an immune milieu consisting of a cytokine environment that includes IL-33, IL-10 and transforming growth factor-β (TGFβ) as well as cells such as $CD103^+$ dendritic cells and regulatory T cells. In the large intestine, compartmentalization also relies on a thick continuous mucus layer[93,94]. HFD feeding and obesity bring about anatomical and functional alterations to the intestinal barrier. Anatomically, humans with obesity exhibit jejunal villus hyperplasia and an increased surface area of exchange with the luminal content, which might be a trigger for changes in the immune compartment. In mice, proinflammatory changes have been observed during HFD feeding, starting in the colon with an increase in IL-17-producing γδ T cells and a concomitant reduction in regulatory T cells[95], and continuing with an increase in interferon-γ (IFNγ)-producing T_H1 cells and $CD8^+$ T cells along with a concomitant reduction in regulatory T cells in both the colon and the small intestine. Another study suggested a microbiome-mediated decrease in T_H17 cells in the ileal lamina propria of mice consuming a HFD at the onset of metabolic disease[96]. Additionally, HFD exposure resulted in a depletion of eosinophils in the small intestine, which may drive increased paracellular permeability[97]. Likewise, in humans with obesity, $CD8^+$ T cells shifted from the lamina propria to the epithelium in the jejunum[98]. These immune alterations coincided with a proinflammatory intestinal cytokine environment in both mice[95,96] and humans[98], which was suggested to reduce intestinal insulin signalling in enterocytes in vitro[98]. Attenuation of this proinflammatory state, either by genetic manipulation or by exogenous administration of an anti-inflammatory agent, led to enhanced intestinal barrier function in mouse models for HFD-induced obesity[95,99]. The cytokine landscape in mice in obesity and insulin resistance is summarized by biogeographical location in a recent review[100].

Containment of the commensal microbiome by the intestinal immune system is crucial to preventing chronic systemic inflammation. Research in mice showed that, in homeostasis, mucosa-associated gut commensals sensed by the Mincle–SYK signalling pathway in small intestinal dendritic cells in Peyer's patches induce IL-6 and IL-23p19 production, which in turn stimulate intestinal T cells and innate lymphoid cells (ILCs) to secrete IL-17 and IL-22 (REF.[101]). Small-intestinal mucosal immune cells (such as RORγt-dependent T_H17 cells and ILC3s) contribute to the induction of antimicrobial peptides (such as REG3γ) and IgA to prevent the translocation of commensal bacteria and systemic inflammation associated with chronic diseases[102–104]. Specifically, $ROR\gamma t^+$ ILCs express membrane-bound lymphotoxin to regulate IgA production by dendritic cell-derived inducible nitric oxide synthase (iNOS; also known as NOS2) induction, and soluble lymphotoxin to regulate IgA production by controlling T cell homing to the small intestinal lamina propria[103]. Interestingly, mice deficient in lymphotoxin genes and fed a HFD were protected from diet-induced obesity due to an altered microbiome composition, which possibly involved segmented filamentous bacteria[105,106]. The contribution of antimicrobial peptides to large intestinal barrier integrity has been recently illuminated in transgenic mice overexpressing the human antimicrobial peptide REG3A in hepatocytes[107]. These mice showed a distinct microbiome composition, which protected them against chemically induced colitis, a trait that was transferrable to wild-type mice by co-housing or by faecal microbiota transplantation. Whether antimicrobial peptide-mediated barrier augmentation may also ameliorate metabolic inflammation merits further studies.

Mice harbouring a genetic aberration in the Mincle–SYK pathway exhibited liver inflammation and impaired lipid metabolism — that is, increased expression of lipogenic genes and accumulation of diacylglycerides and fatty acids[101]. Furthermore, IL-22 and its upstream inducer IL-23 regulate the production of antimicrobial peptides and thereby repress semi-invasive proatherogenic microbiome members, which produce LPS and components of the trimethylamine *N*-oxide (TMAO) biosynthesis pathway in atherosclerosis-prone mice[108]. Interestingly, a similar mechanism was described to protect against intestinal damage and inflammation[109–111]. Of note, a breach in the intestinal barrier by the microbiome has been previously associated with the induction of commensal-specific IgG antibodies, which implies a systemic immune response in metabolic dysbiosis, and indeed circulating IgG against commensals has been reported in humans with obesity and diabetes and in HFD-fed mice[112]. Collectively, this host–microbiome reciprocal signalling circuit serves as a rheostat for immune tuning and microbiome containment, which is pivotal in damping metabolic endotoxaemia.

Host–microbiome interactions are perturbed upon HFD feeding and obesity, and their metabolic aftermath can be recapitulated or corrected in genetically

manipulated mice. For example, mice fed a HFD showed a microbiome-mediated decrease in T_H17 cells in the ileal lamina propria[96] and obese mice showed reduced IL-22 production from colonic ILCs upon immune stimulation[113]. IL-22R1-deficient mice showed increased weight gain and developed glucose intolerance and insulin resistance, which was remediable by IL-22 supplementation. This beneficial effect was mediated, in part, by the reduction of endotoxaemia, and not by microbiome alterations[113]. Additionally, obese mice exhibited increased activity of intestinal and colonic indoleamine 2,3-dioxygenase, which shifted tryptophan metabolism from indole derivative and IL-22 production to kynurenine production, a finding that was also suggested in humans[85]. Mice with genetic deletion of this enzyme maintained gut mucosal barrier integrity, exhibited decreased endotoxaemia, increased insulin sensitivity and regulated hepatic lipid metabolism. This beneficial metabolic phenotype could be transferred between mice by co-housing or by faecal microbiota transplantation[85]. Of note, caution should be practised when attempting to translate these findings from the intestinal niche to other metabolic organs, and from mice to humans.

As opposed to the favourable metabolic effects observed in the mouse gut, T_H17 and T_H22 cells were elevated in adipose tissue of humans with obesity or diabetes[114,115], and IL-17 plasma levels were elevated in women with obesity[116]. IL-17 has been associated with NAFLD progression[117] but also with protection against diet-induced obesity in mice[118]. Additionally, IL-22 was shown to participate in obesity-induced adipose tissue inflammation through IL-1β induction[114] and to instigate insulin resistance in the liver and muscle in vitro[115]. Further studies are warranted to elucidate the role of IL-22 in metabolism and immunity in humans.

Other causative links between the intestinal immune system, the gut microbiome and host metabolism were elucidated by genetic deletion of PRRs in mouse models of the metabolic syndrome. MyD88, an adaptor molecule involved in the activation of most TLRs, has been suggested to have a role in metabolic disturbances, although its effect is ambivalent. Conventional *Myd88*-knockout mice showed increased fat mass and body weight, and exacerbated glucose intolerance and insulin resistance, concurrent with bacterial translocation to mesenteric adipose tissue[119]. However, mice harbouring an inducible deletion of *Myd88* in epithelial cells exhibited enhanced gut barrier integrity, augmented antimicrobial peptide expression and increased abundance of intestinal regulatory T cells, and they were protected against dietary-induced obesity, diabetes and hepatic steatosis[120]. Conversely, mice deficient in TLR5 developed dysbiosis, which prompted low-grade inflammation, impaired insulin signalling and hyperphagia (excessive eating), and other aspects of the metabolic syndrome[121]. Mice deficient in components of the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome exhibited severe NASH following a methionine-choline-deficient diet, which was mediated by dysbiosis[74]; however, other studies showed that NLRP3 inflammasome activation impaired insulin signalling[122], and its inhibition resulted in protection against obesity-induced insulin resistance[123]. The NLRP6 inflammasome was shown to interact with microbiome-associated metabolites to govern the intestinal antimicrobial peptide landscape and prevent dysbiosis and ensuing features of the metabolic syndrome[124,125]. Mice deficient in the PRR nucleotide oligomerization domain-containing protein 1 (NOD1) or CD14 (a co-receptor for the TLR that senses LPS) and fed a HFD were protected from systemic bacterial translocation and the development of glucose intolerance and fat mass gain[119]. However, HFD-consuming NOD2-deficient mice failed to demonstrate the same protective effect[119] and even showed increased obesity, liver inflammation and exacerbated insulin resistance[126]. Metabolic dysregulation was transferrable to germ-free mice through faecal microbiota transplantation, demonstrating a mechanistic role for PRRs that sense the intestinal microbiota and dictate metabolic phenotypes. Similarly, mice deficient in the channel protein ORAI1, which exhibit impaired pancreatic antimicrobial peptide secretion, showed bacterial overgrowth, dysbiosis and increased intestinal permeability, leading to bacterial translocation and mortality[127].

In light of the mucosal proinflammatory milieu in metabolic disorders, a compelling therapeutic approach would be to target and suppress immune pathways. For example, treatment with the anti-inflammatory drug 5-aminosalicylic acid in mice reversed bowel inflammation, reduced gut permeability and endotoxaemia, and ameliorated fasting glucose, fasting insulin and insulin resistance[95].

Factors regulating intestinal barrier integrity and potential therapeutic targets. Dietary composition, and specifically nutrition rich in fat, can impact intestinal permeability, as a HFD promoted intestinal inflammation, which preceded the onset of weight gain in mice[96,128]. It has been shown, in vitro and in mouse models, that in the presence of dietary lipids LPS can be transported through the epithelial barrier via the transcellular route by chylomicrons (rather than the conventional paracellular route)[129,130]. Correspondingly, a study in patients with severe obesity showed that jejunal permeability increased compared with healthy controls upon a lipid challenge[84], and another study found a correlation between jejunal epithelial T cell densities and an unbalanced diet (reduced carbohydrate in lipid-rich diet) in humans with obesity[98], providing a potential explanation to the discrepancies in results observed in human trials and suggesting that factors other than obesity per se mediate intestinal barrier breach in humans. Similarly, mice with hyperglycaemia exhibited increased colonic (and possibly also small intestinal) permeability[131], and mice fed diets rich in fructose, fat or both exhibited impaired duodenal barrier integrity, endotoxaemia and NAFLD[132]. Dietary supplements, such as emulsifiers, may also disrupt the gut barrier integrity by promoting gut microbial dysbiosis and allowing for bacterial encroachment, thereby inducing low-grade inflammation and resulting in features of the metabolic syndrome in mice[133]. The timing and quantity of food also dictate plasma LPS levels, as HFD-fed mice showed diurnal variations in endotoxaemia, which peaked towards the end of the dark phase[78], and short-term starvation attenuated intestinal barrier leakage in

Chylomicrons
Transport vesicles (so-called lipoprotein particles) for absorbed dietary lipids.

humans[134]. Other intraluminal mechanisms accounting for intestinal barrier breach include increased bile acid concentrations (demonstrated in vitro)[135] and the presence of osmotic diarrhoea (demonstrated in mice), which decreases mucus thickness and thus exposes the epithelium to direct contact with bacteria[136].

The gut microbiome is a key player in intestinal barrier disruption, as its absence in antibiotic-treated mice protects against HFD-induced intestinal permeability and its metabolic consequences[131,137]. Some dietary regimens drive microbiome shifts, which in turn contribute to barrier breach in various ways. Mice fed a HFD show an increased proportion of LPS-containing caecal microbiome[78], and mice chronically or intermittently fed a fibre-deprived diet exhibit dysbiosis that favours the use of the colonic mucus as a nutrient source, thus exacerbating pathogen infection[138] and increasing mucus layer permeability, remediable with microbiome transplantation from chow-fed mice[139].

Microbiome-related strategies to ameliorate intestinal barrier permeability involve the administration of microbiome-altering compounds (prebiotics), supplementation of bacteria known to confer beneficial roles on the gut barrier (probiotics) or treatment with bacteria-derived by-products (postbiotics). A plausible prebiotic therapy is the administration of the alkaloid berberine, which ameliorated intestinal barrier function, either directly or by modification of the gut microbiome, attenuating endotoxaemia and improving weight gain, dyslipidaemia and NASH in HFD-fed rats[140]. Oligofructose supplementation to obese mice ameliorated gut barrier integrity and improved systemic and hepatic inflammation, possibly through a GLP2-dependent mechanism[141]. Likewise, curcumin (a substance in turmeric) or non-absorbable antibiotics improved intestinal barrier function and attenuated endotoxaemia, and thereby reduced atherosclerosis and glucose intolerance in HFD-consuming low-density lipoprotein receptor-deficient mice[142].

Several probiotic therapies have been proved effective in reducing intestinal permeability in animal models. *A. muciniphila* supplementation was suggested to augment mucus thickness and tight junction expression and protect against gut leakiness in a mouse model for alcoholic liver disease[143]. Concordantly, the same bacterium, or a specific protein isolated from its outer membrane, enhanced gut barrier integrity, decreased body weight and fat-mass gain, attenuated dyslipidaemia and improved glucose tolerance in HFD-consuming mice[144]. *Lactobacillus rhamnosus* GG supernatant prevented alcohol-induced intestinal leakage, endotoxaemia and liver injury in mice[145]. *Lactobacillus reuteri* improved the features of metabolic syndrome by production of aryl hydrocarbon receptor ligands in mice[146]. *Bifidobacterium longum* NCC 2705 and inulin supplementation to mice consuming a fibre-depleted diet ameliorated mucus growth and reduced mucus permeability, respectively, although there was no improvement in metabolic parameters[139]. *Parabacteroides distasonis* elevated lithocholic acid and ursodeoxycholic acid (UDCA) in the bile of HFD-fed mice and *ob/ob* mice and thereby upregulated ileal tight junction protein expression and reduced endotoxaemia, which in turn decreased weight gain, hyperglycaemia and hepatic steatosis compared with untreated mice[147]. Finally, in humans, treatment of obesity with *Bifidobacterium adolescentis* IVS-1, *Bifidobacterium lactis* BB-12 or the prebiotic galacto-oligosaccharides resulted in improved colonic permeability, although no changes in markers of endotoxaemia ensued[148].

An alternative postbiotic approach is the direct administration of bile acids shown to have a beneficial metabolic effect on the host. Cholic acid ameliorates insulin sensitivity and reduces obesity in mice through the activation of G protein-coupled bile acid receptor TGR5 and downstream NLRP3 inhibition[149]. Similarly, UDCA administration to mice stimulated epithelial cell migration and was protective against intestinal injury[150], and a mixture of UDCA and lithocholic acid increased gut barrier integrity and subsequently improved hyperlipidaemia and hepatic steatosis[147]. Semisynthetic farnesoid X receptor agonists proved to be effective in attenuating epithelial permeability in chemically induced colitis in mice; however, whether such effects contribute to metabolic syndrome mitigation merits further studies[151]. Of note, bile salt-based therapies should be adjusted from mice to humans, as the composition of bile and the metabolism of bile salts differ between organisms[152]. Other postbiotic therapies include aryl hydrocarbon receptor ligands, which alleviated intestinal barrier dysfunction in HFD-fed mice and in vitro[146].

Together, animal research on prebiotic, probiotic and postbiotic therapies holds promise in enhancing gut barrier integrity to combat metabolic inflammation; however, evidence in humans is still sparse and insufficient. Future studies should focus on devising therapeutic strategies that overcome the current shortcomings in this field (see, for instance, REF.[153]).

Gut permeability is enhanced in T2D by other mechanisms that do not necessarily involve the microbiome. For example, hyperglycaemia, either genetically, chemically or diet induced, was shown to compromise intestinal barrier integrity in mice through GLUT2-dependent reprogramming of the intestinal epithelial cell transcriptome and disruption of tight and adherence junctions[131]. Additionally, fatty acid synthase, an enzyme responsible for mucus secretion, was reduced in the colonic epithelium of diabetic mice manifesting as a diminished inner mucus layer, the presence of bacteria in the epithelium and systemic endotoxaemia, which was corrected with insulin[154].

Another pathway modulating gut permeability pertains to the endocannabinoid system, as blockade of cannabinoid receptor 1 modulated tight junction protein localization, reduced endotoxaemia and consequently decreased obesity and glycaemia in mice. Concordantly, administration of a cannabinoid receptor agonist worsened endotoxaemia[155].

In summary, the host–microbiome interface transduces aberrant gut signals derived from the diet or dysbiotic bacterial consortia to local immune responses, resulting in barrier breach, and as such it may be regarded as another hit of metabolic inflammation (FIG. 1). This barrier disruption may contribute, in turn, to systemic chronic inflammation and end-organ dysfunction, leading to metabolic disease.

Berberine
A plant-derived alkaloid of an ancient Chinese herb, *Coptis chinensis*.

Low-density lipoprotein receptor-deficient mice
A mouse model of atherosclerosis caused by a targeted deletion of the gene encoding the low-density lipoprotein receptor (LDLR). In humans, homozygous mutations in *LDLR* cause familial hypercholesterolaemia, a disease characterized by pronounced hyperlipidaemia and accelerated atherosclerotic cardiovascular disease.

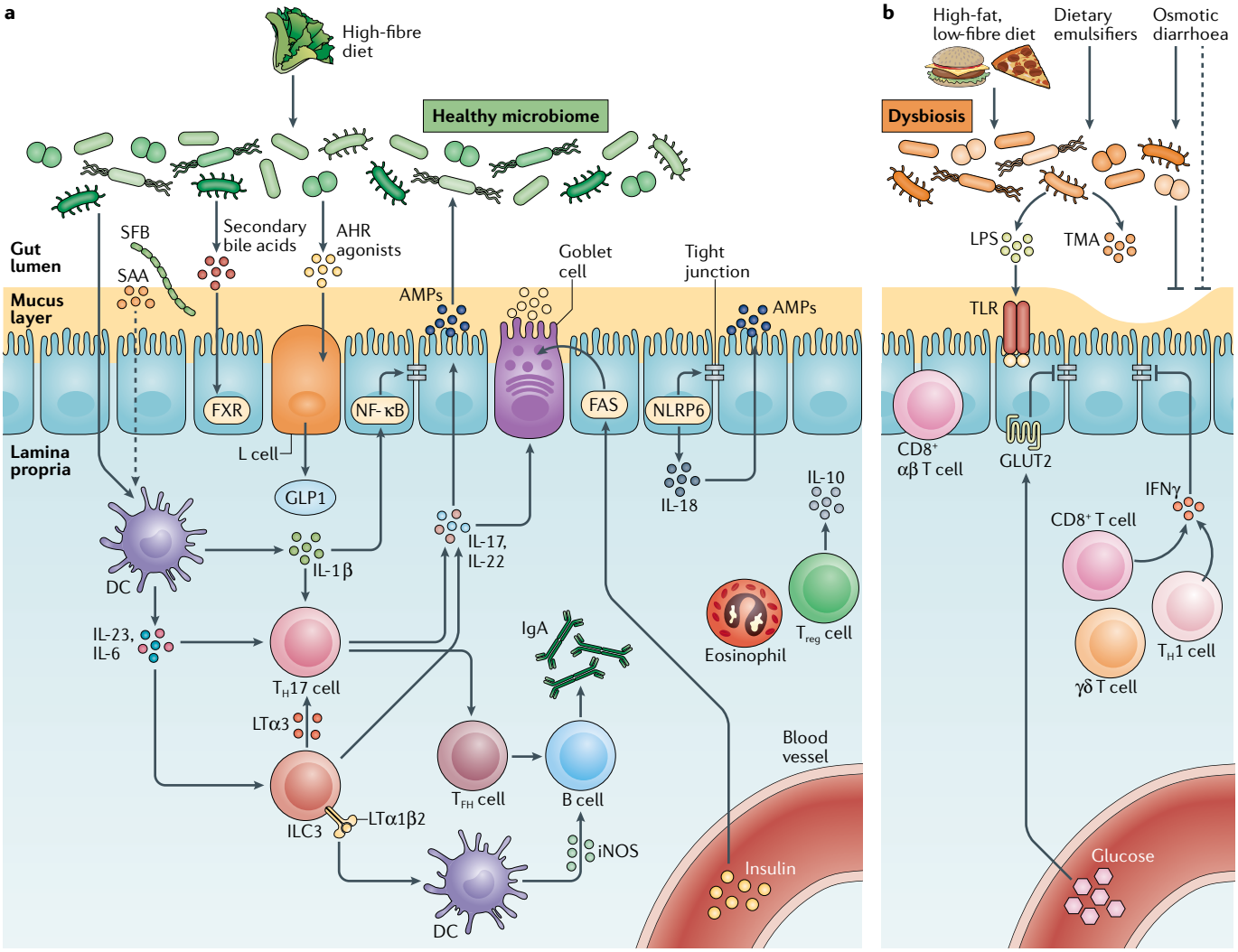

Fig. 1 | **Gut barrier breach in metabolic diseases in mouse models. a** | The integrity of the small intestinal epithelium is maintained by a variety of host exogenous and endogenous factors. Exogenous factors include the gut microbiome and compounds such as intraluminal dietary constituents and microbiome-derived metabolites. In a metabolically healthy state (achieved by a high-fibre diet), the gut microbiome mediates intestinal integrity through direct mechanisms, such as sampling of microbial antigens from the intestinal lumen by dendritic cells (DCs) and triggering RORγt-dependent cell activation and differentiation, leading to secretion of mucus, antimicrobial peptides (AMPs) and IgA. Alternatively, sensing of microbial metabolites by inflammasomes also leads to AMP secretion. These events, and a tolerogenic immune millieu, promote homeostasis with the microbiota. In addition, the microbiome maintains intestinal barrier function through indirect cues such as signalling via secondary bile acids or aryl hydrocarbon receptor (AHR) agonists. Host endogenous factors also determine gut barrier integrity, including blood insulinaemia, which regulates mucus secretion via fatty acid synthase (FAS). **b** | Dysbiosis induced by dietary, mechanical or genetic factors may result in metabolic derangements through increased lipopolysaccharide (LPS) expression, driving Toll-like receptor (TLR) signalling and endotoxaemia by mucus layer degradation or by production of proatherogenic compounds. These events are accompanied by alterations in intestinal lamina propria cell populations and the cytokine landscape. These proinflammatory changes associated with intestinal barrier disruption contribute to metabolic inflammation. Finally, host blood glycaemia induces barrier dysfunction through glucose transporter 2 (GLUT2). FXR, farnesoid X receptor; GLP, glucagon-like peptide; IFNγ, interferon-γ; ILC3, type 3 innate lymphoid cell; iNOS, inducible nitric oxide synthase; LT, lymphotoxin; NF-κB, nuclear factor-κB; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; SAA, serum amyloid A; SFB, segmented filamentous bacteria; T_{FH} cell, T follicular helper cell; T_H cell, T helper cell; TMA, trimethylamine; T_{reg} cell, regulatory T cell.

Systemic microbiota and its metabolites

The liver is a critical gatekeeper of blood flow from the portal vein draining the intestine. Even in health, the liver is constantly challenged by metabolic stress deriving from intestinal bacteria and their metabolites. An intact intestinal epithelial barrier might protect the liver from overwhelming bacterial exposure. Whether hepatic diseases such as steatosis in NAFLD and T2D affect the liver's capacity to detect and respond to bacteria and their derived compounds is currently unknown[156]. However, increased circulating levels of endotoxins in obesity, T2D and NAFLD, as described in this section, support the notion that the steatotic liver might have an impaired capacity to clear bacterial components.

Box 2 | **Concept of microbiota-controlled metabolic inflammation**

An array of bacterial components or pathogen-associated molecular patterns, such as lipopolysaccharides[249], flagellin[250], peptidoglycan[251], ADP-heptose and lipoteichoic acid[252], elicit proinflammatory responses by innate and adaptive immune cells. Pathogen-associated molecular patterns are detected by specialized immune cells, such as tissue-resident dendritic cells and macrophages, which are equipped with a large repertoire of pathogen-recognition receptors such as Toll-like receptors and NOD-like receptors[253,254]. Receptor activation potently triggers the production of proinflammatory mediators (such as cytokines, chemokines and oxidative perturbations[204]) that fuel metabolic inflammation. Other immune cells, such as mucosa-associated invariant T cells, natural killer T cells and regulatory T cells, can modulate metabolic inflammation[255–258]. Immune cells may be primed in the intestine and migrate to distant organs (such as the liver and adipose tissue) or are directly recruited to metabolically active tissues to modulate metabolic inflammation[259].

Besides microbial signals, the immune system may also respond to cellular stress and tissue injury with an inflammatory response in metabolic diseases. For example, DNA damage[260], endoplasmic reticulum stress[261] or damage-associated molecular patterns (such as double-stranded DNA, mitochondrial DNA and ATP) released during tissue injury[262] can perturb immune functions and potentially lead to a chronic inflammatory condition in metabolic disorders.

Bacterial signals may not only modulate the immune system, but also the function of the central nervous system[187]. The study of the gut–brain axis has gained momentum in neurological diseases[231,232], which may also become relevant in behavioural sciences. For example, *Escherichia coli* proteins regulate host satiety in rats[263].

Endotoxin promotes metabolic inflammation. A plethora of PAMPs and bacterial metabolites may contribute to metabolic inflammation and disease in mammals (BOX 2). Several landmark studies have established a major role for endotoxaemia as a driver of metabolic diseases[78–81]. Endotoxaemia and its markers (elevated serum LPS and LPS-binding protein) were associated with increased risk of obesity and T2D in humans[157,158], and postprandial endotoxaemia was suggested to promote the development of T2D[159]. In patients with T2D, endotoxaemia correlated with disease exacerbation. Furthermore, endotoxin administration to mice and healthy humans resulted in systemic and adipose tissue inflammation paralleled by insulin resistance, verifying that endotoxin affects insulin sensitivity[160]. This metabolic dysregulation could be abolished by antibiotic treatment in diet-induced and genetically induced obese mice[137,161]. Likewise, in patients with NAFLD, endotoxaemia was directly correlated with the degree of liver inflammation and fibrosis[162].

One mechanism by which endotoxin modulates metabolic dysregulation could involve adipose tissue homeostasis as metabolic endotoxaemia increased the proliferation of preadipocytes and induced adipose tissue inflammation, which was dependent on CD14 (REF.[163]). The absence of CD14 in *ob/ob* mice mimicked the metabolically beneficial and anti-inflammatory effects of antibiotics[137]. Notably, MyD88-dependent TLR signalling in intestinal epithelial cells promotes diet-induced obesity, diabetes and inflammation[120], and the progression of NAFLD is controlled by TLR4 (REF.[164]).

Several environmental factors, predominantly diet, impact endotoxaemia. A HFD led to the expansion of LPS-containing bacteria and increased plasma LPS concentrations[78]. In humans, high-energy intake was correlated with serum LPS levels in a large French male cohort[165]. Similarly, administration of a 4-week-long HFD (Western style diet) to healthy subjects resulted in a 71% increase in endotoxin plasma levels, which was not observed after a prudent-style diet, suggesting that a HFD-induced barrier dysfunction and/or alteration of gut microbiota might be involved[81]. In contrast, the use of prebiotic oligofructose not only reduced endotoxaemia but also restored intestinal concentrations of *Bifidobacteria* and glucose tolerance[166]. Similarly, functional changes of the gut microbiota by calorie restriction have been shown to lower the expression of key bacterial enzymes required for lipid A biosynthesis, a key component of LPS, and calorie restriction resulted in lower LPS concentrations and metabolic improvements in mice[167].

In summary, these studies support a critical role for endotoxin in metabolic inflammation. Which bacterial strains contribute to metabolic phenotypes remains largely elusive; however, it seems that some bacteria express LPS subtypes that have higher immunogenicity than others[168]. In this respect, Fei et al. isolated *Enterobacter cloacae* B29 from a morbidly obese subject and colonization of this strain in germ-free mice recapitulated metabolic disease[169].

Bacterial components and metabolites control metabolic dysfunction. Studies in the past few years have highlighted a role for various bacterial metabolites in the exacerbation or modulation of metabolic dysfunction[37,170]. Peptidoglycan-based bacterial cell wall components modulate inflammatory responses by stimulating the intracellular sensors NOD1 and NOD2. Signalling through NOD1 primes the innate immune system[171] and expands the number of ileal γδ T cells and promotes the release of the proinflammatory cytokine IL-17A from the mouse intestine, which in turn enhances the lifespan of circulating phagocytes in steady state[172]. These sensors may also contribute to metabolic inflammation and regulate insulin resistance, as HFD feeding of mice increased the levels of circulating NOD1 activators[173]. Injections of a NOD1 ligand to mice induced adipose tissue and hepatic inflammation and systemic insulin resistance, whereas NOD1-deficient mice were protected from obesity-induced inflammation[174], and mice with a specific deletion of NOD1 in haematopoietic cells showed reduced adipose tissue-infiltrating proinflammatory macrophages[173]. Conversely, NOD2-activating muramyl dipeptide triggered anti-inflammatory and insulin-sensitizing effects in HFD-fed or LPS-treated mice, mediated by interferon regulatory factor 4 (REF.[175]).

Dietary nutrients, especially phosphatidylcholine, choline and carnitine, are processed by the intestinal microbiota to produce trimethylamine, which is converted in the liver into TMAO by flavin-containing monooxygenases. TMAO levels correlate with atherosclerosis, cardiovascular complications and clinical outcomes, as this metabolite drives inflammation and platelet activation[176–178]. Increased TMAO levels are indicative of T2D and correlate with glycaemic control, cardiovascular complications and NAFLD[179–183], although the mechanisms of TMAO action may be diverse[184].

Other bacterial products, such as SCFAs, may be metabolically protective[185]. SCFAs are mainly derived from the fermentation of complex carbohydrates (for instance, fibre)

and have emerged as key factors orchestrating host physiology and disease, as recently reviewed elsewhere[186]. SCFAs can directly activate G protein-coupled receptors, inhibit histone deacetylases and serve as a major energy source for intestinal epithelial cells. Their specific binding to G protein-coupled receptors allows them to exert protective immune and metabolic functions[186]. Furthermore, production of the SCFA acetate by the microbiota was shown to activate the parasympathetic nervous system and promote insulin secretion and weight gain[187].

Similarly, flavonoids are diet-derived compounds metabolized by the microbiome and shown to contribute to enhanced energy expenditure in mice by inducing thermogenesis in brown adipose tissue. Consequently, exogenous administration of flavonoids as a postbiotic intervention ameliorated excessive secondary weight gain in mice[53]. Additionally, the gut microbiome can modulate metabolic inflammation by affecting host-derived molecules. For instance, the microbiome has a regulatory role in bile acid synthesis and biotransformation, which in turn affect metabolic processes, such as liver steatosis, adipose tissue browning and insulin signalling, by acting on farnesoid X receptor and TGR5 (REF.[152]).

A recent study investigated the relationship between the plasma and urine metabolomes, faecal metagenomics and the hepatic transcriptome[188]. The degree of hepatic steatosis correlated with a gut microbial signature, characterized by reduced microbial gene richness, and increased relative abundances of Proteobacteria, Actinobacteria and Verrucomicrobia. Reduced microbial gene richness was associated with steatosis and an increase in the amount of branched-chain amino acids. Moreover, phenylacetate, a faecal (mainly bacteria derived) degradation product of essential amino acids was associated with steatosis. Phenylacetate enhanced hepatic lipid accumulation by increasing branched-chain amino acid use. Faecal transfer from obese women with high-grade steatosis into mice promoted hepatic steatosis, similar to feeding with phenylacetate[188].

Koh et al. recently identified the bacterial metabolite imidazole propionate as a factor affecting insulin signalling[189]. In their study, the authors assessed portal vein blood from subjects with and without T2D for metabolically active compounds. They identified imidazole propionate as a histidine-derived microbial metabolite commonly found in patients with T2D. Notably, imidazole propionate impaired insulin signalling in mice in a mechanistic target of rapamycin complex 1 (mTORC1)-mediated pathway, a pathway that was found overexpressed also in livers of patients with T2D[189].

Together, bacterial structural components and metabolites secreted from bacterial processing of diet-derived and host-derived molecules modulate metabolic inflammation through various mechanisms. Of note, metabolic inflammation and the gut microbiome assembly are governed by additional factors, namely dietary habits, exercise and others (BOX 1).

Circulating or tissue microbiome: fact or fiction?

Impaired intestinal barrier function and the influx of bacteria-derived metabolites into the portal system in metabolic disorders have raised the question of whether bacteria or their antigens can reach the circulation or even distant organs. Amar et al. showed that commensal DNA could be detected in the blood and adipose tissue of HFD-fed mice[119]. The same group detected distinct blood bacterial dysbiosis involving a bloom of Proteobacteria in patients with metabolic syndrome and cardiovascular events[190]. The blood fraction and state in which the bacteria can be found (whether intracellularly in dormancy, as previously described[191]) remains to be determined. It has been proposed that bacteria reside in the leukocyte and platelet fractions of whole blood, but not in the plasma, of healthy humans[192]. Likewise, Schierwagen et al. found evidence for a circulating microbiome in the buffy coat fraction of central, hepatic/portal venous and peripheral blood from seven patients with liver cirrhosis undergoing transjugular portosystemic shunting[193]. Particularly, Proteobacteria sequences could be detected in the blood, which was somewhat fraction specific. Importantly, in some patients the authors were able to cultivate bacteria, suggesting that circulating sequences were derived from living bacteria.

A blood microbiome signature for NAFLD-associated liver fibrosis has also been described in a small cohort of patients with severe obesity[194]. A recent study in patients with alcoholic hepatitis portrayed a circulating microbiome signature, characterized by a decrease in Bacteroidetes and an enrichment of *Fusobacteria*, which are mainly present in the oral cavity[195]. This phenomenon was paralleled by exacerbated endotoxaemia and activation of the type III secretion system, which has been associated with Gram-negative bacterial virulence. The presence of bacteria in other extra-intestinal tissues in humans with metabolic diseases has been debated, as they could not be detected in subcutaneous or adipose tissues in 14 individuals with obesity[196].

Notably, the critical role of intestinal barrier breach and microbial translocation extends beyond metabolic diseases and is observed in other chronic disorders. For example, a recent report demonstrated that a specific pathobiont (*Enterococcus gallinarum*) translocates into liver tissue, which triggered autoimmunity similar to systemic lupus erythematosus (SLE)[197]. Treatment of lupus mouse models with vancomycin extended their lifespan and decreased circulating SLE-related autoantibody concentrations. Importantly, *E. gallinarum* could be detected in livers of patients with SLE and autoimmune hepatitis[197]. Similarly, *L. reuteri*, a commensal abundant in some patients with SLE, was found to increase intestinal leakiness, translocate systemically and exacerbate a lupus-like phenotype in TLR7-dependent mouse models of SLE[198]. Furthermore, specific strains of *Klebsiella pneumonia* obtained from patients with the biliary disease primary sclerosing cholangitis and transplanted into mice disrupted their intestinal barrier integrity and led to bacterial translocation of other pathobionts into the mesenteric lymph nodes and to the priming of a T_H17 cell response in the liver[199].

Altogether, these studies shed light on extraintestinal and systemic translocation of the microbiome and its products in metabolic inflammation and reveal exciting perspectives beyond metabolic diseases.

Chronic inflammation and metabolic disease

Chronic low-grade inflammation is a crucial component in metabolic disorders such as obesity, T2D, NAFLD and atherosclerosis and associates with disease complications and prognosis[200–203]. The adipose tissue and liver serve as important sources of inflammatory mediators contributing to chronic inflammation and driving metabolic dysregulation[204], and a wide range of immune cell populations can be found in inflamed metabolic tissues[205]. Prospective studies and meta-analyses linked high-sensitivity CRP (hsCRP) levels with cardiovascular risk[206,207]. Studies have demonstrated that statins induced their lipid-lowering effect in part through inhibition of inflammation and that hsCRP levels were as prognostic of coronary artery disease as low-density lipoprotein serum concentrations[208–210]. Along the same lines, several studies have shown that elevated levels of hsCRP were a reliable prognostic marker of cardiovascular complications in metabolic diseases, including T2D[207]. For atherosclerosis, the detrimental impact of chronic inflammation was underlined in the landmark CANTOS trial, as blockade of IL-1β by canakinumab significantly lowered the rates of recurrent myocardial infarction and cardiovascular mortality in statin-treated patients, proving a key role for this cytokine in the pathogenesis of atherosclerosis[211]. Importantly, the benefits of canakinumab were directly associated with lowering hsCRP and IL-6 serum levels[212,213]. Notably, several preclinical studies demonstrated that 'proinflammatory' consortia of gut microbiota could be transferred to animals, eliciting systemic inflammation and aggravating atherosclerosis[214]. In contrast, bacteria such as *B. vulgatus* or *Bacteroides dorei* ameliorated endotoxaemia and improved atherosclerosis in mice[215], which somewhat conflicts with the clinical observation that *B. vulgatus* was associated with insulin resistance in T2D[59,216] and that the prebiotic inulin-type fructan reduced its abundance in obese women[217].

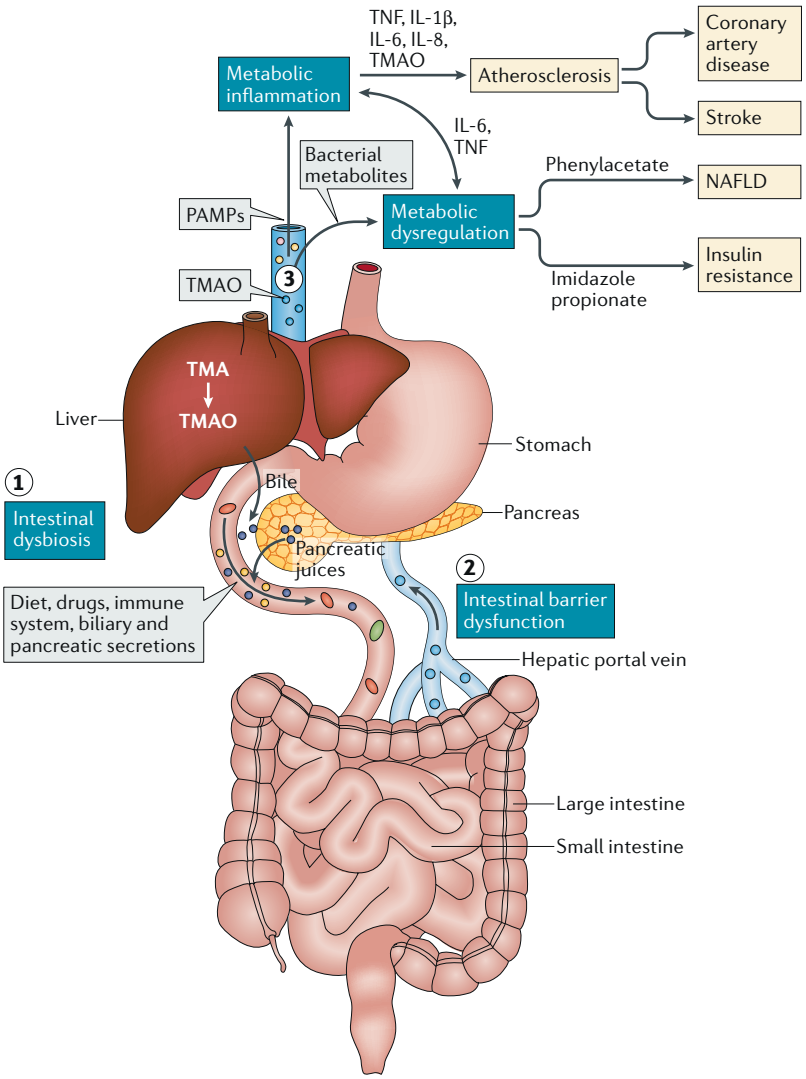

Fig. 2 | **Multiple 'gastrointestinal hits' contribute to metabolic diseases.** The intestinal microbiota is modulated by environmental factors (such as diet and drugs) and host factors (such as the intestinal immune system and pancreatic and biliary secretions). For example, a typical Western diet alters the intestinal microbiota composition and function. Dysbiosis may represent an early event in metabolic diseases (1). A dysbiotic microbiota and dietary factors may trigger intestinal barrier dysfunction (2), which represents a further gastrointestinal hit. These intestinal alterations lead to the translocation of bacterial metabolites, such as phenylacetate, trimethylamine (TMA), imidazole propionate or mediators of metabolic dysregulation, and pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide, which drive chronic low-grade inflammation via the induction of proinflammatory cytokines such as IL-1β. Metabolic inflammation and dysregulation bring about metabolic disease and are tightly interrelated. For example, metabolic inflammation (including increased IL-6 and tumour necrosis factor (TNF)) promotes insulin resistance. Consequently, it may be speculated that a diseased liver has an impaired capacity to clear bacteria and bacterial metabolites from the portal vein (3). This is supported by the presence of bacterial genes and molecular patterns in the systemic circulation and extraintestinal tissues in metabolic diseases. Collectively, the intestine and its resident microbiota emerge as drivers of metabolic inflammation and dysregulation, which are critical hallmarks of metabolic diseases such as obesity, type 2 diabetes, nonalcoholic fatty liver disease (NAFLD) and associated atherosclerosis. TMAO, trimethylamine *N*-oxide.

Chronic inflammation in obesity, T2D and NAFLD might not only contribute to atherosclerosis and cardiovascular complications but also impact the course of the disease, for example, by modulation of insulin resistance in various tissues such as adipose tissue, liver and muscle[5,218–221]. Inflammatory cytokines and transcription factors involved in the regulation of insulin resistance are highly expressed in metabolic tissues[222,223]. Administration of IL-1 receptor antagonist to T2D subjects was able to improve glycaemic control. The improvement in insulin secretion lasted 39 weeks following the withdrawal of this therapy[224,225]. However, canakinumab treatment was not able reduce the incidence of T2D over a median period of 3.7 years and was not associated with long-term benefits on glycated haemoglobin levels[226]. However, this result does not rule out an impact of low-grade inflammation on glucose control. Further clinical studies in atherosclerosis targeting other inflammatory cytokines, such as TNF, are warranted.

Conclusions and future prospects

Mechanisms of chronic inflammation in metabolic disorders are complex and of diverse aetiologies[227]. As outlined in this Review, the gastrointestinal tract and its bacterial inhabitants have emerged as key regulators of metabolic inflammation and dysfunction. Future studies will identify which and how bacterial metabolites regulate immunometabolism. We propose a paradigm by which various 'gastrointestinal hits' such as dysbiosis

and barrier dysfunction may account for the evolution of metabolic diseases (FIG. 2). These events may promote metabolic inflammation and fuel metabolic perturbations[37,228]. Such a cascade of events might be particularly relevant in lean subjects with T2D and NAFLD, in whom classical 'sterile' triggers of lipotoxic inflammation are lacking[229].

Regulatory factors that impact the intestinal microbiota (for example, glucose availability) may similarly affect epithelial integrity[131]. Drawing traditional associations between specific pathogens and diseases (as characterized by Koch's postulates) may require a revision in the context of metabolic disorders, as no single responsible pathogen could be identified or cultured[230]. Such postulates should be challenged, as some commensals could become detrimental to their host (for example, by penetrating the intestinal barrier) under certain circumstances. One might also ask why such 'pathobionts' are not targeted by the immune system, how they trigger a chronic inflammatory response (rather than a self-limited immune response) and how LPS impacts low-grade inflammatory human diseases. In this regard, a strain-specific host immune response was suggested to stem from distinct LPS subtypes in the context of autoimmunity[168].

Studies from the past years have established that chronic inflammation contributes to cardiovascular disease and affects the outcome of metabolic diseases such as obesity, NAFLD and T2D. Targeting inflammatory pathways has become attractive in atherosclerosis and may become pivotal in the treatment of other metabolic diseases in the future[211]. However, it may be more plausible to interfere at the site of origin of inflammation such as the gastrointestinal tract. The gut microbiota–metabolism axis has gained momentum in preclinical settings and opens an exciting new avenue for clinicians, which may similarly become relevant in other (for example, neurological) diseases[231,232].

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