REVIEW

The metabolic impact of bacterial infection in the gut

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Bacterial infections of the gut are one of the major causes of morbidity and mortality worldwide. The interplay between the pathogen and the host is finely balanced, with the bacteria evolving to proliferate and establish infection. In contrast, the host mounts a response to first restrict and then eliminate the infection. The intestine is a rapidly proliferating tissue, and metabolism is tuned to cater to the demands of proliferation and differentiation along the crypt–villus axis (CVA) in the gut. As bacterial pathogens encounter the intestinal epithelium, they elicit changes in the host cell, and core metabolic pathways such as the tricarboxylic acid (TCA) cycle, lipid metabolism and glycolysis are affected. This review highlights the mechanisms utilized by diverse gut bacterial pathogens to subvert host metabolism and describes host responses to the infection.

Introduction

The gastrointestinal tract is the key nutritional interface, which plays a vital role in maintaining metabolic homeostasis [1]. It is contiguous to the external environment and hence, is prone to diseases. Gastroenteritis, the inflammation of the gastrointestinal tract, is associated with various symptoms, including nausea, diarrhoea, fever and abdominal pain. Parasites, viruses and bacteria can cause infectious diarrhoea, and bacterial pathogens are responsible for 20–40% of the total diarrhoeal burden, with children < 5 years suffering the most [2]. There are around 1.7 billion childhood diarrhoea cases globally, with 525 000 deaths annually (https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease). Such infections result in many

physiological responses and include metabolic alterations in both the interacting partners. The host triggers responses aimed at eliminating the invading pathogen. In contrast, the pathogen exploits the presence of host nutrients and other metabolites to fuel bioenergetic and biosynthetic processes needed for rapid replication and transmission.

Interestingly, many reports suggest that intestinal epithelial cells (IECs) undergo metabolic reprogramming upon infection. This leads to an increase in glucose uptake and/or a shift from ATP production in the mitochondria to glycolysis, a phenomenon known as the Warburg effect, a process also seen in proliferating cancer cells [3]. Since the intestinal epithelium is

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composed of many different cell types with various metabolic programmes, the effect of infections on energy homeostasis has significant consequences on the outcome of the disease. Host inflammatory responses against infection also lead to changes in the tissue metabolism [4], which further affect the intestinal epithelial barrier integrity [5]. Therefore, host–pathogen interaction is a complex, interlinked metabolic system and seemingly minor changes could significantly affect infection outcomes.

This review focuses on the metabolic changes induced in IECs by bacterial pathogens. We first describe homeostatic metabolism in IECs and how this changes in response to infection by specific bacterial gut pathogens. An essential contributor to the overall metabolic state is, of course, the host microbiome. This topic has been extensively reviewed in the past [6–10] and will not be discussed here. Another critical player in the context of infection, as mentioned earlier, are the immune cells and the inflammatory responses induced by the immune cells. Again, the effect of infection on immune cell metabolism [11–13] and the role of immune cells on tissue metabolism have been extensively covered elsewhere [14,15]. Since the impact of metabolic changes in IECs could have important implications in the repair process of the gut following infection, we focus on this aspect in this review.

Energy metabolism in intestinal epithelial cells

Structure of the intestinal epithelium

The intestinal lumen is lined with a monolayer of epithelial cells (or mucosa), which perform the primary functions of absorbing nutrients and water and act as a barrier against gut commensals, luminal pathogens and toxic substances [16]. The intestinal epithelium has a highly specialized architecture and consists of various cell types that perform specific functions (Fig. 1). The small intestinal epithelium is organized into millions of crypt–villus units. The villi are finger-like protrusions that increase the surface area for absorption and are surrounded by multiple invaginations called crypts. Multipotent Lgr5$^+$ stem cells are present at the crypt base along with deep crypt secretory (DCS) cells called Reg4$^+$ cells, and antimicrobial peptides-secreting Paneth cells. Transit-amplifying (TA) cells, arising from proliferation and partial differentiation of LGR5$^+$ cells, rapidly divide in the lower half of the crypt before arresting their cell cycle and differentiating into mature cell types as they migrate to the upper surface [17]. Differentiated cell types include absorptive enterocytes,

goblet cells, enteroendocrine cells and tuft cells (tuft1 and tuft2 subsets) [18]. Differentiated cells show a spatial zonation along the crypt-villus axis (CVA based on their functionality and gene expression profiles [19–21]. Intestinal epithelial cells also have immunoregulatory properties enabling them to detect invading pathogens, e.g. they can express pattern recognition receptors and subsequently influence the development of the mucosal immune cell response [22]. Around 3 days after their terminal differentiation, the mature cells reach the villus tip, undergo apoptosis and are ultimately shed into the intestinal lumen [17]. The continuous renewal of epithelial cells along the crypt–villus axis, as a result of the division of the stem cells at the base of the crypt, ensures constant replenishment of cells that are damaged by mechanical forces and commensal or pathogenic bacteria, and this process is critical for the repair of the epithelial layer (Fig. 1).

The large intestinal epithelium is arranged into crypts with no villi, thereby minimizing the potential damage inflicted by transiting semi-solid stool (Fig. 1). Like the small intestine, Lgr5$^+$ stem cells reside at the base of these crypts and give rise to TA cells and differentiate into other types of epithelial cells. Paneth cells are absent in the large intestine.

Metabolism in the intestinal epithelium

Mammalian cell metabolism comprises a complex network of interconnected pathways, summarized in Fig. 2. Metabolism is regulated by a network of nutrient sensors, growth hormone receptors, several signalling pathways and transcription factors [23]. The digestive and absorptive roles of the intestine are highly dependent on energy [24]. Therefore, IECs utilize various sources of energy, including amino acids (like glutamate and glutamine), microbiota-produced short-chain fatty acids (SCFAs) produced at high concentrations in the large intestine and glucose. IECs can take up glucose, glutamine, glutamate and aspartate through the circulatory system [25], while the SCFAs (acetate, butyrate and propionate) and amino acids in general are absorbed directly from the gut lumen [26]. In the absence of SCFA production, metabolism shifts from β-oxidation to lactate fermentation [27]. Importantly, the functioning of Lgr5$^+$ stem cells is also affected by the nutritional state of the organism. For example, fasting and caloric restriction result in an expansion of Lgr5$^+$ cells [28–30], which also occurs during the administration of a high-fat diet [31–33].

Data obtained from intestinal cell lines (HuTu-80, a human duodenal adenocarcinoma cell line, and IPEC-J2, a porcine jejunal epithelial cell line) showed that jejunal cells had higher oxidative phosphorylation
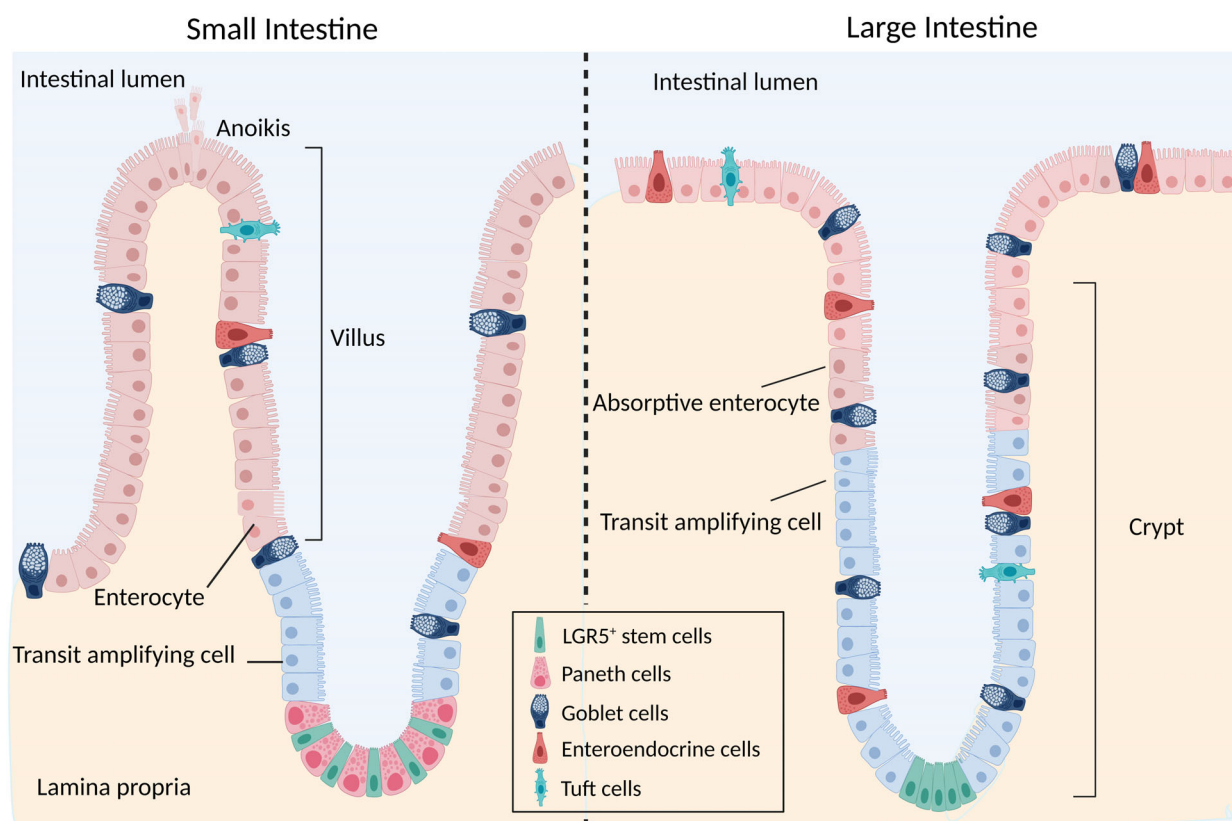
Fig. 1. Structural and cellular organization of the intestinal epithelium. The small intestinal epithelial layer is arranged in villus–crypt units (left panel), while the large intestine has only crypts (right panel). LGR5+ (leu-rich repeat-containing G-protein-coupled receptor 5-expressing) stem cells reside at the base of the crypt and are intercalated with Paneth cells. LGR5+ stem cells give rise to rapidly proliferating transit-amplifying (TA) cells which differentiate into functional cells on the villi that include tuft cells, goblet cells, enterocytes and enteroendocrine cells. Differentiated cells move toward the tip of the villus where they undergo anoikis and shed into the intestinal lumen (left panel). LGR5+ stem cells at the large intestine crypt base generate rapidly proliferating TA cells in the lower half of the crypt and differentiate into goblet cells, enterocytes, enteroendocrine cells and tuft cells (right panel). The figure was created using Biorender.

(OXPHOS) and respiratory capacity [34]. Fatty acid oxidation was most elevated in the duodenum and declined distally. Recently, a transcription factor expressed specifically in the crypt cells of the upper intestinal tract, PRDM16, has been shown to regulate fatty acid oxidation in a region-specific manner [35].

A metabolomic study performed on mice with small intestinal villi and crypt cells reported higher concentrations of lipid–carbon chains in the villus cells than in crypt cells, and transcriptomic analysis revealed higher expression of lipid metabolism genes in the villi [36]. More elevated glucose-, glycogen- and choline-containing metabolites were seen in crypts, while more lactate was present in villi. Other reports independently verified this finding using differential gene expression profiling [37]. Additionally, they showed that proteins involved in nucleotide metabolism, protein processing and folding were increased in the crypt [38].

Enterocytes show high expression of genes involved in lipid uptake and transfer (*Ldl*, *Ldr1*, *Ldr10*, *ApoC-III* and *Slc27a4*) [37], which is consistent with their roles in digestion and absorption of digested fat from the lumen. In a study of porcine IECs [39], an increased expression of proteins involved in glucose, amino acid and fatty acid metabolism, and those in the TCA cycle was observed in villus cells. In contrast, downregulation of proteins related to glutamine metabolism was seen along the crypt–villus axis [39]. These reports suggest that altered metabolism is associated with differentiated cells in the villi and proliferative cells in the crypt.

One major factor that can regulate intestinal metabolism is the availability of oxygen. IECs maintain very low baseline oxygen levels governed by the counter-current blood flow, high energy expenditure-to-consumption ratio and the presence of gut
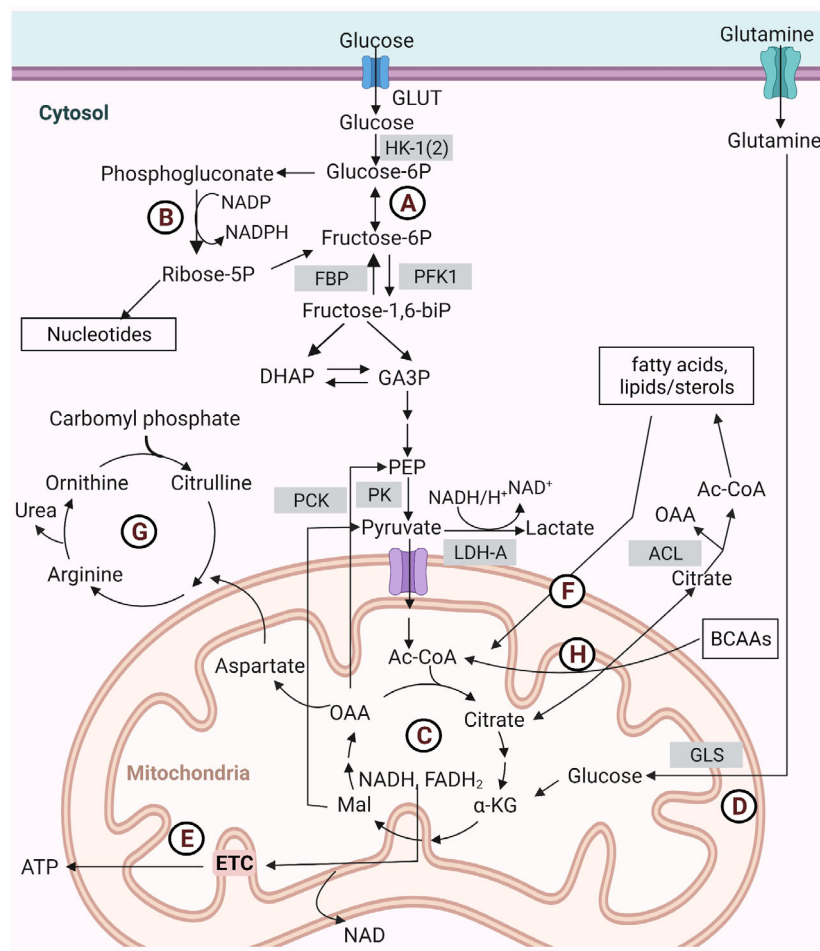
Fig. 2. Key metabolic pathways in mammalian cells. Glucose uptake, glycolysis (A), pentose phosphate pathway (B), tricarboxylic acid cycle (C), glutaminolysis (D), oxidative phosphorylation occurring in mitochondria (E), β-oxidation (F), urea cycle (G) and branched-chain amino acids (BCAA; valine, leucine and isoleucine) metabolism (H) and other related processes are marked by black arrows. Enzymes are highlighted in grey. Abbreviations used: α-KG, α-ketoglutarate; ac-CoA, acetyl CoA; ACL, ATP-dependent citrate lyase; DHAP, dihydroxyacetone phosphate; ETC, electron transfer chain for aerobic respiration consisting of complexes I–IV and ATPase (complex V); FBP, fructose bisphosphatase; GA3P, glyceraldehyde 3-phosphate; GLUT; glucose transporter; HK, hexokinase 1 and 2; GLS, glutaminase; LDH, lactate dehydrogenase; Mal, malate; OAA; oxaloacetate; PCK, PEP carboxylase; PEP, phosphoenolpyruvate; PFK1, phosphofructokinase 1; PK, pyruvate kinase. The figure was created using Biorender.

commensal microbiota [24,40,41]. There are two spatial oxygen gradients in the intestine: longitudinal and radial. There is a steep decrease in the oxygen levels along the longitudinal axis from the small intestine to the colon and from the oxygen-rich intestinal submucosa to the gut lumen along the radial axis [42,43]. Despite the hypoxic nature of the intestine, stem cells switch to OXPHOS to maintain high cell turnover. A metabolomic study on stem cells and Paneth cells showed a higher OXPHOS rate in Lgr5^{+} stem cells, and the glycolytic pathway was more active in the Paneth cells [44]. Lactate produced by the Paneth cells during this glycolysis is used by Lgr5^{+} cells during OXPHOS. Hence, lactate acts as a niche signal that supports and maintains the proper functioning of stem cells.

In the colon, the SCFAs, especially butyrate produced by anaerobic bacteria, serve as the primary fuel for OXPHOS [45,46]. A consequence of hypoxia is the stabilization of the hypoxia-inducible factors (HIFs) [41,47]. Hypoxia-inducible factors is a heterodimer consisting of constitutively expressing HIF-α and HIF-β subunits. Under normal oxygen conditions (normoxia), HIF-α subunits are degraded by proteolytic enzymes. In contrast, the heterodimer is stabilized in the absence of oxygen, allowing it to regulate the transcription of several genes, including those involved in glycolysis, OXPHOS and fatty acid metabolism [41,47], thus fulfilling the energy demands of oxygen-starved IECs [41,48,49]. Butyrate is also responsible for stabilizing HIF levels in the colon [46].

Another essential factor integrating external cues to epithelial homeostasis is mitochondrial metabolism in the IECs [50–52]. The mitochondria define the stem cell differentiation programme, and their dysfunction leads to gastrointestinal disorders like ileitis and IBD [53–55]. Mitochondria sense hypoxic conditions and mitochondrial content reduces progressively from the crypt to the villus tip [20], suggesting that this could be an adaptation to the decreasing oxygen gradient along the CVA.

Alterations in host metabolism in response to gut pathogens

Infection of the gastrointestinal tract by pathogens may dramatically affect gut architecture due to damage to the intestinal epithelium. Infection may also alter the microbiome due to the production by the host. Furthermore, the production of proteins such as lipocalin 2 [56] and calprotectin [57] by the host would result in limited iron, zinc and manganese availability, primarily to prevent microbial growth and virulence. However, this would also result in alterations in metabolic processes that occur in intestinal epithelial cells. Such metabolic responses of the host can be categorized into (a) core host responses and (b) responses induced by pathogen-specific factors.

Core host responses

Generalized responses that differentiate pathogens from normal commensal bacteria are termed core host responses [58,59]. The most common induction programme is regulated by activation of the NF-κB transcription factor [58,60]. NF-κB signalling in intestinal cells is activated when exposed to the luminal microbial contents due to loss of epithelial barrier integrity. NF-κB is activated by cytokines and pathogen-associated molecular patterns (PAMPs, which include cell envelope components, flagella, CpG DNA motifs, dsRNA) via different Toll-like or NOD-like receptors (TLRs or NLRs) [61,62]. NF-κB drives the expression of many target genes involved in immunity and inflammation [63]. Intestinal NF-κB signalling is responsible for maintaining the immune homeostasis of the tissue. Mice develop spontaneous inflammation upon inhibition of NF-κB signalling, specifically in the epithelial cells [64]. Activated NF-κB also plays an important role in regulating energy metabolism [65–67] via intracellular p53 levels [68,69]. Activated NF-κB helps in controlling the mitochondrial respiration and metabolism by suppressing the Warburg effect in colon carcinoma cells [69]. Since intestinal epithelial cells also undergo metabolic reprogramming upon infection [3], NF-κB signalling may play a similar role in this scenario as well. Many amino acids (like GABA, threonine and L-cysteine) are known to suppress the activation of NF-κB signalling [70,71].

Another typical host response is the generation of ROS and reactive nitrogen intermediates (RNI), which have regulatory roles in host defence against invading pathogens. RNIs are antimicrobial molecules that include all the oxidation states of products of nitric oxide synthases like NO^-, NO_2, NO_2^-, N_2O_3, N_2O_4, NO_3^- (nitrate), S-nitroso thiols, peroxynitrite ($OONO^-$) and dinitrosyl-iron complexes. RNIs play important roles in regulating cellular metabolism by the nitrosative modifications of proteins and lipids [72]. Highly reactive oxygen radicals, which include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$), oxidize critical enzymes involved in glycolysis and pentose phosphate pathway (PPP) pathway (GAPDH and PKM2) and reroute carbohydrate flux from glycolysis to the PPP with a subsequent decrease in oxidative stress. This is in addition to the activation of the transcription factor HIF-1 discussed above [73].

Responses induced by pathogen-specific factors

Pathogen-specific responses are triggered to facilitate multiplication and are a counter-measure against host defence responses. For extracellular bacterial pathogens (EBP), effector proteins injected into the host cell via type III or type IV secretion systems (T3SS or T4SS) are critical modulators of host metabolism, along with toxins and adhesins. These factors interact with different host targets, interfering with the host homeostasis [74–77]. In the case of intracellular bacterial pathogens (IBP) before invasion, host modulating factors are similar to EBP (effector proteins, adhesins and toxins). In contrast, bacterial factors directly affect host metabolism after invasion since pathogen proliferation requires nutrients produced or imported by host cells [59,78,79].

The effects of different pathogens on metabolism in intestinal cells are discussed below and reveal that changes they induce are selective and adjusted to both the need of the pathogen to divide and the requirement of the intestinal epithelium to regenerate and heal from the infection.

Adherent pathogens

ETEC

Enterotoxigenic *Escherichia coli* (ETEC) are one of the six diarrhoeagenic categories of *E. coli* pathotypes that produce heat-labile (LT) and/or heat-stable (ST) toxins. ETEC infections are a major cause of acute watery diarrhoea in developing countries, especially in children below 5 years of age [80]. ETEC also causes traveller's diarrhoea in adults [81]. ETEC produces several species-specific colonization factors that mediate bacterial attachment to IECs [82]. Toxins secreted by ETEC bind to their receptors on epithelial cells and activate downstream signalling cascades, ultimately regulating several ion channels leading to ion imbalance, fluid secretion

into the intestinal lumen and diarrhoea [83]. After infection, ETEC rapidly colonize the duodenum, jejunum and ileum. ETEC colonization leads to increased intestinal epithelial barrier permeability [84,85] and induces an inflammatory response in the gut [86–88].

Various *in vitro* studies have been done on IPEC-J2 cells (porcine undifferentiated IECs) to understand the effect of ETEC on the host cells [89–91]. RNA sequencing revealed that most of the differentially regulated genes in the host upon infection by three different strains of ETEC are related to cell cycle progression, amino acid metabolism, apoptosis and immune-related genes [90]. Interestingly, the cell regeneration programme is an innate response of isolated intestinal epithelial cells following infection since repair of the damaged epithelium is a process that occurs following almost all gut infections. ETEC infection also causes an increase in the levels of phosphatidylserine on the outer membrane leaflet of the host cells, which may help in the bacterial adherence [89]. ETEC infection also inhibits the uptake of Vitamin B1 [92] and Vitamin C [93] in human colonic Caco-2 cell lines due to reduced expression of their respective intestinal transporters [92,93]. Vitamins act as cofactors to many enzymes in metabolic pathways. Hence, a decrease in their uptake may increase oxidative stress and overall altered metabolism.

Interestingly, a proteomics study on piglet jejunum after ETEC infection showed similar alterations. A total of 25 out of 92 differentially expressed proteins were related to metabolism [94], indicating that ETEC may modulate the host metabolism for its colonization. Wu et al. [95] analysed the metabolites in jejunal samples of piglets infected with ETEC. Diarrhoeal piglets had higher concentrations of glycine and gamma-aminobutyric acid (GABA) with a significant decrease in glutamine, lysine, tyrosine, ornithine, asparagine and citrulline. The latter three metabolites are associated with the urea cycle suggesting an altered urea metabolism. Gamma-aminobutyric acid (GABA) is a major amino acid that gets accumulated in the jejunum upon ETEC infection [96] and inhibits the ETEC-induced apoptosis of the intestinal epithelial cells via autophagy [97]. There was also a metabolic profile shift in some fatty acids and organic acids [95].

Metabolic variations in ileal mucosa and colon digesta upon ETEC infection in weaned pigs have also been monitored on day 21 post-infection [98]. At this time, ETEC infection is cleared from the gut of these piglets. However, significant metabolic differences were still observed in the ileal mucosa but not in the colon digesta of infected pigs. The most highly upregulated metabolite in ileal mucosa was spermidine, a

polyamine involved in mucosal repair [99]. Upregulation of cytidine and some of the metabolites involved in PPP and glycolysis (fructose-6-phosphate, fructose, ribose, glucose and G6P) [98] were also observed. Taken together, this suggests that the ileal mucosa has altered energy metabolism leading to increased cell proliferation and activated mucosal repair responses even on day 21 post-infection, possibly to restore epithelial barrier integrity. On the other hand, lipid metabolites like lignoceric acid and pentadecanoic acid were downregulated. The levels of these phospholipids are known to be reduced in the plasma of patients with inflammatory bowel disease (IBD) [100,101], suggesting a prolonged chronic inflammation-like condition in the ileum of these pigs some days after they had cleared the bacteria.

Enterotoxigenic *Escherichia coli*-mediated diarrhoea is transitory and can be treated with sufficient hydration and salt replenishment. However, the changes in energy metabolism in the IECs appear to be evident as long as 3 weeks post-infection [98]. Since the epithelial cells renew every 4–5 days, it seems that long-term changes must have occurred in the stem cell population in the gut in consequence to infection. In addition, responses of the IECs to cytokine production post-pathogen clearance could also have triggered prolonged changes in metabolic activity in the IECs [102]. Alternative mouse models that mimic diarrhoea mediated by ST toxins [103] could serve to explore the effects of prolonged intracellular cGMP levels on Lgr5$^+$ cells and metabolic changes in IECs.

EPEC/EHEC and *Citrobacter rodentium*

Enteropathogenic and enterohaemorrhagic *E. coli* (EPEC and EHEC) are human diarrhoeagenic *E. coli* pathotypes posing a significant threat worldwide. EPEC is responsible for infantile diarrhoea, a significant cause of high morbidity and mortality rates in low-income countries [104]. EHEC (particularly serotype O157:H7) express phage-encoded Shiga toxins and can also cause haemorrhagic colitis and haemolytic–uraemic syndrome [104]. These pathogens induce attaching and effacing (A/E) lesions, characterized by intimate bacterial attachment, effacement of the brush border microvilli and formation of actin-rich pedestal-like structures [105,106]. Infection with EPEC and EHEC is facilitated by a Type III secretion system (T3SS), encoded in the LEE pathogenicity island, which injects effectors into IECs that alter cellular functions.

EPEC and EHEC specifically recognize the plasma membrane phospholipid phosphatidylethanolamine (PE) on the outer leaflet of host IECs [107]. Hence,

just like ETEC infection [89], EPEC infection of Caco2 cells also disrupted host phospholipid metabolism, with elevations in PE levels and a corresponding decrease in phosphatidylcholine levels [108], which may help in bacterial attachment.

Mice are inherently resistant to EPEC and EHEC infections [109]. Earlier attempts for EPEC colonization in mice using streptomycin-mediated microbiome disruption [110–113] have provided mechanistic insights into EPEC infection but could not replicate the clinical outcome of diarrhoea. Recently, a more clinically relevant murine model for EPEC infection was developed by disrupting the host microbiota using an antibiotic cocktail [114], which could replicate most of the previously known outcomes of increased intestinal permeability and tissue damage leading to gut inflammation along with diarrhoea. These treatments with antibiotics would have altered the composition of the microbiota, which in turn could have affected host IEC metabolism. Therefore, data obtained may not reflect changes that occur in the IECs on infection, independent of the microbial composition in the gut. Thus, infection is modelled using the murine pathogen *Citrobacter rodentium*, which shares the infection strategy and virulence genes with EPEC and EHEC [115]. Significantly, *C. rodentium* infects immunocompetent mice in the context of the endogenous gut microbiota, thus providing a natural and physiologically relevant infection model.

Following oral inoculation, *C. rodentium* first colonizes the cecum and then spreads to the entire colonic mucosa [116,117]. During this process, the bacteria intimately attach to IECs and inject effector proteins by T3SS into the host cells, which then hijack the entire host cell machinery [118] and establish infection at the epithelial surface leading to colitis [111] and microbial dysbiosis [119].

Apart from a subversion of innate immune responses in IECs [120], the T3SS effector proteins also target mitochondrial functions wherein the effector map disrupts mitochondrial morphology leading to loss of respiratory functions [121]. This disruption of mitochondrial activity leads to increased levels of mucosal O_2 [122].

An in-depth proteomics analysis approach in C57BL/6 mice has shown that infected IECs undergo significant metabolic reprogramming as early as 4 days post-infection (DPI) [122], with significant downregulation of host metabolic pathways, including gluconeogenesis, lipid metabolism, the TCA cycle and OXPHOS, along with simultaneous upregulation of cell cycle and DNA replication pathways and increased abundance of the basolateral glucose importer SLC5A9 [122,123]. One of

the causes for such a shift in host glucose metabolism is a pathogen-derived effector – NleB –, which GlcNAcylates HIF-1α in host cells leading to enhanced HIF-1α transcriptional activity and increased expression of downstream gene targets related to glucose uptake and glycolysis [124]. This leads to an infection-induced shift in bioenergetics towards aerobic glycolysis, an inefficient mechanism to produce ATP. To compensate, the creatine biosynthetic pathway is upregulated [122], resulting in efficient mobilization of cytosolic energy via phosphocreatine production. Aerobic glycolysis facilitates the proliferation of TA cells and colonic crypt hyperplasia to maintain gut barrier functions.

Uniquely, IECs respond to *C. rodentium* infection by simultaneously upregulating cholesterol biogenesis (HMG-CoA reductase), import (LdrR) and efflux (Abca1 and AbcG8) and reverse cholesterol transport (ApoA) [122,123]. Increased cholesterol levels were observed in serum and faeces [122,125]. While elevated cholesterol levels can amplify immune responses, high luminal cholesterol can be utilized by the commensals. This may be one of the reasons for the bloom of Enterobacteriaceae, which have been shown to facilitate the clearance of *C. rodentium* [126]. Therefore, the host response in terms of cholesterol production is a means by which levels of *C. rodentium* in the gut are depleted and, thus, a means of overcoming infection.

Clostridioides difficile

Clostridioides difficile are sporulating, toxin-producing, Gram-positive obligate anaerobic bacteria responsible for antibiotic-associated diarrhoea, especially in hospitalized patients [127]. It is an opportunistic pathogen that colonizes the gut after prolonged antibiotic treatment. The clinical manifestations of *C. difficile* infection (CDI) include diarrhoea, pseudomembranous colitis and megacolon [128]. It is transmitted via the faecal–oral route, where spores enter the host through oral ingestion and germinate under favourable conditions in the intestine to their vegetative form [128]. The bacteria produce two toxins: toxin A (TcdA) and toxin B (TcdB), which cause mucosal inflammation and diarrhoea [129].

Microbial dysbiosis due to prolonged antibiotic use is one of the major drivers of susceptibility to CDI infections [130], without which *C. difficile* cannot colonize. The gut microbiome, especially *Lachnospiraceae* and *Ruminococcaceae* families, is the primary factor contributing towards intestinal colonization resistance of *C. difficile* [131,132]. Antibiotic treatment leads to loss of microbial diversity. Hence, there is a global shift in the metabolome of the host leading to depletion of secondary

bile acids, fatty acids and glucose with a concomitant increase in primary bile acids and sugar alcohols, thereby creating a favourable environment for colonization of *C. difficile* [133]. Hence, the type of antibiotic used dictates the nutritional status of the intestine, and accordingly, *C. difficile* adapts [134–136]. This post-antibiotic host metabolic state is further changed upon CDI [136,137] and is relevant to the discussion here.

An integrated metabolomics and transcriptomics analysis showed the temporal shift of host metabolism, especially amino acid and carbohydrate metabolism, during CDI post-clindamycin treatment [137]. Proline is an essential nutritional requirement for CDI [134,138]. The two major metabolites that significantly changed in the metabolomic study were 5-aminovalerate and trans-4-hydroxyproline [137]. 5-aminovalerate is a fermentation by-product of proline and increased on infection, while trans-4-hydroxyproline, a post-transcriptionally modified form of proline, showed a decrease – suggesting utilization of proline by the pathogen. A significant increase in N-acetylated forms of many amino acids (methionine, threonine and branched-chain amino acids) was observed in the initial hours of infection. Furthermore, upregulation in the expression levels of many proteases and peptidases 24 h post-infection was concomitant with an increase in the levels of free amino acids, carbohydrate and amino acid fermentation products. *Clostridioides difficile* utilizes these products in Stickland metabolism for ATP production and regeneration of NAD$^+$ [139]. A large increase in phospholipids, glycerolipids, sphingolipids, SCFAs, medium-chain fatty acids and long-chain fatty acids was also observed in the later phase of CDI (30 h post-infection) [137]. The authors suggest that since the pore-forming activity of TcdA and TcdB toxins increased at this time, higher lipid levels observed could indicate increased inflammation due to extensive tissue damage.

In summary, *C. difficile* can exploit changes in the microbiome that may have occurred in the host following antibiotic treatment allowing it to colonize the gut. Once established, it also modulates host metabolism to promote its proliferation. The damage caused to the gut would liberate nutrients required for the bacterium to grow and thereby compromise repair pathways. Treatment of *C. difficile* infection involves restoring the host microbiota using probiotics or faecal microbiota transplant or targeting *C. difficile* with antibiotics such as vancomycin or antibodies to *C. difficile* toxin B [140].

Invasive pathogens

Invasive pathogens survive and replicate within the host cell and therefore have direct access to host cellular components for their nutrition. Consequently, it can be anticipated that the changes they induce in host IEC metabolism are more dramatic than those brought about by ETEC and EPEC.

Salmonella Typhimurium

Salmonella enterica serovar *Typhimurium* (*S. Typhimurium*) is a major cause of gastroenteritis in humans and animals [141]. The infection in mice mimics infection by *S. Typhi* and *S. Paratyphi* that are restricted to humans [142]. In susceptible mice, the pathogen invades the intestinal epithelium causing inflammation that supports its growth [143–145]. Once the epithelial layer is breached, bacteria are taken up by dendritic cells where they survive without replication [146,147]. From the dendritic cells, they are transported to the mesenteric lymph nodes (MLNs) and then subsequently spread to other tissues such as the liver, spleen and Peyer's patches [148].

Analysing changes to gene expression in the mouse colon in response to *S. Typhimurium* infection revealed a complete shutdown of the OXPHOS pathway 8 h post-infection. At 4 DPI, a significant downregulation of glycolysis and catabolism of many of the branched-chain amino acids (Val, Leu and Ile) was observed, along with upregulation of the mTOR, NF-kB and p53 signalling pathways [149]. One way in which *S. Typhimurium* may modify carbon metabolism is via AvrA, a T3SS effector, which mediates p53 acetylation [150]. This downregulation of the glycolytic pathway was also shown in several proteome studies using porcine intestinal mucosa [151,152]. The DIGE-based proteomic approach to the small intestinal mucosa of naturally infected pigs showed that the metabolism of carbohydrates, lipids, small molecules, vitamins and minerals was significantly altered following infection [151] with downregulation of retinal dehydrogenase 1, GAPDH, phosphoglycerate mutase1 and aldolase B, which are involved in gluconeogenesis/glycolysis. In addition, a significant downregulation of mitochondrial ATP synthase and other enzymes involved in the TCA cycle and fatty acid oxidation, such as enoyl-CoA hydratase, aconitase 2, citrate synthase and creatine kinase, was also seen. A study on the caecal intestinal mucosa of piglets infected with virulent or attenuated *S. Typhimurium* strains revealed perturbation in energy metabolism and cytoskeleton-related proteins [152]. In contrast, other studies showed reduced levels of enzymes involved in oxidative phosphorylation and GAPDH [151,153].

Measuring the concentrations of metabolites in the intestine and liver showed that sugar metabolism,

including glucose metabolism (glycolysis, gluconeogenesis and PPP), was affected. At the same time, utilization of other carbohydrates, such as fructose, mannose, galactose, starch and sucrose, was decreased in the liver but not in the intestine. Steroid and eicosanoid hormone metabolism was also disturbed, and significantly elevated levels of many of the metabolites involved in the arachidonic pathway (leading to eicosanoid hormones) and the C21-steroid hormone pathway were detected. Some of these hormones are known regulators of carbohydrate metabolism and immune responses [154].

Profiling extracellular metabolites in the caecal contents post-*S. Typhimurium* infection revealed a significant increase in the abundance of lactate in infected mice and identified oxygen as an alternate electron acceptor for lactate metabolism [155]. This increase was observed due to a shift in host metabolism to lactate fermentation caused by *S. Typhimurium* infection-associated dysbiosis. Such a shift in metabolism generally leads to oxygenation of the intestinal lumen that further supports the growth of *S. typhimurium* [156].

Listeria monocytogenes

The Gram-positive facultative anaerobe intracellular bacterium *L. monocytogenes* causes infections ranging from febrile gastroenteritis to encephalomeningitis, sepsis and abortion. *L. monocytogenes* can reside in *Listeria*-containing phagosomes (SLAPs) [157] or replicate in the cytosol of host cells after phagosome escape. Germ-free mice expressing humanized E-cadherin were infected with either the *L. monocytogenes* wild-type strain or the non-pathogenic *L. innocua* strain, and transcriptome analysis of the intestinal epithelia was performed [158]. Significantly enhanced transcription levels of enzymes involved in glycolysis upon infection were reported, with a 10-fold increase in hexose kinase II (*HKII*) levels. HKII produces G6P which is an important carbon source for *L. monocytogenes* when it resides in epithelial cells or macrophages. A concomitant downregulation of genes involved in glutaminolysis was also seen. Interestingly, only transcription of the genes encoding pyruvate dehydrogenase and citrate synthase was upregulated among the TCA cycle and ETC genes. This suggests that the excess citrate may be used for aspartate and fatty acids biosynthesis. Genes involved in serine and asparagine biosynthesis were upregulated, while genes of the cytochrome P450 family were downregulated. This could indicate reduced xenobiotic metabolism in the host cell upon *L. monocytogenes* infection. None of these metabolic changes were triggered by infection with the non-invasive *inlA*, *inlB* mutant strains, suggesting that virulence genes induced only when the organism within the cell contributes to altered metabolism in the host.

A recent study showed that enhanced mitochondrial respiration in the human HCT116 cell line decreased *L. monocytogenes* infection, and bacterial entry is modified by host cell metabolism [159]. Therefore, *L. monocytogenes* gene expression is modulated by host metabolism, and the organism, in turn, alters host cell metabolic pathways. This bidirectional communication suggests the requirement for newer potential therapeutic targets, as current approaches involve the use of antibiotics which may cause mitochondrial dysfunction and aggravate infection [160].

Shigella flexneri

Shigella flexneri is a Gram-negative bacterium that causes the most communicable of bacterial dysenteries, shigellosis. *Shigella* spp. is transmitted by the faecal–oral route and enter the human body via the ingestion of contaminated food or water. The bacteria are highly infectious since as few as 10–100 cells are sufficient to cause disease [161]. After passage through the stomach and small intestine, the bacteria reach the large intestine, where they establish an infection. *Shigella* uses a T3SS system to translocate effector proteins into the host cell cytosol, allowing it to invade the host cell and evade innate immune responses [162].

There is very little information on metabolic responses to *Shigella* infection *in vivo*, possibly because of the severe damage caused to the intestinal epithelium during infection. A human intestinal colonoid *Shigella* model revealed significantly elevated levels of the branched-chain amino acid transporter SLC7A5, perhaps reflecting a response to depletion in amino acid levels in the cell [163]. *Shigella* targets cells of the colonic crypts at early time points in the guinea pig model [164]. Therefore, this suggests that *in vivo*, *Shigella* infects host cells that are metabolically already prepared for supporting intracellular *Shigella* replication.

Campylobacter spp.

Campylobacter jejuni (*C. jejuni*) are spiral-shaped, Gram-negative bacteria. Campylobacteriosis is the most common type of foodborne bacterial gastroenteritis worldwide. Symptoms can include abdominal cramps, fever and diarrhoea, which can be accompanied by intestinal inflammation. Although *C. jejuni* infection is acute and self-limiting, reactive arthritis, Guillain–Barré syndrome or irritable bowel syndrome (IBS) has been associated with post-infection

complications in some patients [165]. With the help of its flagella, *C. jejuni* penetrates the mucous layer of human IECs, via either transcellular or paracellular routes [166] and colonizes the distal ileum, jejunum and colon [167,168]. Recently, using human colon biopsies, epithelial sodium channel (ENaC) was shown to be impaired in *Campylobacter* infection, leading to malabsorption of sodium which caused diarrhoea in these patients [169].

A metabolomics study from the media of Caco2 cells cultured in a microfluidic device showed distinct metabolomic profiles upon *C. jejuni* infection [170]. Infection led to the upregulation of genes involved in branched-chain amino acid metabolism (leucine, iso-leucine and valine), glycolysis and gluconeogenesis (glucose and pyruvate). Interestingly, such changes were not seen when Caco2 cells were cultured in tran-swells, indicating that fluid flow dynamics and sheer stress impacted host cell responses. This has important implications in extrapolating studies performed on cell monolayers to events that may occur *in vivo*, where intestinal cells are subject to peristalsis and other mechanical forces resulting from food passage along the gut.

Campylobacter concisus is another opportunistic pathogen belonging to the Campylobacter family, and is a commensal present in the oral cavity, oesophagus and lower intestinal tract of healthy humans. The organism has, however, been associated with human diseases such as Barrett's oesophagus, gastroenteritis and IBD [167]. Two different pathotypes [adherent toxigenic *C. con-cisus* (AToCC) and adherent invasive *C. concisus* (AICC)], distinguished by their virulence mechanisms, have been classified to date [167]. RNA-seq analysis of Caco-2 cells upon exposure to these two different strains revealed distinct host responses [168]. Upon infection with the AICC strain, increases in nitric oxide synthase 2 (NOS2), glutathione peroxidase 1 (GPX1) and metallothionein-encoding transcripts were seen, leading to generalized oxidative stress responses in these cells. Genes encoding CYP1A1 and CYP1B1, important for xenobiotic metabolism and fluid transport (NPPB and VIPR1), were upregulated in these cells, while those involved in cholesterol metabolism (SQLE, LDLR, VLDLR, HMGCS1 and INSIG1) were significantly downregulated.

Conclusions and prospects

A summary of the metabolic pathways affected following bacterial infection in the gut is shown in Fig. 3. Bacterial pathogens have devised many ways to evade host defence responses and extract nutrients by remodelling host metabolism. The host responds to infection by altering metabolism to amplify immune responses, fuel cell proliferation and feed the microbiome as a means to mount colonization resistance. Bacteria can generate or import more nutrients that are provided
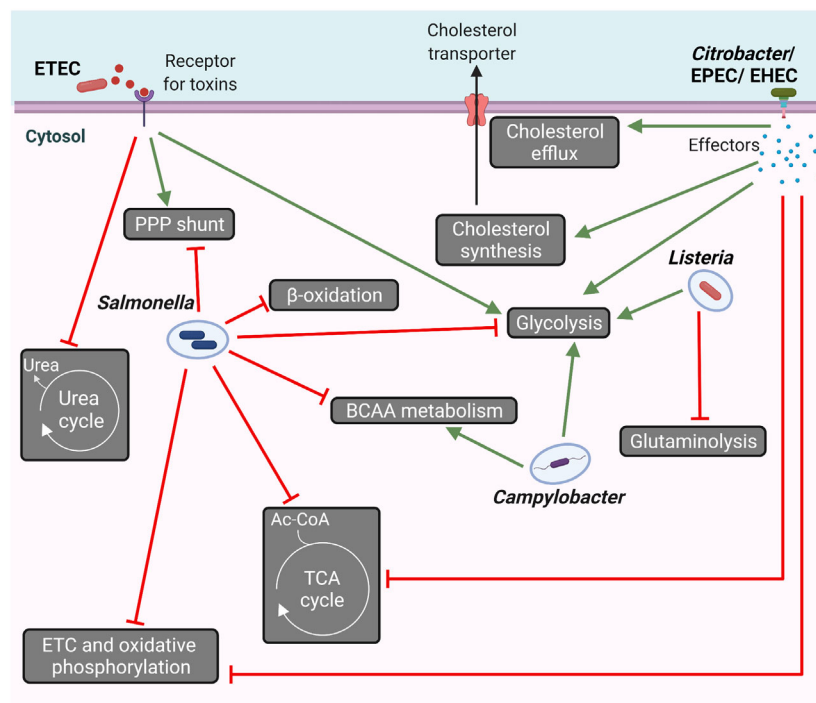

Fig. 3. Host cell metabolic changes in intestinal epithelial cells due to common bacterial infection. An overview of the changes in host metabolic pathways modulated by specific pathogens. Only well-studied examples are shown. The upregulated pathways are marked in green arrows, and red bars denote the downregulated pathways. Abbreviations used are PPP, pentose phosphate pathway; ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; EHEC, enterohaemorrhagic *E. coli*. The figure was created using Biorender.

by this metabolic intervention of the host cells, and this is beneficial for the proliferation and colonization of the pathogen. As shown in Fig. 3, intracellular pathogens inhibit several core metabolic pathways, some due to cell death brought about by these pathogens following infection. In contrast, extracellular and adherent pathogens activate pathways essential for cellular repair, regeneration and nutrient production that the pathogen can utilize to proliferate in the gut.

A limitation in appreciating the range of responses induced by bacteria, specifically in epithelial cells, is the purity of the cell population taken for omics analysis. Epithelial cell preparations from the intestine also contain immune cells present as intraepithelial lymphocytes [171]. The purification process of the EpCAM$^+$ cells [172], either by magnetic sorting or fluorescence-activated cell sorting (FACS), results in significant cell death, and the time taken for the purification would no doubt alter the metabolic states of the IECs. Immune cells undergo substantial metabolic changes during infection [14], and therefore their presence in IEC preparation would no doubt confound the interpretation of changes that occur in IECs.

An approach to ensure a pure epithelial cell population is by infecting intestinal organoids. Traditional organoids generate close cystic architectures, where the lumen, which is the site that is naturally accessible to pathogens, is within the organoid. More recent bioengineering approaches have allowed the construction of tubular intestinal structures, where the lumen is accessible for infection by pathogens [173]. Cells can be harvested from these scaffolds and then be used for metabolomics and transcriptomic analysis. The ability to generate intestinal organoids from induced pluripotent stem cells allows the investigation of responses of human cells to bacterial pathogens [174].

However, many of the host responses in IECs are not directly driven by the pathogen but by cytokines produced during infection. Co-culturing IECs with immune cells in specialized microfluidic devices [175] would be a novel approach to studying the interplay between these two cell types. Alternatively, methods could be employed to fix cells isolated from whole tissue before omics analyses, followed by sorting procedures to separate infected cells from uninfected cells.

It is important to remember that the entire epithelial layer in the intestine renews itself every 4–5 days. Damage to epithelial cells will either be enhanced or reduced depending on the metabolic changes that occur following infection. These could lead to faster cell death or replenishment by the stem cell compartment in the gut. Therefore, long-lasting changes that could affect multiple generations of differentiated

intestinal epithelial cells would need to occur in the stem cell compartment. These metabolic alterations could lead to epigenetic modifications that could impinge on the functioning of differentiated cell types along the gut. Such studies would be of interest to pursue. Indeed, a further understanding of metabolic alterations in IECs and targeting metabolic pathways compromised in host cells may be an effective way of alleviating the damage caused by infection and providing therapeutic approaches in the future.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

All authors contributed to writing, editing and finalizing the manuscript.

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