REVIEW

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Gut permeability, its interaction with gut microflora and effects on metabolic health are mediated by the lymphatics system, liver and bile acid

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There is evidence to link obesity (and metabolic syndrome) with alterations in gut permeability and microbiota. The underlying mechanisms have been questioned and have prompted this review. We propose that the gut barrier function is a primary driver in maintaining metabolic health with poor health being linked to 'gut leakiness'. This review will highlight changes in intestinal permeability and how it may change gut microflora and subsequently affect metabolic health by influencing the functioning of major bodily organs/organ systems: the lymphatic system, liver and pancreas. We also discuss the likelihood that metabolic syndrome undergoes a cyclic worsening facilitated by an increase in intestinal permeability leading to gut dysbiosis, culminating in ongoing poor health leading to further exacerbated gut leakiness.

KEYWORDS
• barrier function • bile acid • gut permeability • liver • lymphatic • metabolic health • microflora

The incidence of obesity, metabolic syndrome and diabetes has risen significantly over the last few decades to epidemic proportions affecting all ages and socioeconomic groups in the developed world [1–3]. In parallel, evidence has been growing to support a causal pathway between diet, the gut microbiota, gut permeability and metabolic dysfunction. Central to this has been the identification, from metagenomic analyses of human fecal samples, of three distinct bacterial enterotypes and their association with obesity [4]. Further evidence has come from animal studies, the link between alterations in the gut microbiota and, more recently, changes in gut barrier function.

The proper functioning of the intestinal barrier is essential to avoid the excessive translocation of inflammatory molecules (e.g., endotoxins such as lipopolysaccharide [LPS]) and their metabolites into the circulation. It has been reported that microbiota can influence the integrity of the intestinal epithelium, functioning of mucosal immunity, and health and disease [5–9], which in turn, can affect gut barrier function and consequently intestinal permeability. Increased leakiness of noxious agents through a defective intestinal barrier can cause excessive activation of the immune system and inflammation [10]. Thus, impaired gut barrier function is associated with the pathogenesis of various intestinal and systemic inflammatory diseases [11,12]. Although disease pathogenesis may be multifactorial, a defective gut barrier function can be the consequence of disease initiation and development [13,14]. This may also be a pathway to poor metabolic health which has been highlighted by the recent report [15] that showed mice fed a high-fat diet had increased gut permeability prior to the onset of obesity. This has prompted us to question how changes in intestinal permeability and microbiota may impact specific organs and normal physiological processes integral to maintain optimal metabolic health [16].

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The objective of this review is to provide a critical assessment of our current understanding of the role of gut barrier function in metabolic health, and to identify knowledge gaps and opportunities for future studies. In the first part we address the factors and some of the mechanisms involved in regulation of gut barrier function. In the second part, we compare the ways in which changes in gut permeability can cause profound effects on metabolic health by influencing remote organs/organ systems: the lymphatic system, liver and pancreas. We also review the changing profiles of the gut microflora in dysbiosis associated with different disease states. In addition, we discuss the likelihood that disease undergoes a cyclic worsening where dysbiosis leads to poor metabolic health resulting in further dysbiosis. Finally, we identify future areas of research, and opportunities to guide development of diagnostics and therapeutics with the overarching goal of promoting metabolic health.

Gut homeostasis depends on gut permeability

The gastrointestinal tract (GIT) is a sophisticated multiorgan system, containing 10^{13}–10^{14} bacteria, as well as digestive enzymes that are capable of degrading most molecules [17]. Although the GIT has been extensively studied for its digestive and absorptive functions, the role that the gut microbes and gut barrier play are currently under increasing scrutiny [18–20] **(Figure 1A)**.

• Tight junctions act as the gatekeepers of the intestinal mucosal barrier

The intestine serves as a selective barrier that consists of the mucus layer and epithelial cell layer. The intestinal epithelial cell layer is made up of two components, the cell membrane, which prevents water-soluble substances from entering the internal environment, and the space between the cells, referred to as the paracellular space [21]. There are two permeability pathways: the transcellular and paracellular pathway. The transcellular pathway is the active absorption and transport of nutrients through the epithelial cell. By contrast, the paracellular pathway is associated with transport in the intercellular space between epithelial cells. This pathway relies on the passive diffusion of solute across the intestinal mucosal barrier, and its functional state depends on the regulation of the tight junctions (TJs) [22]. These TJs act as a seal or gatekeeper, providing a barrier to noxious molecules and a pore for the permeation of ions, solutes and water.

The exact mechanism by which TJs operate is still unknown. However, the discovery of zonula occludens toxin (Zot), an enterotoxin produced by *Vibrio cholerae* that affects the TJs, has provided some insight into the mechanisms of paracellular permeability [23]. Zot has been demonstrated to activate specific proteinase receptor binding and intercellular signaling leading to a reversible opening of TJ [23]. Furthermore, it has been identified that Zot is similar to the human endogenous modulator of epithelial TJs named zonulin [24]. Zonulin has been reported to be involved in intestinal innate immunity [25] and is upregulated in several autoimmune diseases, such as celiac disease and Type 1 diabetes mellitus (T1DM) in which TJ dysfunction has been observed [26].

• Microbes can regulate or disrupt the gut barrier function

An important question arises when it comes to understanding the gut barrier and regulation of intestinal permeability; that is, which factors contribute to an increase in intestinal permeability? Several studies have shed light on the role of microbiota, and it is now well established that the presence of a balanced commensal microbiota contributes to the development of the host innate and adaptive immune system [27], as well as regulating gut barrier function via TJs [28].

Microbiota that symbiotically lives in the intestinal lumen contribute to the maintenance of the intestinal function and health [6,27]. However, pathogenic bacteria that have penetrated the intestines and tissues can disturb the gut barrier function by directly binding to the epithelial cells or by the secretion of toxins [29]. Modulation of intestinal permeability properties is one of the common outcomes of bacterial infection of epithelial cells *in vitro* and *in vivo*, which usually correlates with the induction of diarrhea [30], as well as leading to abnormal electrolytes and fluid transport and subsequently tissue inflammation [25]. Several bacterial pathogens have been shown to modulate epithelial TJs, this is achieved by producing proteins which engage signaling mechanisms in epithelial cells, modulate the actin cytoskeleton

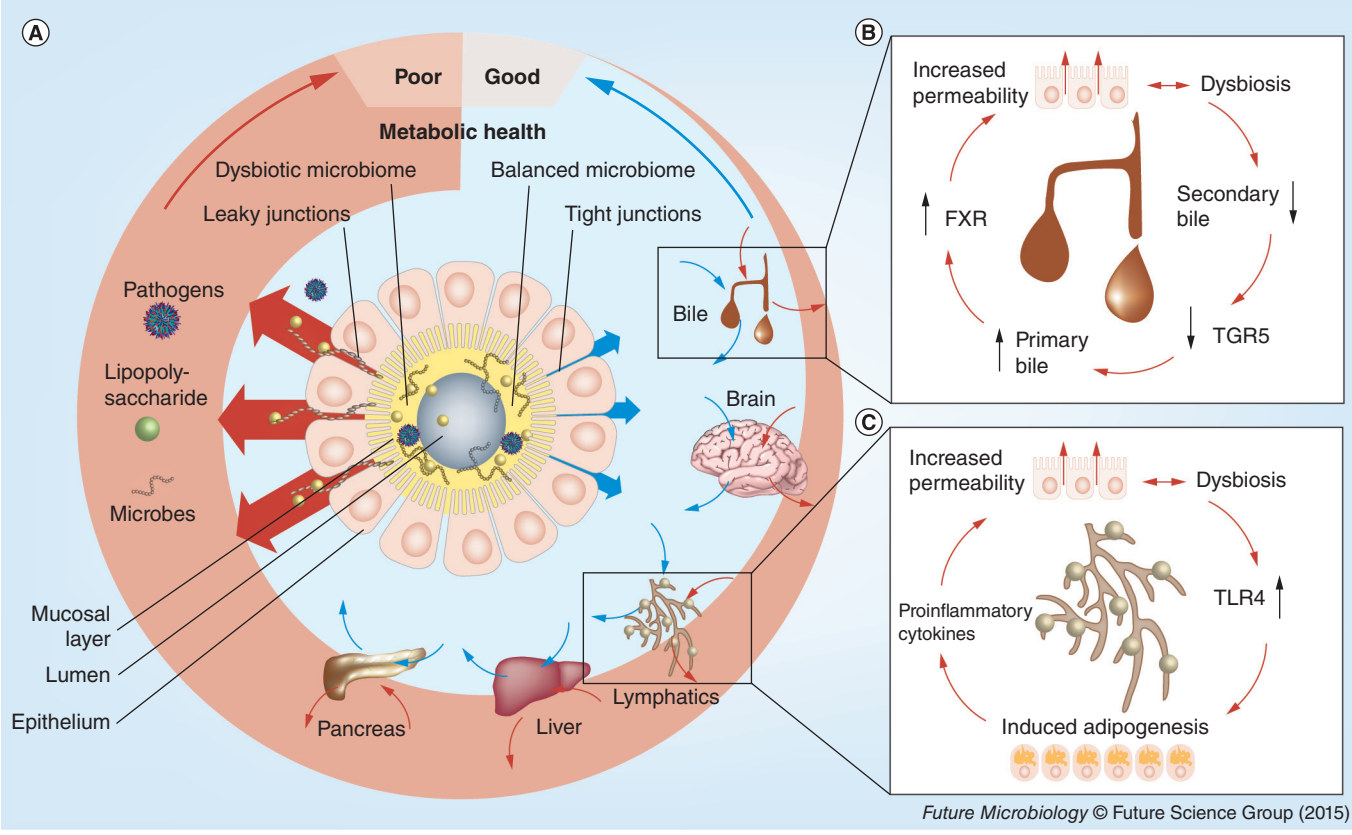

Figure 1. The main figure (A) demonstrates the changes in gut permeability as a result of impaired tight junctions (loss of barrier function), indicated by the red arrows, whereas normal barrier function is manifested by symbiosis and low inflammation, indicated by the blue arrow. The figure also demonstrates the concept of cyclic worsening (indicated by the red arrows) and the profound effects on metabolic health caused by changes in gut permeability by influencing remote organs such as the lymphatic system, liver, bile acid metabolism and pancreas. Subsequent disease progression and development is likely to undergo a cyclic worsening where dysbiosis leads to increased permeability and poor metabolic health leading to further dysbiosis, inflammation and loss of tight junctions. By contrast, improved barrier function leads to symbiosis and reduces inflammation, subsequently better metabolic health outcomes, indicated by the blue arrows. This concept is illustrated in greater detail for two examples: **(B)** Provides a model of cycling worsening focused on bile metabolism. In this proposed model bacterial dysbiosis affects bile metabolism and in turn gut permeability promoting further dysbiosis. Under normal conditions healthy individuals produce a colonic bile acid profile dominated by secondary bile acids, which activates TGR5, promoting gut barrier function. However, in conditions where bacterial dysbiosis has occurred (possibly due to changes in gut permeability) populations of bile acid biotransforming bacteria could be reduced or removed. Examples of this include the loss of *Lactobacillus* and *Bacteroides* species during inflammatory bowel disease or reduction in *Clostridia* during Type 2 diabetes mellitus. **(C)** Provides a model of cycling worsening focused on the lymphatics. In this model, increasing weight gain and body mass, in obese individuals, leads to dysbiosis and increased gut permeability and the release of microbes and their products (e.g., lipopolysaccharide) into interstitial tissue and the lymphatics. The response is an increase in Toll-like receptor 4 and a subsequent induction of adipogenesis and proinflammatory cytokines and the return full circle to further exacerbation of gut leakiness.
For color figure, pelase see online at http://www.futuremedicine.com/doi/full/10.2217/FMB.15.54

or degrade TJ proteins [30]. These findings suggest that opportunistic pathogens can impair the gut barrier function and thereby facilitate the translocation of luminal bacteria into the systemic circulation, which in turn exacerbates the systemic infection, also known as cyclic worsening **(Figure 1A, B & C)**.

Enteric infection with *V. cholerae* and other Gram-negative bacteria, such as pathogens belonging to the genus *Arcobacter*, have been shown to compromise gut barrier function [31]. *V. cholerae* produces several toxins, such as cholerae toxin, hemagglutinin/protease and Zot, which has been reported to disrupt barrier

function [32]. The hemagglutinin/protease produced by *V. cholerae* is a zinc-binding metalloprotease that disrupts the intestinal barrier through TJ degradation [33] by cleaving the extracellular domain of occludin, resulting in the dissociation of zonula occluden-1 from the intracellular domain of occludin [34]. Whereas, Zot has been shown to decrease transepithelial resistance (TER) in small intestines of rats, rabbits and intestinal Caco-2 cells [35].

Infection with either enteropathogenic *Escherichia coli* (EPEC) or enterohemorrhagic *E. coli* also diminishes epithelial barrier function [16]. EPEC disrupts the barrier through direct binding to the intestinal T84 cells and induces paracellular hyperpermeability as measured by TER [36]. Dean and Kenny demonstrated that the EPEC-induced barrier disruption is mediated by *E. coli*-secreted protein F and mitochondrial-associated protein [37]. Enterohemorrhagic *E. coli*, also known as verotoxin-producing *E. coli* or Shiga toxin-producing *E. coli* [38], has been shown to disrupt the TJ barrier with redistribution of zonula occluden-1 and occludin proteins in T84 intestinal cells [39].

Clostridium perfringens, a Gram-positive, anaerobic, spore-forming bacterium is a common cause of food poisoning [40]. It has been demonstrated that *C. perfringens* causes increased intestinal permeability in both cell cultures and animal models as measured by TER [41]. *Salmonella* species, also a major cause of food poisoning, have been shown to increase intestinal permeability, as demonstrated by dysfunction of TJs [42]. Other food-borne pathogens such as *Listeria monocytogenes*, disrupts the gut barrier function in HT-29/B6 colonic cells [43]. In addition, *Staphylococcus aureus*, another common food-borne pathogen, produces the prominent virulence factor alpha-toxin which has also been shown to impair TJ integrity in Caco-2 cells [29].

Rotavirus, reported to be one of the most common causes of gastroenteritis in children, has been shown to increase paracellular permeability in a cell model [44], whereas inconsistencies have been demonstrated in animal studies with [45] and without an associated increased intestinal permeability [46], suggesting further research in this area are warranted. However, only one human study has reported that rotavirus increased intestinal permeability in children and an improvement in intestinal function with probiotic treatment [47].

• Increased intestinal permeability causes cyclic worsening of endotoxemia & inflammation

Impairment of one or several components of the gut barrier function (mucus layer, epithelial cells with their TJs and the mucosa-associated lymphoid tissue) may enhance and sustain systemic inflammation by facilitating bacterial translocation systemically and higher plasma endotoxins (such as LPS), known as endotoxemia. Furthermore, systemic and localized inflammation has been reported to increase intestinal permeability [48]. There is evidence to suggest that obesity and metabolic disorders are linked to chronic low level systemic inflammation [10], possibly due to increased intestinal permeability in obese individuals as reported by Teixeira *et al.* [13].

Conversely, the administration of LPS in healthy subjects resulted in endotoxemia and a fivefold increase in intestinal permeability and this is associated with inflammation [49]. This is also consistent with O'Dwyer *et al.* who found that a single dose of endotoxin increases intestinal permeability in healthy individuals [50]. However, Jorgensen *et al.* reported that there was no effect of intravenous endotoxin, observing no change in colonic permeability, suggesting that this part of the gut is relatively resistant to the systemic inflammation [51]. Nevertheless, it seems plausible that during systemic inflammation, impaired gut barrier function occurs in the whole intestinal tract. Consequently, a vicious circle of inflammation, increased intestinal permeability, leading to toxic mediators release, resulting in a further increase in intestinal permeability and the cycle is repeated [49] **(Figure 1B & C)**.

Hyperpermeability & microbial dysbiosis: its effects on organ systems

• Gut permeability & mesenteric lymphatics: the intestine's drainpipe

The mesenteric lymphatic system plays an integral role in removing fluid, inflammatory cells and molecules, and microbial debris from the abdominal viscera. The lymphatics also have an active role in immunity; transporting immune cells to their site of activity [52]. As outlined in a recent review [53] and here there is accumulating evidence to suggest that a disrupted lymphatic system may contribute to and exacerbate weight gain, obesity, metabolic disruption, inflammatory bowel disease (IBD) and

microbial dysbiosis. In particular, the structure of the lymphatic vasculature plays an important role in the function of the gut [54]. Nearly half of the human body's lymph is produced from the abdominal viscera [54], hence elevated gut permeability will have a significant impact on lymphatic function in this region. We propose that elevated gut permeability and microbial dysbiosis can significantly impact normal lymphatic function contributing to weight gain and poor metabolic health.

We have recently proposed that the 'obesogenic microflora' acts via increased energy harvest from food or via induction of chronic inflammation [16]. However, the role that the lymphatic system plays in weight gain in these circumstances has received little attention. The mesenteric lymphatic system is the primary 'drain pipe' of the gut and has a high exposure to invading microbes and an active role in response to microbial invasions. There is evidence that lymphatic architecture is regulated by and responds to microbes and their byproducts [55]. Vascular endothelial cells express a suite of Toll-like receptors (TLRs) that can respond to microbial components including LPS [56]. In both low and high concentrations, LPS can regulate lymph flow rates [57]. In cultured human lymphatic microvascular endothelial cells, LPS induces production of an array of proinflammatory cytokines (IL-6, IL-8 and VCAM-1 and I-CAM) in response to TLR-4 activation, which promotes inflammation [52] **(Figure 1C)**. The effect of acutely and chronically elevated LPS on lymphatic function, observed in a number of pathologies, including IBD, obesity and metabolic dysfunction, is yet to be discerned and further research is required in this area. We proposed that a shift in the microbiota toward dysbiosis may mediate their effects on weight gain [16], possibly via impacts on the lymphatic system. This may be due to mediating changes in the architecture, inflammatory levels or lymph flow rates that result in an increased pressure on the lymphatic system, elevated lymph stasis and as a result, in adipogenesis. One study in mice demonstrated that a high-fat diet increases the translocation of bacteria from the gut into the mesenteric adipose tissue prior to the onset of diabetes and that within 4 weeks, at the onset of diabetes, bacterial DNA levels are elevated in both the mesenteric adipose tissue and mesenteric lymph nodes [58].

• Disrupted systemic lymphatic structure & function: a cause of weight gain

Lymph contains an adipogenic factor, that when static induces adipogenesis [59]. Hence, factors that contribute to lymph stasis can induce adipogenesis. In mouse models, disruption of lymphatic architecture can cause weight gain and obesity [59]. *Prox1* heterozygous mice have disorganized lymphatic architecture, which causes increased leakage of lymph from lymphatic vessels into the surrounding tissue leading to lymph stasis. This lymph stasis promotes fat accumulation in existing adipocytes and adipogenesis causing weight gain and adult onset obesity in *Prox1* heterozygotes [59]. In humans, cauterization of lymphatic vessels during surgery results in adipose deposition in the immediate area [60]. A recent study has also provided evidence that the integrity (permeability) of lymphatic vessels is an important component to the regulation of adipose accumulation [61]. Mice lacking apelin, a regulator of the integrity of lymphatic vessels, fed a high-fat diet displayed increased size and fat pad mass compared with wild-type controls. Inspection of subcutaneous adipose of apelin knockout mice revealed larger adipocyte size, higher infiltration by macrophages organized in crown like structures and hyperpermeability of lymphatic vessels relative to wild-type controls [61]. Conversely, K14-apelin transgenic mice, overexpressing the apelin cDNA, had smaller lymphatic diameters, reduced permeability of lymphatic vessels and reduced weight gain compared with wild-type controls when fed a high-fat diet [61]. Hence, decreasing stresses on the lymphatic system, by promoting intestinal integrity or by increasing lymphatic integrity (possibly by targeting apelin) may improve metabolic health [61].

Rat models have shown that lymphatic remodeling occurs in metabolic syndrome affecting lymphatic function, reducing lymph load capacity and the intrinsic contractility of the lymphatic vascular impairing the ability to remove lymph from the tissues [62]. In obese individuals, the lymphatic drainage of macromolecules in visceral and subcutaneous adipose is significantly decreased compared with lean individuals, causing inflammation and adipogenesis as inflammatory molecules persist for longer time periods [63]. Furthermore, in lymphedema, a lymphatic obstruction, an increase in adipose deposition occurs in poorly drained areas [64]. Lymphatic remodeling occurs in Crohn's disease

(CD) and ulcerative colitis and is associated with elevated gut permeability [65]. There is growing evidence of the role of lymphatic structure and function in adipogenesis, however, further research is required to understand the association with gut permeability.

• Is visceral adipose accumulation a protective mechanism against the leaky gut?

Adipose is a heterogeneous tissue comprised of mature adipocytes, preadipocytes, immune cells (macrophages and leukocytes), as well as endothelial cells and fibroblasts [66]. It is not only a depot for energy storage, but a tightly regulated active endocrine organ. In healthy individuals, visceral adipose and adipogenesis may be a protective response to gut microbial dysbiosis. Indeed in murine models, high LPS/endotoxemia increases the expression of the inflammatory adipokines IL-6, MCP-1 and TNF-α in adipose tissue [67] and chronic stimulation induces adipogenesis in the lymph node-associated fat pad providing an energy reserve to combat infection [68]. A recent study demonstrated an increase in visceral adipose deposition with gut leakiness in healthy women, suggesting a protective role of healthy visceral adipose in response to gut leakiness [69]. In CD, associated with enhanced gut permeability and elevated LPS, an increase in adipose deposition is also observed. An increase in the number of adipocytes (of small size) surrounding the intestines occur, this is commonly referred to as fat wrapping or creeping fat [70]. This adipose is suggested to play a protective role acting as a barrier against the elevated bacterial endotoxins exposure from the leaky gut limiting intestinal inflammation [70], however, a pathological role of this adipose has also been suggested [53].

In obesity, adipose tissue is considered 'dysfunctional' as adipocytes are hypertrophied and visceral adipose has high levels of oxidative and hypoxic stress, fibrosis and has a higher number of macrophages and crown-like structures [71]. This dysfunctional adipose may have impeded protective response, responding differently to microbial stimuli and signaling, potentially exacerbating disease or resulting in cyclic worsening of disease. We note that these metabolic consequences may not occur in CD as the adipose is structurally different and CD patients often suffer from poor nutrient absorption which could restrict adipose deposition.

The gut microflora & permeability influence liver health

As the liver receives 70% of its blood supply from the large and small intestines via the hepatic portal vein. its resident macrophages, Kupffer cells, are well equipped to deal with 'garbage disposal' of microbes and their products that may have escaped the gut [72]. However, in obesity the gut microflora is altered and the gut barrier function is compromised. We hypothesize that these aberrations combine to compromise the health and functioning of the liver by exposing it to excessive amounts of gut microbes and their byproducts.

The liver is under considerable strain in patients suffering from obesity or metabolic syndrome. For instance, it has been shown that liver function enzymes, aspartate aminotransferase and alanine aminotransferase, are elevated in patients with metabolic syndrome and T2DM. These findings suggest that the relationship between obesity and T2DM may occur through liver disease [73]. Further, disruption of the mucosal barrier results in the translocation of microbial products that also contribute to liver disease by inducing hepatic inflammation [74]. It has been demonstrated that acute liver injury is associated with an early onset of increased intestinal permeability and bacterial translocation that precedes changes in the microbiome [74]. As microbes that enter the liver, once processed, are then either shunted back to the small intestines via the bile or enter peripheral and enterohepatic circulation through metabolic processes, we suggest that this 'recycling affect' exacerbates obesity-induced liver dysfunction in the obese. Furthermore, a recent report has indicated that specialized T-lymphocyte subset (mucosal-associated invariant T cells), that recognizes bacterial ligands and are found throughout the mucosal surfaces and the liver, have been shown to increase with obesity [75].

Nonalcoholic fatty liver disease (NAFLD), a condition where fat is deposited in the liver via steatosis and is associated with obesity, metabolic health syndrome [76–78] and T2DM through insulin resistance [79]. There is mounting evidence that NAFLD could be considered as a new criterion to define metabolic syndrome. NAFLD patients are characterized by increased serum levels of cytokines typical of obese and T2DM patients [80], including the role of adiponectin in obesity-related NAFLD [78]. In humans, NAFLD is associated with increased

gut permeability and this abnormality is related to the increased prevalence of an extreme version of gut microflora dysbiosis; small-intestinal bacterial overgrowth [82]. The increased permeability appears to be caused by disruption of intercellular TJs in the intestine, and it may play an important role in the pathogenesis of hepatic fat deposition [82]. We conclude that alterations in gut microbiota, increased intestinal permeability and metabolic endotoxemia likely play a role in the development of a chronic low-grade inflammatory state in the host that contributes to the development of obesity and associated chronic metabolic diseases, such as NAFLD.

NAFLD can progress into the more serious condition of nonalcoholic steatohepatitis, which is regarded as the major cause of liver cirrhosis through hepatic inflammation and fibrosis. Liver cirrhosis is an extreme pathologic end stage of chronic liver disease, which disturbs intestinal microbiota and innate immunity-related genes, and which contributes to endotoxemia and bacterial translocation [83]. Pathogenic bacteria, such as those of the genera *Enterobacteriaceae* and *Streptococcaceae*, have been shown to increase in abundance, while there is a reduction of beneficial populations such as *Lachnospiraceae* in patients with cirrhosis [84]. Similar to NAFLD, small-intestinal bacterial overgrowth has also been identified to be present in patients with cirrhosis [85]. In fact, cirrhotic patients are at considerable risk of bacterial infections, and it has been concluded that increased intestinal permeability may be a factor contributing to infection risk [86]. There are multiple hypotheses for a causative role for gut microflora as a part of the gut/liver axis in liver disease. For example, gut microflora may cause pathogenesis of NAFLD via any or all of three mechanisms: increase in ethanol production in the gut; metabolism of dietary choline or distribution of LPS (reviewed by [87]). Overall, there is strong evidence that intestinal dysbiosis and the translocation of bacteria and their products across the epithelial barrier drives liver disease progression, which in turn may further increase gut permeability leading to a cyclic worsening of the disease state (see **Figure 1**).

Bile acid dysbiosis: a regulator of gut permeability & metabolic health

Bile acid is an important component of digestion. The end product of cholesterol catabolism [88], formed in the liver where it is conjugated with either glycine or taurine and then stored in the gall bladder and released into the intestinal lumen after eating [89]. Classically, bile acids were considered an emulsification agent necessary for the absorption of lipids and lipid-soluble vitamins [90]. However, research over the last 15 years has established bile acid as a hormone in its own right, playing roles in immune function, glucose homeostasis and bile acid metabolism [90,91].

Bile acids can be classified into primary and secondary types. Primary bile acid is released into the intestinal lumen and is taken up by high affinity transporters in the small intestine [92]. Approximately 5% of the bile escapes this process [93] and moves into the large intestine where it is exposed to large populations of bacteria [91]. In the large intestine the primary bile acid becomes a substrate for bacterial enzymes that deconjugate, oxidate and dehydroxylate the bile acids resulting in the formation of secondary bile acids [94]. The level of secondary bile acid biotransformation in healthy individual feces, is greater than 95% [92]. Germ-free mice, which lack a gut microflora, have >98% primary bile in fecal samples [95], demonstrating the importance of the intestinal bacteria in the biotransformation to secondary bile acids. The majority of bile acid biotransformation is performed by anaerobes of the genera *Bacteroides*, *Eubacterium*, *Clostridium* as well as potentially *Lactobacillus* [96].

Bile acid receptors include the pregnane-X-receptor, vitamin-D receptor, constitutive androstane receptor, farnesoid-X-receptor (FXR) and G protein-coupled bile acid receptor 1 (GP-BAR1, also known as TGR5) [97]. Of these receptors TGR5 and FXR have been most intensively studied. Although FXR and TGR5 display affinity for both primary and secondary bile acids they do so with differing affinities and it is known that FXR is most strongly activated by primary bile acids and TGR5 is most strongly activated by secondary bile acids [98]. It is generally accepted that the affinities these receptors display reflect their biological ligands [97].

It is now well established that bile acids play a role in intestinal inflammation and permeability [99–102] but the exact nature is poorly defined, however recent studies are beginning to shed light on the subject. Suzuki and Hara [100] and Stenman *et al.* [99] have shown that high-fat diets increased intestinal permeability and both groups produced evidence supporting their

hypothesis that increased bile production mediated this change in barrier function. The later study went further attributing the increase in permeability to increased expression of FXR in the intestine. Conversely, TGR5 has been implicated in maintenance of normal intestinal homeostasis, it was shown that its absence increases susceptibility to developing colitis and that its activation can attenuate colon inflammation in a rodent model of colitis [102]. Further to this work, in human cell lines, TGR5 has been shown to play a role in inhibiting the production of CD-associated proinflammatory cytokines [101].

With an understanding of the different functions of FXR and TGR5 as well as the knowledge of their substrate specificity (FXR: primary bile acids, TGR5: secondary bile acids) we and others [103] propose a model whereby bacterial dysbiosis affects bile metabolism and in turn gut permeability further promoting dysbiosis. Under normal conditions healthy individuals produce a colonic bile acid profile dominated by secondary bile acids, which activates TGR5, promoting gut barrier function. However, in conditions where bacterial dysbiosis has occurred (possibly due to changes in gut permeability) populations of bile acid biotransforming bacteria could be reduced or removed. Examples of this include the loss of *Lactobacillus* and *Bacteroides* species during IBD [103] or reduction in *Clostridia* during T2DM [104]. Under such circumstances primary bile acid levels would rise in the colon increasing gut barrier permeability, promoting further inflammation, further dysbiosis and as such generating a feedback loop with the potential to lead to various pathologies as summarized in **Figure 1A, B & C**. This model is much the same as that proposed by Duboc and colleagues [103]. Across two studies, Duboc *et al.* [95,103] produced compelling evidence that linked bacterial dysbiosis and bile acid dysmetabolism (specifically a shift toward more colonic primary bile acids) and the inflammation seen in IBD in human patients. These researchers propose the shift from a bile acid profile dominated by secondary bile acids to one dominated by primary bile acids as generating an 'inflammation loop' which plays a major role in IBD **(Figure 1B)**. This is yet another example of where shifts in gut permeability and microbial composition can lead to a cyclic worsening leading to poor metabolic health outcomes.

Role of the gut barrier & dysbiosis in the healthy functioning of the pancreas

The pancreas is a major organ that is fundamental to metabolic health and while it is distinct from the GIT, the pancreas is nonetheless sensitive to changes in gut integrity and to changes in the gut microbiota **(Figure 1A)**. The pancreas has two very important roles in the maintenance of metabolic health and disruptions to pancreatic function has severe effects on metabolic health. The pancreas functions as a major exocrine gland in producing key enzymes for the digestive system including peptidases, lipases and amylase [105]. The pancreas also functions as an endocrine gland, producing hormones such as insulin and glucagon. In fact, obesity is known to induce insulin resistance due to a dysfunctional glucose transporter 4 and this is the major driver of metabolic syndrome. Since insulin resistance precedes the development of T2DM, it can therefore be viewed as the driving force for our current diabetes epidemic. The precise causes of insulin resistance are varied, but amongst several likely causes a role has been identified for the gut microbiota [106]. Here we explore the impacts of dysbiosis in the gut microflora and alterations to gut permeability on these important roles of the pancreas and hence on pancreatic and metabolic health.

The ability of the gut microbiota to enhance energy harvest from the diet is emerging as an important factor in the pathogenesis of obesity and T2DM [16]. Animal and human studies have gone as far as describing this microbiota as 'obesogenic' [107] and modulation of these factors improves glucose homeostasis, leptin sensitivity and target enteroendocrine cell activity in obese and diabetic mice [108]. This is supported by more recent observations by Vrieze *et al.* [109] where the transfer of intestinal microbiota from lean donors increased the insulin sensitivity in recipients with metabolic syndrome [109].

Early animal studies showed that a relationship existed between the production of digestive enzymes in the pancreas and the presence or absence of caecal microflora [106]. This suggested that regulation of pancreatic exocrine function depended in part on signaling from the caecal microflora or their fermentation products. It is not known if changes in microflora elsewhere in the GIT are also associated with changes in digestive enzyme production but if so, this could represent a general mechanism for signaling between the GIT microflora and the

pancreas. Even if this is not the case, elevated gut permeability can be expected to also disrupt the normal exocrine functioning of the pancreas. There is evidence that this happens via unregulated translocation of bacteria from the gut to the pancreas, followed by endotoxemia and inflammation of the pancreas that results in pancreatitis [110]. In addition, alcoholic endotoxemia, due to increased gut permeability, has been reported [111] to trigger overt necroinflammation of the pancreas.

• Type 2 diabetes mellitus

Increasing evidence has shown an association of the intestinal permeability and T2DM which is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. Importantly, in a diabetes model [112], mice fed a high-fat diet showed increased translocation of live Gram-negative bacteria through intestinal mucosa to blood and mesenteric adipose tissue. Accumulating evidence has indicated that quantitative and qualitative differences in gut microbiota exist between T2DM and nondiabetic individuals and that a causal relationship is likely to exist. In fact, it has been suggested that T2DM can be viewed as a form of gut bacterial dysbiosis [113]. Fecal microbial communities of T2DM patients were examined by quantitative PCR and pyrotagging sequencing and it was found that T2DM is associated with structural changes of the intestinal microbiota [104]. These authors also showed that the relative abundance of *Firmicutes* was significantly lower, while *Bacteroides* and *Proteobacteria* were higher in the diabetic patients compared with their nondiabetic controls. In a separate study [112], metagenome sequencing and functional analysis of gut microbes demonstrated that in T2DM patients there was a decrease of butyrate-producing bacteria and an increase of opportunistic pathogens. Unraveling the mechanism has not yet been completed but it has been shown that binding of LPS to TLR-4 exerts deleterious effect on pancreatic β-cell function [114]. LPS inhibited β-cell gene expression in a TLR4-dependent manner and via NF-κB signaling in pancreatic islets, suggesting that gut microbiota might indirectly affect pancreatic B-cell function.

• Type 1 diabetes

By contrast, in T1DM, there is an absolute insulin deficiency due to destruction of islets of Langerhans. T1DM is an autoimmune disorder resulting from T-cell-mediated destruction of insulin-producing beta cells within pancreatic islets and results in an inability to lower blood glucose levels and its incidence can be affected by the microbial environment [115]. Increased intestinal permeability has been reported both in spontaneous animal models of T1DM and in human T1DM [116]. Given that increased intestinal permeability precedes clinical onset of T1DM, this suggests that the small intestine is an organ participating in the pathogenetic process of T1DM [117]. Furthermore the progression of disease has been shown to involve regulation of the TJ protein, zonulin [26]. These authors tested serum samples from the pre-T1DM phase and showed that elevated serum zonulin was detected in those patients who went on to develop T1DM. The authors concluded that zonulin upregulation preceded the onset of T1DM, and was the link between increased intestinal permeability and the development of autoimmunity in genetically susceptible individuals.

It was also demonstrated that prediabetic NOD mice display increased intestinal permeability and that young NOD mice infected with wild-type *Citrobacter rodentium* suffered intestinal barrier disruption that accelerated development of insulitis [20]. A recent study [118] has also shown that the structure of the gut microbial community changed in children with T1DM and that the quantities of microbes essential to maintain gut integrity were lower in the T1DM children relative to healthy children.

It has been recently reported [119] that a new class of drugs, microbiome modulators that alter microbial populations and their environment in the GIT improve glucose tolerance in adults with prediabetes. Since dysbiosis in the gut contributes to changes in glucose levels in the development of T2DM [120], alternating changes to the microbiome may promote a cyclic worsening and the onset of diabetes.

Conclusion

We have assessed the evidence that metabolic dysfunction can result in altered gut permeability and can have much wider ranging effects on health. We propose that increased gut permeability is the central driver of metabolic health. We further discuss how increased gut permeability and changes in microbiota

can impact the function of different organ systems including the lymphatics, liver and bile acid metabolism and the pancreas. Modulating gut permeability and microbiome represent an avenue for improving metabolic health. The cyclic nature of these detrimental effects further exacerbates metabolic dysfunction and consequently leads to the progressive degradation of metabolic health. Finally, we identify future areas of research, and opportunities to guide development of diagnostics and therapeutics that promote metabolic health. In this fast evolving field, it has not always been easy to clearly distinguish between correlation and causality. However, as more direct evidence is gained from human trials the links will gain greater gravitas.

Future perspective

• Novel biomarkers of TJ barrier function for assessing & monitoring gut permeability & the impact of metabolic health

A better understanding of the molecular mechanisms underlying TJ regulation will lead to the development of effective therapeutic and preventative approaches against diseases such as metabolic syndrome, diabetes and obesity, which are associated with compromised intestinal barrier. These steps may include identification of new markers of gut leakiness, for example, TJs proteins or intracellular modulators thereof present in either blood or urine, for example zonulin.

• Make maintenance of the intestinal barrier function a strategy to treat or prevent diseases

Foods that may cause intestinal inflammation and leaky gut should be avoided and impaired digestive mechanisms should be treated with digestive aids. The mucosal lining of the gut should be rebuilt with foods and supplements, and the gut should be repopulated with beneficial flora to correct dysbiosis.

• Critical organs involved in regulating metabolic health may be used as indicators to characterize poor metabolic health profiles, in identifying at-risk individuals & for monitoring metabolic health in response to interventions & treatments

Treatments that promote intestinal integrity and modulate the lymphatic architecture (e.g., apelin) may positively benefit metabolic health as increased gut permeability and changes in microbiota can impact the function of different organ systems including the lymphatics, liver and the pancreas. Treatments that modulate the microbiome represent an avenue for improving metabolic health. Consideration should be given to measuring bile acids and bile acid receptors (e.g., FXR, TGR5) in obesity and its effect on gut permeability. Potential markers of disease can be investigated in such ways as profiling bile acid ratios, lymphatic function, microflora and permeability in different disease states. Furthermore, we will need to take what we know from animal studies and translate this into better human studies. Such translation will require integrated genomic, transcriptomic assays (of both the host and microbiome) in combination with metabolomic analysis to fully understand this complex interrelated research area.

• Other important areas of future research directions include:

That obesogenic microflora may mediate weight gain via the lymphatics?

A number of recent studies have associated changes in the gut microflora with obesity, that these can be termed 'obesogenic microflora'. When feces were transplanted from obese mice into germ-free mice, a significantly greater increase in total body fat compared with mice colonized with the microbiota from lean donors was observed [121]. Furthermore, these ob/ob mice have a distinct shift in microbial composition with a 50% reduction in the abundance of *Bacteroidetes* and a proportional increase in *Firmicutes* compared with wild type [122]. It has been observed that ob/ob mice have enhanced intestinal permeability and associated metabolic endotoxemia [123], in other words, high levels of circulating LPS derived from Gram-negative bacteria. The lymphatic system remains a crucial factor yet to be assessed in this phenomenon.

That visceral adipose accumulation is a protective response to elevated gut permeability?

Visceral adipose tissue is not only an energy store, but a tightly regulated and active endocrine organ. Adipocytes, preadipocytes and macrophages (components of adipose tissue), express the LPS recognition receptor TLR4 and as such can respond to microbial challenges [66,124]. In

healthy individuals, visceral adipogenesis may be a protective response to chronic inflammation caused by elevated gut permeability and gut microbial translocation. This is supported by a recent study in which an increase in visceral adipose deposition was associated with gut leakiness in healthy women [125].

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EXECUTIVE SUMMARY

Gut homeostasis depends on gut permeability

- The mucosal barrier acts as a seal or gatekeeper, providing a barrier to noxious molecules and a pore for the permeation of ions and solutes.
- Microbiota that symbiotically lives in the intestinal lumen contribute to the maintenance of the intestinal function and health.
- Pathogenic bacteria that have penetrated the intestines and associated tissues can disturb the gut barrier function by directly binding to the epithelial cells or by the secretion of toxins.
- A vicious circle of inflammation, increased intestinal permeability, toxic mediators release, resulting in a further increase in intestinal permeability results in what we term 'cyclic worsening.'

Hyperpermeability & microbial dysbiosis: its effects on organ systems

- Gut permeability & mesenteric lymphatics: the intestine's drainpipe
  - Increased gut permeability and microbial dysbiosis can significantly impact normal lymphatic function contributing to weight gain and poor metabolic health.
  - Lymphatic remodeling occurs in metabolic syndrome affecting lymphatic function, reducing lymph load capacity and the intrinsic contractility of the lymphatic vascular impairing the ability to remove lymph from the tissues.
- The gut microflora & permeability influence on liver health
  - Disruption of the mucosal barrier results in the translocation of microbial products that also contribute to liver disease by inducing hepatic inflammation.
- Bile acid dysbiosis: a regulator of gut permeability & metabolic health
  - Bile acids plays a role in permeability and intestinal inflammation. Increase in permeability is attributed to increased FXR expression in the intestine and TGR5 has been implicated in maintenance of normal intestinal homeostasis.
- Role of the gut barrier & dysbiosis in the healthy functioning of the pancreas
  - Increases in gut permeability triggers bacterial translocation from the gut to the pancreas which results in endotoxemia and inflammation of the pancreas and causes pancreatitis.
  - Dysbiosis has been suggested to play a role in the development of T2DM.
  - Increased intestinal permeability, due to upregulation of zonulin, has been shown to precede clinical onset of Type 1 diabetes mellitus in genetically susceptible individuals.

Conclusion

- Increased gut permeability and changes in microbiota can impact the function of different organ systems including the lymphatics, liver and the pancreas.
- Modulating gut permeability and microbiome represent an avenue for improving metabolic health.

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