VKP 27. 4. 2018

STŘEVNÍ MIKROBIOM VE ZDRAVÍ A NEMOCI

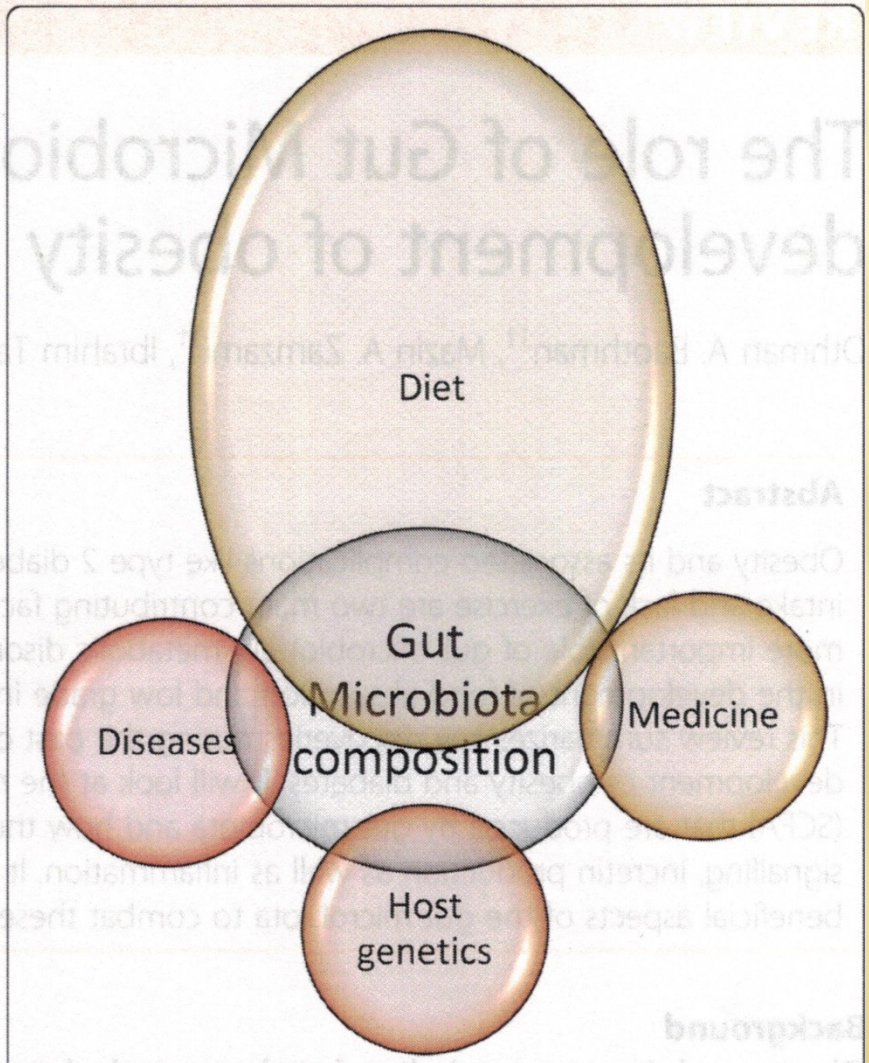


Fig. 1 A diagram showing main factors affecting the gut microbiota composition highlighting the great impact of diet on this composition

EVOLUTION

- Evidence suggests that human evolution, which has taken billions of years of continual interaction with our environment, played a major role in the way we have evolved. Among the environmental factors, intestinal microbes have conferred numerous metabolic and biological functions that we are unable to perform by our own cells.
- Recent data estimate that humans are colonized by trillions of microbes, and the vast majority of them reside in our gut. This tremendous number of microbial cells represents a ratio of approximately 1:1 between human and microbial cells, or even 1:10 if we take into account only the number of human nucleated cells (i.e., excluding red blood cells).

GUT MICROBIOTA

- Gut microbiota describes all organisms living in the gastrointestinal (GI) tract.
- The majority of these organisms reside in the large intestine. These bacteria play important physiological role in vital processes such as digestion, vitamin synthesis and metabolism amongst others. Even though the exact mechanism linking gut microbiota to obesity is far from being very well understood, it's well established that gut microbiota can increase energy production from diet, contribute to low-grade inflammation and regulate fatty acid tissue composition. These processes as well as others have been proposed as the link between obesity and gut microbiota.

GUT MICROBIOTA

- The exact contribution of gut microbiota to the development of obesity and diabetes is not very clear due to many reasons including the complexity and diversity of gut microbes, ethnic variation in studied populations and large variations between individuals studied.
- Modulation of gut microbiota holds a tremendous therapeutic potential to treat the growing obesity epidemic especially when combined with diet and exercise.

MIKROORGANISMY VE STŘEVĚ

- Gut microbiota harbors a vast number of genes that clearly outnumbers our own genome by at least 100-fold. This vast catalog of genes encodes for specific metabolic activities, allowing microbes to adapt to their environment and eventually the energy sources available. Hence, the gut microbiota is considered a massive "organ" able to perform complex functions and thereby produce a myriad of different metabolites.
- Numerous publications have found an association between the microbiota and many diseases (e.g., obesity, diabetes, liver diseases, altered immunity, digestive diseases, cancer, neurodegenerative disorders), but the exact role of the gut microbiota in the onset of diseases remains a matter of debate.

MIKROORGANISMY VE STŘEVĚ

- The microbial diversity (i.e., species richness of the microbiota) is another concept that has been linked with the metabolic functions of the gut bacteria. Indeed, low bacterial richness is consistently appearing in the literature as a risk factor for different diseases (e.g., obesity, low-grade inflammation, intestinal inflammation).
- Aside from the microbial diversity, evidence also suggests that we can classify subjects on the basis of the number of bacterial genes that they harbor in their gut (i.e., microbial gene richness). More precisely, Le Chatelier et al. identified a bimodal distribution of microbial genes leading to the clustering of subjects as either low gene count or high gene count according to the number of genes present in the microbiota. This also seems to be important for the susceptibility to respond to dietary intervention devoted to improving metabolic parameters, since dietary restriction in patients with overweight or obesity is less efficient in low gene count than in high gene count individuals in terms of improving insulin sensitivity and lowering cholesterol and inflammation.

FACTORS AFFECTING GUT MICROBIOTA COMPOSITION

- Composition of gut microbiota is affected by many factors such as diet, disease state, medications as well as host genetics to name a few. As a result, the composition of the gut microbiota is constantly changing affecting the health and well-being of the host such as disease state as well as the use of various medicines such as antibiotics .
- The effect of antibiotics on gut microbiota is well documented showing a long term reduction in bacterial diversity after use of antibiotics.

FACTORS AFFECTING GUT MICROBIOTA COMPOSITION

- Link between antibiotics and weight gain is also well documented in infants as well as in adults.
- Use of antibiotics will cause a decline in the bacterial diversity, stereotypic declines as well as increased abundances of certain taxa.
- Recovery of normal microbiota from certain antibiotic treatment can be long depending on the type of antibiotic and its spectrum. Strong and broad spectrum antibiotics such as clindamycin can have longer affects persisting up to 4 years.
- The stress caused by the disruption of normal flora after antibiotic treatment facilitates the transfer of antibiotic resistance genes to virulent species leading to increased drug resistance.
- Finally, the main contributor to the diversity of the gut microbiota is diet.

Table 2 The effect of various diets on the composition of gut microbiota diversity

Diet Type	Effect on bacteria
High Fat Diet	Decrease of genera within the class Clostridia in the ileum. Increase Bacteroidales in large intestine [130]
	Increase Lactobacillus spp., Bifidobacterium spp., Bacteroides spp., and Enterococcus spp. Decrease Clostridium leptum and Enterobacter spp. [131]
	Increase Firmicutes to Bacteriodetes ratio. And increased Enterobecteriaceae [132]
	increase Bacteroidales, Clostridiales and Enterobacteriales [133]
Vegetarian Diet	Decrease Acteroides spp., Bifidobacterium spp., Escherichia coli and Enterobacteriaceae spp. [134]
	Decrease Enterobacteriaceae and increase Bacteroides [135]
	Increase Bacteroidetes, and decrease Firmicutes and Enterobacteriaceae [136]
Calorie restricted	Decrease Firmicutes to Bacteroidetes ratio [137]

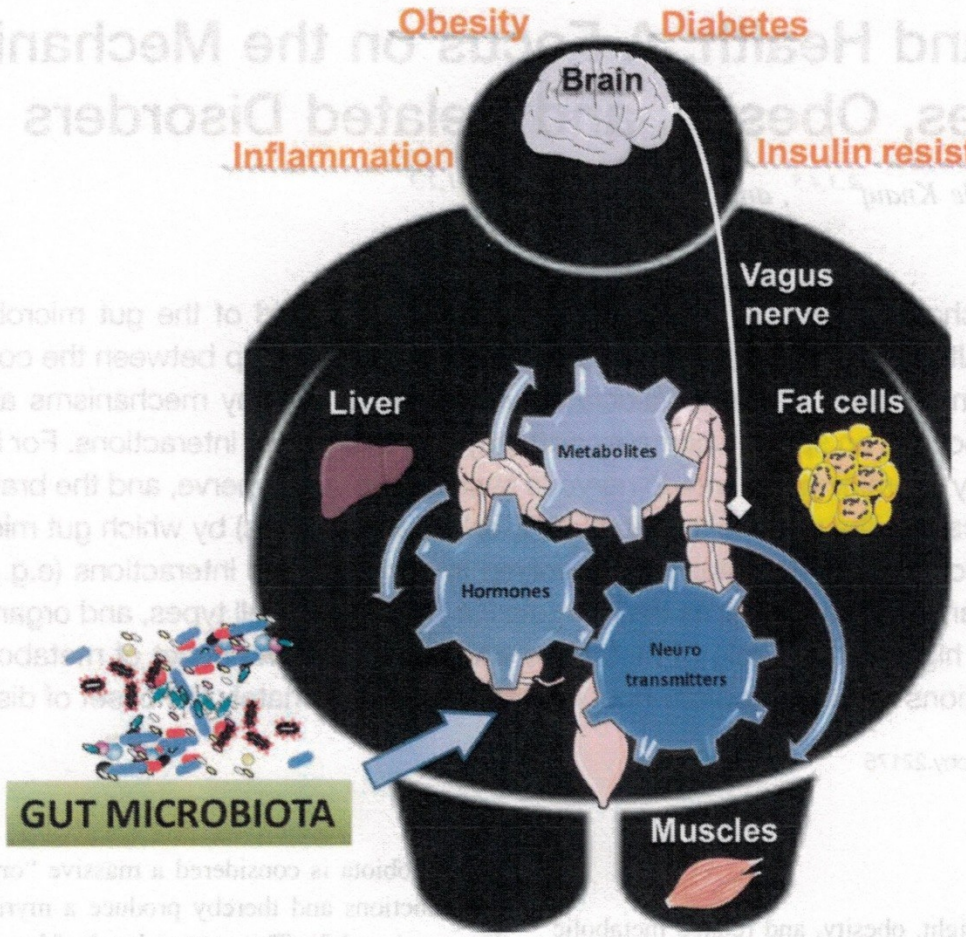


Figure 1 Gut microbiota is involved in a complex interaction with host metabolism. The gut microbiota is involved in complex interaction between food (i.e., dietary ingredients changing the microbiota) and consequently the metabolite produced. Gut bacteria also contribute to the regulation of the production of neurotransmitters, different hormones, and finally host metabolism. Numerous data suggest that the composition and the activity of the gut microbes are responsible for the protection or the onset of diseases associated with obesity, such as insulin resistance, low-grade inflammation, fatty liver, and diabetes. Thus, the gut and microbes are communicating with all the organs via specific metabolites, hormones, and neurotransmitters, acting through direct or indirect pathways (i.e., the vagus nerve).

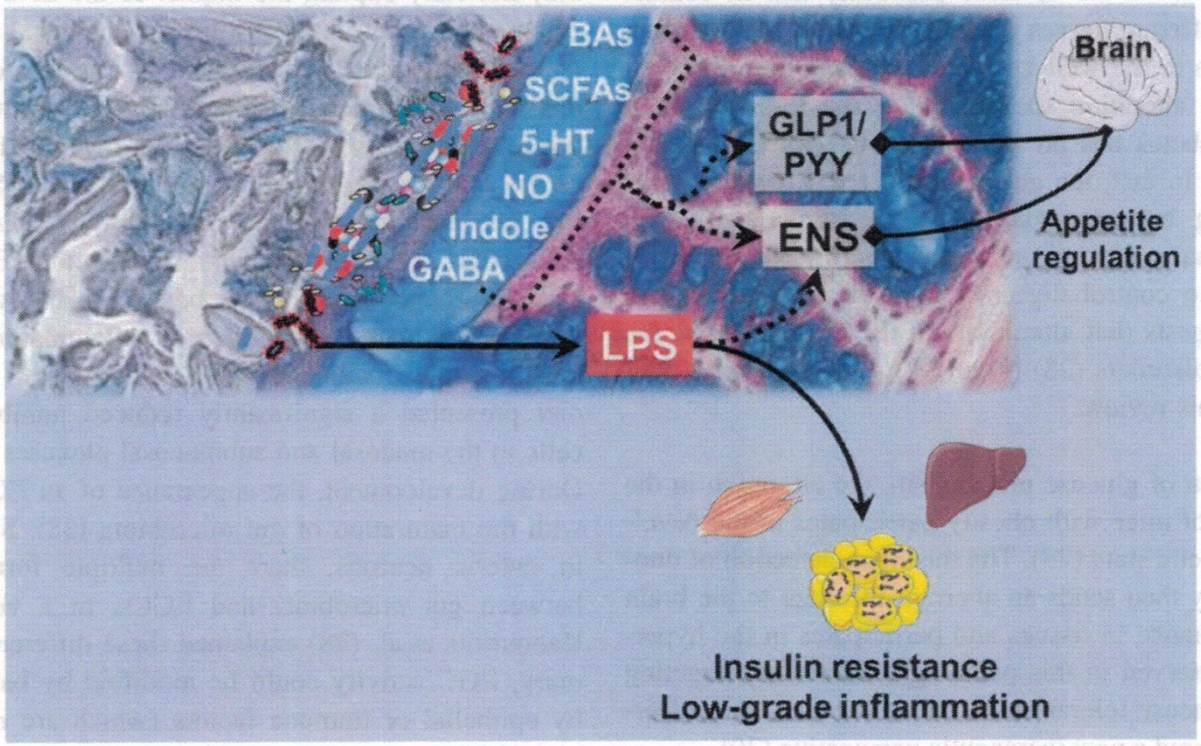


Figure 2 Mechanisms of interaction between bacterial products and host organs: the role of the gut lining. Numerous metabolites are produced upon the metabolic activity of the gut microbes. Most of them are chemically similar to those produced by the host cells (i.e., nitric oxide [NO]; gamma-aminobutyric acid [GABA]; serotonin [5-hydroxytriptamine, (5-HT)]; short chain fatty acids [SCFAs], and indoles), whereas others result from the chemical transformations of host molecules by microbes, namely the bile acids (BAs). All these molecules are recognized by the host cells and may act on specific receptors (both nuclear and membrane receptors) or eliciting the secretion of other hormonal signals such as the gut peptides glucagon-like peptide-1 (GLP-1) or peptide YY (PYY) that both act on energy metabolism by acting through nervous routes or blood relay. Translocation of lipopolysaccharides (LPS) through the gut lining is a hallmark of obesity, diabetes, and related disorders. Leakage of LPS into the blood triggers low-grade inflammation and thereby affects liver, adipose tissue, and muscle metabolism. In addition, those endotoxins can alter the activity of the enteric nervous system (ENS) as well as the gut-brain axis via the vagus nerve, hence affecting appetite regulation.

INERVACE GIT

- The gastrointestinal tract is densely innervated by intrinsic and extrinsic neurons: the differentiation relies on the localization of the soma of the neurons.
- The enteric nervous system (ENS) is composed of various types of neurons, including intrinsic primary afferent neurons and inter- and motor neurons.
- These neurons are in close proximity and in contact with spinal and vagal afferent nerves that send intestinal information to the brain. In addition to the well-known nerve alteration observed in type 2 diabetes, the alteration specifically in the ENS observed during obesity and diabetes has an impact on the control of food intake and metabolism. In fact, the gut is considered a major partner that influences feeding behavior via the ENS.
- Actual cross talk among gut hormones, the ENS, and microbial factors to control digestive motility and food intake, and evidence suggests that alterations in the gut-brain axis are associated with eating disorders were decribed.

- The relationship between the gut microbiota and ENS neurons is relatively complex.
- First, the microbiota can influence the development of the ENS, and this has consequences on ENS activity and neurochemistry (such as neuronal subpopulations).
- Second, gut bacteria can use different modes of communication to talk with ENS neurons, including a direct "sensing" with intrinsic primary afferent neurons or the release of numerous bacterial messengers (e.g., neurotransmitters, bioactive lipids, gaseous factors). Along those lines, it is worth noting that the immune cells infiltrating the gut epithelium may also communicate with the microbiota.

IS THERE A LINK BETWEEN THE GUT MICROBIOTA, THE ENS, AND OBESITY?

- A direct relationship between the gut microbiota, the ENS, and obesity has never been clearly demonstrated. Phenotypic characteristics (e.g., dysbiosis, alteration of gut motility, hyperglycemia) are exacerbated during aging.
- Aging was associated with an increase in excitatory neuronal markers, which could explain intestinal hyper-contractility.
- Dysmotility of the colon during aging could also be explained by the development of fat deposition in the *tunica muscularis* of intestinal smooth muscle cells, which decreases the number of myenteric neurons that express the neuronal nitric oxide (NO) synthase enzyme.

IS THERE A LINK BETWEEN THE GUT MICROBIOTA, THE ENS, AND OBESITY

- The link between obesity and gut microbiota is well established, but researchers have to focus on the capacity of the gut microbiota and its releasing factors to target the ENS in order to propose novel approaches to treat obesity and its associated phenotypes: namely, increase in food intake, intestinal dysmotility, and type 2 diabetes. However, although the link between colonic gut microbiota and the ENS is easily plausible, one may not fully explain the impact of the ENS on glucose absorption or the arrival of nutrients in the duodenal part. For instance, in humans, numerous factors, such as the nutrient composition of the diet and the hormonal response, strongly influence the gastric emptying, which in turn can affect the overall glycemic profile as well as the appetite sensation. In addition to ENS neurons, the cellular link between gut microbiota and obesity could be the enteric glial cells (EGCs).

IS THERE A LINK BETWEEN THE GUT MICROBIOTA, THE ENS, AND OBESITY

- EGCs seem to exert pleiotropic effects throughout the whole body, which could imply various roles in numerous pathologies, such as inflammatory bowel diseases, Parkinson disease, and obesity.
- EGC activity could be modified by bacterial metabolites and by epithelial or immune factors (which are released in response to bacterial recognition by epithelial cells and immune cells, respectively). Deciphering the cross talk among gut microbiota, EGCs, and obesity is thus of major importance.

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) IS

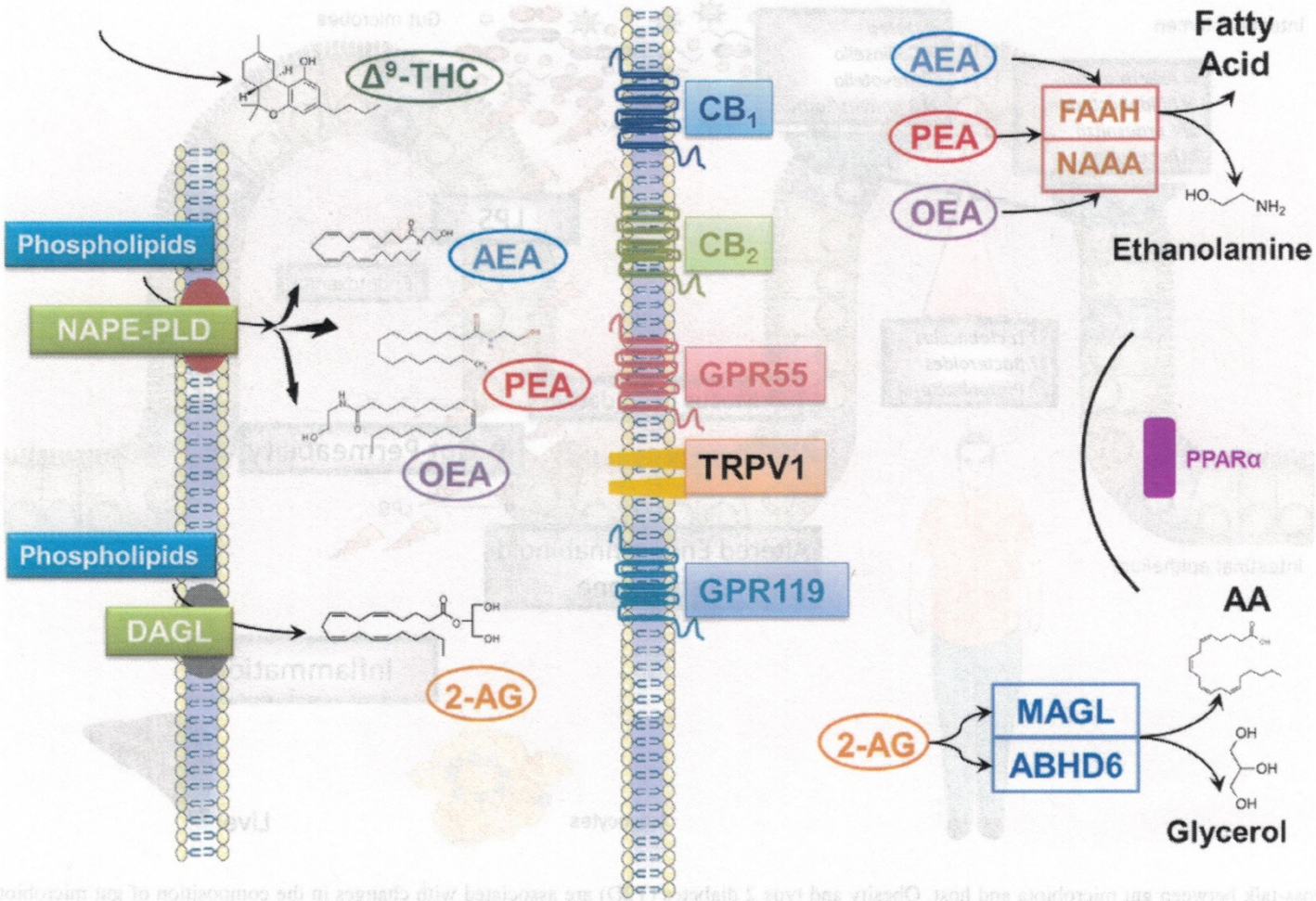
- Glucagon-like peptide-1 (GLP-1) is a key endocrine factor that could participate in the control of the gut-brain axis by gut microbiota because of its location (i.e., released by intestinal L cells).
- GLP-1 could act on ENS neurons to modify the gut-brain axis to control food intake and glucose metabolism.
- GLP-1 has a potential anorexigenic effect in humans with obesity after bariatric surgery. However, whether the appetite and the glycemic impact observed after bariatric surgery are mediated by only the hormone GLP-1 remains a matter of discussion.

ENDOCANNABINOID SYSTEM (ECS)

- In the context of energy homeostasis, the endocannabinoid system (ECS) plays a major role.
- Endocannabinoids (eCBs) are bioactive lipids that are synthesized in and exert their action on several organs involved in metabolism and appetite regulation. Depending on the action exerted by eCBs on the intestinal mucosa, they can be clustered as a "gate opener" (anandamide) and "gate keeper" (palmitoylethanolamine, 2-oleoylglycerol).
- Gut microbiota can modulate intestinal eCB tone. An "obesity microbiota" is associated with an increased intestinal level of anandamide, thus increasing gut permeability.

ENDOCANNABINOID SYSTEM (ECS)

- The daily administration of a key bacterium, *Akkermansia muciniphila*, was found to reverse diet-induced obesity by a mechanism associated with increased intestinal levels of eCBs that control inflammation, the gut barrier, and gut peptide secretion.


Δ9-THC
CB1
CB2
Phospholipids
NAPE-PLD
AEA
PEA
OEA
GPR55
TRPV1
Phospholipids
DAGL
2-AG
GPR119
AEA
PEA
OEA
FAAH
NAAA
Fatty Acid
Ethanolamine
PPARα
AA
2-AG
MAGL
ABHD6
Glycerol

ENDOCANNABINOID (ECB) SYNTHESIS

- Schematic overview of endocannabinoid (eCB) synthesis and degradation. *N*-acylethanolamine (NAE), anandamide (AEA), *N*-palmitoylethanolamine (PEA)and *N*-oleoylethanolamine (OEA) are synthesized on demand from cell membrane phospholipids through *N*-acylphosphatidyl-ethanolamine-specific phospholipaseD (NAPE-PLD).
- 2-Arachidonoylglycerol (2-AG) is also produced from cell membrane phospholipids through the action of diacylglycerol lipase (DAGL). These bioactive lipids activate cannabinoid receptors 1 and 2 (CB1and CB2), also targeted by 9-tetrahydrocannabinol (9-THC), the principal active component of *Cannabis sativa*, or other non-cannabinoid receptors such as PPAR , GPR55, GPR119 and TRPV1. These lipids are hydrolyzed in the cell by several lipases. NAE is mainly hydrolyzed by fatty acid amide hydrolase (FAAH) and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) into ethanolamine and a fatty acid (dependingon the NAE hydrolyzed). 2-AG is hydrolyzed by two serine hydrolases, monoacylglycerol lipase (MAGL) and / -hydrolase domain 6 (ABHD6), into glycerol andarachidonic acid (AA).

METABOLITES PRODUCED BY GUT MICROBIOTA AND ACTING AS SIGNALING MOLECULES

- **Short chain fatty acids**
- Short chain fatty acids (SCFAs) are organic fatty acids containing two to six atoms of carbon and are produced in the cecum and in the colon of the host by the microbiota following the fermentation of nondigestible dietary fibers, proteins, and glycoproteins. Acetate, propionate, and butyrate represent 95% of SCFAs.
- Bacterial SCFAs locally modulate the physiology of the large intestine, but they can also be absorbed (only 5%-10% are excreted in feces) and control the metabolism of other organs (such as adipose, liver, muscle, and brain tissue), thus influencing the energetic homeostasis of the host, including appetite regulation.
- One of the primary roles of SCFAs is the modulation of the activity of histone deacetylase.

METABOLITES PRODUCED BY GUT MICROBIOTA AND ACTING AS SIGNALING MOLECULES

- **Short chain fatty acids** SCFAs induce colon motility.
- SCFA administration increased the luminal release of serotonin (5-HT). It has also been suggested that butyrate, but not acetate or propionate, has a colonic prokinetic effect by increasing the proportion of cholinergic (excitatory) myenteric neurons; it seems that the change in neuronal phenotype is associated with increased acetylation of histone 3.
- SCFAs modulate colonic secretion in response to 5HT: the gut microbiota downregulates 5-HT3 expression via acetate production, thus lowering the host secretory response.
- SCFAs in the gut-brain axis. The finding that the GPR41 receptor is expressed in sensory ganglia (afferent fibers) and in autonomic ganglia (efferent fibers) strongly supports the role played by SCFAs in the gut-brain axis
- Butyrate and propionate activated intestinal gluconeogenesis in the colon via complementary mechanisms. Butyrate increased the expression of intestinal gluconeogenesis enzymes through a cAMP-dependent mechanism, while the same genes were activated by propionate via a gut-brain axis involving GPR41 expressed on periportal neural afferents.

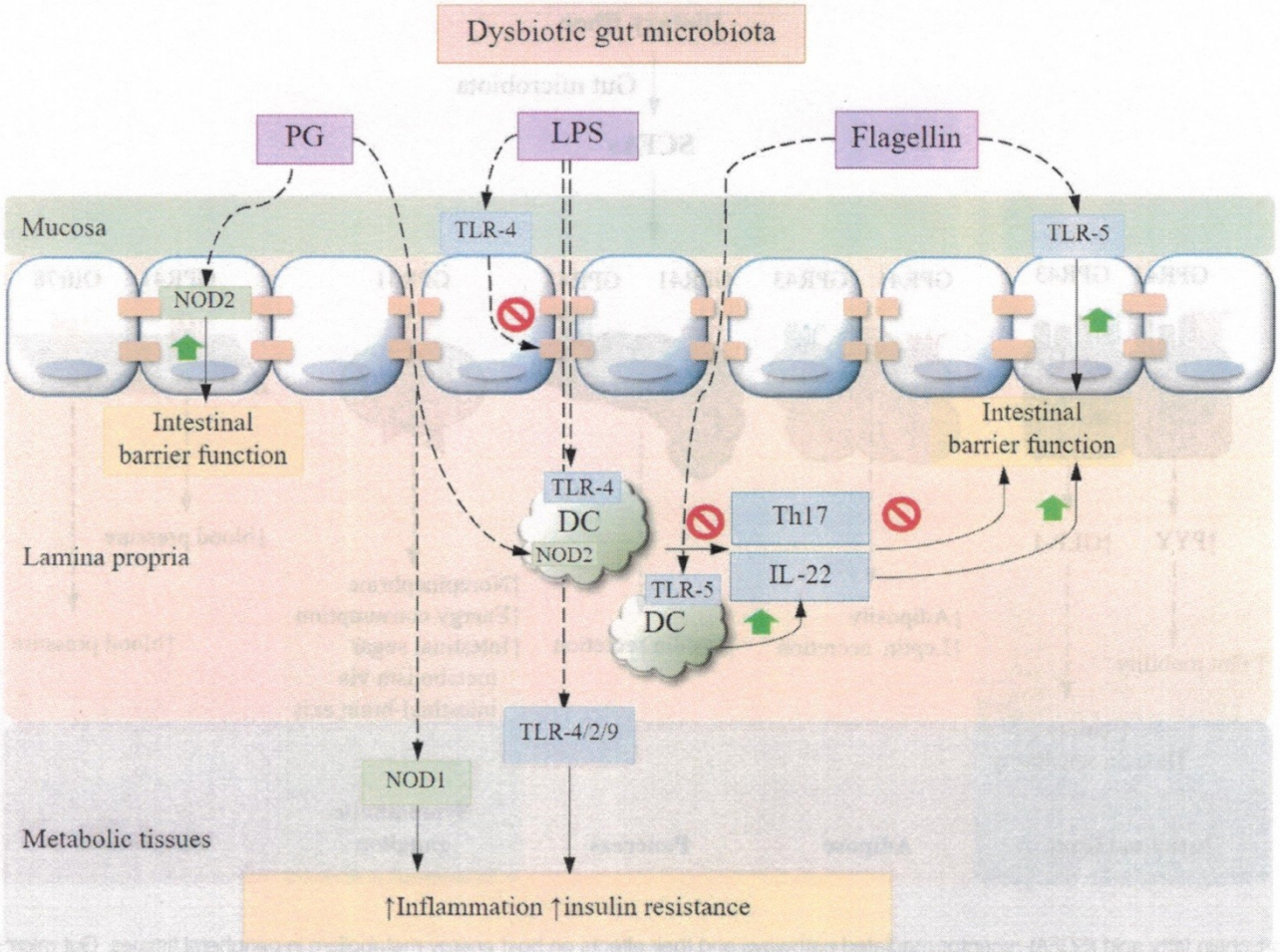


FIGURE 1 | Gut microbiota dysbiosis-driven immune signaling pathways. Bacterial translocation occurs secondary to the mucosal epithelial barrier impairment driven by dysbiotic alterations in gut microbiota, leading to elevated circulating and tissue MAMPs such as LPS and PG. Bacterial LPS can disrupt the expression of epithelial tight junctions and, upon being translocated to peripheral tissues, trigger inflammation, and insulin resistance through toll-like receptors (TLRs). While PG induces tissue inflammation *via* NOD1, its recognition by NOD2 in intestinal epithelium confers protection against gut barrier dysfunction. The cross talk between APC and Th17 cells is also impaired under dysbiotic conditions with a decrease in IL-22. Adversely, the interactions between bacterial flagellin and intestinal epithelium or APC *via* TLR-5 improve gut barrier function. MAMPs, microbe-associated molecular patterns; LPS, lipopolysaccharide; PG, peptidoglycan; APC, antigen-presenting cells; IL, interleukin.

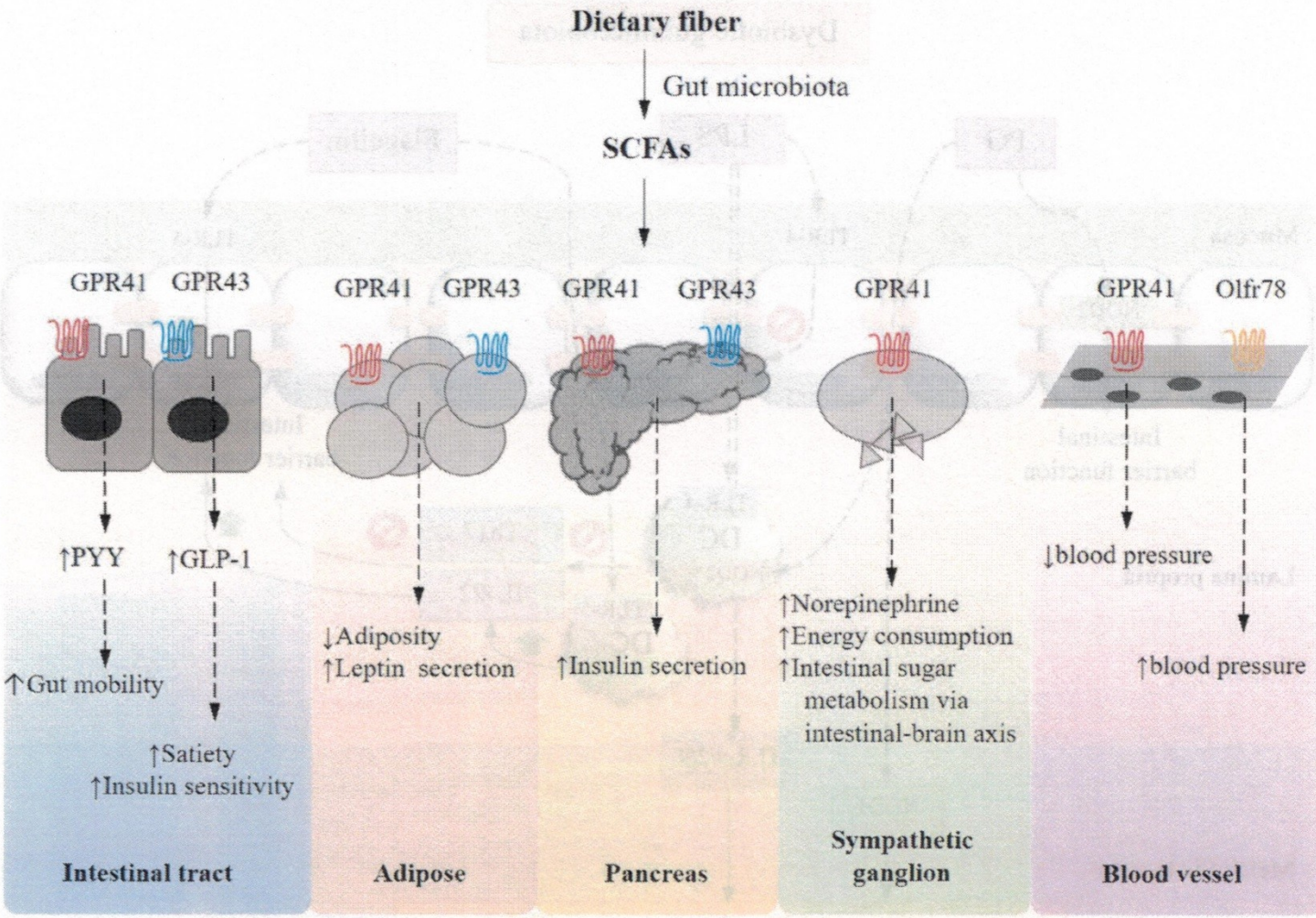
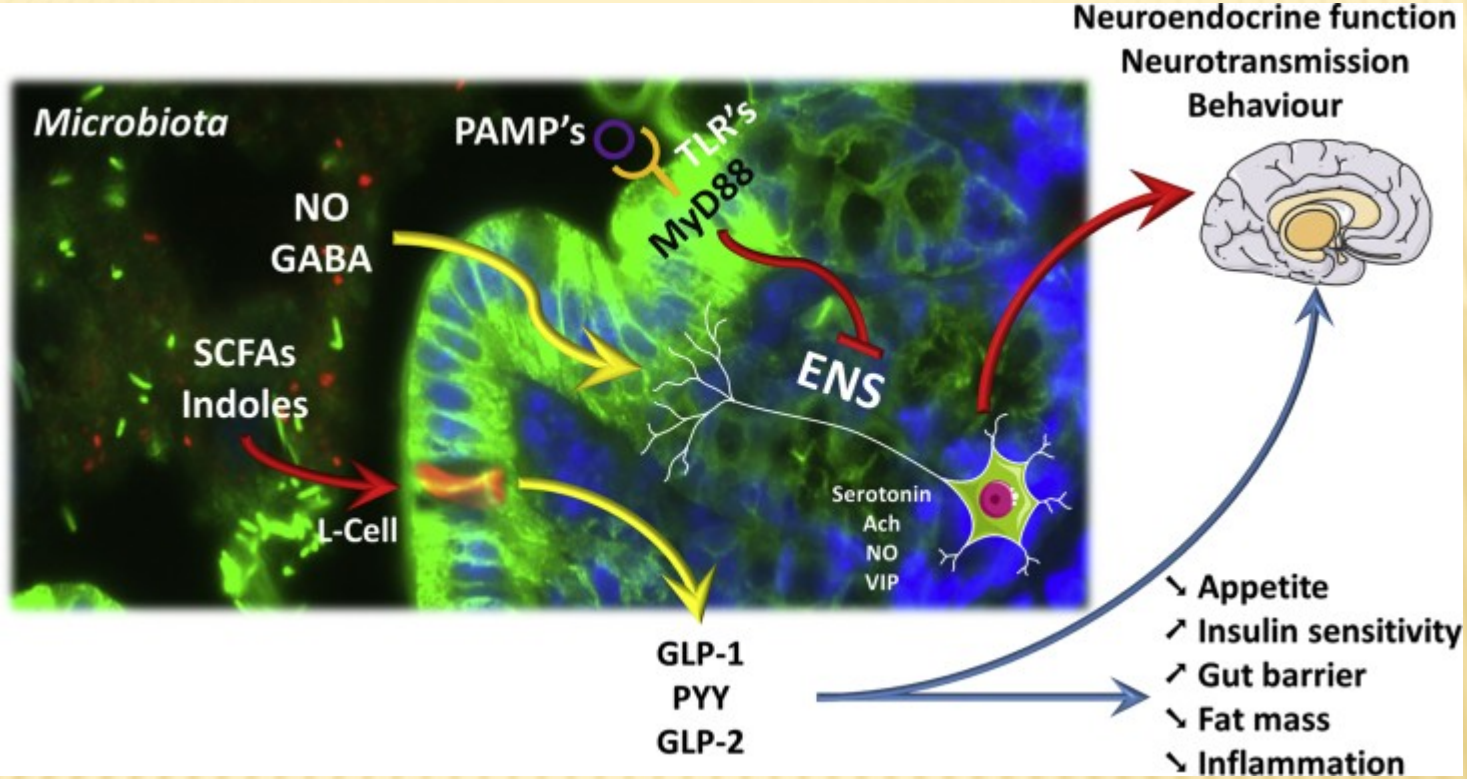


FIGURE 2 | Short-chain fatty acid (SCFA)-receptor-mediated pathways and their effects on host energy metabolism in peripheral tissues. Gut microbes can ferment dietary fiber into SCFAs, which induce an array of G-protein coupled receptor-mediated signaling pathways that are essentially implicated in host energy homeostasis in multiple tissues.

METABOLITES PRODUCED BY GUT MICROBIOTA AND ACTING AS SIGNALING MOLECULES

- Acetate can also influence metabolism via a gut-brain axis. It was demonstrated that fermentable carbohydrates such as inulin altered hypothalamic neuronal activity specifically in the arcuate nucleus (ARC). Intraperitoneal administration of acetate or acetate directly produced by the gut microbiota through fermentation entered the hypothalamus and reduced appetite by increasing the expression of anorectic pro-opiomelanocortin and suppressing agouti-related peptide.
- Acetate production from an altered gut microbiota increased glucose-stimulated insulin secretion, ghrelin secretion, hyperphagia, and other alterations in the metabolism associated with obesity by activating parasympathetic neurons. But, it remains unclear whether the observed effects are attributable to the acetate itself or to other products of the cross feeding.


Neuroendocrine function
Neurotransmission
Behaviour
Microbiota
PAMP's
TLR's
MyD88
NO
GABA
SCFAs
Indoles
L-Cell
ENS
Serotonin
Ach
NO
VIP
GLP-1
PYY
GLP-2
↘ Appetite
↗ Insulin sensitivity
↗ Gut barrier
↘ Fat mass
↘ Inflammation

Overview of the different interactions existing between microbial metabolites, endocrine and nervous routes.

Gut microbes interact with host cells using different mechanisms. SCFAs (short chain fatty acids) are metabolites produced by the microbial fermentation of different nutrients; these SCFAs are recognized by specific G-protein coupled receptors expressed at the surface of enteroendocrine cells such as L-cells, producing GLP-1, GLP-2, and PYY. Indoles are also bacterial metabolites of tryptophan degradation involved in the control of GLP-1 release and appetite control. The secretion of such hormones control appetite, gut barrier, and glucose homeostasis (e.g., insulin sensitivity) via

Overview of the different interactions existing between microbial metabolites, endocrine and nervous routes.

Similar to what is observed in the brain, different neurotransmitters or molecules (produced by intestinal microbes), such as nitric oxide (NO) as well as γ-aminobutyric acid (GABA), act through the enteric nervous system (ENS). Secondary messengers, including NO, serotonin, acetylcholine (Ach) or vasoactive intestinal polypeptide (VIP) release, are involved in the gut to peripheral organ and brain communication, leading to the control of different behaviors (e.g., food intake, anxiety, stress).
Pathogen-associated molecular patterns (PAMPs) are recognized by pathogen recognition receptors such as Toll-Like receptors (TLR's) that are for most of them signaling through the central adaptor molecule myeloid differentiation primary response gene 88 (MyD88). The intestinal abundance of PAMPs and the activation of different TLR's at the intestinal epithelial surface or at the level of the ENS regulate numerous metabolic functions such as for instance leptin sensitivity, gut hormones signaling to the brain, hence controlling whole-body energy homeostasis.

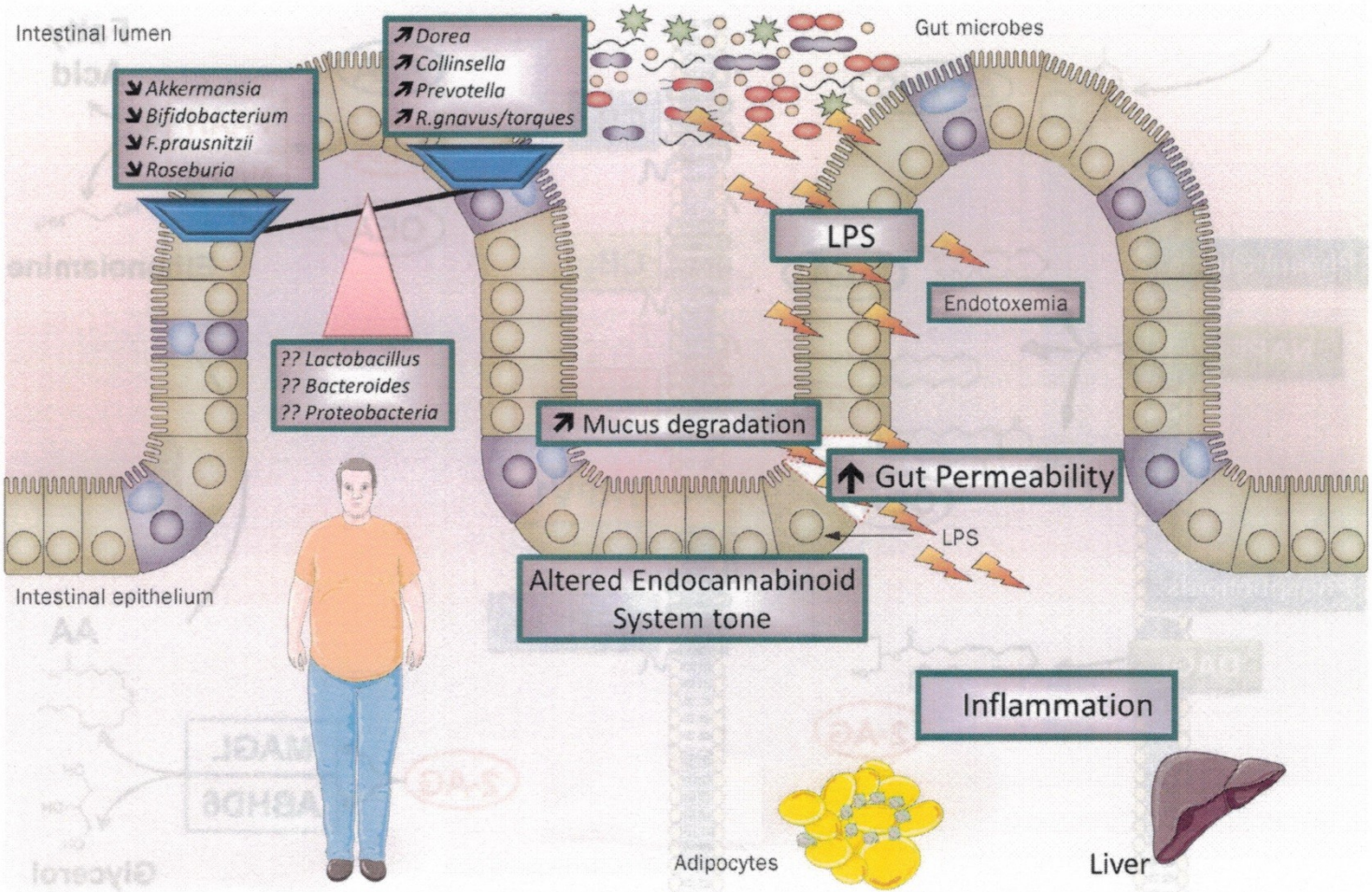


Fig. 1. Cross-talk between gut microbiota and host. Obesity and type 2 diabetes (T2D) are associated with changes in the composition of gut microbiota, leading to increases in some genera and decreases in others (arrow direction indicates either increased or decreased abundance). Some bacteria are positively or negatively associated with obesity and T2D, depending on the study (indicated by question marks). Increased mucus degradation is associated with increased gut permeability and metabolic endotoxaemia, triggering inflammation, macrophage infiltration of adipose tissue and insulin resistance (adapted from Delzenne et al. [111]).

addition to interactions at the level of energy homoeostasis, and different links between the gut microbiota and the eCB system

GUT MICROBIOTA AND DM

- In the Diabetes Prevention and Prediction (DIPP) study it was shown that new-onset T1D subjects had different gut microbiota composition than controls. They showed that in the control group, mucin synthesis was induced by lactate- and butyrate-producing bacteria to maintain gut integrity while mucin synthesis was prevented by the non-butyrate-producing lactate-utilizing bacteria leading to β-cell autoimmunity and T1D.
- Many other studies confirmed the differences observed in gut microbiota composition between T1D and their matched health controls highlighting the need for better understanding of the role that these bacteria may play in the development of this disease.

GUT MICROBIOTA AND DM

- The effect of microbiota on T2D has been proposed to be mediated through mechanisms that involve modifications in the secretion butyrate and incretins.
- T2D patients had moderate degree of gut microbial dysbiosis, a decrease in universal butyrate-producing bacteria and an increase in opportunistic pathogens.
- Similar data were reported by other studies highlighting the role of these bacteria in regulating important T2D pathways such as insulin signaling, inflammation and glucose homeostasis.
- On the other hand, gut microbiota has been shown to affect the production of key insulin signaling molecules such as GLP-1 and PYY through SCFA and its binding to FFAR2.

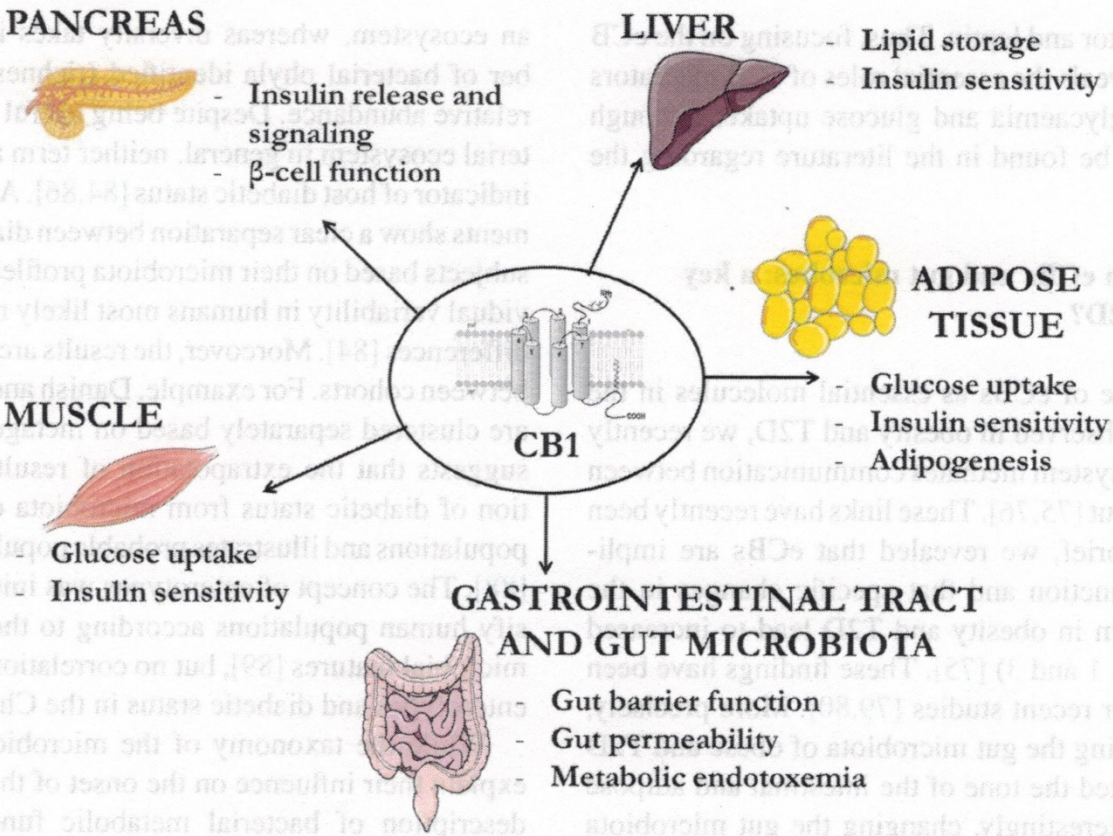


Fig. 3. The endocannabinoid (eCB) system and metabolism. This system plays a central role in the regulation of glucose homoeostasis and insulin sensitivity in several peripheral organs. CB_1 receptor activation induces gut permeability. Gut microbiota composition is associated with intestinal eCB content and CB_1 receptor mRNA expression.

THE TRIALOGUE BETWEEN NUTRITIONAL STATUS, GUT MICROBIOTA, AND IMMUNE SYSTEM REVEALS NOVEL THERAPEUTIC OPPORTUNITIES FOR METABOLIC DISEASES

- Metabolic diseases are characterized by a state of chronic subclinical inflammation in metabolic tissues such as liver, adipose, muscles, and pancreatic islets.
- The causative role of a dysbiotic gut microbiota in this inflammatory status by virtue of engaging diverse signaling transduction pathways and immune responses has been increasingly established in the past decade. In light of the increasingly unraveled trialogue between diet, gut microbiota, and the host immune system, a multitude of therapeutic approaches against metabolic diseases have emerged. One compelling set of mechanisms dictate the translocation of commensal bacteria and bacterial fragments toward metabolic tissues, where they trigger pro-inflammatory responses at the early onset of metabolic disorders.
- Evidence suggests that this translocation is promoted by a diet/microbiota-driven gut barrier impairment in dysbiotic conditions, thereby continuously fueling the host immune machinery that orchestrates the innate and adaptive arms.

GUT MICROBIOTA AND DM

- It's becoming increasingly evident that gut microbiota is contributing to many human diseases including diabetes both type 1 and type 2.
- Type 1 diabetes (T1D) is an autoimmune disease that is caused by the destruction of pancreatic β-cells by the immune system. Even though T1D is mainly caused by genetic defect, epigenetic and environmental factors have been shown to play an important role in this disease. Higher rates of T1D incidence have been reported in recent years that are not explained by genetic factors and have been attributed to changes in our lifestyle such diet, hygiene, and antibiotic usage that can directly affect microbiota.
- It has been shown that diabetes incidence in the germ free non-obese diabetic subjects or patients (NOD) was significantly increased which is in line with the observation that the rates of T1D is higher in countries with stringent hygiene practices. Similarly comparison of the gut microbiota composition between children with high genetic risk for T1D and their age matched healthy controls showed less diverse and less dynamic microbiota in the risk group.

DĚKUJI ZA POZORNOST