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# Role of the microbiome in the normal and aberrant glycemc response

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### SUMMARY

Multiple studies in the recent years suggest that the microbiome is critically important for normal host functions, while impaired host microbiome interactions contribute to the pathogenesis of numerous common disorders. Of these, much attention is recently given to the involvement of the microbiome in the pathogenesis of impaired glucose tolerance, type II diabetes mellitus (T2DM), and other metabolic disorders comprising the 'metabolic syndrome', including obesity, non-alcoholic fatty liver disease and their complications. In addition, alterations in the microbiome have been linked to the pathogenesis of type 1 diabetes mellitus (T1DM), an autoimmune disorder affecting the glycemc response, of distinct pathogenesis than T2DM. In this chapter we will discuss the roles of the microbiome in regulating the normal and impaired glycemc response in both mice and humans, and outline examples of underlying mechanisms by which the microbiome is contributing to diabetes mellitus. We will further discuss means by which the microbiome can be manipulated to develop future therapeutic interventions for hyperglycemia and its adverse effects.

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## 1. Microbiome alterations in type 1 and type 2 diabetes mellitus

The term 'diabetes mellitus' denotes two distinct metabolic disorders that share the common feature of chronic hyperglycemia and impaired glycemic response due to defects in insulin secretion, sensitivity or both. Type 1 diabetes mellitus (T1DM), is an autoimmune condition involving the destruction of beta cells in the pancreas, consequently leading to impaired production of insulin, rendering patients entirely dependent on exogenous insulin. T1DM accounts for ~5–10% of diabetes mellitus cases, and is characterized by abrupt onset in children and young adults. In contrast, type 2 diabetes mellitus (T2DM, previously termed non-insulin dependent diabetes mellitus) is characterized by impaired insulin sensitivity of target tissues (muscle, liver, adipose tissue), leading to impaired glucose uptake into peripheral tissues, a condition termed 'insulin resistance'. During most of the course of T2DM, insulin secretion is increased due to pancreatic beta cell compensation for insulin resistance, and only at late stages of T2DM pancreatic insulin secretion is impaired, leading to exogenous insulin dependency. Due to the gradual nature of this process, T2DM is mostly but not exclusively observed in adults [1]. Despite the differences in epidemiology and pathogenesis, the microbiome is suggested to play a role in the manifestation or progression of both T1DM and T2DM as outlined below.

**T1DM.** In addition to the existing mechanistic knowledge regarding the pathogenesis of T1DM, gut microbiome alterations also seem to be a feature of this autoimmune disorder. The classical murine model for T1DM is the non-obese diabetic (NOD) polygenic mutant mouse, in which animals develop spontaneous insulinitis and consequently depletion of insulin production at about 12 weeks of age. When these mice additionally lack MyD88 (an adaptor for multiple receptors that recognize microbial patterns), they are protected from the development of T1DM, suggesting that microbial stimuli may be involved in disease pathogenesis in this model. Interestingly, deriving these mice as germ-free (GF) or treating them with broad-spectrum antibiotics leads to robust development of T1DM. Moreover, recolonizing NOD GF mice with a defined microbiota reduced the incidence of diabetes, suggesting that certain microbiome compositions may play a protective role in the development of T1DM [2]. This observation and follow up studies have led to the development of the 'balanced signal' hypothesis, in which various members of the microbiome may promote T1DM while others have an inhibitory function [3–5]. T1DM-promoting microbial composition may be induced by early exposure to antibiotics [6] (as discussed in the next section). The downstream microbiome effects on host susceptibility to T1DM may involve microbial-mediated alterations in host sex hormones [7,8], induction of host immune reactivity, mainly the Th17 response [9,10], as well as other, yet unidentified factors.

Support for the conclusions obtained in the above murine models can be found in human trials. In a small-scale prospective case–control study performed in eight children, a significant microbial dysbiosis was apparent early after the onset of autoimmunity, with increase of **Bacteroidetes** (and more specifically, the genus **Bacteroides**) and consequent reduction of **Firmicutes** noted in new onset T1DM cases [11]. Other studies suggested that butyrate producing bacteria may be also reduced in T1D patients [12], possibly leading to altered mucin synthesis and compromised intestinal integrity, leading to systemic influx of microbial antigens inducing autoimmunity. Similar taxonomic differences were reported in a different cohort of children [13]. A recent longitudinal study with 33 infants determined that microbiomes of subjects who progress to T1D are characterized by significant reduction in alpha diversity even before the onset of clinical symptoms. In the children who progress into T1DM, several correlations were determined between distinct microbial taxa and serum or fecal metabolites that were previously associated with diabetes, suggesting that these microbe-metabolite relationships may cooperatively impact T1DM pathogenesis [14]. Despite this increasing number of reports, our knowledge regarding the role of the microbiome in T1D remains mostly descriptive. Further research is needed to fully understand the contribution and the function of the microbiome in the manifestation of T1DM.

**T2DM.** Multiple studies suggested an association between compositional and functional microbiome alteration ('dysbiosis') and the risk of developing metabolic syndrome-associated pathologies such as obesity, T2DM, non-alcoholic fatty liver disease and hyperlipidemia. In mice, induction of insulin resistance may be induced by alteration of the microbiome, mediated by changes in diet and immune dysregulation, as further discussed in the next section.

In humans, several important associations have been suggested between T2DM and microbiome alterations. In a study of 292 Danish individuals, Le Chatelier *et al.* reported that participants with insulin resistance (as well as increased adiposity) are characterized by lower bacterial richness [15] and have higher abundances of **Proteobacteria** and **Bacteroidetes**. Larsen *et al.* also reported higher abundances of the same phyla in diabetic males as well as a positive correlation between **Bacteroidetes-to-Firmicutes** ratio and elevated plasma glucose levels. In contrast, no difference was detected in bacterial alpha diversity [16]. In their cohort of 345 Chinese individuals, Qin *et al.* also reported association between T2DM and higher levels of **E. coli** and **Bacteroides**, as well as **Akkermansia muciniphila** and various **Clostridia** [17]. Functional analysis revealed that the microbiome of these diabetic individuals was enriched for pathways or modules involved in membrane transport, methane metabolism, xenobiotics degradation and metabolism, and sulphate reduction, while pathways of bacterial chemotaxis, flagellar assembly, butyrate biosynthesis and metabolism of cofactors and vitamins were decreased. Consistent with Qin *et al.*, Karlsson *et al.* performed a functional analysis of microbiome from T2DM females and controls [18] and reported that the diabetic group featured enrichment of genes related to membrane transport and oxidative stress. Among the additional commonalities were the enrichment of flagellar assembly and riboflavin metabolism pathways in the non-diabetic controls, and high levels of various **Clostridia** in the diabetic group. Interestingly, analysis of the metagenomic data from the Chinese cohort suggested that not only the microbiome composition and function associate with T2DM, but also the growth dynamics of the microbiome, as for several commensal bacteria, including **E. coli**, **Bacteroides** or **Butyrate-producing bacterium SS3/4**, growth rate was correlated with elevated blood glucose, glycosylated hemoglobin A1c (HbA1c) and T2DM [19].

T2DM treatment may also affect the microbiome. A recent study by Forslund *et al.* [20] investigated the human gut microbiome of T2DM patients obtained from three different human gut microbiome cohorts including the Chinese [17] and the Danish [15] studies mentioned above and another new T2DM and non-diabetic cohort. In all three cohorts, patients clustered by metformin treatment, with metformin causing a shift in the gut microbiome and increasing the microbiome richness. More specifically, metformin treatment increased **Escherichia** and lowered **Intestinibacter** species abundance and led to induction of short chain fatty acids production. Therefore the impact of anti-diabetic medication on the gut microbiome needs to be considered in developing microbiota-based diagnosis and therapy for T2DM. In all, T2DM and its treatment are suggested to have major effects on the gut microbiome, yet despite these associations and several commonalities in microbial composition and function, no distinct microbial “signature” was fully shared between all T2DM patients in these different studies.

## 2. Factors contributing to microbial alterations in T1D and T2DM

The microbiome composition of healthy adult humans is considered to be generally stable over time [21]. Nevertheless, several factors have been suggested to alter and predispose the healthy microbiome to a configuration that contributes to an impaired glycemic response (Table 1). Better understanding of these factors and the mechanisms that shift the microbiome towards a harmful configuration may produce better prevention and intervention strategies.

**Antibiotics.** The use of antibiotics was a critical turn in modern medicine and saved countless lives by treating and preventing pathogenic infection, yet their effects on the gut microbiota may sometimes result in harmful health consequences. In T1DM-susceptible NOD mice, treatment with vancomycin or neomycin or, in a separate study, a broad spectrum combination of streptomycin, colistin and ampicillin starting in early life accelerates the onset of T1DM in adulthood [6,22]. Similar findings were reported in NOD-MyD88 knockout mice, in which treatment with sulfatrim throughout the lifetime of the mouse promotes higher rates of T1DM [2]. Recently, this notion has been expanded to development of diabetes mellitus in humans. In a case-control study of over 1 million participants, Boursi *et al.* [23] observed that repeated treatment with antibiotics (between two and five rounds) early in life increases the risk of developing both T1DM and T2DM, in a dose-dependent manner. Similar findings were reported for T2DM in a case-control study performed in the Danish population by Mikkelsen *et al.* [24]. It is important to note that reverse causality is a potential limit of these studies. Both T1DM and T2DM patients are known to be at higher risk for various infections [25], leading to heavier antibiotic usage

**Table 1**

Factors that alter the microbiome and consequently affect glycemic control.

Factor	Effect on the microbiota	Effect on the host
High-fat diet (HFD)	<b>Increase:</b> Proteobacteria, Firmicutes/ Tenericutes/Mollicutes <b>Decrease:</b> Bacteroidetes, alpha diversity	Endotoxemia, obesity, insulin resistance
Low-calorie diet	<b>Increase:</b> Bacteroidetes <b>Decrease:</b> Firmicutes	Weight loss
Non-caloric sweeteners: Saccharin	<b>Increase:</b> Bacteroides (mice) <b>Decrease:</b> Clostridiales (mice) In humans: Disbiosis in some	Glucose intolerance
Non-caloric sweeteners: Aspartame	<b>Increase:</b> <i>Clostridium leptum</i> <b>Decrease:</b> <i>Clostridium</i> cluster XI (on HFD)	Elevated fasting glucose, impaired insulin tolerance
Dietary emulsifiers	<b>Increase:</b> Mucolytic OTUs (e.g. <i>Ruminococcus gnavus</i> ) <b>Decrease:</b> Bacteroidales	Weight gain, adiposity, impaired glycemic control, colitis
Pregnancy	<b>Increase:</b> Proteobacteria, Actinobacteria <b>Decrease:</b> Butyrate-producers, alpha diversity	Glucose intolerance, increased adiposity and levels of inflammatory markers
Bariatric surgery	<b>Increase:</b> Proteobacteria/ <i>E. coli</i> , Clostridiales <b>Decrease:</b> Firmicutes	Weight loss, reduced adiposity, improved insulin tolerance
Jet lag	Dysbiosis (mice and humans)	Weight gain, glucose intolerance

among pre-diagnosed diabetics, thus possibly making antibiotic use the result, rather than the cause, of diabetes in these patients. One suggested mechanism for a causative antibiotic-diabetes link is antibiotic-mediated changes in microbiome metabolism, leading to reduction of short chain fatty acids (SCFA), which may promote T1DM by increasing gut permeability [12]. Given these interesting yet inconclusive correlations, the association between antibiotic use and the risk of either T2DM or T1DM merits further prospective validation.

**Diet.** The type and amount of consumed foods play an important role in both establishing the metabolic syndrome, and in shaping the gut microbiome [26–28]. It is therefore surprising that causative association between different types of diets, the gut microbiome, and health consequences is currently sparse. The most well studied diet in the context of microbiome and risk for metabolic syndrome is a diet rich in fat; this diet alters the gut microbiome and promotes metabolic syndrome when microbiome from obese mice is transplanted to GF recipients [29–32]. Weight reduction diets alter the composition of the microbiome (i.e. less **Firmicutes**, more **Bacteroidetes**), yet this may be a result of the changes in weight rather than a direct effect of the diet on the microbiome [33]. Interestingly, the effect of the microbiome on host metabolism is dependent not only on the amount of fat but also on its source; compared to mice fed a diet containing fish oil, mice fed a diet with lard exhibited exaggerated weight gain, white adipose tissue inflammation and impaired insulin sensitivity. These detrimental effects of the lard were due to its association with a distinct microbial composition, which led to induction of adipose tissue inflammation [32]. It remains to be determined how other diets affect the microbiome, and how the microbiome-diet interactions govern metabolic homeostasis.

**Food additives.** Modern human diet has been dramatically altered in the last decades, including the addition of multiple synthetic ingredients into human nutritional repertoire. One such example is non-caloric artificial sweeteners, which are consumed by millions worldwide to maintain the sweet taste of foods without the associated calories. While replacing caloric with non-caloric sweeteners is widely endorsed [34], studies in both humans and rodents associate their consumption with the counter-intuitive effect of promoting insulin resistance and weight gain (reviewed in [35]). We have recently demonstrated that mice consuming saccharin, sucralose or aspartame develop glucose intolerance, which was ameliorated by antibiotics treatment. Saccharin significantly altered the composition and function of the microbiome, with concomitant increase in **Bacteroides** and pathways involved in the degradation of glycans [36]. Importantly, the dysbiotic microbiome had a causative role in the

phenotype, as its transplantation into GF mice promoted glucose intolerance in the recipients. Similar to our results, Palmnäs *et al.* also reported impaired insulin-stimulated glucose disposal following aspartame consumption, as well as microbial dysbiosis [37]. In humans, we have reported in a small-scale preliminary experiment that some individuals show microbiota-dependent development of glucose intolerance following acute consumption of saccharin. The full scope of this susceptibility in the population and the effect of chronic long-term consumption of various non-caloric sweeteners merits further research.

Another example of recently introduced food ingredients whose effects on the microbiome may contribute to metabolic consequences in the host, are dietary emulsifiers, heavily used by the modern food industry. Chassaing *et al.* [38] showed that two commonly used dietary emulsifiers derived from processed food alter the composition of the gut microbiome and predispose to increased intestinal permeability and elevated circulating LPS levels. Emulsifier supplementation to mice led to enhanced adiposity and food intake and elevated blood glucose levels. Importantly, these metabolic phenotypes were abrogated in GF mice, while transfer of microbiota from conventional mice treated with emulsifiers into GF recipients resulted in development of the metabolic disorders. This study suggests that dietary emulsifiers may cause a combination of microbial dysbiosis and impaired host intestinal barrier function, promoting obesity-induced metabolic manifestations.

**Pregnancy.** Several metabolic changes that occur throughout pregnancy are also characteristic of metabolic syndrome, including insulin insensitivity. Koren *et al.* [39] characterized pregnant women and determined that the fecal microbiome of the third trimester is significantly altered, with increase of **Proteobacteria** and **Actinobacteria** and reduction in some butyrate-producers and alpha diversity. To determine whether these alterations may contribute to the metabolic parameters, fecal microbiota from the third trimester were transplanted into GF mice. Recipient GF mice showed glucose intolerance, as well as increased adiposity and inflammatory markers. In contrast to the above study, a recent study by DiGiulio *et al.* [40] examined the microbiota composition in the vagina, stool, saliva and mouth of pregnant women who undergo full term deliveries compared to preterm cases. The study suggested that during pregnancy the microbiota composition remains stable in all the sites. In women who subsequently delivered prematurely the vaginal community was modified characterized by increased **Peptoniphilus**, **Prevotella**, and **Anaerococcus** while decreased **Lactobacillus** species. Further studies may investigate possible population and obstetric differences that may resolve these different pregnancy-related results. It will be interesting to determine whether microbiome alterations are associated with some pregnancies, and whether they persist after pregnancy and contribute to the development of the metabolic syndrome later in life.

**Immune response.** Innate immune signaling may directly cause dysbiosis and induce metabolic disorders in response to obesity [41]. Toll-like receptors (TLRs) are one of the central innate immune sensors, activating a pro-inflammatory pathways involving Myd88 and NF- $\kappa$ B, leading to transcription of inflammatory cytokines and other factors [42]. However, conflicting results were reported about the effect of TLR signaling on glucose and weight control. Obese and insulin resistant mice [32] as well as T2DM patients [41] showed increased TLR2 and TLR4 expression, which was associated with the severity of insulin resistance. Accordingly mice with mutated CD14, which is the TLR4 co-receptor [43], and mice with ablation of TLR4 in hematopoietic cells [44] featured decreased obesity-induced glucose tolerance and insulin resistance. However, mice lacking Myd88 [45] and TLR5 [46] showed increased body weight and glucose intolerance. Deleterious TLR5 signaling caused dysbiosis and the metabolic effect was transferable to GF mice, while antibiotics abrogated the metabolic perturbations, suggesting that the microbiome plays a central role in the pathogenesis that governs the progression of the metabolic syndrome abnormalities [46].

A recently identified family of cytoplasmic innate sensors is the nucleotide-binding-and-oligomerization domain and leucine-rich-repeat-containing (Nod-like receptor, NLR) family. Pathogen- and damage-associated molecular patterns are able to induce a cytoplasmic multi complex termed inflammasome composed of specific NLR, which may include the adaptor protein ASC (PYCARD), promoting proximity cleavage of Caspase-1 and catalytic activation of IL-1 $\beta$  and IL-18, as well as a cell death termed pyroptosis. Expression of Nod-like receptor-3 (NLRP3) inflammasome is enhanced in adipose tissues and livers of obese mice and humans and correlates with the severity of T2DM, whereas reduced adipose NLRP3 inflammasome is associated with improved insulin sensitivity

[47]. Deletion of NLRP3 [47] and the adaptor protein ASC [48] augmented insulin resistance and glucose tolerance but ablation of other inflammasome components such as Caspase1 led to improved glucose homeostasis [49]. Mice with defective NLRP3, NLRP6 and ASC inflammasome exhibited dysbiosis and developed nonalcoholic fatty liver disease (NAFLD) [48]. Importantly, the metabolic phenotype was directly caused by modified gut microbiota configuration associated with exacerbated fatty liver via influx of TLR4 and TLR9 ligands into the portal circulation, leading to hepatic inflammation and development of steatosis [48]. Similarly, ASC-deficient mice develop increased obesity and loss of glycemic control on high-fat diet feeding and the metabolic effect was mediated by the gut microbiota since it was transferable to wild-type mice and abrogated by antibiotics [48].

All together, TLR and NLR innate sensors modulates gut microbiota composition and function, which in turn may control whole body glycemic response in steady state and in obesity and its associated metabolic disorders.

**Alterations in gut permeability.** A change in gut permeability and immune cells composition was recently noticed in response to obesity. In obese mice modified gut permeability [50] and a shift in intestinal pro-inflammatory adaptive immune cells [51] led to changes in the gut microbial composition and function and contributed to insulin resistance and glucose response through changes in intestinal barrier function, endotoxemia and adipose tissue inflammation. Treatment of obese mice with the gut-specific anti-inflammatory agent mesalamine (5-ASA), which is used as therapy for inflammatory bowel disease (IBD), reversed the activation of gut adaptive immune cells, microbial dysbiosis, adipose inflammation, rescued systemic endotoxemia and improved insulin sensitivity [51].

**Bariatric surgery.** Bariatric surgical procedures are currently considered an effective treatment for sustained weight loss and reduction of obesity-related comorbidities, including T2DM [52]. Several studies have reported that in addition to improvement of clinical and metabolic markers, Roux-en-Y gastric bypass (RYGB) operations also alter the composition of the gut microbiome in correlation with the improved metabolic parameters. A common trend is observed in these studies, namely the post-operation reduction in relative abundance of **Firmicutes** and increase in the abundance of **Proteobacteria** (or more specifically, *E. coli*) [53–56]. Nevertheless, the majority of studies characterizes the short-term effects of the surgery on the microbiome, and do not demonstrate a causal link between microbial alterations and improvement in metabolic markers. In a recent study, Tremaroli *et al.*, followed operated-women 9 years post-operation (RYGB or vertical banded gastroplasty) with comparison to obese or BMI-matched controls, and reported that some of the microbial alterations persist for long-term, including the increase in *Escherichia* and some *Clostridia* [57]. Transplantation of microbiota from post-operation patients and control obese subjects to GF mice demonstrated reduced adiposity and improved metabolism (measured as respiratory exchange rate) in recipients receiving the post-operated microbiota. These findings suggest that controlling the microbiome composition after bariatric surgery may aid in the maintenance of the improved clinical and metabolic parameters.

**Disturbances to circadian behavior.** Chronic jet lag and shift work are behavioral patterns that involve the disruption of normal circadian rhythmicity, and are associated with increased risk for obesity, diabetes, and cardiovascular disease. We and others have recently reported that the gut microbiome composition and function oscillate in a rhythmic fashion due to dietary and host cues in both mice and humans [58–61]. These oscillations are abrogated in mice that experience circadian rhythm impairment, consequently leading to obesity and glucose intolerance. In humans, similar sleep-wake alterations (induced by severe jet lag) alter the composition of the microbiome, and when the microbiota was transplanted to GF mice they developed glucose intolerance and gained more weight [58]. Feeding mice a high-fat diet also perturbs microbiome circadian oscillations, while forced night time feeding has a protective effect in preserving a normal microbiome composition and preventing diet-induced obesity and glucose intolerance [59,60]. Together, these studies suggest that timing of feeding, in addition to the feeding content, has significant effects on the microbiome, while changes in circadian behavior may alter the microbiome in ways which may promote risk for glucose intolerance and T2DM.

### 3. Mechanisms of microbiome-mediated regulation of the glycemic response

Microbiota-related metabolites play pivotal role in regulation of host glucose homeostasis. Microbiota composition and function can integrate the functional states of food intake, affecting whole body

energy homeostasis, insulin tolerance and glycemic response in steady state and during progression of obesity and T2DM [62]. Microbiota modification and production of diet metabolites and inflammatory factors plays a central role in controlling whole body glucose homeostasis. The most well studied microbiota-derived metabolites affecting the glycemic response are short chain fatty acids and bile acids. In this section, we will discuss how these metabolites regulate the glucose response of the gut, liver, pancreas and adipose tissues, influencing systemic glycemic response in steady state and in obesity-induced T2DM (Fig. 1). We will further highlight inflammatory factors produced or induced by the microbiota that affect the host immune response, thereby contributing to insulin resistance and impaired glycemic response.

**Short chain fatty acids.** Short chain fatty acids (SCFA) including acetate, propionate, and butyrate are widely studied metabolites that are produced by bacterial fermentation of polysaccharides. Diet supplementation with SCFA leads to improved glucose tolerance and insulin sensitivity in both lean and obese and diabetic humans and rodents [63,64]. The SCFA butyrate and propionate bind the G-protein coupled receptors GPR41 (FFAR3), GPR43 (FFAR2), and GPR109A mainly expressed in colonic epithelium, adipose tissue and pancreatic beta cells. The cells in the colonic epithelium utilize the SCFA produced by the bacteria as an energy source, while GF mice have low SCFAs and are energy deprived with deficit in mitochondrial oxidative phosphorylation and ATP levels leading to autophagy [65]. SCFA act through GPR41 and GPR43 to induce secretion of the incretin glucagon like peptide-1 (GLP1) from L cells and thus can contribute to glucose control [66] and gastric emptying [67]. The effect can be mediated by coupling of the receptors to  $G\alpha_q$  protein, resulting in increased intracellular calcium levels [66].

GPR43-deficient mice gained more weight on a normal diet with increased adiposity, weight, glucose intolerance and insulin resistance in response to obesity, and mice with GPR43 overexpression in adipose tissues exhibited no change in weight gain in response to high-fat diet feeding [68]. Importantly, GF mice that were deficient of GPR43 displayed a normal weight and glycemic phenotype, suggesting that bacterial SCFA induce GPR43 activation leading to reduced insulin-mediated fat accumulation, promoting lipids and glucose homeostasis [68]. GPR43 is a potent inhibitor of inflammatory response [69] expressed in immune cells. Since the inflammatory response has a substantial role in driving obesity-associated pathologies including T2DM [70], it is possible that regulation of immune function via GPR43 may contribute to improved glucose control and insulin sensitivity mediated by SCFAs. Mice lacking GPR41 were leaner compared to control mice and showed reduced expression of the enteroendocrine hormone PYY that normally inhibits gut motility, with reduced energy harvest from the diet. No difference in weight was observed in GF GPR41 null mice, suggesting that the effects of GPR41 on weight and energy balance is mediated by the microbiota.

De Vadder *et al* [64] showed that butyrate and propionate could also promote intestinal gluconeogenesis by two different mechanisms to advance energy metabolism. Butyrate induces transcription of intestinal gluconeogenic genes in enterocytes via cAMP. Propionate acts on GPR41 to induce a gut-brain neural circuit leading to conversion of propionate to glucose, resulting in modified gut microbiota composition, reduced adiposity and improved glucose control [64].

Another receptor for  $\beta$ -D-hydroxybutyrate (the major ketone body in circulation) is GPR109A, which is a niacin receptor. GPR109A induce anti lipolytic effect decreasing free fatty acids levels, whereas the role of butyrate-GPR109A in glucose regulation has not been elucidated to date [71]. Butyrate is a well known histone deacetylase (HDAC) inhibitor [72], yet the connection to its beneficial effect on energy metabolism remains unknown. Although the presented data propose a pivotal function of SCFAs and their cognate receptors in metabolism, SCFAs can enter the cell by diffusion or transporters, suggesting the metabolic effects mediated by SCFA can occur via mechanisms independent of their receptors.

**Bile acids.** Bile acids are derived from hepatic cholesterol catabolism. Newly synthesized bile acids are conjugated and transported into the gallbladder and postprandial contraction of the gallbladder empties the bile acids into the intestinal lumen [73–75]. In addition to their role in dietary fat digestion, bile acids are now recognized as important regulators of lipid metabolism, energy homeostasis and glycemic control.

Bile acids strongly interact with the gut microbiota. In the intestine microbial enzymes can modify the primary bile acids into secondary bile acids, facilitating the digestion and absorption of dietary fats. The central role of gut microbiota in regulating bile acid conjugation is demonstrated in mice treated

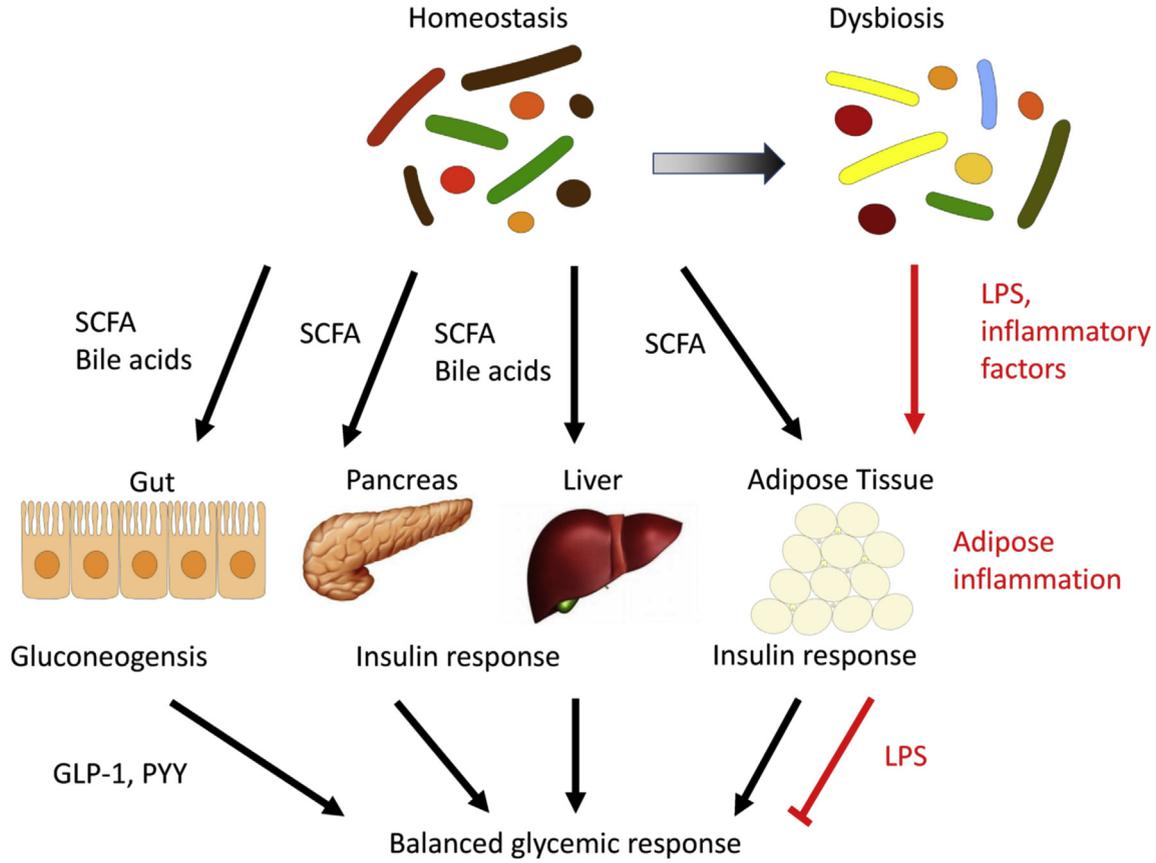


Fig. 1. Microbiota effects on the glycemic response.

with antibiotics or GF mice, which showed reduced secondary bile acids and decreased bile acid diversity [76–79]. Sayin *et al.* [78] showed that the gut microbiota mediate their effect on bile acids mainly via regulating the nuclear receptor farnesoid X receptor (FXR, also known as NR1H4) and its target gene fibroblast growth factor 15 (Fgf15) [78]. In the liver Fgf15 act together with hepatic Fgf4 to inhibit the expression of cholesterol 7- $\alpha$ -hydroxylase (Cyp7a1), which is a rate-limiting enzyme in bile acid biosynthesis. Obese and insulin resistant mice show decreased gut microbiota diversity accompanied with reduction in bile acids composition and abundance, increased FXR and Fgf15 expression in the ileum and decreased hepatic Cyp7a1 [80]. The role of FXR in obesity development was demonstrated in mice lacking both leptin and FXR [81] and mice with over-expression of hepatic Cyp7a1 [82] that exhibited protection from development of obesity and insulin resistance. Ryan *et al.* [83] showed that the beneficial effects of bariatric surgery on glucose and weight metabolism were associated with changes in the gut microbial communities and were diminished in FXR-deficient mice. Together, these studies propose that the gut microbiota plays a dominant role in regulating bile acids diversity via FXR signaling, which in turn affecting obesity and glycemic control. A recent paper published by our group [84] shows that the bile acid component taurine modifies the microbiome composition, leading to activation of NLRP6 inflammasome signaling and secretion of anti-microbial peptides. Taurine administration to mice led to amelioration of acute DSS-colitis and the effect depended on the microbiota and inflammasome activation. It will be interesting to check the effect of taurine and other bile acid components on the microbiota composition and function in controlling the glycemic response in homeostasis and in metabolic disorders.

**Immune response.** Chronic inflammation of visceral adipose tissues in obesity has become a prominent pathological mechanism in which the fat is populated with both adaptive and innate immune cells contributing to systemic insulin resistance and glucose intolerance [70]. Microbiota-derived pro-inflammatory factors may have direct effect on adipose inflammation, adiposity, insulin sensitivity and whole body glycemic control. High levels of gram-negative bacteria-derived lipopolysaccharide (LPS) that induces endotoxemia were detected in T2DM patients [85] and in obese and insulin resistant mice [31]. Administration of LPS to high-fat diet fed mice led to increased serum insulin and adipose inflammation [43], whereas antibiotics treatment of obese and insulin resistant mice significantly changed microbiota composition, abolished adipose tissue inflammation, reduced LPS levels and improved insulin sensitivity [31,86]. Moreover, GF mice introduced with *E. Coli* strain that produce immunogenic LPS led to increased macrophage infiltration to adipose tissues and augmented adipose inflammation [87]. A recent study by Caesar *et al.* [32] reported that gut microbial changes derived from lard diet resulted in induction of Ccl2 and TLR4 signaling in adipose tissues, development of adipose inflammation, with increased serum LPS and adiposity. The metabolic effect was transferrable to GF mice, whereas gut microbiota from fish-oil diet given to lard-fed mice counteract the metabolic phenotype.

Collectively these studies suggest that microbiota-derived or –induced inflammatory factors such as LPS, Ccl2 and TLR4 may directly contribute to obesity and its associated metabolic disorders, through induction of adipose tissue inflammation, which in turn drives insulin resistance and glucose intolerance.

#### 4. The microbiota as a therapeutic target for improvement of glycemic control

Unlike the human genome, the microbiome may be more readily manipulated and thus may be considered as a potential therapeutic target. This is an important advantage when considering microbiome-targeting interventions in a variety of microbiome-associated or –driven pathologies, such as those related to impaired glycemic control. Microbiota-modulating therapies may aim to ‘correct’ the detrimental microbial composition or function, either by administration of antibiotics or probiotics, or by transplant of healthy fecal microbiota, which will replace the disease-associated configuration. Other therapies may attempt to modulate the immune response, by targeting microbial antigens or inflammatory signaling cascades impacting the microbiome or impacted by the microbiome. Examples of these various therapeutic modalities and related open questions will be further addressed in this section.

**Antibiotics.** Repeated antibiotics exposure in early life has been suggested to promote both T1D and T2DM. However, Membrez *et al.* demonstrated that modulation of the gut microbiome with norfloxacin and ampicillin improved glycemic control in both diet-induced and genetically-susceptible hyperglycemic (ob/ob) mice [88]. Similar findings were reported by Carvalho *et al.* [86] with high-fat diet fed mice treated with ampicillin, neomycin and metronidazole, including improvement of glucose and insulin tolerance, along with reduction in circulating LPS and inflammatory cytokines levels. This suggests that modulation of a dysbiotic gut microbiota may improve insulin signaling and glucose tolerance by reducing inflammation. In a model of viral-induced T1DM, treatment with trimethoprim/sulfamethoxazole had a protective effects on the rats, possibly by preventing viral-induced alterations to the gut microbiome [89]. Information regarding the efficacy of antibiotics as a therapeutic tool in humans is sparse and is somewhat in contrast to the aforementioned findings [90]. When considering antibiotics as therapeutics for a non-infectious disease, it is important to bear in mind that they act non-specifically on the microbiome and eliminate not only the bacteria of interest, but also other 'beneficial' bacteria that are critical for healthy function of the various host systems. In addition, antibiotics use increases the risk of expansion of pathobionts (commensals that become pathogenic under specific conditions) and opportunistic infections [91]. Finally, frequent antibiotics use contributes to the emergence of antibiotics-resistant pathogens. Taken together, it seems unlikely in our view that antibiotics will serve as a widely used therapeutic tool for diabetes mellitus. Nevertheless, the aforementioned studies in rodents suggest that a more refined strategy that targets specific aberrant members of the microbiota in diabetes may hold a therapeutic potential as microbiome-targeting treatment in these disorders.

**Fecal microbiome transplantation (FMT).** FMT has recently gained much attention as an efficient treatment for recurrent *Clostridium difficile* infections. In this method, upon transplantation, the fecal microbiota of a healthy donor is assumed to replace or correct the aberrant composition that underlies certain pathology in the recipient. In a currently stand-alone, proof-of-concept study, Vrieze *et al.* [92] performed small intestine infusions of fecal microbiome to obese individuals. Participants that received microbiota from lean donors demonstrated improvement of insulin tolerance, as well as compositional changes in both the small and large intestine microbiota, namely expansion of butyrate-producing bacteria. No improvement was observed in obese participants that received autologous microbiota. Although promising, transplantation of a whole microbiome is not risk-free. Even after screening for pathogens, there is a potential of unwanted effects of the transplanted microbial community on other, unrelated conditions. A more targeted approach would be to transplant selected microbes that improve insulin tolerance, or administer metabolites produced by the microbiome. In mice, supplementing the diet with butyrate protects against high fat diet-induced insulin resistance [93]. It remains to be determined whether such approach has therapeutic potential in humans.

**Probiotics and prebiotics.** Probiotics can be considered a more specific approach than transplantation of an entire fecal microbiota community, as they are comprised of a defined combination of several so-called 'beneficial' bacteria. However, the efficacy of probiotics in promoting health benefits remains questionable. Several studies determined a beneficial effect for probiotics comprised of **Lactobacilli** and/or **Bifidobacteria** on both T1DM and T2DM onset and progression in mice [94,95]. In humans, however, supplementation with probiotics does not always yield similar effects [96], and in a recent meta-analysis of human trials the authors concluded that probiotics may only modestly affect glycemic control [97]. An additional interesting bacterium that can be considered as probiotic in the context of glycemic control is **A. muciniphila**. Treating high-fat diet fed mice with *A. muciniphila* prevents many of the diet-induced metabolic derangements, including glucose intolerance [98,99]. A novel approach utilizes probiotic bacteria as vectors for local expression of beneficial metabolites. Chen *et al.* fed mice with an *E. coli* strain transformed to express NAPES (N-acyl-phosphatidylethanolamines, compounds with known efficacy against obesity in mice). Mice treated with this strain had reduced levels of obesity and insulin resistance [100].

Prebiotics is a term denoting the use of various foods/nutrients that, upon their supplementation to the diet, modify the composition of the microbiome to a beneficial one. In the context of diabetes, feeding high fat diet fed mice with oligofructose increases the abundance of **Bifidobacterium** which is positively correlated with improved glycemic control [101]. The establishment of both pre- and probiotics as efficient treatments for glucose intolerance still requires further validation in additional

large-scale human trials, that will address both their efficacy in the general population and their mode of action to improve glycemic control.

**Personalized dietary interventions.** Dietary intake is a significant determinant of blood glucose levels, highlighting the importance of making food choices that induce normal post-prandial glycemic responses (PPGR) in prevention and treatment of diabetes. Nevertheless, the PPGR to the same food is different between individuals, rendering global dietary recommendations that are based solely on intrinsic properties of the food (such as carbohydrates content) inefficient. A recently reported study denoted a significant inter-individual differences to identical foods in a cohort of 800 individuals. By integrating blood parameters, dietary habits, anthropometrics, physical activity, and gut microbiota features from this cohort, a machine learning based algorithm was devised that accurately predicted PPGRs in an independent cohort. A dietary intervention based on predictions by this algorithm resulted in significantly lower postprandial responses [102].

In a dietary intervention study performed by Kovatcheva-Datchary *et al.*, participants supplemented their diet with barley kernel-based bread for three days. Following this supplementation, microbiota-dependent improvement in post-prandial glycemic and insulinemic responses were observed, as response to the beneficial effect of the supplement was dependent on diet-induced bloom of *Prevotella* [103]. Considered together, these reports suggest a promising role for microbiome data in designing personalized diets for prevention and management of hyperglycemia.

## 5. Perspective

Recent technological advances in the fields of next-generation sequencing and gnotobiology led to the accumulation of a significant body of data regarding the involvement of the microbiome in common multi-factorial disorders, including those involving an aberrant glycemic response. As the field advances, changes in the microbiomes are no longer considered merely correlative to these diseases, but in some cases are suggested to play a causative role in disease pathogenesis. In this review, we highlighted multiple environmental and genetic factors that may alter the microbiome in a way that impacts the glycemic response. We also describe how the aberrant microbiota may exert its adverse effects on glycemic response through bacterial metabolite secretion, modulation of mucosal and systemic immunity or through other, yet unidentified mechanisms. In this regard, the microbiome can thus be considered a signaling hub, integrating both endogenous and exogenous signals to promote net effects on the host glucose homeostasis. There are still many open questions regarding these interactions. We currently lack a detailed understanding of the nature and mechanism of activity of the myriad of factors that affect the microbiome, and are only beginning to unravel the network of microbiome-host interactions and their contribution to the pathogenesis of diabetes mellitus.

One critical factor that may impact microbiome research in years to come is its heterogeneity among individuals, which may provide insights to the individualized responses of people to environmental stimuli including nutrition, wake-sleep alterations, and medications. Moreover, it is now appreciated that inter-individual variability in the composition and function of the human microbiome extends far beyond the healthy state, and particularly in T1DM or T2DM, in which, despite several commonalities, no unique bacterial signature is identified across studies. Furthermore, several human trials of various bacteria-targeted therapeutics featured mixed results, which may possibly be the outcome of inter-individual microbiome variability. It is plausible that clinical heterogeneity and individualized treatment responsiveness in both T1DM and T2DM may be influenced by inter-individual variability in microbiome composition and function. Of equal importance is heterogeneous microbiome isolation, sequencing and analysis methods by different groups that at times may affect the obtained results. Thus, one of the critical steps as part of the 'maturation' of the microbiome field must involve standardization of procedures that would allow for better reproducibility allowing the integration of microbiome data into clinical practice. Future research in the field may involve personalized microbiome-targeted approaches, utilized for personalized diagnostics and therapeutics of an altered glycemic response and its complications in afflicted individuals.

## Conflict of interest

None declared.

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