Current Diabetes Reports (2018) 18: 98
https://doi.org/10.1007/s11892-018-1057-6

PATHOGENESIS OF TYPE 2 DIABETES AND INSULIN RESISTANCE (M-E PATTI, SECTION EDITOR)

Altered Gut Microbiota in Type 2 Diabetes: Just a Coincidence?

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Published online: 13 September 2018
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Abstract

Purpose of Review In the last decade many studies have suggested an association between the altered gut microbiota and multiple systemic diseases including diabetes. In this review, we will discuss potential pathophysiological mechanisms, the latest findings regarding the mechanisms linking gut dysbiosis and type 2 diabetes (T2D), and the results obtained with experimental modulation of microbiota.

Recent Findings In T2D, gut dysbiosis contributes to onset and maintenance of insulin resistance. Different strategies that reduce dysbiosis can improve glycemic control.

Summary Evidence in animals and humans reveals differences between the gut microbial composition in healthy individuals and those with T2D. Changes in the intestinal ecosystem could cause inflammation, alter intestinal permeability, and modulate metabolism of bile acids, short-chain fatty acids and metabolites that act synergistically on metabolic regulation systems contributing to insulin resistance. Interventions that restore equilibrium in the gut appear to have beneficial effects and improve glycemic control. Future research should examine in detail and in larger studies other possible pathophysiological mechanisms to identify specific pathways modulated by microbiota modulation and identify new potential therapeutic targets.

Keywords Dysbiosis · Inflammation · LPS · SCFAs · Insulin resistance · Probiotics

Introduction

Trillions of microorganisms reside in the human gut in a complex ecosystem which operates as a "hidden organ" [1]. This microbial community and its genome (microbiome) include not only bacteria but also protozoans, viruses, and archaea collectively termed microbiota.

Gut microbiota exerts diverse physiological features such as modulation of immune and inflammatory response; regulation of neuronal signaling; regulation of integrity and mobility of the gut barrier; biosynthesis of vitamins, steroid hormones, and neurotransmitters; and metabolism of branched-chain aromatic amino acids, bile salts, and drugs [2].

In the last decades, improvement of analytical methods allowed researchers to show that alteration of microbiota is associated with many human diseases: gastrointestinal diseases [3], cancer [4], metabolic disease [5, 6], neurodegenerative disorders [7], cardiovascular [8], renal [9], and lung diseases [10]. Although experimental data in mice support the hypothesis of a possible causal role in the etiology of these diseases, human data that favor causality are often insufficient or conflicting.

T2D is thought to arise at the intersection of genetic factors, sedentary lifestyle, poor diet, excessive visceral obesity, and other environmental exposures throughout life [11]. While the precise causes of disease are not completely clear, increasing evidence supports a role for the intestinal microbiota in development of T2DM [12••]; however, the pathophysiological

This article is part of the Topical Collection on *Pathogenesis of Type 2 Diabetes and Insulin Resistance*

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mechanisms that link the microbiota to T2D have not been well elucidated.

In this review, we provide an overview of the most recent findings, with the aim to understand more about pathophysiology and identify new potential therapeutic approaches.

Gut Microbiota: a Dynamic Ecosystem

Analytical Methods

Since it is very difficult to identify intestinal microorganisms through culture-based methods, alternative techniques allow a much more comprehensive mapping of bacteria. For the study of microbial DNA from fecal samples, considered representative of the distal gut microbiota composition, culture-independent methods have benefited from evolution of next-generation sequencing technology, such as 16S ribosomal RNA gene amplicon sequencing and shotgun metagenomics sequencing [13].

The 16S rRNA gene sequencing provides information on the composition of microbial communities. With this method, polymerase chain reaction (PCR) is used to amplify a specific region in the 16S gene; this product is subsequently sequenced. By contrast, shotgun metagenomics sequencing is able to analyze the entire genomic content of a community, by using direct sequencing of microbial RNA without prior amplification. A subsequent taxonomic assignment requires assembly tools and updated databases [14]. In recent years, both methods have improved substantially, with increased throughput and reduced costs. However, specific bioinformatics tools and up-to-date databases continue to evolve. Although each method has its own advantages and limitations, new data suggest that shotgun sequencing provides a higher resolution representation of the microbial composition and allows better characterization of complexity as compared to 16S rRNA amplicon sequencing [15]. Further studies comparing the two methods are required.

Microbial Changes in Humans with T2D

Although the human gut microbiota composition differs in different parts of intestinal tract, six main phyla are dominant: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Fusobacteria*, *Verucomicrobia*, and *Proteobacteria* [16•]. Over 90% of the 1000 prevailing bacterial species belong to the *Firmicutes* and *Bacteroidetes* phyla. Recent reports show that interindividual variation in the composition of communities is very high, potentially related to age, diet, illness, genetic and environmental factors, and medication [17••, 18••].

The first study describing a substantial difference in the composition of gut microbiota between individuals with T2D and healthy individuals dates back to 2010 [19]. In this small study from Denmark, the authors analyzed the fecal bacterial composition of 18 men without and with T2D using 16S rRNA amplicon sequencing. T2D was associated with changes in the intestinal microbiota composition (dysbiosis) at a phylum level, with a reduction in the proportion of *Firmicutes* and a slight increase in *Bacteroidetes* and *Proteobacteria.* However, these results were not confirmed in two large metagenome-wide association studies conducted in China and Europe [20, 21]; moderate dysbiosis was observed in T2D but the differential microbial composition varied between two studies. This could be due to ethnic and dietary heterogeneity in the populations studied, differences in antidiabetic drugs or other medications, or disease status, as well as different sequencing techniques used. Nonetheless, both the Chinese and European studies found butyrate-producing bacteria (*Roseburia intestinalis* and *Faecalibacterium prausnitzii*) concentrations lower in T2D while certain *Lactobacillus* species and some opportunistic pathogens, such as *Bacteroides caccae*, *Clostridium hathewayi*, *Clostridium ramosum*, *Clostridium symbiosum*, and *E. coli*, were higher in T2D. Increased expression of the microbial genes involved in oxidative stress and the pro-inflammatory state typical of T2D was also observed in the two larger studies, but not the smaller Danish study [22].

Using 16S rRNA-based high-throughput sequencing analysis, Zhang et al. [23] found decreased abundance of *Akkermansia muciniphila* inindividuals with prediabetes and newly diagnosed T2D suggesting that low concentrations of this bacteria in the gut could be a biomarker for glucose intolerance [24]. *A. muciniphila* is a mucin-degrading bacterium that colonizes the intestinal mucous layer and constitutes 3–5% of the human intestinal microbial composition. Interestingly, daily treatment with viable *A. muciniphila* in mice with dietary obesity yielded decreased metabolic endotoxemia, insulin resistance, adipose tissue macrophage infiltration, and improvement in fasting glycemia [25].

Another study of Danish individuals with insulin resistance demonstrated increases in serum levels of branched-chain amino acid (BCAA), potentially linked to both increased production by intestinal microorganisms (*Prevotellacopri* and *Bacteroides vulgatus*) and reduced transport within the bacterial cell [26]. This is intriguing, as increased plasma BCAA are associated with a greater future probability of developing insulin resistance and T2D in both children and adults [27, 28]. Thus, these data support the hypothesis that the intestinal microbiota could play a fundamental role in systemic metabolism and insulin resistance.

Stool analysis of 50 individuals with T2D from Japan showed both increased total counts of *Lactobacillus*, together with a reduction of *Clostridium coccoides* and *Prevotella*as compared with the control group. Intestinal bacteria were also found in the blood in patients with T2D, suggesting a translocation from the intestine to the bloodstream [29].

Recent studies based on the analysis of 16S rRNA gene using next-generation sequencing (Illumina MiSeq) found that *Firmicutes/Bacteroidetes* ratio in patients with T2D was significantly higher than in controls [11, 30]. These results are in contrast with those obtained in Danish, Chinese, and European studies in which patients with T2D had a lower *Firmicutes/Bacteroidetes* ratio compared to controls. Egshatyan et al. [30] identified *Blautia* as the most represented genus; its representation was higher in patients with T2D and prediabetes than in subjects with normal glucose tolerance. It is noteworthy that some *Clostridia* and *Blautia coccoides* can stimulate the secretion of TNFα and inflammatory cytokines to a greater extent than LPS [31].

In summary, the results to date suggest that patients with T2D show evidence of gut dysbiosis. Discrepancies between studies are numerous, probably due to various confounding factors such as different study populations, different sequencing techniques used, and differences in dietary intake and medication use. Large studies that take into account these different variables are necessary. A cross-sectional and observational prospective study in patients with T2D is ongoing (*ClinicalTrials.gov* ID: NCT03204799).

Bacteria, Metabolities, and Host Interactions

In the last 10 years, low-grade inflammation has been hypothesized to be the link between microbiome and T2D risk, via mechanisms related to bacterial toxins, short-chain fatty acids, bile acids and BCAA metabolism (Fig. 1).

Low-Grade Inflammation and Bacterial Toxins

Animal and human studies suggested that a high-fat diet (HFD) can change the intestinal ecosystem and increase the circulating levels of proinflammatory mediators. Caniet al. [32] defined "metabolic endotoxemia" as a condition characterized by a two- to threefold increase in circulating lipopolysaccharide (LPS) levels; this may result in low-grade systemic inflammation and contribute to insulin resistance. LPS is a component of the gram-negative bacterial wall which can activate local immune response via high-affinity binding to specific receptors (e.g., Toll-like receptors (TLRs), the NLRP3 inflammasome and NOD like receptors (NLRs)) expressed at high levels on the surface of macrophages and dendritic cells [33]. In blood and tissues, LPS also activates the TLR4/MyD88/NF-κB pathway, triggering an inflammatory response through release of pro-inflammatory molecules TNF-α, IL-1, IL-6, and iNOS. With activation of this inflammatory cascade, activated serine kinases (JNK and IKK) can induce IRS (insulin receptor substrate) serine phosphorylation, which inhibits insulin signaling, resulting in cellular insulin resistance (IR) [34]. In human muscle cell lines TLR4 inhibitors suppressed inflammation and decreased LPS-induced insulin resistance [35].

N-acetyl cysteine (NAC) is a potent antioxidant that exerts anti-inflammatory activity via inhibition of NF-kB, while reducing glucose intolerance and insulin resistance in T2D and inhibiting the growth and adhesion of some pathogens [36]. Zheng et al. recently evaluated the effects of NAC (1 mg/mL, in drinking water) on the microbiota of HFD-fed mice. After 5 months of treatment, NAC improved glucose tolerance and reduced fasting glucose, body weight, and plasma endotoxin levels, while increasing the prevalence of beneficial bacteria such as *Akkermansia*, *Lactobacillus*, and *Bifidobacterium* [37•]. Since systemic levels of NAC were not measured, the precise site of action is uncertain.

Patients with T2D have higher blood levels of LPS (a component of the gram-negative bacterial wall) compared to healthy subjects. This may seem like a paradox, because in the microbiota of patients with T2D, there is a decrease in the percentage of gram-negative and increase in gram-positive *Firmicutes*. The explanation is that high endotoxemia is directly related to increase of intestinal permeability [38••]. Increases in LPS may even precede the development of T2D; indeed, the CORDIOPREV study [39••] demonstrated that postprandial LPS levels were higher in individuals who developed T2D over a median of 60 months, as compared with those who remained free of T2D during a median follow-up of 60 months.

Intestinal permeability is usually regulated by tight and adherence junction proteins between intestinal epithelial cells, which create a barrier that prevents bacteria, toxins and intestinal lumen products from reaching the circulation. In mice fed a high-fat diet, reduced expression of zonula occludens-1 (ZO-1), occludin, and claudin-1 leads increased translocation of bacteria and LPS into the circulation [40]. A recent paper showed that in mice with streptozotocin-induced diabetes, hyperglycemia could reduce tight and adherence junction integrity via a direct effect on reprogramming of intestinal epithelial cells through GLUT2-dependent mechanism [41••].

Taken together, these data suggest that hyperglycemia could lead to an increase in intestinal permeability, favoring translocation of proinflammatory bacteria and toxins which in turn impairs glucose metabolism.

Short-Chain Fatty Acids

One mechanism potentially mediating the impact of intestinal dysbiosis to regulate systemic metabolism and T2D risk is related to alterations in short-chain fatty acids (SCFAs). SCFA acetate, propionate, and butyrate are the most abundant microbial metabolites derived from fermentation of non-digestible carbohydrates introduced with diet. SCFAs play diverse roles including cell growth and differentiation,

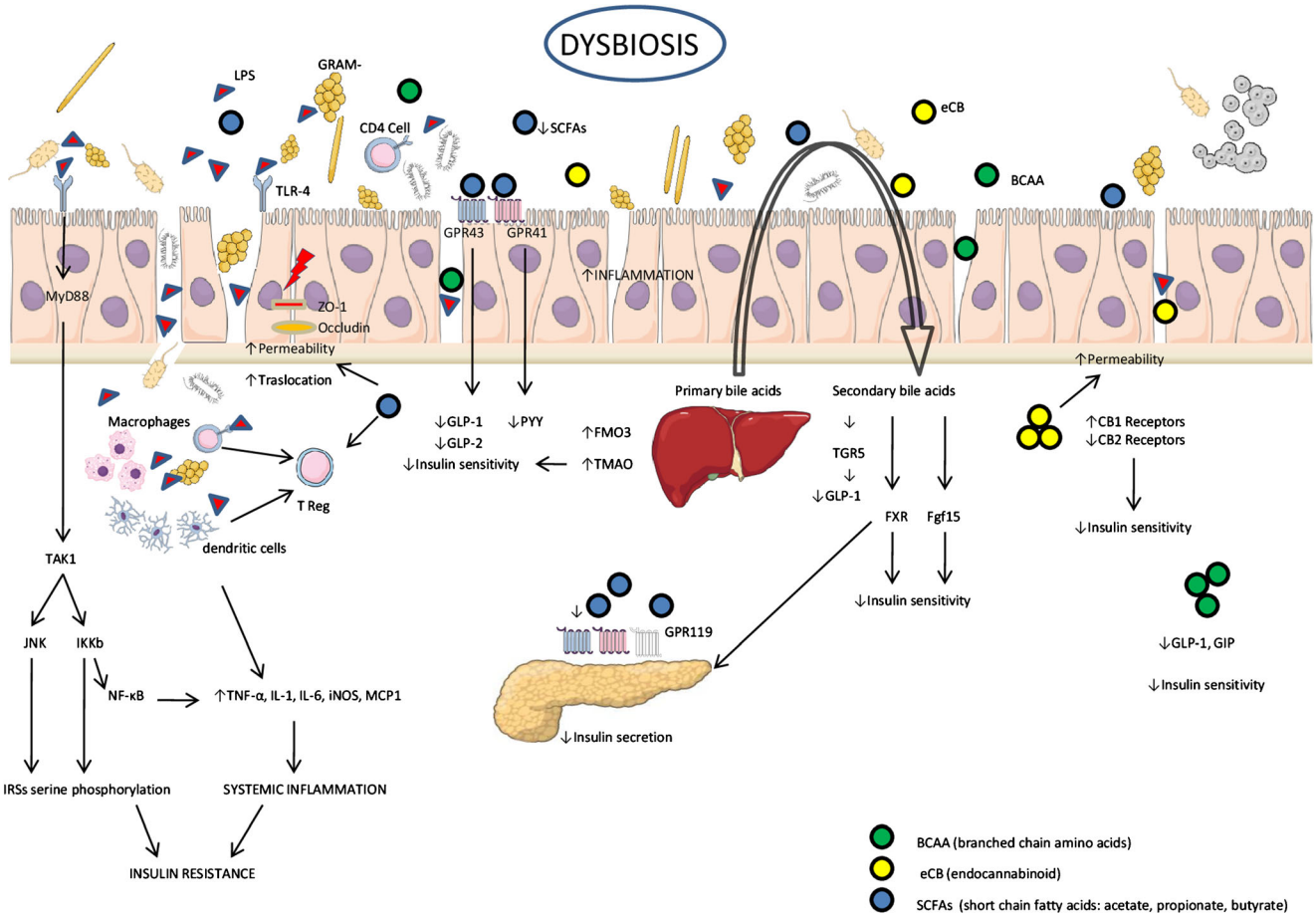

Fig. 1 Influence of the gut microbiota in promoting insulin resistance and T2D. TLR-4 Toll-like receptor 4, MyD88 myeloid differentiation protein 88, TAK1 transforming growth factor B-associated kinase 1, GPR43 G-protein-coupled receptor 43, GPR41 G-protein-coupled receptor 41, TMAO trimethylamine N-oxide, FMO3 flavin monooxygenase 3, LPS lipopolysaccharides, NF-kB nuclear factor-kappa B, GLP-2 glucagon-like-peptide 2, IL interleukin, TNF tumor necrosis factor, eNOS endothelial nitric oxide synthase, MCP-1 monocyte chemoattractant protein-1, GLP-1 glucagon-like peptide-1, IRS insulin receptor substrate, ZO-1 zonula occludens-1, PYY intestinal peptide YY, TGR5 G-protein-coupled bile acid receptor 1, FXR farnesoid X receptor, Fgf15 fibroblast growth factor 15, GIP glucose-dependent insulinotropic polypeptide, CB endocannabinoid receptor, eCB endocannabinoid (yellow circles), SCFAs short-chain fatty acids (blue circles), BCAA branched-chain amino acids (green circles)

promotion of gut epithelial integrity, anti-inflammatory and immunomodulatory functions [42], and regulation of pancreatic β cell proliferation and insulin biosynthesis [43]. SCFAs have also been shown to modulate intestinal inflammation by Treg cells and influence fluid secretion, motility, and duodenal barrier function through mechanisms that may also involve GLP-2 secretion [44]. SCFA principally bind G-protein-coupled receptors 43 and 41 (GPR43/FFA2 and GPR41/FFA3) expressed not only on enteroendocrine and intestinal epithelial cells but also in the islets of Langerhans [45]. In turn, animal data show that GPR41 regulates intestinal gluconeogenesis, food intake and energy expenditure, and stimulate secretion of the intestinal peptide YY. This regulates appetite and energy intake with direct effects on the central nervous system [46]. Similarly, GPR43 stimulates production of glucagon-like peptide-1 (GLP-1), a gut hormone that increases glucose-dependent insulin secretion and inhibits glucagon secretion [47]. SCFAs can also bind GPR119, another receptor expressed in intestinal L-cells and pancreatic β-cells. GPR119 agonists reduce blood glucose by promoting intestinal secretion of GLP-1, improving pancreatic β-cell function and insulin secretion [48]. Thus, activation of G-protein-coupled receptors by SCFAs may have beneficial effects via reducing food intake, improving insulin sensitivity, inhibiting fat accumulation, and reducing systemic inflammation. Decreases in SCFA-producing bacteria may reduce these beneficial effects and promote the development of insulin resistance and T2D.

Nevertheless, there are animal [49] and human studies [50] in which an increased concentration of SCFAs in feces was associated with higher body weight and fat gain (via GPR41 receptor action) and insulin resistance. The role of SCFAs is thus controversial and needs further investigation in this context.

Bile Acid Metabolism

Intestinal bacteria play an important role in the conversion of primary bile acids to secondary bile acids, and can also influence their composition. However, bile acids, in turn, could regulate the composition of the gut microbiota because of their antimicrobial activity [51]. Previous reports found that bile acid composition is different between control and germ-free mice, and probiotic administration changes gut microbiota composition and increases bile acid deconjugation [52]. Secondary bile acids can stimulate GLP-1 secretion through G-protein-coupled bile acid receptor 1 (TGR5), modulate expression of farnesoid X receptor (FXR) and fibroblast growth factor 15 (Fgf15), thus regulating hepatic glucose metabolism and insulin sensitivity [53].

Branched Chain Amino Acids

Gut microorganisms are a potential source of circulating BCAA through both biosynthesis and by modifying nutrient absorption. Although many of the underlying mechanisms have not yet been identified, several authors suggest that microbial amino acid metabolism may play multiple roles in the genesis of insulin resistance [54]. Human studies have demonstrated that both dietary intake of BCAA and high plasma levels of BCAA are associated with an increased risk of T2D [55]. Multiple mechanisms may contribute to elevations in BCAA in this context. Firstly, insulin resistance may cause reduced suppression of proteolysis [56]. Moreover, obesity and proinflammatory states are linked to reductions in BCAA catabolism in peripheral tissues, potentially mediated at least in part by reduced adiponectin secretion [57]; this effect may precede insulin resistance. BCAA may affect insulin, glucagon, GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) secretion [58].

A latest meta-analysis of three genome-wide association studies provides genetic evidence linking IR with elevated circulating BCAAs levels [59]. While these data do not provide conclusive data about cause and effect relationships, it is important to note that experimental reductions in dietary BCAA results in increased energy expenditure and improved insulin sensitivity in rodents [60].

TMAO

Trimethylamine (TMA) is an organic compound synthesized exclusively by gut microbiota from dietary nutrients including phosphatidylcholine, choline, and carnitine. After being absorbed, it is converted in the liver by flavin monooxygenase 3 (FMO3) to form trimethylamine N-oxide (TMAO). Higher TMAO plasma levels are associated with an increased risk of T2D [61] and cardiovascular disease [9]. Overexpression of FMO3 in human hepatoma cell lines elicited a significant increased glucose production and insulin resistance probably through the PPARα and Kruppel-like factor 15 pathways; in mice, its deletion conferred protection against obesity [62]. In the POUNDS Lost trial [63], the author highlight that dietary changes can modify plasma levels of TMAO, choline and L-carnitine, and their reduction is associated with improved insulin sensitivity.

Endocannabinoid System

Another emerging mechanism involved in intestinal permeability, glucose metabolism, and energy homeostasis is alteration in the endocannabinoid (eCB) system. Gut metabolites can activate several pathways via cannabinoid CB1 and CB2 receptors, an important target in the context of inflammation, T2D, and obesity [64]. In rodents, CB1 receptor blockade improves intestinal barrier function while stimulation of CB2 receptors improves insulin resistance [65].

Microbiota Modulation: a New Therapeutic Strategy for Diabetes?

Diet

More than 10 years ago, the results of the CARDIA study highlighted how high-fat diet and lower consumption of dietary fibers are associated with weight gain and insulin resistance while the consumption of dairy products improved glycemic control [66]. Animal-based diets may reduce the levels of *Firmicutes* that metabolize the plant-derived polysaccharides and produce beneficial SCFAs [67].

Emerging data indicate that dietary interventions can indeed change the composition of the bacterial community. A meta-analysis evaluated the efficacy of dietary interventions, finding modulation of intestinal microbiota in parallel with improved blood glucose control, as measured by HbA1c, while having little or no effect on fasting blood glucose, fasting insulin, insulin resistance, inflammation, and SCFA levels compared to the control group [68••].

In another recent clinical study, high dietary fiber intake improved hemoglobin HbA1c levels and increased GLP-1 production. Shotgun metagenomics and metabolomic analysis showed increase abundance of SCFA-producing bacteria and lower levels of deleterious metabolites such as indole and hydrogen sulfide [69•]. Therefore, diet represents an important factor contributing to the composition of the intestinal microbiome. However, responses to intervention can vary substantially between individuals; for example, abundance of several *Firmicutes* species at baseline, predicted the responsiveness to intervention in one study [70]. Large RCT will be required to fully test whether dietary modifications aimed at

changing the microbiome could represent a new therapeutic target for the prevention and management of T2D.

Probiotics

Probiotics are defined as "live microorganisms that, when administered in proper amounts, may exert health benefits to the host" [71]. While the type and composition of bacteria in various commercial products differ considerably, the most common probiotic products contain *Lactobacillus* sp., *Enterococcus* sp., *Bifidobacterium* sp., *and Streptococcus* sp. A promising technique could be to use recombinant bacteria to improve glycemic control: recently, a genetically modified strain of *Lactococcus lactis* was shown to enhance insulin secretion and improve glucose tolerance in mice [72]. The beneficial effects of probiotics on T2D have been extensively demonstrated in animal studies, with reduction of *Firmicutes/Bacteroidetes* ratio, increased abundance of SCFA-producing bacteria, decreased levels of inflammatory molecules TNFα, IL-1, IL-6; increased levels of GLP-1; and improved insulin resistance [73]. Furthermore, probiotics reduce inflammatory phenotypes, improve β-cell dysfunction, and can have beneficial effects on the intestinal wall, reducing intestinal permeability and preventing translocation of bacterial LPS [74]. Experimental data suggest also a potential immunomodulatory role: *B. infantis* can induce T regulatory (Treg) cells, stimulate production of CD25+ lymphocytes, stimulate human dendritic cells (DCs), and induce the production of IL-10 [75].

While the limited studies conducted in humans are generally concordant with animal studies (Table 1), there are some exceptions. Ivey et al. did not find any effect of probiotic supplementation for 6 weeks using a capsule containing *L. acidophilus* La5 and *B. animalis* subsp. *lactis* Bb12 on glycemic parameters in overweight subjects [80]. In another RCT, 31 glucose-tolerant patients were enrolled to evaluate the efficacy of *L. reuteri SD5865* administered over 4 weeks; increases in GLP-1 and insulin secretion were found, without changes in insulin sensitivity [82]. More recently, Mobini et al. [84] evaluated the effects of *Lactobacillus reuteri DSM 17938* over 12 weeks in patients with T2D on insulin therapy: probiotic supplementation improved insulin sensitivity in a subgroup but did not affect overall glycemic control measured by HbA1c.

Several systematic reviews and meta-analyses concluded that probiotics could have beneficial effects on glycemic control in T2D, but effects on HbA1c, anti-inflammatory and anti-oxidative benefit are inconsistent [89–93]. Limitations of the RCT conducted thus far include: heterogeneity of the groups studied (ethnicity, metabolic state, drugs, duration of diabetes), different bacterial strains used, short period of treatment, different methods of analysis, and small sample sizes. Therefore, given the considerable disparities in both the design and findings of the studies, and substantial interindividual variation in response to probiotic integration, future long-term, multicenter RCT utilizing consistent methods will be required to determine their usefulness as prevention or as synergistic approach in T2D treatment. Several trials are ongoing (IRCT201511032321N2; ACTRN12613001378718).

Fecal Transplantation

Fecal microbiota transplant (FMT) consists of administering fecal matter, taken from a healthy donor, via endoscopy or enema. FMT is a technique that has produced good results in the treatment of *Clostridium difficile* infection when medical therapy is ineffective, and it is a promising treatment for a variety of diseases [94]. Thus far, there has been only one human study evaluating the effects of FMT in patients with metabolic syndrome [95]. In this work, FMT from healthy subjects to adults with metabolic syndrome led to increased butyrate-producing bacteria in the stool of the recipient microbiota and improvement in peripheral insulin sensitivity. This study, while promising, was limited by small sample size and absence of data on glycemic control and inflammation markers. Given the limited data available at present, future studies will be required to fully define the adverse effects of such treatment, including transplantation of potentially pathogenic microorganisms, and to critically evaluate the risk/benefit ratio. Several RCTs are ongoing (ChiCTR1800014569; NCT03127696; NCT01790711; NTR 5141). Ultimately, it is hoped that defining the microbiota mediating beneficial effects will allow administration of cultured bacterial treatments.

Impact of Diabetes Treatments on the Microbiome

Different drugs, including antidiabetic medications, can affect the intestinal microbiota [96].

Metformin, currently the cornerstone of T2D treatment, has long been recognized to improve insulin sensitivity and reduce hepatic glucose production [97]; it also alters the composition of the microbiota: increase abundance of *A. muciniphila*, *Lactobacillus*, and *Escherichia* spp. and decrease abundance of some pathogens [98]. Metformin also promotes the production of SCFAs, regulates bile acid turnover, improves intestinal permeability, reduces endotoxin levels, and stimulates the activity of endocrine cells by enhancing release of GLP-1 and PYY peptides [99].

Incretin-based therapies can also affect the composition of the microbiota in rodents, but results differ across studies. In mice fed a HFD, the GLP1RA liraglutide reduces

Table 1 Overview of randomized clinical trials performed to evaluate the effects of probiotic strains

Probiotic	Source	Patient	Duration of treatment (weeks)	Sample size (intervention/control)	Positive effects	Negative or no effects(≡)	References
Lactobacillus acidophilus NCFM	Capsules	T2D	4	21/24	Preserved insulin sensitivity	≡Inflammatory markers	Andreasen et al. [76]
L. acidophilus La5 and *Bifidumbacterium lactis* Bb12	Probiotic or conventional yogurt	T2D	6	30/30	↓FBG and HbA1c ↓ antioxidant status	≡Insulin concentration and erythrocyte catalase activity	Ejtahed et al. [77]
L. acidophilus, *L. casei*, *L. rhamnosus*, *L. bulgaricus*, *B. breve*, *B. longum*, and *Streptococcus thermophilus*	Capsules	T2D	8	27/27	Prevented rise in FBG ↓hs-CRP ↑GSH	↑Serum insulin ↑HOMA IR (but lower than that in the placebo group)	Asemi et al. [78]
L. acidophilus, L. bulgaricus, L. bifidum, and *L. casei*	Capsules	T2D	6	16/18	↓MDA, IL-6 and HOMA IR (not statistically significant)	≡FBS ↑ hs-CRP (not statistically significant)	Mazloom et al. [79]
L. acidophilus La5, *B.animalis* subsp. *lactis* Bb12	Probiotic yogurt ± probiotic capsule; control milk ± probiotic capsule	Overweight subject	6	Yogurt 40/37 Milk 39/40		↑HOMA-IR ↑FBG ≡Fasting insulin and HbA1c	Ivey et al. [80]
L. casei, *L. acidophilus*, *B. lactis*	600 mL/day probiotic fermented milk (kefir) vs. conventionally fermented milk	T2D	8	30/30	↓FBG, HbA1c		Ostadrahimi et al. [81]
L. reuteri SD5865	Capsules	Glucose-tolerant humans	4	11/10	↑GLP-1, GLP-2 release ↑Insulin and C-peptide secretion	≡Peripheral and hepatic insulin sensitivity ≡Circulating cytokines	Simon et al. [82]
L. acidophilus, *L. casei*, *L. lactis*, *B. bifidum*, *B. longum*, and *B. infantis*	Powder	T2D	12	68/68	↓HbA1c and fasting insulin ↓HOMA IR	≡hs-CRP	Firouzi et al. [83]
L. reuteri DSM 17938	Powder	T2D	12	29/15	↑ISI and DCA (in subgroup with higher microbial diversity at baseline)	≡HbA1c	Mobini et al. [84]
L. acidophilus La5 and *B. animalis* subsp. *lactis* BB-12	Probiotic fermented milk vs. conventional fermented milk	T2D	6	25/25	↓HbA1c and fructosamin levels ↓TNF-α and resistin	≡IL-10 ↑Acetic acid	Tonucci et al. [85]
Lactobacillus planetarum A7	Probiotic soy milk	T2D	8	20/20	↓LDL ↑HDL	≡FBG, adiponectin, TNF-α and hs-CRP	Feizollahzadeh et al [86]
Lactobacillus casei	Capsules	T2D	8	20/20	↓FBG, insulin, HOMA-IR ↑SIRT1; ↓fetuin-A ↓HbA1c (not significant)		Khalili et al. [87]
14 probiotic bacteria genera *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Propionibacterium*	Sachet formulation	T2D	8	31/22	↓HOMA-IR, HbA1c (only in probiotic responders) ↓TNF-α, IL-1β, IL-6	≡BFG, IL-8, γ-INF	Kobyliak et al. [88]

FBG fasting blood glucose, *HbA1c* hemoglobin A1c, *HOMA-IR* homeostasis model of assessment-insulin resistance, *hs-CRP* high-sensitivity C-reactive protein, *MDA* malondialdehyde, *GSH* glutathione, *ISI* insulin sensitivity index, *DCA* secondary bile acid deoxycholic acid, *LDL* low-density cholesterol, *HDL* high-density cholesterol, *SIRT1* sirtuin 1

Bacteroidetes, *Proteobacteria*, and *Actinobacteria* and increases *Firmicutes* abundance. Similar results were obtained in obese rats after treatment with saxagliptin. On the contrary, in HFD-fed mice sitagliptin induces an increase in the relative abundance of *Bacteroidetes* and *Proteobacteria* and decrease in *Firmicutes* [100]. It is not clear whether these discrepancies are due to the model (rat vs. mouse) or to the drug. The effect of DPP-4i on the microbiota could be related to an enhancement of intestinotrophic effect of GLP-2, which improves intestinal mucosal barrier integrity and thus reduces permeability.

SGLT2 inhibitors may also impact the microbiome. In db/db mice, treatment with SGLT2i yields an anti-inflammatory effect and increases SCFA levels in cecum [9]. There are no published data on humans thus far.

Various studies to evaluate changes in the composition of the intestinal microbiota before and after the use of diabetes medications agents are underway (eudract_number: 2015-000199-86; ChiCTR-OPC-17010757; ClinicalTrials.gov ID: NCT02900417; ChiCTR-OPC-17010757).

Bariatric Surgery

Bariatric surgery has proved to be one of the most effective interventions for severe obesity. Moreover, there are several weight loss-independent effects of "metabolic surgery" including beneficial effects on intestinal glucose metabolism, insulin sensitivity, β-cell function, changes in bile acids, and gut microbiota composition [101]. A recent systematic review of 12 animal and 9 clinical studies [102] demonstrated an increase of *Bacteroidetes*, *Fusobacteria*, *Verrucomicrobia*, *Proteobacteria*, and a decrease of *Firmicutes*, *Clostridiaceae*, *Clostridiales*, *Blautia*, and *Dorea*. Three studies showed an increase in abundance of *A. muciniphila*. The mechanisms responsible for these changes are unknown but may include intestinal remodeling, antibiotic use, and dietary changes.

Conclusion

In recent years, the study of human gut microbiota has led to interesting discoveries that open the frontiers for innovative treatments for many diseases including T2D. Available data suggest that human microbiota plays an important role in the onset and maintenance of insulin resistance in patients with T2D. Given the personal and societal impact of T2D worldwide, large-scale randomized trials will be required to fully assess whether microbiome modulation may be a therapeutic option to improve glycemic control and reduce the risk of complications.

Funding Source This manuscript has no funding source, and no author received payments from any pharmaceutical company or other agency to write this article.

Compliance with Ethical Standards

Conflict of Interest Antonio Sircana, Luciana Framarin, Nicola Leone, Mara Berrutti, Francesca Castellino, Renato Parente, Franco De Michieli, Elena Paschetta, and Giovanni Musso declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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