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Review Article

CONSIDERATIONS FOR GUT MICROBIOTA AND PROBIOTICS IN PATIENTS WITH DIABETES AMIDST THE COVID-19 PANDEMIC: A NARRATIVE REVIEW

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ABSTRACT

Objective: To review data implicating microbiota influences on Coronavirus Disease 2019 (COVID-19) in patients with diabetes.

Methods: Primary literature review included topics: "COVID-19," "SARS," "MERS," "gut microbiota," "probiotics," "immune system," "ACE2," and "metformin."

Results: Diabetes was prevalent (~11%) among COVID-19 patients and associated with increased mortality (about 3-fold) compared to patients without diabetes. COVID-19 could be associated with worsening diabetes control and new diabetes diagnosis that could be linked to high expression of angiotensin-converting enzyme 2 (ACE2) receptors (coronavirus point of entry into the host) in the endocrine pancreas. A pre-existing gut microbiota imbalance (dysbiosis) could contribute to COVID-19–related complications in patients with diabetes. The COVID-19 virus was found in fecal samples (~55%), persisted for about 5 weeks, and could be associated with diarrhea, suggesting a role for gut dysbiosis. ACE2 expressed on enterocytes and colonocytes could serve as an alternative route for acquiring COVID-19. Experimental models proposed some probiotics, including *Lactobacillus casei*, *L. plantarum*, and *L. salivarius*, as vectors for delivering or enhancing efficacy of anti-coronavirus vaccines. These *Lactobacillus* probiotics were also beneficial for diabetes. The potential mechanisms for interconnections between coronavirus, diabetes, and gut microbiota could be related to the immune system, ACE2 pathway, and metformin treatment. There were suggestions but no proof supporting probiotics benefits for COVID-19 infection.

Conclusion: The data suggested that the host environment including the gut microbiota could play a role for COVID-19 in patients with diabetes. It is a challenge to the scientific community to investigate the beneficial potential of the gut microbiota for strengthening host defense against coronavirus in patients with diabetes. **(Endocr Pract. 2020;26:1186-1195)**

Abbreviations:
ACE2 = angiotensin-converting enzyme 2; **ARI** = acute respiratory infection; **CoV** = coronavirus; **COVID-19** = Coronavirus Disease 2019; **DM** = diabetes mellitus; **GI** = gastrointestinal; **HFD** = high-fat diet; **MERS** = Middle East respiratory syndrome; **NOD** = nucleotide-oligomerization domain; **SARS** = severe acute respiratory syndrome; **T1D** = type 1 diabetes mellitus; **T2D** = type 2 diabetes mellitus

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) infection emerged at the end of 2019 and progressed to a pandemic (1-3). The cause of COVID-19, a novel coronavirus (CoV)-2 zoonotic virus with predominant reservoir in bats, was found to have the largest known viral RNA genome among about 30 coronaviruses capable of infecting humans and animals (1-3). Four human coronaviruses (OC43, 229E, NL63, and HKU1) instigated predominantly

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mild respiratory infections and, after rhinoviruses, were a leading cause of common colds (10 to 30% of cases) (1-3). However, coronaviruses could cause severe and highly contagious disease, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and Coronavirus Disease 2019 (COVID-19) (1-5). The genetic sequence of the COVID-19 coronavirus showed approximately 80% and 50% shared identity with SARS and MERS coronaviruses, respectively (1-5).

The natural course of COVID-19 varies from the asymptomatic carrier to acute upper respiratory infection (ARI), pneumonia, acute respiratory distress syndrome, and multi-organ failure (1-5). The reported mortality rate was greater in MERS and SARS than COVID-19; however, total death toll could be higher in COVID-19 due to the larger number of populations being affected by the COVID-19 pandemic. Severity of disease was positively associated with older age and the presence of comorbid conditions, including diabetes mellitus (DM) (1-6).

Gut bacteria (gut microbiota) implicated in diabetes and immune responses could be important for modulating the response to coronavirus via interactions with receptors used by coronaviruses as points of entry into the host, angiotensin-converting enzyme 2 (ACE2) for COVID-19 and SARS and dipeptidyl peptidase 4 (DPP4) for MERS (7,8). The same receptors played a significant role in pathophysiology and treatment of diabetes (7,8). There were no randomized controlled trials (RCTs) investigating use of beneficial bacteria (probiotics) for COVID-19; however, valuable insights could be gained by evaluating data from other coronavirus infections. This review summarizes existing data on the interplay of COVID-19 with diabetes, microbiota, and probiotics and provides some insights into the possible role of the gut microbiota for coronavirus infection in patients with diabetes.

METHODS

We searched manuscripts from PubMed, Science Direct, and Google Scholar. The terms and keywords included combinations of the following specific terminologies: "COVID-19," "SARS-CoV-2," "SARS-nCoV," "SARS-CoV," "MERS-CoV," "coronavirus," "diabetes mellitus," "type 2 diabetes (T2D)," "type 1 diabetes (T1D)," "gastrointestinal (GI) symptoms," "comorbidity," "mortality," "gut microbiota," "microbiome," "innate and adaptive immunity," "mechanisms," "vaccination," "probiotics," "prebiotics," and "synbiotics." Articles published in English between January 2002 and August 15, 2020, were retrieved and reviewed, as were relevant articles from the reference lists. The majority of studies for coronavirus did not differentiate between T2D and T1D; therefore, we used terms "diabetes," "T1D," and "T2D" as appropriate for available information. The method was consistent with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (9).

RESULTS AND DISCUSSION

The published manuscripts were predominantly in the following topic areas: coronavirus epidemiology, clinical course, comorbidity and mortality; spontaneous coronavirus infection in animals; microbiota; use of probiotics; and the role of immune system, ACE2, and metformin in coronavirus infection. Below, we summarize the key findings from reviewed manuscripts, highlighting the findings that in our opinion were of the greatest importance for the field.

COVID-19 AND DIABETES

Diabetes is a highly prevalent disease throughout the world, with a particular predominance of over 90% T2D in the middle aged and elderly. In the world, approximately 463 million (9.3%) individuals have diabetes, the majority being older than 65 years (10). In the U.S., about 34 million people (10.5%) have T2D, with 27% in those 65 years or older (10). Diabetes and older age are both risk factors for severe disease and increased mortality in patients with COVID-19 (Table 1) (1-6). The Centers for Disease Control of China reported that among 44,672 confirmed cases, the crude case fatality rate was 2.3% (1,023 deaths), while it was 14.8% in those age 80 years or older (2). The crude case fatality rate for patients with no comorbidities was approximately 0.9%, whereas it was 10.5% for those with cardiovascular disease and 7.3% for those with diabetes (2). In the U.S., a Centers for Disease Control report estimated an 11% prevalence of diabetes among COVID-19 patients, while it reported that mortality was 6.2%, 28.8%, and 41.7% for patients without diabetes, with diabetes, and with uncontrolled hyperglycemia (blood glucose >180 mg/dL), respectively (3). In previous epidemics of SARS and MERS, diabetes was positively associated with risk for infection, development of complications, and higher mortality (Table 1) (4,5). There was evidence suggesting that infection could directly impact pancreatic endocrine function. Newly diagnosed diabetes was reported in COVID-19 and SARS (6,11). In addition, receptors for coronaviruses were identified in endocrine pancreas with expression of ACE2 in β- and δ-cells (insulin- and somatostatin-producing cells, respectively) (6) and DPP4 in β- and α-cells (glucagon producing) (12). Overall, these data suggested that coronavirus-induced pathophysiologic changes and/or direct impairment of pancreatic islets could be contributing to adverse outcomes of coronavirus infection in patients with diabetes.

COVID-19 AND THE GUT MICROBIOTA

There could be multiple potential interactions of coronaviruses with gut microbiota, as shown by data from human and spontaneous animal infection and experimental studies (Fig. 1). The coronaviruses could cause microbiota

Table 1
Comparison of Patients With Coronavirus Infection

Characteristic	COVID-19[a]	SARS[a]	MERS[a]
Year	2019-2020	2002-2003	2012, 2015
Countries	Pandemic	31	27
Person-to-person transmission	Highly efficient	Efficient	Not efficient
Source of origin	Bats, pangolins	Bats, civet cats	Bats, camels
Upper respiratory infection	Common	Common	Common
Diabetes prevalence, %	11	8-11	50
Mortality overall, %	2-6	11	35
Mortality in diabetes, %	7-29	37-64	35-61
CoV receptors in pancreas	ACE2: β-, δ-cells	ACE2: β-, δ-cells	DPP4: β-, α-cells
Gastrointestinal symptoms, %	5-22	38-70	26
CoV in stool, %	15-60	16-97	15
CoV persistence in stool, weeks	5	3-12	3
Microbiota evaluation	In gastrointestinal tract	In lung	NR
CoV receptors in colon	ACE2: colonocytes	ACE2: colonocytes	DPP4: colonocytes

Abbreviations: ACE2 = angiotensin-converting enzyme 2; CoV = coronavirus; COVID-19 = Coronavirus Disease 2019; DPP4 = dipeptidyl peptidase 4; MERS = Middle East respiratory syndrome; NR = not reported; SARS = severe acute respiratory syndrome.
[a]Data are from hospitalized patients with severe infection and may not represent patients in general population.

imbalance (dysbiosis), disrupt the gut barrier, and promote secondary bacterial invasion, whereas the microbiota could modify coronaviruses entry into the host (including lung) and modify the immune response to coronaviruses (13,14). In addition, alteration of the gut microbiota with antibiotics and/or other therapeutics could modify resistance against viral and bacterial pathogens as well as drug metabolism and pharmacokinetics (15). Finally, beneficial microbiota (probiotics) from food and/or supplements could modify the gut microbiota and influence clinical course (16).

Human Coronavirus Infection

Coronavirus was a common pathogen in stool specimens of 331 patients seen in general practice with an ARI (17). In these patients, laboratory-confirmed compared to nonconfirmed coronavirus infection was associated with almost 3-fold increased GI symptoms, suggesting that coronavirus could be associated with dysbiosis in patients with common ARI (17). Comparably, all three epidemic-related coronaviruses were identified in stool samples and were associated with similar GI symptoms (i.e., nausea, vomiting, and diarrhea), suggesting that the GI tract could serve as an alternative route for acquiring coronaviruses, including those causing COVID-19 (18), SARS (19), and MERS (14) (Table 1). In a prospective study comparing COVID-19 patients with healthy controls and pneumonia controls, COVID-19 disease severity and fecal viral load correlated with gut microbiota changes, and dysbiosis persisted after patients' recovery (20). The baseline (antibiotic-naïve) abundance of microbiota was increased for multiple opportunistic pathogens known to cause bacteremia. The top two bacteria correlating strongly positively with disease severity were *Clostridium ramosum* and *Clostridium hathewayi*. Conversely, the top two bacteria correlating strongly negatively with disease severity were *Alistipes onderdonkii* and *Faecalibacterium prausnitzii*, known as beneficial for immune homeostasis and anti-inflammatory properties (20). The data suggested a potential role for gut microbiota in determining host response to COVID-19. A study comparing bronchoalveolar lavage fluid from 8 patients with COVID-19, 25 patients with community-acquired pneumonia (CAP), and 20 healthy controls showed that the microbiota in COVID-19 and CAP patients were similar and either dominated by pathogens or had elevated levels of oral and upper respiratory commensal bacteria (21). In the upper respiratory tract (collected from oropharynx by using swabs), a comparison of microbiota from 57 healthy asymptomatic people with that from 59 patients acutely sick with corona and other viruses found that the healthy harbored primarily *Streptococcus*, whereas the acutely sick had an enrichment of *Haemophilus* or *Moraxella* (22). The differences in microbiota were not associated with the virus type but were rather linked to patient age, with *Moraxella nonliquefaciens* exhibiting unprecedentedly high abundance in children younger than 6 years (22).

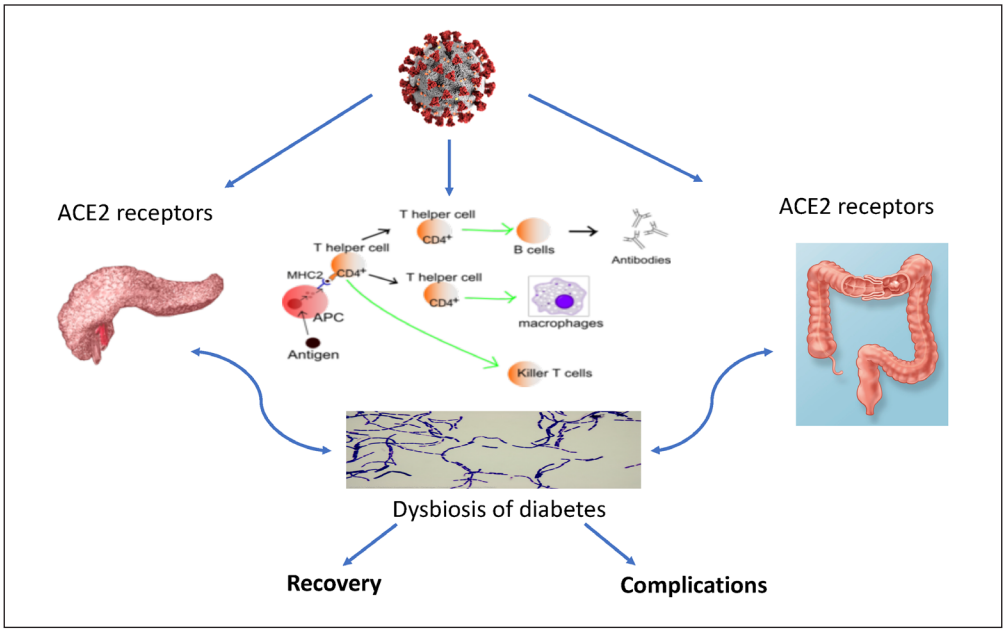

Fig. 1. Coronavirus Disease 2019 virus interactions with gut microbiota in patients with diabetes. *ACE2* = angiotensin-converting enzyme 2; *APC* = antigen-presenting cell.

Animal Coronavirus Infection

The studies investigating spontaneous coronaviral diseases and gut microbiota in animals frequently interacting with people (cats, dogs, pigs, calves, and horses) showed similarities with human infection (Table 2) (13,23-28). Coronavirus infection in animals varied from asymptomatic to severe and was predominantly asymptomatic in young horses (24), similar to rare COVID-19 in children (29). The alterations in intestinal histology as well as local intestinal and systemic immunology also resembled those reported in human infection (Table 2) (13,24). Severe gut dysbiosis was shown in multiple animal studies, with changes in microbiota occurring at each taxonomic level (Table 2). In cats and pigs, coronavirus disease was associated with a low *Bacteroidetes* to *Firmicutes* ratio (23,28), similar to patients with diabetes (30), suggesting that pre-existing dysbiosis of diabetes could possibly contribute to COVID-19–related complications. Overall, the data of microbiota differences between healthy adults and patients with diabetes (30), between adults and youngsters (22), and those reported for coronaviruses (23,24,28,29) suggested that coronavirus and microbiota interactions could potentially be contributing to variable coronavirus infectivity and clinical presentation in patients with diabetes.

COVID-19 and Gut Microbiota-Targeted Therapies

There were no clinical trials investigating probiotic use in coronavirus infection in people (31,32). A cohort of 70 Italian patients hospitalized for COVID-19 was treated with similar standard therapy, and in addition, a group of randomly chosen patients was treated with a probiotic (33). The probiotic and control groups were comparable for anthropometric and COVID-19–related factors. The probiotic compared to control group had fast improvement of symptoms, 8-fold lower estimated risk of developing respiratory failure, and all patients in this group survived (vs. ~9% mortality in controls) (33). The formulation administered in this study contained a mixture of bifidobacteria and lactobacilli: *Streptococcus thermophilus* DSM 32345, *L. acidophilus* DSM 32241, *L. helveticus* DSM 32242, *L. paracasei* DSM 32243, *L. plantarum* DSM 32244, *L. brevis* DSM 27961, *B. lactis* DSM 32246, and *B. lactis* DSM 32247 (Ormendes SA, Lausanne, Switzerland gifted the product Sivomixx^{R} called SivoBiome^{TM} in U.S.A.) (33). Probiotics were suggested yet not proven as potentially helpful for ventilator-associated pneumonia (34).

Probiotics were proposed as an empiric supplement for spontaneous coronavirus infection in domestic animals; however, no studies provided proof of their efficacy. There were several reviews of in vivo (15,35) and in vitro (15) models of coronavirus infection. Particularly promising in vivo models appeared to be genetically engineered (using the CRISPR-Cas9 gene-editing tool) mouse models that could expedite preclinical development of candidate COVID-19 vaccines and drugs (15). Ex vivo three-dimensional organoid systems, mini-tracheobronchial tree (15) and mini-gut (with morphological and functional properties of in vivo GI tract) (14), and various cells lines (15) were used for facilitating mechanistic insights and therapeutics investigations. Probiotics were used in experimental studies predominantly for developing anti-coronavirus vaccines rather than direct treatment of coronavirus infection. Animal models and cell line experiments showed that some probiotics including strains of *L. casei*, *L. plantarum*,

Table 2
Coronavirus Infection in Domesticated Animals and Associated Changes in Gut Microbiota

Animal	Clinical presentation	Gut microbiota changes
Pigs	CoV caused devastating disease with vomiting, diarrhea, dehydration and death in PED and TGEV (13,23).	CoV infection: Low B/F ratio (23), decreased butyrate-producing bacteria (23) in PED, as well as decreased *Lactobacillus* and increased Enterobacteriaceae in TGEV (13).
Horses	CoV was frequently asymptomatic (4-80%), whereas symptoms included fever, anorexia, and lethargy (80-90%), diarrhea (20%), and encephalitis (3%) (24). Horses <1 year old rarely had symptomatic CoV infection (24).	CoV infection: Increased abundance of ammonia-producing microbiota (24).
Calves	CoV was frequently asymptomatic (46%) yet was found in 64% fecal samples of calves presenting with diarrhea (25).	CoV diarrhea was associated with changes in commensal *Escherichia coli* that acquired pathogenic features (genes for curli, cellulose, and fimbriae and antimicrobial resistance) (26).
Dogs, cats	CoV found in stool of 40% cats and 5% dogs presenting with diarrhea (27,28).	CoV diarrhea in cats was associated with low B/F ratio (28).
Pathology	Intestinal histology varied from mild to severe inflammation (13,24).	
Immunology	Local intestinal immunity and barrier function were impaired and characterized by decreased number of IgA-positive cells, $CD3^+$ T cells, and dendritic cells (13,24). Systemic immunity was characterized by enhanced mRNA expression levels of cytokine IL-1β, IL-6, TNF-α, IL-10, and TGF-β. Impairment of intestinal integrity and immune function resulted in increased pathogenic bacterial loading and facilitated secondary infections (13,24).	

Abbreviations: B/F = Bacteroidetes to Firmicutes ratio; CoV = coronavirus; IL = interleukin; PED = porcine epidemic diarrhea; TGEV = transmissible gastroenteritis virus; TGF = transforming growth factor; TNF = tumor necrosis factor.

and *L. salivarius* could be helpful for developing anti-coronavirus vaccines and diabetes treatment (Table 3) (36-51). The mechanisms of these beneficial effects were dependent on specific strains and involved improved circulating glucose and glucose tolerance as well as potential beneficial effects on gut microbiota ecology, barrier function, and inflammation (Table 3). Of interest, a majority of probiotics were found among *Lactobacillus* and *Bifidobacterium*. Both species had been major components of the human gut microbiota and recognized for their antibacterial and antiviral activities (52). Multiple RCTs and meta-analyses showed that probiotics could decrease the incidence of upper respiratory infections (53) and enhance the immune response to influenza vaccination (54). In everyday food, *Lactobacillus* and *Bifidobacterium* are mostly present in fermented dairy products (e.g., yogurt, kefir, cheese) as well as sourdough bread and sauerkraut, which can be included in a balanced diet (55).

COVID-19 and Gut Microbiota-Related Mechanisms

Mechanistic insights into the pathophysiology of COVID-19 are evolving and can be changing with further exploration into the unique biology of SARS-CoV-2 (Fig. 1). Multiple potential mechanisms, connections, and interactions between the host and the virus influence COVID-19 infection risk, severity of the course, and clinical outcomes in patients with diabetes. Among these mechanisms, an important role is played by metabolic endotoxemia characteristic of T2D (56), as well as by interactions in the gut-lung and gut-brain axes that have been implicated in neuroinflammation and the lung inflammatory response in COVID-19 (33,57-59). This review, however, will focus on three possible mechanisms of interconnections for coronavirus, diabetes, gut microbiota, and probiotics, including the immune system, ACE2 receptors, and the antidiabetes drug metformin.

Mechanisms Related to the Immune System

The interaction between coronaviruses and the immune system portends the severity of infection (Fig. 1). Coronavirus antigen is recognized by the cells of the innate immune system, and downstream of this pathway, cells of the adaptive immune response produce antibodies against the virus. The proper response of innate and adaptive immunity stops viral replication, prompts viral clearance, promotes tissues repair, and stimulates antiviral immunity (1-3). In severe infection, coronaviruses can trigger secretion of high amounts of inflammatory cytokines ("cytokine storm"), contributing to respiratory and multisystem failure (1-6). The emerging data suggest that immune system dysregulation could be a link connecting COVID-19 infection severity, diabetes, and the microbiota. Both innate and adaptive immunity are dysregulated in T2D (60,61) and T1D (62,63), although the mechanisms of immune dysregulation are different. Viral clearance assessed by viral RNA measurement is delayed in T2D patients with COVID-19, suggesting attenuated immune function in these patients (60). Natural killer (NK) cells, well-known

as defense against viral pathogens and immunoregulators, are also associated with the development of diabetes (T1D and T2D) and with changes in the gut microbiota (62,63). Specifically, the number of intestinal NK cells secreting IL-17 is correlated with high Bacterioidales and low Clostridiales microbiota abundance in the gut of nonobese diabetic mice that spontaneously develop T1D (63). In a T1D nonobese diabetic mouse model, a single early-life antibiotic exposure produced gut dysbiosis and accelerated T1D development (62). In this model, gut dysbio-

Table 3
Probiotics With Potential Effects in Coronavirus Infection and Diabetes

Probiotic	Effects in coronavirus infection	Effects in DM	Relevant mechanisms of action
Lactobaccillus casei	Recombinant[a] oral vaccine induced efficient anti-PED immune responses in piglets (36). Recombinant *L. casei*[b] enhanced mucosal and systemic immune response to CCV vaccination of Beagle puppies (37). *L. casei* Shirota[c] had anti-TGEV activity in cell lines (38).	T2D patients: decreased FBG, serum insulin, insulin resistance (41). *L. casei* Shirota prevented HFD-induced insulin resistance (42).	CoV: *L. casei* Shirota anti-TGEV activity involved increased production of ROS (38). T2D patients: increased serum SIRT1 and decreased fetuin-A (41), decreased total count of blood bacteria (45). DM mice and rats: reversed dysbiosis (46,47), increased B/F ratio (46,47), enriched *Bifidobacterium*, *Lactobacillus*, and SCFA-producing GMB: *Allobaculum* and *Bacteroides* (46), *Butyricimonas* (47).
L. plantarum	Recombinant[a] oral vaccine induced efficient immune anti-TGEV responses in piglets (39). *L. plantarum* Probio-38 had anti-TGEV[d] activity in cell lines (40). *L. plantarum* PCA236 had anti-TGEV[c] activity in cell lines (38).	T2D/PreDM patients: decreased FBG and insulin resistance (43).	T2D/PreDM patients: decreased pro-inflammatory cytokines (IL-6, MCP-1) (43). DM mice: increased GMB alpha diversity, reversed gut dysbiosis and decreased pro-inflammatory markers expression: pJNK and iNOS in the intestine, Fmo3 in the liver, IL-1β in Kupffer cells, and ICAM in the aorta and liver (48). DM rats: decreased serum pro-inflammatory IL-1β, IL-6, CRP, increased anti-inflammatory IL-10 and downregulated the inflammasome signaling pathway (49).
L. salivarius	*L. salivarius* Probio-37 had anti-TGEV[d] activity in cell lines (40).	T2D patients[e]: decreased insulin resistance (44).	DM mice: reversed dysbiosis, increased mucosal antibacterial proteins (e.g., Reg3β), decreased endotoxin levels and *Klebsiella pneumoniae* translocation (50).
Multistrain[f] SivoBiome™	Multistrain lowered the risk of respiratory failure and mortality in a cohort of COVID-19 patients (33).	T2D/PreDM patients: decreased FBG and insulin resistance with similar multistrain (51).	DM mice: reduction of oxidative stress and inflammation via activation of transcription factor Nrf2 system and its target gene HO-1 leading to NF-kB inhibition (33).

Abbreviations: B/F = Bacteroidetes to Firmicutes ratio; CCV = canine coronavirus; COE = core neutralizing epitope; CoV = coronavirus; COVID-19 = Coronavirus Disease 2019; CRP = C-reactive protein; DM = diabetes mellitus; FBG = fasting blood glucose; GMB = gut microbiota; GM-CSF = granulocyte macrophage colony-stimulating factor; HFD = high-fat diet; HO-1 = heme oxygenase-1; ICAM = intercellular adhesion molecule; IL = interleukin; iNOS = inducible nitric oxide synthase; LAB = lactic acid bacteria; MCP-1 = monocyte chemotactic protein-1; NF-kB = nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2 = nuclear factor erythroid 2p45-related factor; PED = porcine epidemic diarrhea; pJNK = phosphorylated c-Jun kinase; PreDM = pre-diabetes mellitus; ROS = reactive oxygen species; T2D = type 2 diabetes; TGEV = transmissible gastroenteritis virus; SCFA = short-chain fatty acid; SIRT1 = sirtuin 1; SOD = superoxide dismutase; TLR = toll-like receptor.

[a]A fusion protein was developed comprising of CoV COE antigen and dendritic cells targeting peptide (DCpep). The intestinal dendritic cells were used since these cells were known as antigen-presenting cells, connecting humoral and cellular immune responses. Probiotic lactobacilli were administered orally to deliver fusion protein.

[b]A recombinant *L. casei* produced biologically active canine GM-CSF.

[c]The potential anti-TGEV activity of multiple LAB was investigated using animal and human intestinal and macrophage cell line models of nontumor origin. Highest protection effects were shown for known probiotic *L. casei* Shirota and *L. plantarum* PCA236 with mechanism involving increased production of ROS.

[d]A total of 310 bacterial strains isolated from the porcine gastrointestinal tract were tested for their activity against TGEV and other enteric pathogens. *L. plantarum* Probio-38 and *L. salivarius* Probio-37 were selected as potential probiotics for in vivo use based on anti-TGEV and anti-enteric bacterial pathogens activity as well as bile tolerance, high survival in gastric juice, and antibiotic resistance.

[e]*L. salivarius* included as part of multistrain probiotic.

[f]Multistrain probiotic containing: *Streptococcus thermophilus* DSM 32345, *L. acidophilus* DSM 32241, *L. helveticus* DSM 32242, *L. paracasei* DSM 32243, *L. plantarum* DSM 32244, *L. brevis* DSM 27961, *Bifidobacterium lactis* DSM 32246, *B. lactis* DSM 32247.

sis was persistent, induced changes in genes regulating innate and adaptive immunity, and was not due to antibiotic effects (62). Moreover, evaluation of the gut microbiota showed that four taxonomic groups (Enterococcus, Blautia, Enterobacteriaceae, and Akkermansia) were significantly overrepresented, and four other groups (S24-7, Clostridiales, Oscillospira, and Ruminococcus) were significantly underrepresented in intervention mice versus controls, implicating an important role for early life gut microbiome perturbations in T1D development (62).

Correspondingly, in a mouse model of T2D, hyperglycemia and insulin resistance were controlled by a mechanism comprising the adaptive immune system and gut microbiota (61). In this model, the mouse immune system was triggered by a process of 'immunization,' subcutaneous injection of a homogenized diluted gut microbiota extract from the diabetic mice. A month later, two groups of mice (immunized and nonimmunized) were fed a high-fat diet (HFD) for 2 months in order to challenge the development of metabolic features. Immunization prevented hyperglycemia and insulin resistance in a dose-dependent manner in response to the HFD. The transfer of immune cells harvested from the spleen of the microbiota-immunized mice to naïve mice produced immune system changes, CD4+ and CD8+ T cell proliferation, cytokine production (including IL-17) and antibody secretion, and protected the recipients from HFD-induced impairments. Also, HFD-induced dysbiosis was partially corrected with favorable reversal toward normal abundance for specific genera, *Anaerotruncus*, *Lachnospiraceae incertae*, and *Mucispirillum* (61). Similarly, studies in mice showed that the gut microbiota per se could affect and counteract a genetically determined condition that predisposes mice to the T2D phenotype (64). The gut microbiota changes in these murine models indicated lower richness, alpha diversity, and a depletion of *Allobaculum*, *Lactobacillus*, and enrichment with *Bacteroides* genera in T2D mice compared to wild-type mice. The intestinal IL-17–producing Th17 cells were involved in limiting gut dysbiosis, lipopolysaccharide translocation to visceral adipose tissues, and reducing obesity and T2D in murine models (64).

Mechanistic studies in models of T1D and T2D showed that the gut microbiota and/or its components activate receptors on gut endothelium such as toll-like receptors and nucleotide-oligomerization domain (NOD)-like receptors, which results in activation of IL-17–producing Th17 cells suggested to be critically involved in the pathogenesis of T1D and T2D (63-65). The downstream pathway, including multiple intermediaries and receptors (e.g., interferon-γ, Foxp3, NOD1, and NOD2), is involved in empowering microbiota Th17-related mechanisms (64,65). Th17 cells also play a role in the gut-lung axis. A constituent of the gut microbiota, segmented filamentous bacteria, stimulate the migration of Th17 cells to the lung, enhancing the autoimmune response and aggravating lung inflammation in a mouse model (57). Overall, the data demonstrate the causality of microbiota-stimulated adaptive immune system modifications for pathogenesis and protection against diabetes.

Mechanisms Related to the ACE2 Pathway

ACE2 is a point of cellular entry for COVID-19 and SARS viruses (6), is important in diabetes and its complications (7), and is linked to the gut amino acid transport and microbiota ecology (66). ACE and ACE2, two different enzymes with multiple roles in health and disease, are best known for their role in the renin-angiotensin system (RAS). ACE converts angiotensin-I to angiotensin-II (Ang-II), the main bioactive molecule of the RAS, which stimulates vasoconstriction. By contrast, ACE2 hydrolyzes Ang-II into an inactive metabolite, thereby promoting vasodilation. ACE2 counteracts many functions of ACE with antifibrotic, antiproliferative, and anti-inflammatory effects (7,66-68). Studies of mouse models lacking ACE2 receptors (ACE2 knockout, ACE2-KO) confirmed the receptor's role in the pathophysiology of diabetes complications (67). The gut of ACE2-KO mice showed reduced epithelial integrity and leakage of bacterial products into the circulation and a marked increase in potentially deleterious peptidoglycan-producing bacteria (66,67). Of interest, in a cohort of 1,128 adults with COVID-19 and hypertension, all-cause mortality was lower in users compared to nonusers of ACE inhibitors and ACE receptor blockers (adjusted hazard ration, 0.42; $P = .03$) (68). Presently, ACE2 is under active investigation for potential development of an anti–COVID-19 therapy (15).

Mechanisms Related to Metformin

Metformin, a known regulator of bowel function and gut microbiota (69), was associated with reduced mortality from lower respiratory tract disease in patients with T2D (70), with decreased all-cause mortality in patients with T2D, and with reduced all-cause mortality and diseases of aging, independent of its effect on diabetes control (71). Metformin could be connected to coronaviruses and ACE2 via the silent information regulator T1 (SIRT1) pathway. SIRT1, an enzyme of the sirtuin family of deacetylating proteins acting on >80 substrates, was proposed as an important mechanistic link in diabetes, with positive impacts on glucose homeostasis, insulin sensitivity, energy metabolism, chronic stresses (e.g., oxidative stress), as well as acting as a promoter of longevity (41,72-74). Metformin is a direct stimulator of SIRT1 (72), while SIRT1 is involved in controlling ACE2 expression (74) and functionally linked to MERS-CoV expression (75). SIRT1 had antiviral effects against influenza virus infection in a study of senescent human bronchial epithelial cells and dermal fibroblasts (76). SIRT1-microbiota interactions appear to play a role in diabetes. The use of *L. casei* probiotic resulted in improved fasting glucose and insulin resistance and was associ-

ated with increased circulating SIRT1 in *L. casei*–treated patients compared to the placebo group in a double-blind RCT of T2D patients (41). Correspondingly, lack of SIRT1 catalytic activity (SIRT1Y/Y mice) resulted in modulation of the gut microbiota (73). In SIRT1Y/Y compared to SIRT1+/+ mice fed normal chow, gut *Fusobacteria* were decreased and *Prevotella* were increased, while Firmicutes and Bacteroidetes were the dominant phyla in both mouse strains. After HFD feeding, the relative proportion of phylum Firmicutes increased, whereas that of Bacteroidetes decreased in both strains, but the shift occurred faster in SIRT1Y/Y mice. Ruminococcaceae and *Alistipes* showed the same trends as the above phyla. The HFD was associated with an increase in the genus *Enterococcus* only in the SIRT1Y/Y mice, whereas the Verrucomicrobia phylum, including the *Akkermansia* genus, disappeared completely in the SIRT1Y/Y mice after only 1 week on the HFD, a result consistent with data showing decreased *Akkermansia* in obesity and T2D (73). The mechanism of the anti-DM effect of metformin also involves, among many other actions, attenuation of endotoxemia, activation of the anti-oxidative nuclear factor erythroid 2p45–related factor 2 (Nrf2) system, and increased abundance of the beneficial bacteria *Lactobacillus* and *Akkermansia muciniphila* (77). The activation of the Nrf2 system and its target heme oxygenase-1 was similarly suggested as a mechanism for the beneficial effect of multistrain probiotics used in a cohort of Italian COVID-19 patients (33,59). Taken together, these data suggest that a metformin-related mechanistic pathway (including microbiota) could modulate the coronavirus course in patients with diabetes.

CONCLUSION

The available data suggest that the environment within the human gut, including the microbiota, could play an important role in the development and severity of COVID-19 in patients with diabetes. There are no data and no scientific rationale to suggest that probiotics could provide protection and/or treatment of coronavirus infection. Based on data of probiotic benefits for viral and other infections and diabetes, a balanced diet inclusive of probiotics could be proposed as relevant for controlling glucose and supporting the immune system during the COVID-19 pandemic. It is a challenge to the scientific community to uncover the beneficial potential of the gut microbiota for strengthening host defenses not only against COVID-19 but also against other novel infections. Future studies of the multifaceted network of interplay between the microbiota, probiotics, and the host could lead to new discoveries in the pathogenesis and management of coronavirus and other infections in patients with diabetes.

DISCLOSURE

The authors have no multiplicity of interest to disclose.

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