The Microbiota–Gut–Brain Axis and Diabetic Cognitive Impairment: A Memorable Journey

ABSTRACT
Objective: Diabetes mellitus (DM) is associated with complications affecting the quality of life. Interestingly, the gut microbiota is closely related to glucose metabolism. This narrative review introduces the characteristics of the gut microbiota in DM, describes the modulation of host glycemic control by the gut microbiota, characteristics of intestinal permeability, mechanisms of diabetic cognitive impairment (DCI), and the role of brain–gut–microbiota axis in DM.

Materials and methods: The literature search was performed in Medline, Scopus, WOS, and PubMed databases using the keywords gut microbiota, DM, intestinal permeability, and DCI.

Results: Dysbiosis of gut microbiota causes intestinal barrier disruption resulting in the entry of intestinal bacteria and their metabolites into the circulatory system, which may disturb insulin sensitivity, glucose metabolism, and immune homeostasis. Gut microbiota plays a critical role in regulating systemic insulin sensitivity and energy metabolism. Intestinal barrier dysfunction induced by hyperglycemia is considered to be the underlying mechanism of systemic infection and inflammatory response in patients with diabetes. Both dysbacteriosis and cytokines will lead to the intestinal barrier and blood–brain barrier dysfunction, facilitating harmful substances (advanced glycated end products) to access neurons, and thus contribute to the development of DCI. The modulation of intestinal permeability through nutritional interventions may represent a potential prevention target for DM.

Conclusions: The clinical evidence for the association between hyperglycemia and intestinal barrier dysfunction in humans is scarce. Further clinical studies are needed to provide more insight by studying the intestinal barrier integrity markers and glycemic status and their association with cognitive status. (Clin Diabetol 2023; 12; 4: 261–271)

Keywords: gut microbiome, intestinal permeability, diabetic cognitive impairment, diabetes mellitus

Introduction
Type 2 diabetes (T2D), which is characterized by hyperglycemia, is emerging as a leading health challenge of the cases still remain undiagnosed. The projected estimate of people with diabetes in India would be 69.9 million, which appears to be the tip of the iceberg as most [1, 2]. Etiopathogenesis of T2D is multifactorial, involving the interaction of both genetic and environmental factors [3]. Genetics, obesity, infection, diet, and immune disorders play a major role in the pathogenesis of T2D. Recently, the gut microbiota, the so-called “forgotten organ” which harbors trillions of microorganisms, has been elevated to unprecedented importance due to its possible role in the pathogenesis of T2D mellitus and its complications [2].
Gut microbiome

The human genome project was accomplished in 2003 and decoded the human genome to understand human biology better. The “second human genome project” was conceived recently to study the importance of trillions of organisms of gut microbiota [3]. The gut microbiota, the “hidden organ” or “organ within an organ,” has been in the limelight since sequencing and complementary data analysis methods have enabled the discovery of its composition [4]. The human intestine is home to 500 to 1000 different bacterial species and 100 trillion (10^{14}) bacteria, which share a harmonious relationship with the human body [5].

Four main microbiota families (phyla) reside in the gut — Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria [6]. The majority of the bacterial species of the adult gut belong to phyla Bacteroidetes (Gram-negative) and Firmicutes (Gram-positive) [7].

Microbiome composition in healthy cohort and T2D and its implications

There are 64% Firmicutes, 23% Bacteroidetes, 8% Proteobacteria, 3% Actinobacteria present in healthy cohorts [8]. Larsen et al. [9] studied the composition of gut microbiota in T2D and observed a decreased number of Firmicutes, increased number of Bacteroidetes, increased B/F ratio and increased number of Proteobacteria. The increased ratio is associated with an increase in plasma glucose following a glucose load. An increased number of Firmicutes and Proteobacteria, a decreased number of Bacteroidetes and increased F/B ratio were observed by Sedighi et al. [10].

A decrease in butyrate-producing microbes Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus, and Subdoligranulum was evidenced in T2D. Butyrate possesses anti-inflammatory properties and reduces oxidative stress. Decrease in levels of Bacteroides, Prevotella, and Bifidobacterium will enhance intestinal permeability and reduce systemic inflammation.

An increase in Lactobacillus species and a decrease in Clostridium species was observed in T2D. Animal studies have shown a reduction in insulin resistance and inflammation, which may prove beneficial in T2D when supplemented with strains of Clostridium butyrium [11]. Akkermansia muciniphila and Faecalibacterium prausnitzii species appear to provide protection against the development of T2D. Akkermansia muciniphila maintains the mucin layer and plays a significant role in decreasing inflammation. Supplementation with Faecalibacterium prausnitzii results in a decrease in systemic inflammation and improves insulin resistance [12, 13].

Dysbiosis and T2D

Dysbiosis, characterized by alteration in the composition and function of gut microbiota, is known to play a critical role in the pathogenesis of T2D. Dysbiosis results in the excessive production of genes that encode enzymes involved in carbohydrate metabolism regulation [14].

Mechanisms of modulation of host glycemic control by the gut microbiota

Chronic low-grade inflammation is one of the characteristic features of T2D. It is also associated with the release of many mediators that promote inflammation [15]. Gut microbiota activates host inflammation through the release of lipopolysaccharide (LPS). Innate toll-like receptor 4 (TLR-4) gets activated which results in stimulation of nuclear-kappa B factor (NF-κB), the intracellular pathway which in turn favors the release of proinflammatory cytokines which induces phosphorylation of insulin receptor substrate (IRS) serine through activation of kinases (JnKand IKK) promoting the progression of insulin resistance, hence worsening of diabetes [16–18]. Increased intestinal permeability results in endotoxemia due to the transport of products from gut microbiota [19]. Gut microbiota plays a pivotal role in glucose metabolism by influencing glucose homeostasis and insulin resistance [20]. Gut dysbiosis results in the disruption of the integrity of the intestinal mucosal cells leading to a “leaky gut” that results in increased intestinal permeability promoting inflammation and dysregulation of the normal immune response [21] (Fig. 1).

Role of gut microbial metabolites

Microbial metabolites such as short-chain fatty acids, branch-chain amino acids, indole, imidazole, and succinate are produced during fermentation which acts as a mediator in microbe-to-host signaling pathways.

Short-chain fatty acids

Clostridium, Bacteroides, Lactobacillus, and Bifidobacterium are the important microbes in the gut that modulate the synthesis and signaling of bile acids. Gut microbiota dysbiosis in T2D alters the production of these microbial metabolites leading to disturbances in the microbe-host signaling pathways [22] (Fig. 2).

Bile acids

Bile acids produced from cholesterol have antimicrobial properties, hence suppressing bacterial growth in the intestine. Bile acids interact with FXR and G-protein receptor-S (TGR-5), decrease gluconeogenesis,
increase glycogenesis, inhibit GLP-1, and promote fibroblast growth factor (FGF-19) [23, 24].

**Intestinal permeability in T2D**

The gastrointestinal epithelial barrier and mucous layer, along with the lamina propria, act as a gatekeeper that prevents the luminal contents from coming in contact with the systemic circulation. [25]. Gut microbiota can regulate the expression of the Muc2 gene, which in turn can affect the structure and function of the mucous layer [26]. Microvilli on the epithelial cell act as a physico-chemical barrier to prevent the entry of microbes and their metabolites. The tip of the microvilli secretes intestinal alkaline phosphatase, which acts on the LPS of the bacterial cell wall, thereby lysing it [27]. Epithelial cells are held together by a complex junctional system comprising tight junctions, adherent junctions, and desmosomes. The arrangement of barrier cells facilitates the crosstalk between the intestinal microbiome and the underlying immune system of the host [28]. There are around 20 tissue-specific proteins in the tight junction, adherent junction, and desmosomes of intes-
Dysbiosis of gut microbiota causes barrier disruption, and emerging evidence suggests that structural and functional impairment of the intestinal barrier is an important factor in the pathogenic process of T2D [31]. Tight junctions of the intestinal epithelial cells were significantly damaged during hyperglycemia in experimental mice [32]. This can lead to an influx of microbes and their metabolic products into the bloodstream, causing their dissemination and further consequences such as systemic infections and inflammations. [33]. The entry of microbes or their metabolites can also disturb insulin sensitivity, glucose metabolism, and immune homeostasis through the NF-κB pathway and JNK signal transduction pathway [34]. Efficient strategies to rejuvenate the barrier structure and function help in diabetes prognosis. In a cohort of 7169 subjects of the FINRISK97 study, metabolic endotoxemia predicted the incidence of diabetes during the study period of 10 years [35]. Cox et al. [36] have also reported that the risk of T2D is high in subjects with increased intestinal permeability. The intestinal mucosal barrier is disrupted in individuals with T2D who have poor short-term glucose control. Also, increased intestinal permeability was found to be independently associated with the magnitude of blood glucose variations, according to multivariable linear regression analysis [37].

A study by Horton et al. [38] demonstrated increased intestinal permeability in 20 T2D patients by chromium (51Cr)-EDTA urinary recovery compared to age, sex, and BMI-matched healthy controls. Genser et al. [39] demonstrated increased jejunal permeability in obese subjects, which was correlated to T2D and inflammation. *Faecalibacterium prausnitzii* constitutes 4% of the butyrate-producing microbiome in a normal gut. The levels of these microbes were significantly reduced in the diabetic gut. Microbial anti-inflammatory molecule (MAM), a metabolite of *F. prausnitzii*, can be used as a marker of intestinal permeability as well as a therapeutic agent to restore the damaged gut barrier in diabetes subjects [31].

**Figure 3. Dysbiosis of Gut Microbiota and Intestinal Permeability in Type 2 Diabetes**
Markers of intestinal permeability
Lipopolysaccharide (LPS)
All gram-negative bacteria have an outer membrane that contains LPS, and the serum level of LPS is a marker of bacterial translation. As LPS can enter the bloodstream through the damaged intestinal barrier and can cause inflammation in the host, the serum level of LPS can indirectly determine intestinal barrier integrity [31]. In comparison to the matched control group, the LPS level was greater in 25 middle-aged T2D patients [40]. According to Marius Troseid et al. [41], HbA1c levels correlated with LPS levels in patients with diabetes, and there was a drastic reduction in LPS after performing bariatric surgery for glycemic control.

Zonulin
Zonulin is a protein that regulates the zonula occludens (tight junctions) in the intestinal barrier epithelium. Being expressed most at the duodenum and jejunum of the small intestine, increased circulating levels of zonulin indicate more permeability of the barrier. Many autoimmune diseases such as celiac disease, Crohn’s disease, type 1 diabetes (T1D), and inflammatory bowel disease reported elevated zonulin levels in serum. Zonulin can reversibly loosen the tight junctions of the intestinal epithelium, and consequently, it can also regulate innate immunity. Serum zonulin correlated with lactose mannitol ratio in urine, thus, it is a biomarker of barrier integrity. There was a visible increase in serum zonulin level in T2D patients when compared to BMI-matched impaired glucose tolerance and normal glucose tolerance subjects [42]. But studies on the association of insulin resistance with zonulin reported controversial results, with some studies showing a good correlation while others did not. Moreno-Navarrete et al. [43] published a positive association of zonulin with IR, which is mediated through interleukin 6 (IL-6). A study performed on Asian Indians also showed an elevation of LPS and ZO-1 in T2D patients, which correlated with tumor necrosis factor alfa (TNF-α), IL-6, and HbA1c [44].

Intestinal fatty acid binding protein (i-FABP)
Intestinal fatty acid binding protein (i-FABP), encoded in chromosome 4, is expressed only in the intestinal epithelial cells. Epithelial cells in the intestine host i-FABP, and elevated serum levels of i-FABP indicate barrier dysfunction. I-FABP correlated with the duration of diabetes but not with the severity of hyperglycemia in a study conducted on subjects with diabetes. Also, islet beta cell function negatively correlated with serum levels of i-FABP. Patients with complications such as diabetic retinopathy had higher serum levels than those without complications, which further stresses the chronic effect of hyperglycemia on intestinal barrier integrity [45]. Serum zonulin, LPS, and i-FABP were found to be elevated, indicating impaired barrier function in subjects with T2D, and the barrier function worsened with chronic complications of DM [46].

Lipopolysaccharide binding protein (LBP)
Lipopolysaccharide binding protein (LBP) is a protein of the acute phase, which binds LPS in circulation. The LPS-LBP complex is subsequently recognized by soluble or membrane-bound CD14 and binds to toll-like or other receptors on the membrane of innate immunity cells [30]. Diamino oxidase (DAO) is an intracellular enzyme predominantly present in the intestinal barrier cell. It metabolizes bacterial metabolites such as putrescine, histamine, and cadaverine. Damage to the barrier leads to the release of DAO into the lumen, which further enters the lymphatic vessels and bloodstream, thereby elevating the plasma DAO levels. Shen et al. [37] reported that there is no significant difference in the DAO levels in T2D patients when compared to controls.

Diabetic cognitive impairment (DCI)
Cognitive impairment is defined as difficulty in learning, decision-making, poor memory, and lack of concentration. Cognitive impairment is an important complication that has been recently in the limelight in diabetes. Diabetic cognitive impairment refers to cognitive impairment caused by T1D or T2D [47]. Studies have demonstrated that diabetes patients, especially those with T2D, develop cognitive problems such as dementia and Alzheimer’s disease, irrespective of gender. Diabetes is associated with a 60% increased risk of dementia and 19% greater cognitive decline [48]. Glycemic control seems to influence the extent of cognitive dysfunction in both type 1 and type 2 diabetes patients [49]. Approximately 11% of T2D patients exhibit cognitive impairment. In a population of nearly 2000 postmenopausal women studied by Yaffe et al. [50], HbA1c > 7% exhibited a 4-fold increased risk of cognitive impairment. This is further supported by the evidence of brain abnormalities such as reduced hippocampal volumes observed in both animals [51] and human models [52]. Significant oxidative stress levels in the hippocampus and cerebral cortex are believed to be the contributing factors to the development of DCI [53].

The exact pathophysiology underlying DCI is not fully elucidated. However, oxidative stress, advanced glycosylated end products (AGEs), and inflammation seem to contribute to the development of DCI [54–55]. Reactive oxygen species (ROS) stimulated by persistent hyperglycemia downregulate tight junction proteins
to damage the blood–brain barrier (BBB) and increase BBB permeability [56]. Oxidative stress seen in persistent hyperglycemia overproduces AGEs that cause oxidative damage and injure neurons. The hyperglycemia-mediated excess AGEs and oxidative stress comprise a vicious cycle that damages neurons and vascular endothelium leading to cognitive dysfunction [57]. AGEs destroy BBB basement cells through the secretion of vascular endothelial growth factor and matrix metalloproteinases-2 (MMP-2) from cerebral vascular endothelial cells and transforming growth factor-β (TGF-β) from the outer membrane of BBB, suggesting their prominent role in DCI development [58]. Further, the release of inflammatory cytokines predisposes to inflammatory DCI [59].

Diabetes has been associated with poor memory, poor attention, dementia, impaired attention, and poor processing speed [60]. Determining which cognitive domain gets maximally affected in diabetes will assist in the implementation of rehabilitation therapies such as lifestyle modifications, cognitive exercises, aerobic exercises, intranasal administration of insulin, and use of glucagon-like peptide-1 (GLP-1) analogs to overcome the contributing risk factors. Intranasal administration of insulin may be more promising for improving cognition in T1D as it reduces intracellular amyloid plaque, promotes tau hypophosphorylation, which stabilizes microtubules, and promotes tubulin polymerization [61]. It has been demonstrated that intranasal (IN) insulin administration might be used as a therapeutic method to administer insulin directly to the brain without subjecting it to its side effects [62]. The trigeminal nerve’s perineural spaces carry the IN-delivered insulin to the brain, where it disperses across the cerebral perivascular spaces and stimulates the brain’s insulin receptors to provide its therapeutic effects without disrupting the peripheral systems [63].

The memory of healthy people and the metabolic integrity of AD patients were both found to be improved by intranasal insulin administration [64]. Moreover, a recent study showed that following an IN insulin infusion, the hippocampal cells’ intracellular insulin signaling pathways were activated [65]. Glucagon-like peptide-1 (GLP-1) analogs that have neuroprotective effects have been studied [66]. GLP-1 analogs such as liraglutide and lixisenatide improved memory prevented synapse loss and reduced beta-amyloid plaque count in the brain cortex of mouse models with Alzheimer’s disease [67]. Dipeptidyl peptidase IV (DPP-IV) inhibitors such as vildagliptin and sitagliptin restore brain mitochondrial function and alleviate cognitive impairment [68]. Human clinical trials with other GLP-1 analogs and other antidiabetic medications, such as rosiglitazone and pioglitazone, have also recently been completed, but data is not currently reported. In conclusion, both T1 and T2 diabetes showed an association with cognitive dysfunction and improved diabetes control, and decreased diabetic complications seem to be associated with less cognitive dysfunction. However, further studies are essential to understand the magnitude of this association. Studies on the association of diabetes with cognitive function in different populations are shown in Table 1.

**Brain–gut–microbiota axis in T2D**

Blood and the central nervous system (CNS) have been found to include metabolic by-products produced by microbiota, and these by-products appear to be the critical regulators of gut–brain communication. The vagus nerve, which the enteric nervous system uses for bidirectional communication with CNS, has been shown to have a strong link between gut bacteria and brain behavior. The vagus nerve was shown to be implicated in the pathophysiology of the anxiety-like behavior through observations of chemical colitis-induced anxiety-like behavior in the mice, which explained the positive effects of the probiotic *Bifidobacterium longum* NCC300 [78]. The CNS function is altered by the neurotransmitters, short-chain fatty acids, and folate released by the microbiota influenced by environmental factors such as stress, diet, and medications. These factors shift the microbiota profile resulting in adverse health effects collectively known as dysbiosis.

The pathophysiology of cognitive impairment in connection with gut dysbiosis is heavily influenced by inflammation. Increasing the number of gram-negative bacteria may cause a long-lasting, low-grade GIT inflammation that damages the intestinal epithelium and results in the so-called “leaky gut” [79]. As a result, the circulation of pathogen-associated molecular patterns such as LPS may increase systemic inflammation and oxidative stress [80].

Toll-like receptors (TLR) and immune cells are stimulated by LPS and bacterial lipoprotein to release pro-inflammatory cytokines. In the CNS, peripheral cytokine signaling can affect astrocytes, microglia, and neurons. This happens by stimulating the hypothalamic–pituitary–adrenal axis (HPA) axis at the anterior pituitary or hypothalamus, binding to cytokine receptors associated with the vagus nerve, activating cells lining the cerebral vasculature (endothelial cells and perivascular macrophages), active transport through transport molecules, and recruitment of activated cells such as monocytes and macrophages from the periphery to the brain [81]. The systemic changes have an impact on the brain and favor the neurodegenera-
One putative mechanism through which gut bacteria imparts hippocampal dysfunction involves increased activity of glial cells in the inflammatory process. Astrocytes, microglia, and oligodendrocytes are constituents of the CNS glia. To promote CNS healing, minimize initial damage, eliminate toxic debris, and return the CNS to homeostasis, astrocytes, and microglial over-activation in the hippocampus, and increased amyloid-beta deposition [82].

Table 1. Studies on the Association of Diabetes with Cognitive Function in Different Population

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study design</th>
<th>Participants</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antal et al.</td>
<td>Cross-sectional study</td>
<td>1,012 type 2 diabetes, 19,302 healthy control UK participants</td>
<td>Type 2 diabetes accelerated atrophy of gray matter, and brain aging and this increased with advanced age T2DM was associated with defects in executive functioning and processing speed</td>
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<tr>
<td>Naguib et al.</td>
<td>Cross-sectional study</td>
<td>262 Saudi Arabia diabetes participants</td>
<td>80.3% had cognitive impairment, 33.8% had severe cognitive impairment. Advanced age, females, low education level, and low income, lower duration of diabetes, higher HbA1c, and ophthalmic complications associated with cognitive impairment</td>
</tr>
<tr>
<td>Varghese et al.</td>
<td>Cross-sectional study</td>
<td>400 participants with diabetes and 400 participants without diabetes from Thiruvalla, India</td>
<td>Cognitive impairment was present in 63.8% of the patients with diabetes compared to 10.8% of patients without diabetes, with an odds ratio of –8.78 (CI: –4.47–17.22). Diabetes patients had significant deficits in visuospatial function, language, attention, language, and memory compared to people without diabetes. Cognitive impairment was associated with higher RBS, longer duration of diabetes, blood pressure, and macro vascular diseases (p &lt; 0.05)</td>
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<tr>
<td>Han et al.</td>
<td>Cross-sectional study</td>
<td>2,032,689 diabetes Korean patients</td>
<td>Diabetes patients with a history of hypoglycemia have a higher risk for dementia</td>
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<tr>
<td>Malik et al.</td>
<td>Cross-sectional study</td>
<td>332 Pakistani diabetes patients</td>
<td>24.4% of diabetes patients had cognitive impairment. Cognitive impairment in T2D is associated with the advancing age of diabetes patients irrespective of gender</td>
</tr>
<tr>
<td>Frison et al.</td>
<td>Cohort study</td>
<td>2,323 French participants with cognitive impairment of which 254 participants had diabetes</td>
<td>Diabetes associated with lower cognition as a result of neurodegeneration</td>
</tr>
<tr>
<td>Lin et al.</td>
<td>Cross-sectional study</td>
<td>863 elderly diabetes Taiwanese patients</td>
<td>18.5% of diabetes patients had cognitive impairment and associated with poor glycemic control, advanced age, and lower eGFR</td>
</tr>
<tr>
<td>Dove et al.</td>
<td>Cohort study</td>
<td>682 cognitively impaired and 1,840 cognitively healthy elderly Swedish participants without dementia</td>
<td>Increased HbA1c levels, diabetes, cardiac disease and inflammation doubled the risk of cognitive impairment with even higher risk of progression to dementia</td>
</tr>
<tr>
<td>Sun et al.</td>
<td>Cross-sectional study</td>
<td>120 Chinese type 2 diabetes patients</td>
<td>In T2D, duration of diabetes, blood glucose, cholesterol, triglyceride, glutamate and glucose level, advanced age, and education level were identified as independent risk factors for cognitive impairment. Cognitive impairment associated with macro and microvascular diseases</td>
</tr>
</tbody>
</table>

CI — confidence interval; eGFR — estimated glomerular filtration rate; HbA1c — glycated hemoglobin; RBS — random blood sugar; T2D — type 2 diabetes; UK — United Kingdom
permeability is also associated with dysbacteriosis of candidate for mechanism research. Altered intestinal ome and diabetes, intestinal permeability is a promising impairment. As an interface between the gut microbi-

Dysbiosis can trigger and further aggravate metabolic secondary complications with significant cost savings. (FMT) should be investigated in order to ensure early prebiotics, probiotics, and facilitated microbial transfer interventions. The effect of treatment regimens such as may also serve as a tool to identify new therapeutic targets. This contributes to a pro-inflammatory environment that may impact neuronal cell death, neurogenesis, and synaptic connections in the CNS via the so-called “gut–brain axis” [90].

Summary

The modulation of gut barrier function (intestinal permeability) through nutritional and other interventions may represent a potential prevention and treatment target for metabolic diseases mainly T2D. Identification of gut microbiota profile could serve as a possible microbial biomarker along with glycemic parameters in an artificial intelligence model to identify individuals at risk of developing T2D. This model may also serve as a tool to identify new therapeutic interventions. The effect of treatment regimens such as prebiotics, probiotics, and facilitated microbial transfer (FMT) should be investigated in order to ensure early intervention for individuals at risk, thus preventing secondary complications with significant cost savings. Dysbiosis can trigger and further aggravate metabolic impairment. As an interface between the gut microbiome and diabetes, intestinal permeability is a promising candidate for mechanism research. Altered intestinal permeability is also associated with dysbacteriosis of the gut microbiome, which is one possible mechanism for the development of DCI in T2D.

Conflict of interests

Dr Raveendran AV is serving as Associate Editor of the Clinical Diabetology.

REFERENCES


