INTRODUCTION

There has been a dramatic increase in the global prevalence of type 2 diabetes mellitus (T2DM) over the last three decades, and this increase has been accompanied by decreased life expectancy and quality of life among affected populations. As of 2015, more than 415 million adults have diabetes mellitus and this number is estimated to increase to 642 million by 2040. Type 2 diabetes mellitus constitutes more than 95% of all cases of diabetes in adults worldwide. India represents one of the global “hotspots” of diabetes and is second only to China in the number of people with diabetes. A recent National study, the Indian Council of Medical Research-India Diabetes, estimates that 62.4 million people in India have diabetes and 77.2 million have prediabetes. Despite the availability of clinically effective therapies for diabetes, achievement and maintenance of good glycemic control in these patients remains a challenge. Poor glycemic control can have devastating consequences, including the development of microvascular complications (diabetic neuropathy, retinopathy, and nephropathy) and macroangiopathy (ischemic heart disease, stroke, and peripheral vascular disease).

Impaired glucose homeostasis characteristic of T2DM develops due to a combination of impaired pancreatic beta cell dysfunction and diminished insulin sensitivity, in varying proportions. While factors such as genetic predisposition, poor physical activity, fetal programming, and obesity, have been shown to increase the risk of developing T2DM, recent interest has focused on the role of alterations of the microbial community in the gut—the microbiota. In this context, the evidence base is growing to suggest that gut microbiota may play a key role in the onset and development of diabetes. Therefore, there is an imperative need to study the link between gut microbiota and T2DM in all possible ways in order to target therapeutics. This review is an attempt to give a glimpse of current happenings in this field with a focus on future perspectives.

ROLE OF GUT MICROBIOTA IN HUMAN METABOLISM

The gut microbiota plays a major role in maintaining the physical and biochemical integrity of the intestinal barrier. The human gut hosts more than 100 trillion bacteria belonging to over 1,000 species. The gut microbiome which exerts several trophic, metabolic, and protective functions on the host exceeds the size of the human genome by 100-fold. The gut microbiome functions like a virtual organ system, and consists of around 200 common species and 1,000 less prevalent ones. While firmicutes, bacteroidetes, and actinobacteria constitute more than 95% of the total microbiota, the composition of the gut microbiota may vary based on affected by diet, host genetics, and the immune status. While the core functions of the gut microbiota are similar in all human beings, they differ in specialized functions. As a consequence, some communities are linked to human diseases and obesity more than others.

THE LINK BETWEEN GUT MICROBIOTA AND TYPE 2 DIABETES

As understanding grows, it is being realized that the gut is not only associated with digestion and excretion but also that gut hormones may have an important therapeutic
potential. This is increasingly drawing the attention and interest of medical researchers. The "gut connection" to T2DM received even more attention in recent years because of the fact that there is an intricate relationship between intestinal microbiota and development of metabolic diseases with special reference to diabetes. Recent research has also focused on the use of the human microbiome as a biomarker for the early detection of metabolic disorders in general and diabetes in particular.

Studies on germ-free mice suggest that gut microbiota might be linked to alterations in glucose metabolism. Recent human studies using metagenomic techniques on Chinese and Swedish individuals suggest that T2DM is characterized by dysbiotic gut microbiota. Both studies reported a paucity of butyrate-producing bacteria (Roseburia species and Faecalibacterium prausnitzii) in individuals with T2DM. Earlier studies in humans as well as in mice reported that obesity and impaired glucose metabolism were associated with an altered ratio between the two major phyla in the human gut, firmicutes and bacteroidetes. However, neither the Swedish nor the Chinese study confirmed these findings. An abundance of bifidobacteria has been reported as a possible characteristic of diabetes. When obese and lean individuals with/without T2DM were evaluated for the presence of F. prausnitzii, a prominent member of the human gut microbiota belonging to the firmicutes group, it was observed that there was a reduction in the levels of F. prausnitzii in patients with T2DM. The reduction was associated with an increase in inflammatory markers indicating that an increase in the number of F. prausnitzii may alleviate inflammation and insulin resistance. An Austrian study has also reported on the differences in the characteristics of the microbiota in T2DM and obesity.

The landmark Chinese study elucidated the association of intestinal flora with T2DM. Type 2 diabetes mellitus was associated with moderate gut dysbiosis, differential abundance of around 60,000 microbial genes decrease in the abundance of butyrate-producing bacteria and an increase in various opportunistic pathogens. Distinct changes in gut microbiota have also been reported in individuals with impaired glucose tolerance (IGT) and patients with T2DM compared to subjects with normal glucose tolerance (NGT). Interestingly in this study also, butyrate-producing bacteria were more abundant in individuals with NGT. In addition, verrucomicrobia was postulated as a potential marker of T2DM.

Recently, Karlsson et al. have used shotgun sequencing to characterize the fecal metagenome of 145 European women with NGT, IGT, or T2DM. They have identified "metagenomic clusters" that accurately predicted T2DM. Classification of women with IGT into subgroups with T2DM- or NGT-like metabolism appears to offer a potentially new approach to identify individuals at high risk of developing T2DM. Interestingly, the data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR) study has recently linked dysbiosis of blood microbiota to the onset of cardiovascular events.

There is little published evidence on the effects of antidiabetic medications on gut microbiota. In the Swedish study, metformin use was associated with increased levels of enterobacteiraceae and decreased levels of Clostridium and Eubacterium, however, the significance of these observations was not clear. Metformin increases the levels of Akkermansia muciniphila species in high-fat diet-fed mice in parallel to its beneficial effects on glucose metabolism. Oral administration of A. muciniphila has been reported to result in improvements of glucose tolerance, metabolic endotoxemia, and adipose tissue inflammation in mice. These findings point toward novel glucose-lowering mechanisms of metformin and may also represent future potential targets for altering glucose regulation by means of bacteriotherapy.

GUT MICROBIOTA AND TYPE 1 DIABETES MELLITUS

Type 1 diabetes mellitus (T1DM) results from autoimmune destruction of the pancreatic β-cells in genetically predisposed individuals. Beta cell destruction involves innate and adaptive immune responses and when around 80% of the β-cells are affected, the first signs of diabetes become manifest. While a link between gut microbiota and the development of T1DM has been postulated, the exact mechanisms underlying this relationship remain unclear. Increased intestinal permeability may facilitate the absorption of antigens which can elicit an immune response that then damages pancreatic β-cells. The functioning of the intestinal mucosal barrier has been shown to be defective in individuals susceptible to T1DM and other autoimmune diseases. However, the mechanisms resulting in the development of a "leaky gut" prior to development of T1DM have not yet been fully elucidated.

While analyzing the metabolic profiles of T1DM patients, Oresic et al. observed that certain metabolites correlated well with the disease. These differences were observed even before the onset of the first autoantibodies and thus are a potential biomarker of the disease. Since
many of these metabolites were produced by the gut microbiota, alterations in these could promote the development of T1DM. In fact, the study by Giorgi et al.\textsuperscript{30} has identified alterations in gut microbiota associating with progression toward T1DM. Mining 16S ribosomal ribonucleic acid (RNA) data from T1DM patients showed an association of gut microbiota alterations with development of autoimmunity.\textsuperscript{31} Significant differences have been reported in the gut microbiota of children with T1DM as compared to that of healthy children.\textsuperscript{32} This emphasizes that more studies are needed to explore the role of the gut in T1DM development.

While probiotics have been shown to have multiple health benefits, their widespread use in prevention of T1DM is not justified at the present time due to lack of knowledge of the normal intestinal microbiota. In addition, studies using newly developed techniques in proteomics and metabolomics to evaluate intestinal microbiota are certainly needed.

**GUT MICROBIOTA AND THE HOST METABOLISM**

Gut microbiota are under control of host genetics as documented in knockout studies in mice.\textsuperscript{33} One of the mechanisms by which microbiota impact gene regulation in the host appears to be mediated through microbial circulating noncoding RNA.\textsuperscript{34} Also gut microbiota are known to affect the innate immune response of the host, e.g., through the intestinal uptake of bacterial lipopolysaccharide (LPS), resulting in metabolic endotoxemia, i.e., a systemic low-grade inflammation and insulin resistance.\textsuperscript{35} Similarly, bacterial metabolites including short chain fatty acids (SCFAs), trimethylamine-N-oxide, and hippurate have biological activities that regulate host functions. For instance, SCFAs (majorly acetate, propionate, and butyrate), which are produced by bacterial fermentation of dietary fibers have been shown to stimulate the release of anorectic hormones like glucagon-like peptide 1 and peptide tyrosine tyrosine.\textsuperscript{36}

Lipopolysaccharide is an integral component of the cell wall of Gram-negative bacteria. Consumption of a high-fat diet has been shown to increase circulating levels of LPS, by modulating the gut microbiota and increasing uptake of LPS by chylomicrons by way of increased intestinal permeability. Lipopolysaccharide have been associated with metabolic endotoxemia and release of proinflammatory cytokines.\textsuperscript{37-39} While several complementary mechanisms underlie the effect of gut microbiota on glucose and lipid metabolism, a common feature appears to be an altered intestinal integrity of the gut epithelial wall. While there is very little information available on these aspects, it is important to know whether these microbial components and metabolites serve as systemic biomarkers in relation to changes in gut microbiota in metabolic disorders.

Measurement of LPS activity, therefore, holds promise as a new biomarker to estimate metabolic risk profile in humans.\textsuperscript{40} In this context, we have recently demonstrated the association of increased LPS levels and LPS activity in patients with T2DM.\textsuperscript{41} Increased circulatory levels of LPS and zonulin (a marker of gut permeability) in patients with T2DM appear to expand our understanding of the emerging role of gut and gut microbiota in the pathogenesis of diabetes. The biomarker potential of LPS is also inferred by several other studies. An increase in plasma LPS occurs in healthy individuals after a high-fat meal,\textsuperscript{38} whereas a chronic state of low-grade endotoxemia as measured by plasma LPS\textsuperscript{37} or LPS-binding protein\textsuperscript{62} is evident in patients with obesity and insulin resistance. Once elevated in circulation, LPS can target various tissues (macrophages, adipose, skeletal muscle, and liver) where they can interact with specific receptors (toll-like receptors and pattern-recognition receptors), induce the secretion of inflammatory cytokines, and negatively regulate insulin signaling. Interestingly, LPS-producing *Enterobacter cloacae* isolated from a morbidly obese patient was shown to trigger obesity and insulin resistance in germ-free mice.\textsuperscript{43}

**CONCLUSION**

The bulk of currently available evidence supports the concept of alterations in gut microbiota in diabetes. However, available data is insufficient to conclude whether these alterations are a cause or consequence of T2DM. These alterations may predispose to metabolic disorders by altering gut permeability, triggering systemic immune alterations, altering signaling pathways, and promoting low-grade inflammation eventually leading to insulin resistance and T2DM.

The first step could be implementation of well-designed prospective studies, ideally with carefully phenotyped prediabetic individuals. If such longitudinal studies support a link between the gut microbiota and future development of T2DM, microbiota in those prediabetic individuals who develop T2DM and in those who do not develop T2DM should be further tested and characterized in mechanistic mice experiments. Finally, to establish causality, proper randomized clinical
intervention trials are needed in which subjects are randomized to receive treatment with health-promoting bacteria or placebo. Future research should also focus on the complex hormonal, immunomodulatory, and metabolic mechanisms that influence host-microbiota interactions and evaluated through well-designed studies that measure specific endpoints.

REFERENCES


