



## Role of Probiotics in Type 2 Diabetes Mellitus

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### ABSTRACT

Recently, it has been found that human gut microbiota plays important role in health & disease. The microbiota associated with chronic inflammation has been shown to contribute Type-2 Diabetes mellitus (T2DM). Moreover the microbiota is altered in T2DM. This review summarizes altered microbiota in the pathogenesis of T2DM & role of probiotics in T2DM.

**Keywords:** Type 2 Diabetes mellitus, probiotics, microbiota, endotoxemia.

### INTRODUCTION

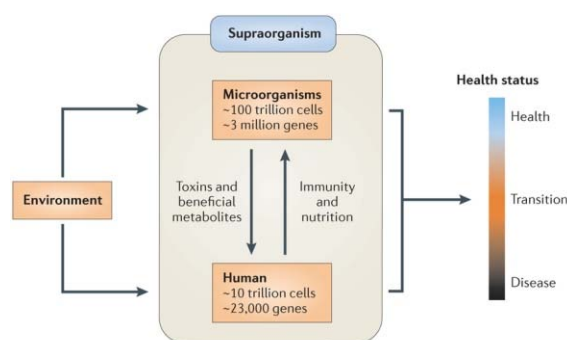
The prevalence of Type-2 Diabetes Mellitus (T2DM) has increased dramatically in the past half century & is accompanied by various morbidities<sup>1</sup>. It also decreases quality of life & life expectancy of millions of people worldwide. In 2011, the International Diabetes Federation (IDF) estimated that 61.3 million people in India had T2DM of which 31.3 million were undiagnosed<sup>2</sup>. A recent National study (ICMR-INDIAB) also showed that the similar projection for India would be 62.4 million people with diabetes & 77.2 million people with prediabetes<sup>3</sup>.

T2DM (formerly called non-insulin-dependent DM/adult onset DM) is a metabolic disorder that is characterized by hyperglycemia in the context of insulin resistance & relative lack of insulin<sup>3</sup>. It has been known that several risk factors like obesity, unhealthy diet, physical inactivity, increasing age, high blood pressure, family history of diabetes are associated with diabetes.

Recently, it has been found that, the gut microbiota has been found as potential contributor of this epidemic<sup>4</sup>. The resident microbiota associated with chronic inflammation has been shown to contribute to the onset of T2DM. Moreover the gut microbiota is altered in the development of T2DM & its comorbid disease condition. So there is a need to study the link between gut microbiota and type 2 diabetes and to explain the role of probiotics in type 2 diabetes.

#### The Gut Microbiota

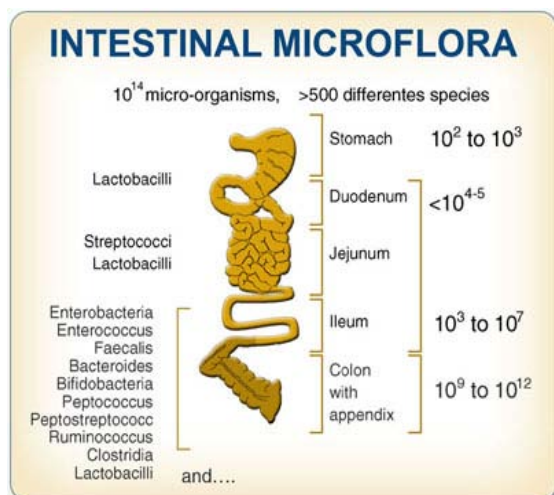
Julian Davies suggested, a full understanding of human biology requires to properly accounting for the gut microbiota<sup>5</sup>. Each human body is host to 100 trillion bacteria, a number 10 times greater than total no of human cells in the body<sup>6</sup>. The genetic material of the intestinal microbes, collectively named the microbiome, exceeds the magnitude of the human genome over 100 times<sup>7-10</sup>. Medical literature therefore refers to the gut microbiota as an 'exteriorized organ'<sup>10</sup>.



The phyla that account for the vast majority of all gut microbes include the gram negative Bacteroides, Proteobacteria and Verrucomicrobia as well as gram positive Firmicutes and Actinobacteria<sup>7,10-12</sup>. As we know that they mediate mucosal barrier integrity, modulate tight junctions & epithelial permeability, trophicity of the epithelium & cellular renewal, immune maturation, stimulation of immune cells such as paneth cell & defensins, supplying B & K vitamins to the host. They also mediate gut brain cross-talk, as recently supported by animal & human studies<sup>13</sup>.

The microbial population of the gut increase in density as we proceed from stomach to small intestine. The microbial population of the gut is very sparse in the stomach due to luminal acidity & vigorous peristalsis. The microbial population increases in density as we proceed from duodenum ( $10^1$ - $10^3$  organisms/ml) to jejunum ( $10^4$ - $10^6$  organisms/ml) to ileum ( $10^5$ - $10^7$  organisms/ml). Microbial concentrations in the large intestine reach  $10^{11}$ - $10^{12}$  organisms/g of stool. The nature of the resident microbiota also changes as we proceed from the stomach to the colon. In the proximal bowel, aerobic microbes are likely to predominate, while in the colon, due to anaerobic luminal conditions, the microbiota is composed of strict anaerobes in large numbers & facultative anaerobes in smaller numbers<sup>6</sup>. Studies in the 1970s indicated that the fecal microbiota of healthy Indians was composed of a large number of cultivable anaerobic organisms, predominantly Bacteroides, Bifidobacteria,

Enterobacteriaceae and Enterococci<sup>14</sup>. The same study evaluated the gut microbiota at various levels of the gastrointestinal tract from the mouth to the colon and found that the luminal fluid from the upper jejunum contained a mean of  $10^5$  organisms/ml predominantly Streptococci, Fusobacteria, and Veillonella. Enterobacteria dominated in the lower jejunum & upper ileum, while Bacteroides & Bifidobacteria were the dominant flora in the feces.



In the early 1990s, it has been recognized that the largest part of human intestinal microbiota remains inaccessible by culture and therefore molecular techniques are developed for a culture independent assessment. The bacteria were quantified by qPCR targeted at the 16S ribosomal RNA (16S rRNA) sequences, sequence based method<sup>15</sup>. Advances in sequencing technologies now have resulted in the widespread application of whole genome sequencing (WGS) technologies for metagenomic analysis of gut microbiome<sup>16</sup>. The significance of this functional WGS provide opportunities to find out which metabolic pathways are affected & how the microbiome may contribute mechanistically to health & disease states. Large consortia, such as the National Institute of Health's Human microbiome project<sup>17</sup> and the European Union's MetaHIT<sup>16</sup>, together with many other microbiome projects on different scales worldwide, are providing us a first impression of the diversity of the human microbiome. These projects not only allow us to understand the microbes that we harbor and how these microbes assemble into healthy communities but also the genes involved in specific microbial functions.

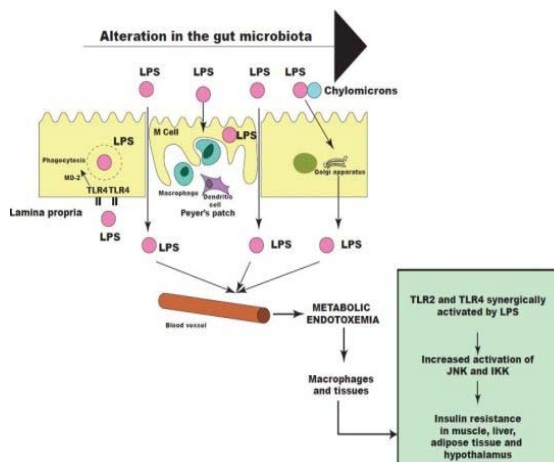
### Gut Microbiota & Type 2 Diabetes Mellitus

The 'gut connection' to T2DM received 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. It is well known that the gut microbiota is involved in the process of energy harvest accounting for the development of obesity. Several studies showed that changes in the gut microbiota are correlated with the development of obesity, insulin resistance & diabetes.

The possible mechanisms (Fig-1) include the ability of the microbes to extract energy from the diet<sup>18</sup>, altered fatty acid metabolism within the adipose tissue & liver<sup>19</sup>, changes in the level of the gut hormones<sup>20</sup>, activation of lipopolysaccharide toll-like receptor-2<sup>21</sup>, and changes in the intestinal barrier integrity<sup>22</sup>. High-fat or high-calorie diet associated with exaggerated post-prandial spikes in glucose and lipids generate excess free radicals that can trigger a biochemical cascade resulting in chronic, low grade systemic inflammation<sup>23</sup>. Experimental studies have described how circulating free fatty acids trigger increased inflammatory responses by activating toll-like receptor-4 (TLR-4) or nuclear factor-kappaB (NF-kB), while studies on glucose metabolism have focused on glycolysis, pyruvate accumulation in mitochondria favoring free radical generation and inflammation<sup>24</sup>. Inflammation thus induced has been found to play key role in the pathogenesis of obesity, metabolic syndrome, insulin resistance, type 2 diabetes<sup>25</sup>. Pro-inflammatory cytokines induce insulin resistance by activating the suppressor of cytokine signaling (SOCS) family of proteins, which interfere with insulin receptor substrates 1 (IRS-1) and 2 (IRS-2), tyrosine phosphorylation or increase proteosomal degradation and decrease availability of receptor substrate for insulin activation<sup>26</sup>. Besides all these intestinal alkaline phosphatase may also be involved in altered intestinal permeability & pathogenesis of insulin resistance. Furthermore, the presence of gut microbiota resulted in an increase in hepatic lipogenesis and suppression of the lipoprotein lipase (LPL)-inhibitor angiopoietin-like 4 (ANGPTL4), which was formerly known as fasting-induced adipocyte factor (FIAT)<sup>27,28</sup>. Several reviews<sup>29,30</sup> are available with discussions mostly on animal studies that associate gut microbiota alterations with obesity/insulin resistance/T2DM. Cani in 2008<sup>31</sup> showed that mice fed a high fat diet were more prone to inflammation & exhibited endotoxemia both underlying factors for the development of T2DM. Analysis of the cecal content of the high fat fed mice revealed reduced level of Bifidobacteria compared with control mice fed standard food. Larsen reported that the gut microbiota in human adults with T2DM differed from non-diabetic adults<sup>32</sup>. The authors reported that there was a significant reduction in the relative number of Firmicutes & Clostridia in adults with T2DM. This study has also demonstrated that in addition to the decreased abundance of Firmicutes, the Beta-proteobacteria levels were significantly increased in diabetic patients compared to non-diabetic controls & their abundance significantly correlated with their plasma glucose concentrations.

Wu reported a reduced abundance of Bifidobacteria in T2DM<sup>33</sup>. When obese & lean individuals with/without T2DM were evaluated for the presence of Faecalibacterium prausnitzii (a prominent member of the human gut microbiota belonging to the Firmicutes group) it was observed that there was a reduction in the Faecalibacterium prausnitzii in patients with T2DM. The

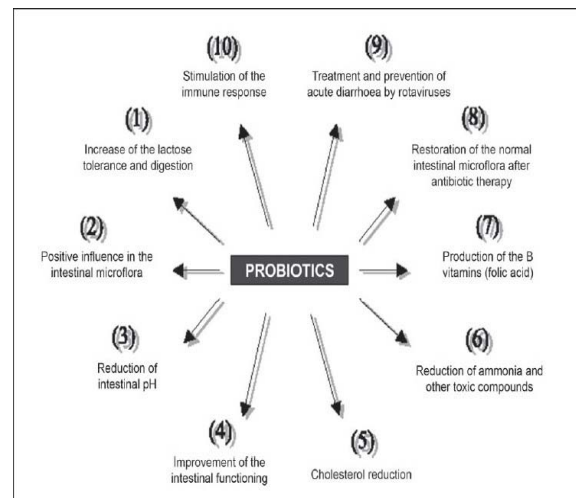
reduction correlated with increased levels of inflammatory markers indicating that an increase in the number of *Faecalibacterium prausnitzii* may alleviate inflammation & insulin resistance. Zupancic studied Amish men & women with metabolic syndrome; found that metabolic syndrome can be affected by certain members of the gut microbiota that can contribute to the metabolic syndrome<sup>34</sup>. Data from the Epidemiological Study of on the Insulin Resistance Syndrome (DESIR) also reported that bacterial phylotypes present in blood when identified by pyrosequencing of the overall 16S rDNA gene content predicted the risk of diabetes<sup>35</sup>. A recent landmark showed the involvement of intestinal flora in a Chinese cohort<sup>36</sup>. Approximately 60,000 microbial genes were found to be differentially abundant among T2DM patients who were also characterized by a moderate degree of gut microbial dysbiosis, a decrease in the abundance of some universal butyrate-producing bacteria and an increase in various opportunistic pathogens. Zhang has also reported distinct gut microbiota changes in subjects with impaired glucose tolerance and patients with T2DM compared to subjects with normal glucose tolerance<sup>37</sup>. This study also shows that butyrate-producing bacteria are abundant in subjects with normal glucose tolerance than others. In addition, Verrucomicrobiae was identified as potential marker of T2DM.



### Probiotics in T2DM -

Dr Elie Metchnikoff, a Nobel Prize winner for his work on phagocytosis, created the concept of 'probiotic'-microbes being 'useful for life'. The term 'Probiotic' was first introduced in 1953 by Werner Kollath & means 'for life'. According to the universally accepted definition given by the FAO & WHO in 2002, probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'<sup>38</sup>. Probiotics are becoming increasingly popular worldwide for offering health benefits<sup>39</sup>. Use of oral probiotic cultures has been shown to restore the gut microbiota beneficially in certain metabolic derangements<sup>40</sup>. It has been confirmed that some probiotic strains are able to modulate blood glucose homeostasis and hence improve T2DM. Some probiotics have been confirmed to prevent onset of

diabetes through down-regulating inflammatory IFN and IL-2 or IL-1 or enhancing anti-inflammatory IL-10 production in diabetic animal studies<sup>41</sup>.



At present, several clinical trials are going and probiotics of different phyla available. The phylum Firmicutes with the order Lactobacillales, genus Lactobacillae. Some of the lactobacilli are *Lactobacillus casei* and *Streptococcus*. The phylum Actinobacteria in the genus *Bifidobacterium* e.g. *Bifidobacterium animalis* is another probiotic available. The first discovered probiotic an *Escherichia coli* strain Nissle is part of the phylum Proteobacteria. There is also a probiotic from the yeast domain, *Saccharomyces boulardii*. Probiotics, especially *Lactobacillus* & *Bifidobacterium*, have been associated with number of health benefits. Specific probiotic strains may alter the gut microbiota, beneficially affect intestinal integrity, and reduce lipopolysaccharide (LPS) transmission from the gut lumen to the systemic circulation, which would eventually reduce the production of pro-inflammatory cytokines that is the root cause of many chronic non-communicable diseases<sup>42</sup>. Specific strains of *L. rhamnosus* and *L. plantarum* exerted beneficial effects on diet-induced obesity in mice, which has been attributed to conjugated linoleic acid (CLAs) produced by these strains<sup>43,44</sup>. Feeding fermented skimmed milk containing *L. gasseri* has been shown to reduce the size of adipocytes in visceral adipose tissues of rats, which has been speculated to be due to the inhibition of dietary fat absorption<sup>45</sup>. VSL # 3, a mixture of viable lyophilized strains of *Bifidobacteria*, *Lactobacilli* and *Streptococcus thermophilus*, increased natural killer T-cells, reduced inflammation and improved diet induced obesity and insulin sensitivity in mice<sup>46</sup>. *Lactobacillus paracasei* ST11 was found to reduce diet-induced obesity by enhancing lipolysis in white adipose tissues and thermogenesis in brown adipose tissues in rats<sup>47</sup>. The results of these animal studies indicate that specific probiotic strains may have a critical role in diet-induced obesity and its related disorders<sup>46,47</sup>. In humans, *L. plantarum* strain (LP14), used as a starter strain for pickles, has been reported to reduce body fat in a randomized, placebo-controlled, double-blind trial<sup>48</sup>.

*Lactobacillus salivarius* alone and in combination with fructo-oligosaccharides (FOS) was supplemented to determine its effect on colonization ability, lipid profile, insulin sensitivity and immune response in a study conducted in normal human volunteers, aged 20-35 years. *Lactobacillus salivarius* alone and in combination with FOS reduced serum low-density lipoprotein (LDL), triglycerides, total cholesterol, pro-inflammatory markers (IL-6, IL1 $\beta$ , TNF- $\alpha$ , CRP), increased HDL, and improved insulin sensitivity in normal young adults. Recently, *Lactobacillus reuteri* GMNL-263 have been demonstrated to suppress serum glucose, insulin, leptin, C peptide, glycated hemoglobin, GLP-1 level, inflammatory IL-6 and TNF in adipose tissues and PPAR and GLUT4 gene expression in high fructose-fed rats<sup>49</sup>.

## CONCLUSION

Gut microbiota provide different functions useful for the host and play a major role in maintaining human metabolism and homeostasis. The gut microbiota beneficial in counteracting the adverse effects of high fat diet, maintenance of intestinal integrity and amelioration of inflammation by regulating endotoxemia. In this review, we have discussed that administration of probiotics may be a possible dietary strategy to regulate gut microbiota, reduce inflammation & associated metabolic disorders. So a regular intake is needed to get a sustained functional benefit.

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