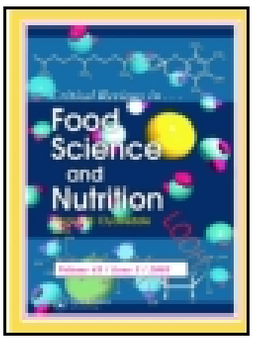

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Clinical Application of Probiotics in Diabetes Mellitus: Therapeutics and New Perspectives

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ABSTRACT

The characterization of gut microbiota has become an important area of research in several clinical conditions, including type 2 diabetes. Changes in the composition and/or metabolic activity of the gut microbiota can contribute to human health. Thus, this review discusses the effects of probiotics and gut microbiota on metabolic control in these individuals. Relevant studies were obtained from electronic databases such as PubMed/Medline and ISI Web of Science. The main probiotics used in these studies belonged to the genera *Lactobacillus* and *Bifidobacterium*. We found seven randomized placebo-controlled clinical trials and thirteen experimental studies directly related to the effect of probiotics on metabolic control in the context of type 2 diabetes mellitus. The hypothesis that gut microbiota plays a role in the development of diabetes indicates an important beginning, and better results had been observed in animal studies. In clinical trials, the use of probiotics in glycemic control presented conflicting results, and only few studies have attempted to evaluate factors that justify metabolic changes, such as markers of oxidative stress, inflammation, and incretins. Thus, further research is needed to assess the effects of probiotics in the metabolism of diabetic individuals, as well as the main mechanisms involved in this complex relationship.

Keywords: Gut Microbiota, Type 2 Diabetes, Probiotics, Oxidative Stress, Inflammation.

INTRODUCTION

Currently, there has been a progressive increase in the global prevalence of type 2 diabetes mellitus (T2DM) and its complications. According to the World Health Organization (WHO), 346 million people worldwide have diabetes (World Health Organization, 2012). On an average, 8% of adults living in developed cities and more than 10% living in developing countries are diagnosed with T2DM. Nowadays, China and India lead the world rankings of the prevalence of T2DM and Brazil is at the fifth place (Scully, 2012).

The mechanisms and factors that trigger T2DM have been subjected to intense discussion. Genetic factors, high caloric intake, and physical inactivity are well established as major risk factors for the T2DM (Lyssenko et al., 2008). Currently, studies aimed at investigating the importance of other factors such as gut microbiota, inflammatory markers and oxidative stress (Jonietz, 2012).

At birth, the gut is sterile and is colonized immediately. Gut microbial composition among healthy humans is complexy and the distribution of microorganisms throughout the gastrointestinal tract is not homogenous. The colon provides optimal conditions for the growth of microorganisms due to absence of digestive secretions, slow peristalsis and abundant nutritional supply (Neish, 2009).

Many external factors influence the composition of the microbiota, especially the diet, antibiotics, hygiene conditions and it appears that organic disease and drugs can modulate microbiome composition and activities (Nicholson et al., 2005).

The unbalance gut microbiota seems to be able to influence the metabolism of the host through utilization of nutrients and production of metabolites, promoting susceptibility to

metabolic disorders such as insulin resistance (Cani et al., 2007b) and metabolic syndrome (Petruzzelli and Moschetta, 2010; Vijay-Kumar M et al., 2010). Initial data have shown the differences in the composition of the adult intestinal microbiota among those with diabetes mellitus and control subjects (Larsen et al., 2010; Qin et al., 2012) and suggest that the composition of intestinal microbiota may influence the energy extraction of ingested foods, mucosal immunity, intestinal permeability and transit-time and systemic inflammation (Backhed et al., 2004; Cani and Delzenne, 2007; Gravitz, 2012). These factors have also been highlighted as triggers in the development and progression of T2DM and its complications (Ceriello and Motz, 2004; Dandona et al., 2004; Larsen et al., 2010; Stephens et al., 2009).

Interestingly, administration of probiotics and prebiotics has been reported to be one of the most widely used approaches to modulate intestinal microbiota and may subsequently prevent or delay diabetes incidence (Steer et al., 2000). This possibility was demonstrated in a study in mice supplemented with prebiotics. These mice exhibited increased levels of *Bifidobacterium,* which were associated with improved glucose tolerance and decrease in inflammation (Cani et al., 2007a). Thus, probiotics particularly lactobacilli and bifidobacteria have recently emerged as a potencial biotherapeutics for diseases prevention and/or treatments (Panwar et al., 2013).

The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) defined probiotics as “live micro-organisms”, which when administered in adequate amounts confer a health benefit on the host (FAO and WHO, 2002). The term prebiotic is defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth of bacterial species residing in the colon, which has a positive influence

on the intestinal microbiota composition (Schrezenmeir and de Vrese, 2001). The bacterial genera most commonly used in probiotic preparations are *Lactobacillus* and *Bifidobacterium*.

Many clinical trials examining the effects of different probiotic strains and their formulations on various intestinal disorders such as diarrhea, peptic ulcers, inflammatory bowel disease, colorectal cancer, atopic dermatitis, and allergies have shown a positive influence (Ritchie and Romanuk, 2012). However, few studies have investigated the role of probiotics in patients with diabetes.

With the increasing annual growth of industrial production of food-containing probiotics around the world, the interest to find out how the changes in the intestinal microbiota as a result of probiotics ingestion could serve as a new way of regulating metabolism in subjects with chronic diseases is increasing.

The present review discusses the effects of probiotics on metabolic control in T2DM subjects as well as the main mechanisms involved, with an emphasis on the involvement of gut microbiota, to better understand its clinical application, effectiveness, and safety.

GUT MICROBIOTA AND DIABETES *MELLITUS*

The human gut houses trillions of bacteria representing more than 500 species belonging to four major phyla: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Among them, ~ 60% of the total gut bacteria belong to the phylum Firmicutes, with more than 250 genera of Gram-positive bacteria, including *Lactobacillus, Streptococcus, Staphylococcus, Clostridium,* and *Mycoplasma*. Among the phylum Actinobacteria (Gram-positive bacteria), the major genus found is *Bifidobacterium* (Diamant et al., 2011).

Recently, the presence of three main enterotypes has been reported to characterize the gut microbiome according to their co-occurrence in healthy adult European, North American, and Japanese subjects. The changes in the levels of *Bacteroides* (Gram-negative bacteria, phylum Bacteroidetes, enterotype 1), *Prevotella* (phylum Bacteroidetes, enterotype 2), and *Ruminococcus* (phylum Firmicutes, enterotype 3), and the respective classes of microorganisms that use different routes for energy generation have been suggested to affect their synergistic relationship with the human host. However, the three enterotypes were not found to be significantly correlated with age, sex, body mass index (BMI), or nationality, except enterotype 1, which were more in the Japanese subjects (Arumugam et al., 2011).

As mentioned previously, T2DM is a result of complex gene–environment interactions, and several risk factors have been identified, including age, family history, diet, physical inactivity, and obesity. Statistical models indicate that human genetics has little contribution, whereas environmental factors have major influence (Noble et al., 2011).

The gut microbiota has been considered as an environmental factor involved in the complex web of gene–environment interactions that can influence the development of obesity (Backhed et al., 2004; Cani et al., 2007b; Turnbaugh et al., 2009), insulin resistance (Cani et al., 2007b), diabetes mellitus (Larsen et al., 2010), and metabolic syndrome (Vijay-Kumar M et al., 2010).

In 2004, Fredrik Bäckhed et al. found that germ-free mice had lower body weight, when compared with conventional mice. However, transplantation of feces of the conventional mice into the germ-free ones induced weight gain and decreased the glycemic control in germ-free mice (Backhed et al., 2004). These results were also observed in obese humans and patients with

metabolic syndrome, who, after 6 weeks of allogeneic or autologous fecal transplant from normal individuals, exhibited an improvement in insulin sensitivity (Vrieze et al., 2012). Since then, the study of gut microbiota in models of diabetes and obesity, either in animals or humans, has attracted the interest of many researchers (Gravitz, 2012).

Studies that characterize the gut microbiota of diabetics and evaluate the possible correlations between the abundance of certain groups and metabolic aspects are fundamental to clarify and strengthen the role of microbiota in this clinical condition. Some studies have reported that patients with T2DM have a high number of opportunistic pathogenic bacteria (*Clostridium clostridioforme*, *Bacteroides caccae*, *Clostridium hathewayi*, *Clostridium ramosum*, *Clostridium symbiosum*, *Eggerthella sp.,* and *Escherichia coli*) and a low number of butyrate-producing bacteria (*Clostridiales* sp. SS3/4, *Eubacterium rectale*, *Faecalibacterium prausnitzii*, *Roseburia intestinalis,* and *Roseburia inulinivorans*) (Karlsson et al., 2013; Larsen et al., 2010; Qin et al., 2012). The increase in *Roseburia* spp. and butyrate levels have been reported to be associated with improved insulin sensitivity (Vrieze et al., 2012). These evidences suggest the importance of butyrate-producing bacteria in glycemia regulation. Moreover, these bacteria potentially stimulate bacterial defense mechanisms against oxidative stress (Qin et al., 2012).

Larsen et al. (2010) found a low number of bacteria belonging to the phylum Firmicutes and a high number of Betaproteobacteria in diabetic patients, when compared with those in non-diabetics. The Betaproteobacteria levels were positively correlated with the plasma glucose levels. In addition, the ratios between Firmicutes and Bacteroidetes, Bacteroidetes and *Prevotella* spp., and *Clostridium coccoides* and *E. rectale* were observed to be higher in diabetic patients and positively and significantly correlated with plasma glucose, but not with the BMI. Thus,

these bacteria were more specific than T2DM for obesity. A previous study showed that the decrease in the number of *Prevotella* spp. was significantly associated with the decrease in metabolic endotoxemia and inflammation in ob/ob mice with glucose intolerance (Cani et al., 2008). On the other hand, the diabetes progression was observed to increase in association with the decrease in the number of Firmicutes and Bacteroidetes over time (Giongo et al., 2011).

Different bacterial phyla exert different effects on carbohydrate metabolism and have the ability to influence glycemic control. Carbohydrates are an essential dietary component for mammals as well as their gut microbiota. Mammals absorb simple sugars in the proximal jejunum and hydrolyze disaccharides (sucrose, lactose, and maltose) in their monosaccharide constituents or degrade starch into monosaccharides. However, they have limited ability to hydrolyze other polysaccharides (Hooper et al., 2002). As a result, undigested polysaccharides (cellulose, xylan, and pectin) and partially digested starches reach the gut flora in the distal gut, where they are metabolized by bacterial enzymes (Musso et al., 2011).

Monosaccharides hydrolyzed from polysaccharides are converted by bacteria to pyruvate through glycolysis, resulting in the production of adenosine triphosphate (ATP). In a highly anaerobic environment of the intestinal lumen, distal carbons and greater energy are obtained from microbial fermentation of pyruvate. To recover the nutritional value of the degraded polysaccharides, mammals have developed mechanisms for the uptake and utilization of products of bacterial fermentation such as short-chain fatty acids (SCFA), the main end product of bacterial fermentation.

Acetate, propionate, and butyrate have different metabolic pathways. Butyrate is the preferred energy source for the epithelial cells of the colon, where it is converted to ketone

bodies or oxidized to carbon dioxide (Louis et al., 2007). The colonic epithelium takes 60–70% of energy from butyrate (Louis and Flint, 2009). Propionate and acetate are absorbed into the hepatocytes, which use most of these compounds for gluconeogenesis and lipogenesis. The SCFA have important effects on other aspects of intestinal physiology: they decrease the pH of the proximal colon and significantly affect the composition of the colonic microbiota. At pH 5.5, a higher concentration of ethyl butyrate was observed in the colon, when compared with that at pH 6.5. Furthermore, at pH 6.5, higher propionate production was noted, which induced changes in the composition of the microbiota, reducing the number of *Roseburia* spp. and increasing the number of *Bacteroides* spp. (Walker et al., 2005).

It is also noteworthy that the SCFA can improve insulin sensitivity owing to its metabolic effects in reducing the levels of free fatty acids through inhibition of phosphorylation of insulin receptor substrate. In addition, butyrate supplementation has been reported to improve glycemic control and insulin resistance in C57BL/6J mice (Gao et al., 2009). Thus, the SCFA, so far considered only as an indirect nutrient supplying energy to the bacteria and intestinal enterocytes, are regarded as regulators of other metabolic processes.

Firmicutes are usually involved in the transport of nutrients, and facilitate the absorption and fermentation of SCFA in non-digestible carbohydrates (Turnbaugh et al., 2009). Among the main phenotypic characteristics of *Bifidobacterium* spp., production of lactic acid and acetic acid as the main products of carbohydrate utilization in bowel is noteworthy (Ishibashi et al., 1997).

The following paragraphs briefly present a review of the efficacy of probiotics in the management of T2DM in vivo conditions. Studies included in this systematic review were conducted with people already diagnosed with diabetes or animals models of diabetics. To

broaden the search, additional articles were sought from the references cited in the selected articles. To confirm the number of experimental studies and clinical trials in each database, we selected the advanced search options "clinical trial" or "animal species" after typing the descriptors. The keywords searched were: Gut microbiota, Probiotics, *Lactobacillus,* and *Bifidobacterium*, used individually or in combination with Insulin sensitivity, Diabetes, Insulin resistance, Oxidative stress, and Inflammation.

MAIN EFFECTS OF PROBIOTICS CONSUMPTION IN T2DM

Experimental studies

Studies using animal models of diabetes were the first to show that different species of bacteria such as *Lactobacillus acidophilus* and *Lactobacillus casei* reduce oxidative stress and exhibit antidiabetic effect (Harisa GI et al., 2009; Kim et al., 2013; Yadav et al., 2007). In diabetic animal models, oxidative stress caused by the accumulation of free radicals leads to the damage of multiple tissues, including the β-cells of the pancreas, as well as to the distortion and dysfunction of several organs, including the liver and kidney (Hamden et al., 2008).

In non-diabetic C57BL/6J mice fed on fat diet was demonstrated that *Lactobacillus rhamnosus* (10^8 CFU/mL) administered for 13 weeks was able to achieve glycemic control by enhancing insulin sensitivity and increasing GLUT4 expression and adiponectin production (Kim et al., 2013). Similar results were also observed in diabetic mice using the same strain of bacteria administered for 6 weeks (Honda et al., 2012).

Male Sprague–Dawley rats were fed a high-fructose diet with or without *Lactobacillus reuteri* GMNL-263 administration for 14 weeks. The levels of serum glucose, insulin, leptin, C-

peptide, glycated hemoglobin, GLP-1, liver injury markers, lipid profile in serum and liver were significantly increased in high-fructose-fed rats. However, after Lr263 administration, the elevation of these parameters was significantly suppressed. Furthermore, concentrations of IL-6 and TNF-α in adipose tissue which were elevated in high fructose treatment were markedly decreased after Lr263 feeding and decreased levels of PPAR-γ and GLUT4 mRNA after high fructose treatment were significantly enhanced by Lr263 administration. Interestingly, Lr263 consumption significantly increased the number of *bifidobacteria* and *lactobacilli*, and on the contrary, decreased the number of *Clostridia* in the feces of treated rats (Hsieh et al., 2013). These results implicated that *L.reuteri* administration might exert its therapeutic effect on diabetes via increasing the beneficial as well as decreasing the harmful gut flora species. However, the relevant mechanisms underlying this phenomenon needed further investigation.

In another study, the effect of dahi, a fermented milk product containing *Lactobacillus acidophilus* NCDC14 and *L. casei* NCDC19 (10^8 CFU/g) on progression of streptozotocin (STZ)-induced diabetes in rats for 28 days was investigated. Feeding of probiotic dahi significantly suppressed STZ-induced oxidative damage in pancreatic tissues by inhibiting the lipid peroxidation and formation of nitric oxide, and preserving antioxidant pool such as glutathione content and activities of superoxide dismutase, catalase and glutathione peroxidase (Yadav et al., 2008). The feeding of the same probiotic dahi to the fructose-induced diabetic rats significantly decreased the blood glucose and glycosylated haemoglobin, free fatty acids and trigycerides (Yadav et al., 2007).

Interestingly, *L. acidophilus*, *Bifidobacterium lactis* and *L. rhamnosus* has been reported to reduce blood glucose levels and further improve the bioavailability of gliclazide, a

sulphonylurea drug used to treat T2DM in alloxan-induced diabetes rats (Al-Salami et al., 2008a). A possible explanation for the increase in gliclazide systemic absorption is that probiotics activate the efflux drug transporter, Mrp2 (Al-Salami et al., 2008b).

Recently, the oral administration of *Lactobacillus plantarum* TN627 was noted to significantly improve the immunological parameters, reduce the pancreatic and plasmatic a-amylase activities and level of plasma glucose in Alloxan-induced diabetic rats. Furthermore, this probiotic treatment was observed to markedly reduce serum triglyceride and LDL-cholesterol rates and to increase the level of HDL-Cholesterol (Bejar et al., 2013). In a different previous study, a synbiotic product containing *Enterococcus faecium* or *Lactobacillus helvetians* and yacon to diabetic animals for 7 weeks and observed improvement in the lipid profile, but no effects on glycemic control was observed (Roselino et al., 2012). Improvement in the lipid profile, even if not accompanied by improved glycemic control, may be important effect in patients with T2DM.

A dose-dependent effect on control glycemic has also been reported after ingestion of probiotics. The blood glucose levels decreased from 4480 to 3620 mg/L (with 10^7 CFU/d) and 3040 mg/L (with 10^9 CFU/d) in STZ-induced DM animal models treated with *L. reuteri* GMN-32. Probiotic treatment also reduced the changes in the heart caused by the effects of DM (Lin et al., 2014).

The main features and results obtained in studies involving diabetic animals are shown in Table 1. The study period ranged from 2 to 14 weeks. *Lactobacillus* spp. was the most evaluated in the experimental studies. In some cases, the results regarding glycemic control were dependent on the strain used, although in most of the studies, an improvement in some

parameters (insulin, glucose, or glycated hemoglobin) related to glycemic control was observed. (Honda et al., 2012) showed that only *L. rhamnosus* GG, and not *Lactobacillus bulgaricus,* was able to improve glycemic control in fasting and postprandial diabetic mice.

Clinical trials

A few clinical trials assessing the effects of probiotics on diabetics have been reported (Table 2). Initially, studies were performed in healthy subjects (Naruszewicz et al., 2002; Songisepp et al., 2005). The effects of probiotics on glycemic control have also been reported in pregnant women, who are predisposed to changes in glucose metabolism. Luoto et al. (2010) reported a lower prevalence of gestational diabetes in the pregnant group (n = 256) who received *L. rhamnosus* GG and *Bifidobacterium lactis* Bb12 (16%), with a significant difference (P = 0.003), when compared with the control group (36%) who received normal diet according to the dietary guidelines. No adverse effects were observed in relation to probiotics consumption throughout the pregnancy. On the other hands, probiotic capsule treatment (*Lactobacillus salivarius* UCC118, 10^9 CFU) of 4 weeks during pregnancy did not influence maternal fasting glucose, the metabolic profile, or pregnancy outcomes in 175 obese women (Lindsay et al., 2014).

Recently, a randomized controlled trial with 156 overweight men and women does not support the hypothesis that *L. acidophilus* La5 and *B. animalis* subsp *lactis* Bb12 for 6 week, either in isolated form or as part of a whole food, benefit short-term glycaemic control. Indeed, there is weak data for an adverse effect of these strains on glucose homoeostasis (Ivey et al., 2014).

In subjects with normal or impaired insulin sensitivity, the effects of oral supplementation with the probiotic bacterium *Lactobacillus acidophilus* NCFM (capsule – 10^{10} CFU) on insulin sensitivity and the inflammatory response were investigated. Forty-five males with type 2 diabetes, impaired or normal glucose tolerance were enrolled and allocated to a 4 week treatment course. The results showed that *L. acidophilus* NCFM was detected by denaturating gradient gel electrophoresis in 75% of the faecal samples after treatment with the probiotic bacterium. Insulin sensitivity was preserved among volunteers in the probiotic group ($P < 0.01$), whereas it decreased in the placebo group. Both baseline inflammatory markers (and the systemic inflammatory response (TNF, IL-6, IL-1ra and C-reactive protein) were, however, unaffected by the intervention (Andreasen et al., 2010). The authors highlight that the effect on insulin sensitivity of *L. acidophilus* NCFM is not related to its anti-inflammatory properties, at least not through any apparent effects on circulating cytokines, and your findings lend no support to the contention that insulin sensitivity is improved through probiotic-induced anti-inflammatory mechanisms. Alternatively, the variation in plasma cytokines due to the intervention may be so discreet that no significant changes during the treatment period were detected with the current number of volunteers included in the present study.

In a different, study was observed that improve fasting blood glucose was associated with an improvement in antioxidant status after intake of probiotic yogurt. In this study, sixty-four patients with T2DM were assigned to two groups. The patients in the intervention group consumed probiotic yogurt containing *Lactobacillus acidophilus* La5 (10^6 CFU) and *Bifidobacterium lactis* Bb12 (10^6 CFU) and those in the control group consumed conventional

yogurt for 6 week (Ejtahed et al., 2012). The antioxidative properties of probiotic strains in T2DM will be discussed later in a specific topic.

In another study evaluated the effect of a symbiotic drink, a combination of both probiotics and prebiotics, in 20 diabetic (ten for placebo group and ten for symbiotic group) over a total test period of 30 days on glycemia and cholesterol levels. The results of the symbiotic group that consumed *L. acidophilus* (10^8 CFU/ml), *Bifidobacterium bifidum* (10^8 CFU/ml), and 2 g oligofructose showed a significant increase ($P < 0.05$) in HDL cholesterol, non-significant reduction ($P > 0.05$) in total cholesterol and triglycerides and a significant reduction ($P < 0.05$) in fasting glycemia. However, no significant changes were recorded in the placebo group (Moroti et al., 2012).

Besides the effects on glucose metabolism, probiotics have also been investigated for possible impacts on lipid metabolism. Such assessments are important because diabetics have greater risks for cardiovascular diseases. Randomized trials conducted with hypercholesterolemic subjects also reported a significant reduction in total cholesterol after 6 weeks of yogurt intake with *L. acidophilus* and/or *B. lactis* (Anderson and Gilliland, 1999; Ataie-Jafari A et al., 2009). However, these results are still controversial, especially with respect to evaluation in healthy subjects without hypercholesterolemia, and only a few studies have reported improvement in lipid profile in diabetic subjects (Ejtahed et al., 2011; Greany et al., 2008; Moroti et al., 2010; Moroti et al., 2012).

A meta-analysis involving clinical trials using milk products containing probiotics (*E. faecium* and *Streptococcus* spp.) reported that probiotic treatment for 4–8 weeks led to 4% decrease in total cholesterol and 5% decrease in LDL-C in healthy subjects (Agerholm- Larsen L

et al., 2000). Recently, other meta-analysis examined the effects of probiotics on low-density lipoprotein cholesterol (LDL-C) and to assess the potential of probiotic intake as a therapeutic lifestyle change dietary option. Twenty-six clinical studies and two meta-analyses are reviewed. The results showed a significant LDL-C reduction was observed for four probiotic strains: *Lactobacillus reuteri* NCIMB 30242, *Enterococcus faecium*, and the combination of *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12. Two synbiotics, *L. acidophilus* CHO-220 plus inulin and *L. acidophilus* plus fructo-oligosaccharides, also decreased LDL-C. Of the probiotics examined, *L. reuteri* NCIMB 30242 was found to best meet therapeutic lifestyle change dietary requirements by 1) significantly reducing LDL-C and total cholesterol, with robustness similar to that of existing therapeutic lifestyle change dietary options, 2) improving other coronary heart disease risk factors, such as inflammatory biomarkers, and 3) having "generally recognized as safe" (GRAS) status (DiRienzo, 2014) .

The clinical trials that examined the effect of probiotics on the metabolism of diabetic subjects, which have been cited in this review, are shown in Table 2. The duration of intervention employed in studies that evaluated the relationship between consumption of probiotics and metabolism in diabetic patients was 4–6 weeks. The primary endpoints were fasting glucose, insulin, glycated hemoglobin, lipid profile, and markers of oxidative stress and/or inflammation. The probiotics used in the clinical trials were *L. acidophilus*, *Lactobacillus sporogenes*, *Bifidobacterium bifidum,* and *B. lactis*, used alone or in combination. Among them, only one study quantified the bacteria in the feces of the study population (Andreasen et al., 2010). It is important to analyze of the faecal samples once the increase of bacteria tested can

provide evidence of the presence of the strain in significant amounts in the gastrointestinal tract. Such results indicate a satisfactory compliance of participants, as well as an ability of the bacterium to survive gastrointestinal passage. Moreover, only a few clinical trials had qualitatively and quantitatively evaluated the nutrient intake, which can influence the interpretation of the results.

Recently, the protocol of a double-blind, randomized, placebo-controlled clinical trial developed by researchers in Saudi Arabia was published. This study will be conducted with 120 individuals recently diagnosed with diabetes, who will be administered with probiotics in one formulation (*B. bifidum*, *B. lactis*, *L. acidophilus*, *Lactobacillus brevis*, *L. casei*, *Lactobacillus salivarius*, and *Lactococcus lactis* – 10^9 CFU/g) for 26 weeks. Subsequently, the effects of probiotics consumption on the levels of plasma endotoxin and inflammatory cytokines will be evaluated (Alokail et al., 2013). Although the published protocol is interesting and well controlled, the inclusion criteria comprise a wide age range (20 –75 years).

Some exclusion criteria such as time of T2DM diagnosis, insulin use, the type of medication used, and treatment duration are important and should be standardized, especially for those trials that involve determination of markers of oxidative stress (Choi et al., 2008). Pioglitazonas and rosiglitazones are the hypoglycemic agents that have been demonstrated to produce intracellular antioxidant effects (Dobrian et al., 2004) and affect inflammatory markers such as C-reactive protein and interleukin-6 (IL-6) (Agarwal, 2006). However, such effects have been observed 12 weeks after the initiation of treatment, and have not been evaluated after long periods of use.

T2DM is a condition that often requires treatment with various drugs such as statins and anti-hypertensives, in addition to hypoglyceminates (sulfonylureas, biguanides, thiazolidinediones, and/or insulin). This limits the clinical trials because large number diabetics cannot be included in such studies, and the sample size becomes increasingly smaller, not representing the present context, because most of the people with diabetes have other associated pathologies.

PRIMARY EFFECTS OF THE GUT MICROBIOTA ON HOST METABOLISM

Currently, studies have been focused on understanding how the gut microbiota can influence the host metabolism and thus contribute to the development of diabetes and its complications. The metabolites derived from bacterial metabolism and reactions of bacterial cells with the host immune system represent the triggers for the development of metabolic abnormalities (Gravitz, 2012). Furthermore, changes in the gut microbiota can influence the levels of gut hormones involved in the regulation of satiety and glycemic control, such as glucagon-like peptide-1 (GLP-1) (Baggio and Drucker, 2007; Tolhurst et al., 2012; Yadav et al., 2013). The GLP-1 is a hormone secreted by the L-cells of the small intestine and distal colon, which produces antidiabetic effect by stimulating insulin secretion from pancreas, which can be modulated by SCFA, particularly, butyrate, after the intake of prebiotics (Tolhurst et al., 2012; Yadav et al., 2013). In addition, in a previous study, the consumption of probiotics by diabetic rats was observed to increase the bioavailability of gliclazide, an oral sulfonylurea class antidiabetic drug (Al-Salami et al., 2008a).

Probiotic consumption has also been observed to decrease the oxidative stress and inflammatory markers. This is an interesting effect because individuals with diabetes often

exhibit changes in the levels of these markers, which are known to be involved in some way in glycemic control, and thus, in the pathogenesis and progression of T2DM (Ceriello and Motz, 2004; Stephens et al., 2009). Inflammation and oxidative stress may be induced by bacterial components such as lipopolysaccharide (LPS), the major component of the extracellular membrane of Gram-negative endotoxin. The increase in the circulating levels of endotoxin is currently known as "metabolic endotoxemia," and can occur owing to changes in intestinal permeability. The decrease in the number of *Bifidobacterium* spp. and *Lactobacillus* spp. in the intestine, together with the derangement of intestinal cell junctions, have been noted to increase the intestinal permeability (Cani et al., 2007b; Cani et al., 2008), suggesting the role of microbiota in modulating intestinal permeability. Figure 1 shows the major mechanisms involving the intestinal microbiota and the main metabolic changes observed in diabetic subjects.

The general protective mechanisms involving probiotics, which are the most commonly reported are as follows: competition among pathogenic microorganisms for intestinal epithelial receptors; release of antimicrobial compounds that fight pathogens, such as SCFA (lactic, acetic, and propionic acids), hydrogen peroxide, free fatty acids, and bacteriocins; stimulation of mucin secretion for binding of probiotics to the intestinal mucosa and hindering pathogens; and stimulation of host immunity by inducing the production of interleukins as well as stabilization and improvement of the intestinal barrier associated lymphoid tissue (O'Hara AM and Shanahan F, 2006; Williams NT, 2010). Thus, the biological activity of the probiotic bacteria is partly associated with its ability to adhere to enterocytes, which inhibits the binding of enteric pathogens through competitive exclusion. The attachment of probiotic bacteria to the cell surface receptors of enterocytes also initiates signaling events, resulting in the production of cytokines.

Moreover, butyric acid production by some probiotic bacteria affects the turnover of enterocytes and neutralizes the activity of carcinogens such as nitrosamines (Kailasapathy and Chin, 2000).

PROBIOTICS AND OXIDATIVE STRESS

A major characteristic of T2DM is the systemic increase inoxidative stress (Rains and Jain, 2011). One way to counter balance the increase of reactive oxygen species and depletion of antioxidants is to supplement the latter through diet. In addition to the polyphenols, intestinal bacteria such as *Bifidobacterium* species and dietary interventions with probiotics containing *Lactobacillus acidophilus* LA5 and *B. animalis* subsp. *lactis* BB12 have improved glucose tolerance and total antioxidant status in T2D patients (Ejtahed et al., 2012).

Therefore, previous studies have shown that different species of lactic acid bacteria exhibit antioxidant activity (Lin and Chang, 2000; Uskova MA and Kravchenko LV, 2009). The most frequently reported lactic acid bacteria exhibiting antioxidant activity are *Lactobacillus* spp., *Bifidobacterium* spp., *Saccharomyces boulardii*, *Streptococcus thermophilus*, *Bacillus cereus,* and *E. faecium* SF68. Only the genus *Lactobacillus* comprises more than 120 species, and *L. acidophilus, L. rhamnosus, L. casei, L. bulgaricus, Lactobacillus plantarum,* and *Lactobacillus reuteri* are the most commonly used in experimental and clinical trials .

Studies examining the relationship between oxidative stress and consumption of probiotics were first reported in healthy smokers (Naruszewicz et al., 2002; Songisepp et al., 2005). When compared with the control group, consumption of a functional drink containing *L. plantarum* 299v by smokers for 6 weeks resulted in a significant reduction in arterial blood pressure, levels of leptin and fibrinogen, as well as plasma levels of F2-isoprostane (37%) and

IL-6 (42%) (Naruszewicz et al., 2002). Furthermore, Songisepp et al. (2005) evaluated the effect of the intake of goat's milk, goat's milk fermented with *Lactobacillus fermentum* ME-3 (10^9 cfu/mL, n = 19), or capsules containing only the probiotic (10^8 cfu/g, n = 21) on healthy subjects. After 3 weeks of consumption, a significant increase in the number of *Lactobacillus* spp. in the feces, along with a significant improvement in the capacity and total antioxidant activity of plasma, were observed in both the groups that received the probiotic. On the other hand, a reduction in the ratio of oxidized glutathione/reduced glutathione was observed only in the group that received fermented milk containing *L. fermentum* ME-3. In another study with healthy subjects resistant to lipoprotein oxidation, reduced levels of peroxidized lipoproteins (oxidized LDL), 8-isoprostane, and oxidized glutathione/reduced glutathione ratio, and increased plasma total antioxidant capacity were noted after 3 weeks of consumption of fermented goat's milk containing *L. fermentum* ME-3 (10^9 cfu/mL) and *L. plantarum* LB-4 (10^8 CFU/mL). Furthermore, the use of probiotics was also observed to change the prevalence and proportion of lactic acid bacteria (*Lactobacillus* spp.) in the gut microbiota (Kullisaar et al., 2003).

In the context of diabetes mellitus, experimental and clinical studies have also demonstrated that different species of bacteria reduce oxidative stress, showing antidiabetic effect (Harisa GI et al., 2009; Yadav et al., 2007). A randomized, double-blind, placebo-controlled study, showed that consumption of yogurt containing *B. lactis* BB-12 and *L. acidophilus* LA-5 for 6 weeks significantly reduced the levels of blood glucose and glycated hemoglobin (HbA1), and increased the levels of erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GPx) activity, and total antioxidant capacity, when compared with consumption of conventional yogurt. Furthermore, the concentration of malondialdehyde (MDA)

was significantly reduced in both the groups, whereas the insulin concentration and activity of erythrocyte catalase remained unchanged (Ejtahed et al., 2012).

Besides the presence of probiotics, fermented milks have been described as dietary sources of natural antioxidants, and have been reported to contain bioactive peptides that are capable of promoting changes in the concentration of oxidative stress markers in the absence of probiotics (Fabian and Elmadfa, 2007). Most of the identified bioactive peptides derived from casein have been noted to be able to reduce free radicals and inhibit lipid peroxidation enzyme and non-enzymatic reactions (Muro Urista et al., 2011). Furthermore, the antioxidant peptides from whey protein rich in cysteine and glutathione precursor amino acid have been observed to act as a potent antioxidant (Hayes et al., 2007). Although fermented milk may exert antioxidant effects through its bioactive peptides, some studies have shown that milk beverages containing probiotics are most effective in improving antioxidant activity than conventional fermented dairy beverages (Sabeena Farvin et al., 2010).

The precise mechanisms involved in the effects of probiotic drinks on glycemic control can be partly explained by the effects on oxidative stress as a result of inhibition of ascorbate autoxidation, metal-ions chelation, and activity reduction and excretion of free radicals such as superoxide anion and hydrogen peroxide (Lin and Chang, 2000; Wang et al., 2013). Furthermore, in the context of improving oxidative stress, the importance of increasing activity of catalase and GPx, which should be higher than SOD activity, to remove reactive oxygen species has also been observed (Maritim et al., 2003).

PROBIOTICS AND LOW-GRADE SYSTEMIC INFLAMMATION

The intestinal microbiota may be crucial to the development and homeostasis of the immune system since most immune cells in the body are conditioned in the gut and the gut microbiota interact closely with intestinal immune cells (Magrone and Jirillo, 2013).

According to the ‘‘metainflammation’’ hypothesis, T2DM is also considered as a state of chronic, systemic and low grade inflammation (Hotamisligil, 2006). Circulating levels of several inflammatory mediators such as acute-phase protein, cytokines and markers of endothelial activation are elevated in T2DM patients (Kolb and Mandrup-Poulsen, 2005).

Low-grade systemic inflammation is characterized by constant high levels of proinflammatory cytokines in the circulatory system such as TNF-α, IL-6, β kinase inhibitor (IKKβ), and Jun N-terminal kinase (JNK). All these molecules can phosphorylate insulin receptor substrate (IRS) and turn them into serine, which exerts a negative effect on insulin signaling and can cause insulin resistance (Carvalho-Filho et al., 2005; Wang et al., 2013). The major cytokines that are related to glycemic control are tumor necrosis factor-α (TNF-α), IL-6, resistin, adiponectin, and IL-10 (Esposito et al., 2002; Pickup et al., 2000; Spranger et al., 2003; Wang et al., 2013). Thus, the intestinal mucosa can contribute to or facilitate the development of inflammatory disorders when the signaling cascade involves increased levels of pro-inflammatory signaling molecules (interleukins and neutrophils) and stress-mediators (norepinephrine and corticosterone) (Maslowski et al., 2009), leading to the endotoxemia concept (Cani and Delzenne, 2007). Accordingly, subjects with T2D present an altered microbiota reported to be enriched in gram-negative bacteria (Larsen et al., 2010; Wu et al., 2010) which express lipopolysaccharides (LPS).

Growing evidence suggests that cross-talk between gut bacteria and host is achieved through specific metabolites (such as short-chain fatty acids - SCFA) and molecular patterns of microbial membranes (lipopolysaccharides) that activate host cell receptors (such as toll-like receptors and G-protein-coupled receptors). Furtermore, the endocannabinoid (eCB) system is an important target in the context of T2DM, once insulin is a potent regulator of eCB metabolism. It has been demonstrated that eCB system activity is involved in the control of glucose and energy metabolism, and can be tuned up or down by specific gut microbe (Cani et al., 2014; D'Eon et al., 2008).

In this way, dietary intervention has represented an attractive way to restore immune function (Chandra, 1992; Santos et al., 1996). Different probiotic strains have been shown to enhance the intestinal barrier function, decreasing the translocation of microorganisms and their derivatives such as LPS (Cani et al., 2007a). When LPS enters the bloodstream, it activates Toll-like receptor 4 (TLR4), which is located at the surface of immune cells, leading to the release of proinflammatory cytokines and inflammation (Guha and Mackman, 2001).

Interestingly, evidence suggests that LPS is associated with marked changes in glucose metabolism. The activation of TLR4 signalling can induce both insulin resistance and pancreatic b-cell dysfunction (Tsukumo et al., 2007). In addition, LPS inhibited insulin secretion and insulin gene expression in isolated islets of Langerhans and in b-cell lines (Garay-Malpartida et al., 2011; Kiely et al., 2009). In LPS-treated mice, a decrease in both inflammation and mortality was reported when the plasma glucose level was strictly maintained at normal values, suggesting that appropriate/tight glucose control is a major determinant of outcome. Whether abnormalities

in glucose control may vary according to the endotoxemic insult is unknown, and the molecular mechanisms involved are poorly understood (Nguyen et al., 2014).

Recently, the insulin response to experimental hyperglycemia was studied in mice with LPS mediated inflammation. The authors demonstrated that LPS increased glucose-stimulated insulin secretion (GSIS), which has been shown to be due, at least in part, to an increase in the level and activity of glucagon-like peptide 1 (GLP-1) (Nguyen et al., 2014).

Addicionaly, microbiota components account for the production of SCFA, wich are endowed with anti-inflammatory (inhibition of NF-κB) and anti-neoplastic activities, also exerting a protective function in favor of intestinal epithelia (De Vuyst and Leroy, 2011). The lactic acid producing bacteria may provide clinical benefits for specific populations. However, reports on the ability of lactic acid bacteria to modulate immune and inflammatory condition of low grade in T2DM have been limited.

Reductions in *Bifidobacterium* spp. and *Lactobacillus spp*. during the onset of insulin resistance in hight fructose diet rats was related to increased plasma LPS (Cani et al., 2007b). Therefore, SCFA decrease may lead to an impaired secretion of mucins and easier entry of pathogens into the intestinal mucosa, especially Enterobacteriaceae. These Gram negative bacteria are able to release LPS or endotoxins, which, in turn, aggravate the inflammatory condition (Schiffrin et al., 2010).

In the context of cytokines, certain strains of lactobacillus and bifidobacteria have been shown to be able to modulate the production of cytokines by monocytes, and lymphocytes, presenting ability to regulate the immune system favorable for human health (Castellazzi AM et al., 2007; Kankaanpää et al., 2003). For example, *L. casei* shirota, *L. acidophilus* X37, and *B.*

bifidum S131 were observed to increase the activity of NK cells in humans (Fink et al., 2007; Takeda and Okumura, 2007). In addition, *B. lactis* HN019 was proved to be effective as a probiotic dietary supplement, improving some aspects of cellular immunity in the elderly after 3 weeks of consumption (Gill et al., 2001). The beneficial effects of different bacterial strains are primarily based on their ability to differentially regulate the production of anti-inflammatory cytokines (IL-10) and proinflammatory cytokines (IL-6, TNF-α, IL-12, and INF-γ) and balance the T helper cells (Th) 1/Th2 (Cross et al., 2004; Drago et al., 2010; Ghadimi et al., 2008). However, there is still little evidence on the role of probiotics in the regulation of immune response mediated by T cells and NK cells and also in immune response related cytokine production (Dong et al., 2012; Paineau et al., 2008).

SAFE USE OF PROBIOTICS

Several researchers have not evaluated the adverse effects (AEs) of products containing probiotics because probiotics have been historically used in the manufacturing process of some of these products. Many microorganisms to which we ascribe probiotic effects have origins in dairy/fermented foods and have long been consumed as constituents of these foods without any apparent ill effect for centuries (Wallace and MacKay, 2011). Despite their widespread use in foods and dietary supplements, the incidence of bacteremia attributable to probiotic strains remains extremely low (Salminen et al., 2002).

In recognition of the importance of assuring safety, even among a group of bacteria that is Generally Recognized as Safe (GRAS), the Working Group (FAO/WHO) recommends that probiotic strains be characterized at a minimum with the following tests: determination of

antibiotic resistance patterns, assessment of certain metabolic activities (e.g., D-lactate production, bile salt deconjugation), assessment of side-effects during human studies, epidemiological surveillance of adverse incidents in consumers (post-market), assessment of toxin production and determination of hemolytic activity if the strain under evaluation belongs to a species with known hemolytic potential. The assessment of lack of infectivity by a probiotic strain in immunocompromized animals demonstrate a measure of confidence in the safety of the probiotic (FAO and WHO, 2002).

Recently, some systematic reviews have been published on the safety of probiotics used in research to reduce the risk of, prevent, or treat disease (Hempel et al., 2011; Whelan and Myers, 2010).

A meta-analysis involving 622 clinical trials (a total of 24,415 reviews) after consumption of different genera of microorganisms (*Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Enterococcus*, and *Bacillus*: 10^5–10^{11} CFU/mL), used alone or in combination, found that the relative risk (RR) of any AE did not differ among the groups receiving probiotics (RR: 1.06; 95% CI: 0.97–1.16; P = 0.201), when compared with the control group. However, only 5% of the studies had evaluated the safety of long-term use of probiotics (more than 1 year). Of the 622 articles evaluated, 38% reported no AEs observed, only indicating that the intervention was "well tolerated." Most of the studies using *Lactobacillus* spp. and/or *Bifidobacterium* spp. as the probiotic had reported that the most common AEs were related to gastrointestinal effects, followed by infection and "other" AEs (Hempel et al., 2011). The case studies indicated that fungemia, bacteremia, sepsis, and other infections may be associated with the administration of probiotics, such as *L. rhamnosus* and *Bacillus subtilis,* mostly in

immunocompromised patients and/or patients with short gut syndrome (Kunz et al., 2004; mDe Groote et al., 2005; Zein EF et al., 2008). Among clinical trials, no infections were observed. Gastrointestinal effects, when present, had been observed during the first 3 days of probiotic intake (Hempel et al., 2011). The study concluded that despite the substantial number of publications, the current literature is not well equipped to answer questions on the safety of probiotic interventions with confidence. It is important for the scientific community to recognize that this conclusion should not put the safety of these microorganisms into question but provide further evidence to advance the notion that the 6 genera included in the study have minimal safety concerns, because no adverse events were reported (Wallace and MacKay, 2011).

In patients receiving nutritional support, a systematic review including 72 publications reported low occurrence of bloodstream infections after consuming probiotics. There were 20 case reports of adverse events in 32 patients, all of which were infections due to *Lactobacillus rhamnosus* GG or *Saccharomyces boulardii*; the risk factors included central venous catheters and disorders associated with increased bacterial translocation. There were 52 articles reporting 53 trials in which 4131 patients received probiotics. Most trials showed either no effect or a positive effect on outcomes related to safety (eg, mortality and infections). Only 3 trials showed increased complications, which were largely noninfectious in nature and in specific patient groups (eg, transplant and pancreatitis). In 2 of these trials, the probiotic was administered through a postpyloric tube. The main risk factors for infection were the presence of a central venous catheter and immunosuppression (Whelan and Myers, 2010). Therefore, it is recommended that probiotics be used with caution in critical ill patients.

To explore the question "are probiotics safe?" using a drug based framework assumes that the literature will include drug like safety and toxicology data. The scientific community report the need to consider that traditional foods and food components are not studied in the same way as drugs. Additionally, many probiotics pose a low risk in the general population, as shown by their native colonization in the gastrointestinal tract of humans. Researchers should recognize that in the absence of drug-like safety data, the safety of traditional foods should be based on the totality of evidence in healthy populations (Wallace and MacKay, 2011).

CONCLUSION AND PERSPECTIVES

The proposal that intestinal microbiota plays a role in the development of T2DM is important, but further consideration needs to be given to the potential influences of probiotics on glucose metabolism due to lack of sufficient scientific evidence in support of the health claims. Nevertheless, data from animal studies encourage to believe that probiotics do have the potential to prevent and reduce the severity of T2DM and other metabolic syndromes possibly through modulating gut microbiota, immune response and other mechanisms. In clinical trials, the use of probiotics in glycemic control of diabetic individuals has presented different results, and only a few studies have attempted to evaluate the markers of oxidative stress and inflammation which may explain possible links between glycemic control and intestinal microbiota. Furthermore, no clinical trials have been carried out to evaluate GLP-1 and LPS. The evaluation of probiotic efficacy in human population is, however, far more complex than under controlled experimental conditions because many of the confounding factors such as diet profile, use of drugs, body mass index and endotoxin content of ingested food may also affect gut microbiota, glucose

metabolism, insulin secretion, energy balance and other gut hormones such as incretins. Thus, the design of future research should attempt to neutralize such factors in order to better understand the effects of probiotics on the metabolism of diabetic individuals as well as the main mechanisms involved in this complex relationship. Such studies could lead to future nutritional interventions focused on glycemic control and other metabolic disorders commonly present in chronic diseases, especially T2DM.

Conflict Of Interest

The authors declare no conflict of interest related to the contente of the present paper. All authors contributed equally to all aspects of the article.

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Table 1 Experimental studies on the effects of probiotic consumption on glycemic control in diabetic animals

References	Animals models	Probiotic Strain / dose	Duration (wk)	Main outcomes after ingestion of probiotics
(Matsuzaki et al., 1997)	KK-A^y mice	*L. casei* (0.05%)	16	↓ blood glucose and insulin ↓ IL-2 and INF-y
(Tabuchi et al., 2003)	Streptozotocin	*L. rhamnosus* (CFU - not reported)	9	↓ HbA1c and improvement in glucose tolerance ($P < 0.05$)
(Yadav et al., 2007)	Rats/ High-fructose diet	*L. acidophilus* and *L. casei* (CFU - not reported)	8	Lower elevations in: HbA1c, insulin, blood glucose, TC, TG, LDL-C and NEFA ($P < 0.05$) HDL-C decreased slightly Lower values of thiobarbituric acid-reactive substances and higher values of reduced glutathione in liver and pancreatic tissues ($P < 0.05$)

(Yadav et al., 2008)	Rats/ Streptozotocin	*L. acidophillus* NCDC14 *L. casei* NCDC19 (10^8 CFU/mL)	4	Suppressed the incremental peaks and area under the curve and delayed reduction of insulin secretion during OGTT Suppressed STZ-induced oxidative damage in pancreatic tissues by inhibiting the lipid peroxidation and formation of nitric oxide, and preserving antioxidant pool such as GPx, SOD and catalase ↓ TC, TG, LDL-C and VLDL-C and ↑ HDL-C levels ($P < 0.05$)
(Harisa GI et al., 2009)	Rats/Streptozotocin	*L. acidophilus or L. acidophilus* + acarbose (10^7 CFU/mL)	2	↓ fasting blood glucose, HbA1c, TG and MDA ($P < 0.001$) after administration

				of *Lactobaillus* alone or with acarbose.
(Yun et al., 2009)	db/db mice	*L. gasseri* BNR17 or rosiglitazone (10^{10} CFU/mL)	12	↓ fasting and postprandial (2h) glucose levels ($P < 0.05$) after intake of L. gasseri and rosiglitazone ($P < 0.001$) ↓ HbA1c ($P > 0.05$)
(Roselino et al., 2012)	Rats/Streptozotocin	*Enterococcus faecium* CRL 183 + *L. helveticus* 416 + soybean and yacon extract (25%) (10^{8} CFU/mL)	7	No change was observed in blood glucose ↑ 23.7% in HDL-C and ↓ of 33.5% in TG levels in symbiotic groups
(Honda et al., 2012)	KK-A^{y} mice	*L. rhamnosus GG ou L. bulgaricus LB3 (0.5%)*	6	↓ fasting and postprandial blood glucose and HbA1c ($P < 0.05$) of the *L. rhamnosus* group

(Bejar et al., 2013)	Rats/alloxan	*L. plantarum* TN627 (10^9 CFU/mL)	4	↓ plasma glucose ($P < 0.05$) ↓ serum TG and LDL-C ($P < 0.05$) ↑ HDL-C ($P < 0.05$)
(Kang et al., 2013)	Mice/ High-sucrose diet (50%)	L. *gasseri* BNR17 (10^{10} CFU/mL)	10	↑ GLUT4 mRNA expression ↓ leptina ($P < 0.01$) and insulin ($P < 0.05$) ↓ body weight and white adipose tissue weight ($P < 0.01$)
(Hsieh et al., 2013)	Rats/ High-fructose diet	L. *reuteri* GMNL-263 (10^9 CFU/mL)	14	↓ fasting blood glucose, insulin, leptin, C-peptide, HbA1c, GLP-1, liver injury markers, lipid profile in serum and liver ↓ IL-6 and TNF-α in adipose tissue ↑ PPAR-γ and GLUT4 mRNA expression ↑ number of

				Bifidobacterium and Lactobacillus and ↓ *Clostridium* in faeces
(Lin et al., 2014)	Rats/Streptozotocin	*L. reuteri* GMN-32 (10^7 and 10^9 CFU/day)	4	↓ blood glucose ($10^9 > 10^7$ CFU) ↓ the changes in the heart caused by the effects of DM.

KK-Ay, a model of genetic type 2 diabetes. IL-2, interleucin 2. INF-y, interferon-y. HbA1c, glycosylated hemoglobin. TC, plasma total cholesterol. TG, triacylglycerol. LDL-C, low-density lipoprotein cholesterol. NEFA, free fatty acids. HDL-C, high-density lipoprotein cholesterol. STZ, streptozotocin. OGTT, oral glucose tolerance test; GPx, glutathione. SOD, superoxide dismutase. MDA, malondialdehide. GLP-1, *glucagon like peptide*. IL-6, interleucin 6. TNF- α, tumor necrosis factor α. PPAR - γ, peroxisome proliferator-activated receptor γ; GLUT4, glucose transporter 4. ↑, increase; ↓, decrease.

Table 2 Clinical trials on the effects of probiotic consumption on the metabolism of diabetics subjects

References/ Design	Sample	Strain / daily dose	Duration (wk)	Main outcomes after ingestion of probiotics	Methodological limitations
(Andreasen et al., 2010) R, PC, DB	IG: 21 CG: 24	L. a*cidophilus* NCFM (10^{10} CFU) in capsule form	4	L.*acidophilus* was detected in the faeces of 75% of the probiotic group. Insulin sensitivity (clamp) was preserved only in probiotic group ($P < 0.05$). No change was observed ($P > 0.05$) in IL-6, TNF-α, CRP, IL-1 and insulin plasma levels.	Inclusion of individuals with newly diagnosed T2DM and normal tolerance glucose. Not evaluated the characteristics of the diet during the study.

(Ejtahed et al., 2011) R, PC, DB	IG: 28 CG: 28 (DM2 and LDL-C ↑)	*L. acidophilus* LA-5 and *B. lactis* BB-12 in yogurt (10^6 CFU)	6	↓ levels of TC and LDL-C ($P < 0.05$) No change was observed in HDL-C plasma levels ($P > 0.05$).	-
(Ejtahed et al., 2012) R, PC, DB	IG: 30 CG: 30	*L. acidophilus* LA-5 and *B. lactis* BB-12 (10^{10} CFU) in yogurt	6	↓ FPG ($P < 0.01$) and HbA1c ($P < 0.05$). ↓ SOD, GPx and TAC ($P < 0.05$). ↓ MDA ($P < 0.05$) compared with the baseline value in both groups. No significant changes from baseline were shown in insulin	-

				and CAT.	
(Moroti et al., 2012) R, PC, DB	IG: 10 CG: 10	*L. acidophilus,* *B. bifidum* (10^8 CFU) and 2g of oligofructose	4	↓ TC and TG ($P < 0.05$) ↓ FPG ($P < 0.05$) and ↑ n-HDL-C ($P < 0,05$) after ten days of symbiotic ingestion	Small number of subjects per group. Inclusion of individuals with impaired glucose tolerance. Inclusion of smokers. Not evaluated the characteristics of the diet.
(Asemi et al., 2013) R, PC, DB	IG: 27 DM2 CG: 27 DM2	Multispecies probiotic supplement (> 10^9 CFU) and 100 mg fructo-oligosaccharide	8	Lower elevations in fasting blood glucose ($P = 0.01$) ↑ serum insulin and LDL-C levels in both groups. ($P < 0.05$) ↑ HOMA-IR in both groups ($P =$	-

				0.001). ↑ plasma GPx levels compared to placebo (P = 0.03).	
(Mazloom et al., 2013) R, PC, SB	IG: 16 CG: 18	L. acidophilus, L. bulgaricus, L. bifidum, and L. casei. (CFU – not reported)	6	No change was observed in: FPG, insulin, lipids profile, IL-6, MDA e CRP.	CFU not reported Not evaluated the characteristics of the diet. Some subjects were using statins. Control group received 2.0g of magnesium as placebo. Waist circumference was greater in the intervention group.
(Asemi et al., 2014) R, PC, CO	IG: 62 DM2 CG: 62	*Bacillus coagulans* (10^7 CFU) + 1.08g of	6	↓ insulin (P = 0.03) and CRP (P = 0.01)	Used only the CRP as a marker of

	DM2	inulin	↑ GPx (P < 0.001) No change in the HOMA-IR, FPG, LDL-C e TAC levels (P > 0.05)	inflammation.

R, random study. PC, placebo-controlled. DB, double-blind. IG, intervention group. CG, control group. IL-6, interleucin 6. TNF- α, tumor necrosis factor α. CRP, C-reactive protein. FPG, fasting plasma glucose. TC, plasma total cholesterol. SOD, erythrocyte superoxide dismutase. GPx, glutathione peroxidase activities. TAC, total antioxidant capacity. MDA, malondialdehyde. CAT, catalase activit. TG = triacylglycerol. n-HDL-C = non HDL-C. CO, crossover. SB, single-blinded. ↑, increase; ↓, decrease.

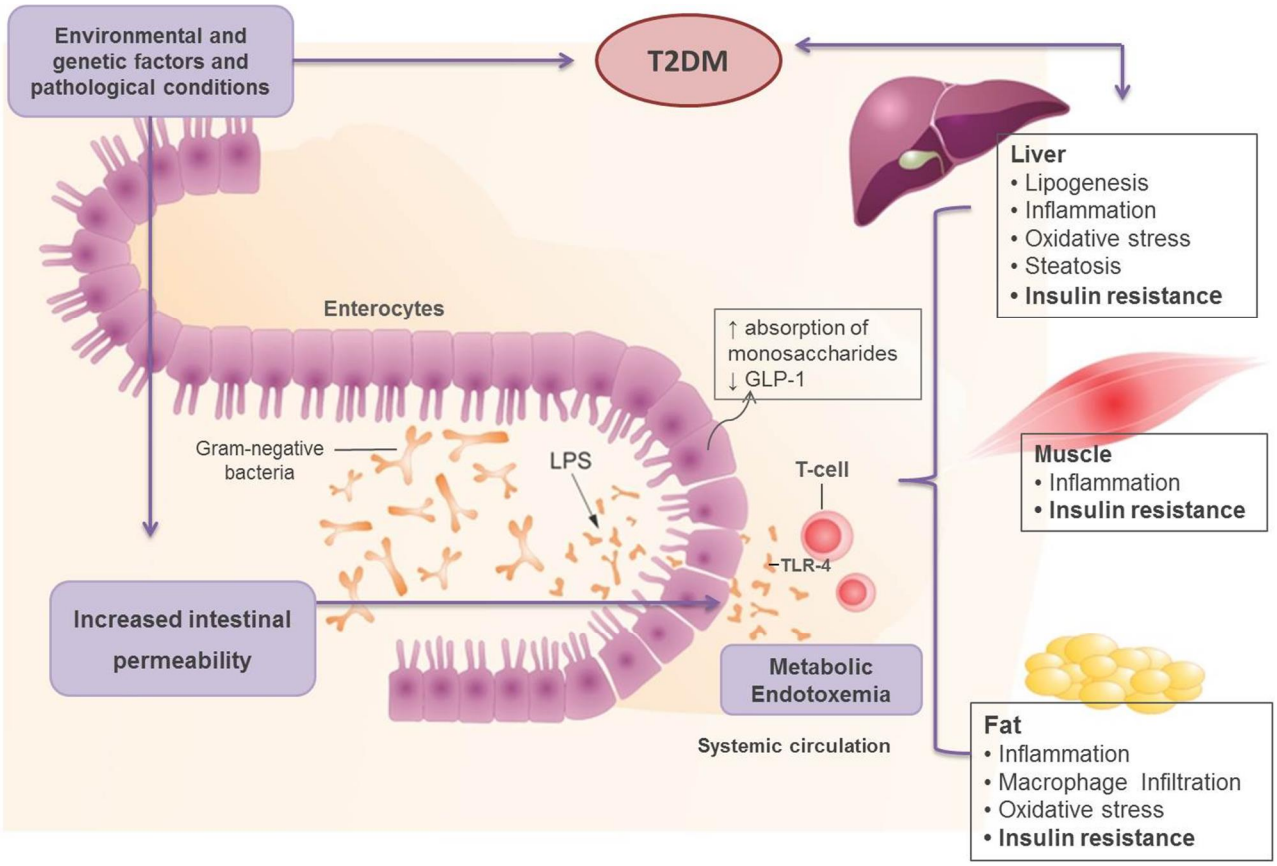

Fig. 1. The low grade, systemic and chronic inflammation often associated with metabolic diseases such as T2DM may develop due to influences of the gut microbiota. The origin of metabolic diseases is multifactorial and new evidences have demonstrated that gut microbiota could be an importante factor in the development of metabolic diseases. The diet is able to influence intestinal microbiota composition, increasing level of Gram-negative bacteria, and also the absorption of molecules from the intestinal lumen. These bacterias can influence the assembly of proteins from the tight junctions in intestinal epithelium, leading to an increased

intestinal permeability (leaky gut). This in turn may favor the translocation of lipopolysaccharides (LPS), a component of gram-negative cell wall, from the intestinal lumen to the circulation. Once in the circulation, LPS can bind and activate toll-like receptor-4 expressed by different cell types (immune cells, adipocytes and Kupfer cells), triggering inflammatory responses such as the release of cytokines (TNF-α, IL-6) and oxidative stress. In consequence, insulin signaling is impaired, leading to the development of insulin resistance. An increased ability to ferment substrates from the diet may augment the availability and absorption of monosaccharides and short chain fatty acids. Microbiota can also influence the secretion of hormones such as decreasing the glucagon-like peptide (GLP-1), which is well known to increase insulin sensitivity. Therefore, gut microbiota composition is an environmental factor with great influence on host metabolism. Strategies aimed at influencing its composition may offer benefits to the host.