GUT MICROBIOTA AND METABOLIC DISEASES

Bachelor's thesis (TFG)

Bibliographic research

Carla Gras Guiteras
Universitat de Barcelona
Facultat de Farmàcia i Ciències de l'alimentació
Departament de Biologia, Sanitat i Medi Ambient
Secció de Microbiologia
5 de Febrer de 2021

ABSTRACT

Nowadays, it is becoming increasingly apparent that gut microbiota plays an important role in the prevention and development of metabolic diseases. Several bacterial species habit the human intestine and live in symbiosis with the host. During the last decades, abundant evidence arose confirming an active role of the microbiota in human metabolism. Hence, the disruption of the gut ecosystem might promote the development of metabolic disorders. This review aims to elucidate the current evidence regarding the mechanisms through which the gut microbiota may contribute to the protection or development of metabolic diseases. It specifically focuses on obesity, type-2 diabetes mellitus (T2DM), cardiovascular disease (CVD), and the potential interventions involving microbiota for preventing metabolic diseases.

Keywords: microbiota, metabolic disease, obesity, type-2 diabetes mellitus, cardiovascular disease, probiotics, prebiotics

RESUMEN

Actualmente, cada vez es más evidente el rol de la microbiota intestinal en la prevención y desarrollo de las enfermedades metabólicas. Existen una gran variedad de especies bacterianas que habitan el intestino humano y viven en simbiosis con el huésped. Durante las últimas décadas, ha surgido nueva evidencia confirmando el rol activo de la microbiota en el metabolismo humano. Por lo tanto, la disrupción del ecosistema intestinal parece que puede promover alteraciones metabólicas. El objetivo de esta revisión es elucidar la evidencia actual respecto los mecanismos a través de los cuales la microbiota puede contribuir en la protección o desarrollo de enfermedades metabólicas. Concretamente, se centra en la obesidad, la diabetes

mellitus tipo 2, las enfermedades cardiovasculares y las potenciales intervenciones para prevenir estas enfermedades metabólicas a través de la microbiota.

Palabras clave: microbiota, enfermedad metabólica, obesidad, diabetes mellitus tipo 2, enfermedad cardiovascular, probióticos y prebióticos.

INTRODUCTION

The human gut contains several microorganisms which are referred to as the microbiota. Colonization by these microbes seems to start prenatally, through transmission from mother to fetus (1). However, it varies along life due to different factors such as diet, environment, age-related factors, antibiotics, exercise, or pathologies (2).

The human gut harbors trillions of microorganisms. It is believed that a standard man has around 38 billion bacteria in the colon, the part of the gut where most of the microbiota lives (3). Moreover, it is estimated that the microbiome has 150-fold more genes than the human genome, which is accompanied by a huge microbial diversity in the intestine (4). Regarding the similarity of the DNA sequences of the gene 16s rRNA, it is possible to classify the bacteria from the gut in five different phyla. *Firmicutes* and *Bacteroidetes* represent the two main phyla, involving 90% of the gut microbiota (3). However, there are three more phyla that include *Actinobacteria*, *Proteobacteria, Fusobacteria,* and *Verrucomicrobia*, this last one being recently discovered. *Firmicutes* are anaerobic, gram-positive bacteria that form spores and they mainly involve the genera *Ruminococcus, Lactobacillus, Blautia, Clostridium,* and *Faecalibacterium.* On the other hand, *Bacteroidetes* are mainly represented by *Prevotella* and *Bacteroides*, which are gram-negative, aerobic bacteria and they do not form spores. As *Table 1* shows, the main genres of

Actinobacteria, Proteobacteria, Verrucomicrobia, and *Fusobacteria* are *Bifidobacterium, Enterobacteriaceae*, *Akkermansia,* and *Fusobacterium* respectively (5,6)

Microbiota composition is highly influenced by diet. The different gut microbial patterns are called enterotypes and they can be classified depending on the predominant bacterial cluster: *Bacteroides* (enterotype 1), which is the most prevalent among the population and it is associated with a diet rich in fat and proteins, *Prevotella* (enterotype 2), which is linked to a diet rich in carbohydrates (CH), and *Ruminococcus* (enterotype 3). Each enterotype includes bacteria that share similar functions (3). Although it is known that diet plays an important role in shaping the microbiota composition, it is difficult to establish which components of the food are more beneficial for microbial diversity (7).

These last decades, microbiota and its composition have been a subject of study and debate. It is known that there is a microbial-host symbiosis which contributes to several metabolic and biological functions (8). Therefore, the disruption of the gut ecosystem can promote a wide variety of physiological disorders, leading to the development of metabolic diseases (1).

METHODOLOGY

This research was conducted by consulting three different databases: PubMed, Scopus, and Cochrane library. In order to establish the theoretical framework, the search was limited to systematic reviews and metanalysis from the last six years. However, this review includes scientific literature from the past 21 years with the purpose of providing wide coverage of the topic. The search was conducted in English and different terms and Boolean operators were used: microbiota AND metabolic diseases, microbiota AND obesity, GLP1 AND microbiota, nutrition AND microbiota AND GLP1, GLP1 AND microbiota AND type-2 diabetes, artificial sweeteners

AND microbiota AND obesity, GLP1 AND incretin effect, gut microbiota AND cholesterol, gut microbiota AND cardiovascular disease, prebiotics OR probiotics AND metabolic diseases, diet AND microbiota. Among all the results obtained, only the most relevant and suitable were selected by reading the title or the abstract in order to be included in the review. Considering that most of the evidence regarding gut microbiota and metabolic disorders comes from animal studies, these were included in the present manuscript. Notwithstanding, randomized clinical trials were prioritized aiming to obtain a higher level of scientific evidence.

MICROBIOTA AND OBESITY

Microbiota composition

Several studies support the idea of obesity being associated with changes in the composition of the two predominant phyla in the gut. Obese individuals seem to have an increased proportion of *Firmicutes* and a decreased abundance of *Bacteroidetes* (9,10). On the contrary, weight loss seems to be linked to a reduction of the *Firmicutes* to *Bacteroidetes* ratio (11). *Firmicutes* include several butyrate-producing species, which contributes to increasing energy harvesting from the diet in obese people (12). Furthermore, obese individuals have lower bacterial diversity and their gut microbiota is altered (9).

What can also explain the dysbiosis associated with obesity is the variation of specific genera or bacterial species in the gut. Various studies found a reduced abundance of *Bifidobacterium* in obese individuals (13,14). On the other hand, Everard *et al.* (2013) showed that increased amounts of *Akkermansia muciniphila* inversely correlates with weight gain in mice and humans. They also found that its levels were decreased in type 2 diabetic mice (15). As it can be observed in *Table 2,* not only *Akkermansia* but also *Faecalibacterium, Coprococcus, Bifidobacterium,*

Butyrivibrio Methanobrevibacter, and Lactobacillus were typically found in individuals with a lean phenotype and an increased bacterial richness. In contrast, *Campylobacter, Bacteroides, Anaerostipes, Dialister, Porophyromonas, Parabacteroides, Staphylococcus,* and *Ruminococcus* were more prevalent in obese subjects with reduced bacterial richness (16). All these findings suggest that microbiota composition might contribute to obesity development or protection as well as obesity may affect microbiota composition.

Energy extraction from the diet

Most of the evidence regarding the role of the gut microbiota in metabolic diseases comes from germ-free (GF) animal models (17). For instance, various of these studies have confirmed the association between microbiota and weight gain. Turnbaugh *et al.* (2006) proved that conventionally raised (CR) mice developed more body fat than GF mice. Besides, body fat increase was higher in GF mice colonized by "obese microbiota" than in GF mice colonized by "lean microbiota", which indicates that the first one has a higher capacity to harvest energy from the diet. (18).

The gut microbiome has a special enzyme called glycoside hydrolase, which cannot be found in the human genome. This enzyme hydrolyzes non-digestible carbohydrates, contributing to increasing the energy that the host obtains from the diet, which is linked to an increment of weight gain. The products of the fermentation of carbohydrates (CH) are called short-chain fatty acids (SCFAs), the main ones being acetate, propionate, and butyrate. SCFAs constitute an important energy source for colonocytes and they also play a role in metabolism regulation (7). Not all the microorganisms in the gut have the same capacity of extracting energy from the diet. Obese microbiota is composed of bacterial species which have a greater capacity to harvest energy from the diet, which can easily lead to weight gain and obesity (4).

Low-grade chronic inflammation and obesity

Obesity is a metabolic disease that is characterized by a low-grade chronic inflammation (17). Gut microbiota and permeability of the intestinal barrier play an essential role in its development. Lipopolysaccharide (LPS), which is a component of the cell-wall of gram-negative bacteria, can enter into the systemic circulation and cause endotoxemia. If the integrity of the gastrointestinal barrier is compromised, LPS can cross it through the leaky tight junctions. Nonetheless, LPS can also enter into circulation through chylomicrons, which are the responsible lipoproteins for dietary fat absorption (1). Once LPS crosses the gut epithelium, it activates toll-like receptor 4 (TLR4), which triggers the release of pro-inflammatory cytokines and leads to activation of several inflammatory processes. Together, it results in insulin desensitization, inflammation of adipose tissue, increased intestinal permeability, and oxidative stress (19).

Various studies have confirmed the association between diet and metabolic endotoxemia. Cani *et al.* (2007) proved that a 4-week high-fat diet (HFD) considerably increased LPS levels in plasma and LPS-containing gut microbiota. Their findings showed that inflammation caused by LPS can lead to body weight gain and diabetes (20). After treatment with antibiotics, metabolic endotoxemia was reduced in *ob/ob* mice and in mice fed with an HFD, followed by a reduction in glucose intolerance, inflammation, and weight gain (21).

GUT MICROBIOTA AND TYPE 2 DIABETES

Gut microbiota not only plays a role in obesity but also in other metabolic disorders. For instance, changes in gut microbial composition have been reported in type-2 diabetic patients. A Chinese cohort study conducted by Qin *et al.* (2012) established an association between T2DM and microbial dysbiosis. It was detected a reduction of butyrate-producing species such as

Eubacterium rectale, Roseburia intestinalis, Roseburia Inulinivorans, Faecalibacterium prausnitzii, and *Clostridiales* species (spp.) SS3/4, accompanied by an increase in opportunistic pathogens in type-2 diabetic patients (22). In addition, Zhang *et al.* (2013) also observed depletion of the abundance of butyrate-producing bacteria in pre-diabetes and T2DM patients (23). Considering that butyrate is an essential component for the maintenance of the integrity of the intestinal epithelium, it is reasonable to claim that the impairment of butyrate production detected in type-2 diabetic patients might be associated with the low-grade chronic inflammation which characterizes this disease. Such a link between gut microbiota and T2DM can be better appreciated in *Figure 1.*

Incretin effect of GLP-1

Incretins are a group of gut hormones which are secreted in response to food ingestion and they cause an increase in insulin release in a glucose-dependent manner. There are two incretin hormones secreted by the human gut: glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). Once they are released into the bloodstream, they interact with β-pancreatic cells stimulating the secretion of insulin and they inhibit hepatic gluconeogenesis reducing the secretion of glucagon (24–26). Even though the incretin effect is reduced or absent in type-2 diabetic patients, the pancreas seems to remain responsive to GLP-1 but not to GIP (25). While supraphysiological dosages of GLP-1 administered intravenously can increase the secretion of insulin in diabetic subjects and improve glucose homeostasis, GIP does not cause the same response (24).

Besides acting as an incretin hormone, GLP-1 has a wide variety of effects on the organism. For instance, it diminishes blood pressure, increases satiety, and reduces appetite, thus being considered an anorexigenic peptide (25). Moreover, the activation of GLP-1 receptor seems to

reduce the food reward, avoiding overeating and preventing weight gain (27). What is more, GLP-1 reduces gut motility and gastric emptying, which slows glucose absorption, thus decreasing the peak of postprandial blood glucose levels (28). Therefore, considering the effects that GLP-1 has on glucose metabolism, scientific advances have led to the development of antidiabetic drugs based on the action of GLP-1, for instance, GLP-1 receptor agonists and dipeptidyl peptidase-4 inhibitors.

GLP-1 and gut microbiota

GLP-1 is encoded by the proglucagon gene and it is secreted by L-cells, which are enteroendocrine cells (EEC), in response to different stimuli. Nutrients of the diet can trigger its secretion, as well as hormonal factors, some dietary polyphenols (curcumin and anthocyanin), and some specific microbial metabolites (24). However, this review will only focus on the release of GLP-1 through the mechanism that involves gut microbiota.

SCFA

SCFA can interact with L-cells through specific G protein-coupled receptors (GPCR) and promote GLP-1 secretion. More specifically, SCFA are ligands of GPR41 and GPR43 (29). These receptors are highly expressed in L-cells and their activation by SCFA leads to the secretion of GLP-1. Tolhurst *et al.* (2012) observed that GPR43 and GPR41 knockout mice had reduced GLP-1 secretion in vivo and in vitro, together with glucose tolerance impairment (30). Furthermore, a cross-sectional study conducted by Müller *et al.* (2019) showed a positive association between circulating SCFA and GLP-1 concentration, lipolysis, and enhanced insulin sensitivity (31). Finally, Wang *et al.* (2020) reported that administration of probiotics to a group of db/db mice increased the proportion of SCFA-producing bacteria such as *Roseburia, Lactic*

177 *acid bacteria, Bifidobacterium,* and *Clostridium leptum*. As a result, the insulin secretion was
178 increased due to enhanced production of GLP-1 (32). Hence, the gut microbiota exerts a positive
179 impact on glucose metabolism through SCFA.

180 Secondary bile acids

181 Bile acids (BAs) are molecules synthesized from cholesterol that are released into the gut aiming
182 to facilitate the solubilization and absorption of dietary lipids and fat-soluble vitamins after meal
183 ingestion (33). Once they are released into the intestinal lumen, microbial-derived bile salt
184 hydrolases (BSH) deconjugate and dehydroxylate them, leading to the synthesis of secondary
185 BAs. These play an important role in glucose metabolism through the activation of two receptors
186 called farnesoid X receptor (FXR) and TGR5, which are expressed in EEC. The activation of
187 FXR seems to have a positive impact on the regulation of peripheral insulin sensitivity through a
188 mechanism which does not involve GLP-1 secretion. Nonetheless, the activation of TGR5 by
189 secondary BA triggers the release of GLP-1, thus promoting insulin secretion and inhibiting
190 glucagon release (28,33).

191 **GUT MICROBIOTA AND CARDIOVASCULAR DISEASE**

192 Nowadays, CVD is the main cause of mortality and disability in developed countries (34). As
193 already known, gut microbiota interacts to target organs through the release of bacterial
194 metabolites which can act like hormones. Consequently, dysbiosis contributes to the development
195 of different metabolic disorders such as CVD (35). The exact mechanisms underlying this
196 association have not been fully elucidated. Nonetheless, various links, which are summarized in
197 *Table 3,* have been found between gut microbiota and CVD.

198 **Impact of gut microbiota on cholesterolemia**

Cholesterol metabolism and primary bile acid synthesis

Hypercholesterolemia is one of the main risk factors associated with CVD. The liver and the gut are the two organs responsible for cholesterol homeostasis (36). In the liver, cholesterol can be converted to primary BAs. These are synthesized through two different mechanisms: the classical pathway, which produces most of BAs and is regulated by cholesterol 7α-hydroxylase (CYP7A1), and the acidic pathway (37). The expression of the key enzymes involved in BA production can be modulated by gut bacteria, mainly *Lactobacillus* and *Bifidobacterium,* which suggests a possible role of intestinal microbiota in the reduction of total cholesterol (TC) (38).

The primary BAs produced in human hepatocytes are cholic acid (CA) and chenodeoxycholic acid (CDCA). Previous to excretion into the bile they are conjugated with glycine or taurine (37). Then, they are stored in the gallbladder and secreted into the duodenum after food intake. Most of the BAs are actively reabsorbed in the ileum via apical Na^+-dependent transporter and released by OST-α/β into the portal vein, which will transport them back to the liver (39). This cycle is called enterohepatic circulation and it is repeated 4-5 times daily. Each cycle leads to the excretion of 5% of the BAs after bacterial modification in the colon. In order to compensate for the loss and maintain the BA pool size, an equivalent amount of BAs are consequently synthesized in the liver from cholesterol, thus leading to a reduction of TC (40).

Secondary bile acids

As already commented, some of the primary BAs secreted into the gut can undergo a series of modifications due to the activity of microbial enzymes, resulting in secondary BAs. Some of the genera which have been identified to have BSH activity are *Bacteroides, Enterococcus*, *Bifidobacterium, Clostridium,* and *Lactobacillus* (36). Such enzyme converts CA to deoxycholic

acid (DCA) and CDCA to lithocholic acid (LCA) (37). As a result of these modifications, the hydrophobicity of BAs increases as well as their pk_a, thus facilitating their excretion through feces. Since they are less efficiently reabsorbed, the amount of BAs excreted needs to be replaced by de novo synthesis from cholesterol (41).

Conversion of cholesterol into coprostanol by gut microbiota

Cholesterol absorption can be diminished via its conversion to coprostanol by gut microorganisms. Due to its structure, coprostanol is poorly absorbed in the gastrointestinal tract and easily excreted through feces (36). The rate of cholesterol-to-coprostanol conversion is highly influenced by gut microbiota composition, which explains why it exists interindividual variation (42). *Bacteroides spp.* strain D8 was shown to reduce cholesterol to coprostanol (43), as well as different strains of *Lactobacillus* and *Bifidobacterium* (44). What is more, some members of *Lachnospiraceae* and *Ruminococcaceae* families have also been associated with coprostanoligenic activity (45). However, bacterial enzymes involved in this biotransformation are still unknown (36). All in all, coprostanol has been linked to cholesterol elimination from the body, thus leading to a reduction of CVD risk (46).

Role of gut microbiota in the development of atherosclerosis

Gut microbiota is involved in the formation of trimethylamine (TMA), which can be oxidized in the liver to produce a pro-atherogenic compound called trimethylamine-N-oxide (TMAO). Intestinal bacteria can synthesize TMA from dietary precursors such as choline, betaine phosphatidylcholine, γ-butyrobetaine, crotonobetaine, carnitine, and glycerophosphocoline (34), which are mainly found in red meat, eggs, and dairy products. In addition, fish and other seafood are rich in TMA and TMAO (47). The hepatic enzymes responsible for the conversion of TMA

into TMAO are flavin monooxygenases (FMOs). FMO3 is the main isoform in the liver and it is also the one which shows the highest activity to produce TMAO (48). Clara *et al.* (2017) showed that subjects with higher TMAO production presented lower gut microbial diversity and higher *Firmicutes*:*Bacteroidetes* ratio (49). What is more, Kymberleigh *et al.* (2015) found that species belonging to *Firmicutes* and *Proteobacteria* phyla showed higher conversion activity from choline to TMA (50). These findings suggest that a low gut microbiota diversity*, Firmicutes* and *Proteobacteria* are associated with TMA production and therefore, to higher TMAO synthesis.

Higher plasma TMAO levels have been clearly linked to atherosclerosis and increased cardiovascular risk (35). A recent metanalysis concluded that subjects with high TMAO levels have 62% more risk of suffering major adverse cardiovascular events (MACE) and 63% increased risk for all-cause death than subjects with low TMAO levels (51). What is more, Fu *et al.* (2017) investigated TMAO levels of patients with coronary artery disease and found that subjects with plaque rupture had higher concentrations of this compound than those with nonplaque rupture (52). Besides, it was observed that patients with unstable plaques showed an increase of *Collinsella* and a decrease of *Eubacterium* and *Roseburia* (53).

Different mechanisms have been proposed to explain the association between TMAO and increased CV risk. For instance, Zhu *et al.* (2016) found that TMAO induces platelet aggregation in human cells *in vitro* (54). Furthermore, it leads to inflammatory gene expression and endothelial cell adhesion of leukocytes (55). On the other hand, TMAO seems to upregulate two scavenger receptors (SR), cluster of differentiation 36 (CD36) and SR-A1, which increase the uptake of modified LDL, thus promoting foam cell formation (56). Additionally, it was found that dietary TMAO supplementation in rodents not only decreases the expression of the key BA synthetic enzymes CYP7A1 and cytochrome P450 27A1 (CYP27A1), but also downregulates the

hepatic BA transporters expression. This effect causes a reduction of the bile acid pool size and results in lower reverse cholesterol efflux (57).

Metabolic endotoxemia and its role in CVD

CVD is characterized by increased intestinal permeability and higher levels of circulating LPS. It is known that LPS can trigger an inflammatory response which might enhance the formation of atherosclerotic plaque. (58). Low-grade chronic inflammation caused by increased endotoxemia has been previously linked to CVD (59). For instance, Mclntyre et al. (2011) observed higher peripheral endotoxemia in patients with major CVD burden (60). Nevertheless, it is still not clear if CVD is the cause or the consequence of dysbiosis, gut barrier disruption, and the associated metabolic endotoxemia.

POTENTIAL INTERVENTIONS

There are different potential interventions for the prevention or treatment of metabolic diseases which involve the gut microbiota. In this review, only the role of probiotics, prebiotics, and diet will be deeply considered.

Effect of probiotics in metabolic diseases

It is well known that the consumption of probiotics has different benefits for the host. Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), define probiotics as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (61). A recent review concluded that specific strains of *Lactobacillus* such as *L. casei, L. rhamnosus, L. plantarum,* and *L. gasseri,* and some strains of *Bifidobacterium* including *B. Breve, B. Infantis* and *B. longum,* have anti-obesogenic effects and cause a reduction in body weight, body fat mass and white adipose tissue in several animal

studies and in human studies (62). On the other hand, *Pediococcus pentosaceus* and *Bacteroides uniformis* CECT 7771 proved to reduce several obesity parameters in DIO mice (63,64). What is more, *Akkermansia muciniphila* was also identified to reduce fat-mass gain, insulin resistance, metabolic endotoxemia, and adipose tissue inflammation in rodents (15). Regarding the evidence in human subjects, *Pediococcus pentosaceus* and different strains of *Bifidobacteria* and *Lacobacillus* combined or on their own have proved to diminish body weight, fat mass, waist circumference and, BMI in human adults (65–69). Therefore, all the evidence suggests that certain probiotics have anti-obesogenic effects.

Notwithstanding, some probiotic strains also ameliorate different parameters related to T2DM, especially those belonging to *Bifidobacterium* and *Lactobacillus* spp. For instance, supplementation with *B. adolescentis or L. rhamnosus* GG improved insulin sensitivity in HF-diet-fed mice (70,71). Furthermore, supplementation of HFD-fed rats with *B. longum* reduced metabolic endotoxemia diminishing plasma LPS levels (72). Some other probiotics such as *L. rhamnosus* NCDC 17, *L. casei* CCFM419, *L. plantarum* MTCC5690 and *Clostridium butyricum* CGMCC0313.1 also proved to play an important role in the prevention of T2DM by increasing GLP-1 secretion in mice (73–76).

Even though the current evidence is not as vast as in animal models, the anti-diabetic effects of certain probiotics have also been tested in humans. First of all, different randomized clinical trials (RCTs) confirmed that consumption of probiotic yoghurt containing *B. animalis* subsp *lactis* BB-12 and *L. acidophilus* La-5 or *L acidophilus, Lb. casei,* and *B. bifidum,* reduced several parameters related to diabetes such as Hb1Ac, fasting blood glucose levels, TG, TC, and antioxidant status (77,78). Another RCT proved that consumption of fermented milk containing *B. animalis* subsp *lactis* BB-12 and *L. acidophilus* La-5 during 6 weeks improved glycemic

control in T2DM patients (79). On the other hand, consumption of *L. reuteri* DSM 17938 during twelve weeks enhanced insulin sensitivity in diabetic humans, as well as *Lb. acidophilus* NCFM (80,81). Altogether, this evidence leads to the conclusion that several probiotic strains might exert anti-diabetic effects both in animals and humans.

On the other hand, certain probiotics seem to modulate some parameters related to cardiovascular risk such as low-grade chronic inflammation, obesity, hypertension, and hypercholesterolemia.

According to Deng *et al*. (2017), the administration of *Bacillus subtilis* and *Bacillus licheniformis* attenuated the inflammation response caused by LPS in rats (82). On the other hand, several probiotic strains seem to exert hypocholesterolemic effects. One of the main mechanisms through which probiotics could reduce cholesterol levels is via BSH activity. Degirolamo *et al.*, (2014) showed that administration of a mixture of probiotic strains called VSL#3 (*B. breve, B. infantis, B. longum, L delbruekckii* spp. *bulgaricus, L. acidophilus, L. plantarum, L. casei,* and *Streptococcus salivarum* spp.) in mice, increased BA deconjugation and fecal excretion. (83). What is more, an RCT proved that administration of yoghurt containing microencapsulated BSH-active *L. reuteri* 30242 to hypercholesterolemic adults, reduced LDL-cholesterol, TC, and apoB-100 (84). Considering the evidence, BSH-active bacteria are currently being used as supplements aiming to reduce cholesterol levels and CVD risk (36,85).

Finally, certain probiotics can diminish CV risk by reducing blood pressure. This ability is thought to result from the generation of bioactive peptides like ACE inhibitory peptides when fermenting some food products such as fermented milk, soymilk, yoghurts and cheese (85,86). It was detected that *L. helveticus* has anti-hypertensive effects (87–89). Similar results were observed with consumption of *L. acidophilus* and *B. longum* strains (90), *L. casei*, spp.

rhamnosus (91), *L. bulgaricus* FTCC 411, and *L. fermentum* FTD 13 (92). All in all, current evidence suggests that these probiotic strains might reduce CV risk by decreasing blood pressure.

Effect of prebiotics in metabolic diseases

FAO/WHO stated that "a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health" (93). There are several types of food ingredients which are considered prebiotics. The most important include inulin, oligosaccharides, galactooligosaccharides (GOS), fructooligosaccharides (FOS), xylooligosaccharides, resistant starch, and non-starch polysaccharides such as pectins, gums, mucilages, celluloses, and hemicelluloses (94). There is increasing evidence that consumption of foods rich in prebiotics might be beneficial for the prevention of metabolic diseases. The ingestion of these compounds can modulate the gut microbiota composition, mainly leading to an increase of *Bifidobacterium* spp. (5). It was reported that resistant starch presents a bifidogenic effect (95), which is negatively correlated with the development of obesity and T2DM (96). Furthermore, it improves gut barrier integrity, thus preventing LPS translocation and associated disorders (97).

Evidence in humans is not as consistent as in animal studies since some contradictions arise. According to Cani *et al.* (2009), daily prebiotic consumption during 2 weeks, enhanced plasma concentrations of GLP-1 and peptide YY in healthy subjects, which might reduce appetite sensation and improve insulin response after a meal (98). Administration of GOS to a group of overweight adults resulted in an improvement of TG, TC, and insulin levels as well as an increase in the abundance of *Bifidobacterium* spp. (99). In addition, oligofructose-enriched inulin caused a decrease in body weight, body fat, and fat trunk in overweight and obese children, accompanied by a significant increase in *Bifidobacterium* spp. (100). Interestingly, inulin supplementation

proved to reduce fasting blood sugar, HbA1c, insulin resistance, and inflammatory markers such as hs-CRP, TNF-alpha, and LPS in diabetic females (101). Even though these results suggest that consumption of prebiotics might be protective against metabolic disorders, other studies show no effect on such parameters (102,103). Therefore, further studies in humans are needed in order to elucidate the role of prebiotics as a potential intervention for metabolic diseases.

Gut microbiota modification by diet and its effect on metabolic diseases

Several elements have been identified to alter the microbiota composition. Nevertheless, one of the main factors which can modulate gut microbiota is the diet, thus having an impact on the prevention or development of metabolic diseases.

For instance, a protein-rich diet seems to improve gut bacterial richness, which has been previously linked to a healthy metabolic status (104,105). According to David *et al.* (2014), the ingestion of a diet based on animal protein increases the levels of *Alistipes, Bactoroides,* and *Bilophila* and it reduces the abundance of some *Firmicutes* spp. such as *Ruminococcus Bromii, Roseburia,* and *Eubacterium Rectale* (106). On the other hand, it was observed that pea protein significantly elevates the number of *Lactobacillus* and *Bifidobacterium* spp. as well as increases the levels of SCFA, which are beneficial for the colonic epithelium (107).

Regarding the consumption of fats, there is wide evidence suggesting that an HFD increases the risk of metabolic disorders. Nevertheless, the type of fat needs to be differentiated. Fava *et al.* (2013) conducted a large-scale dietary intervention which showed that a low-fat diet reduced cholesterol levels and fasting glucose concentrations and elevated fecal *Bifidobacterium.* In contrast, consumption of a high-saturated fat diet increased *Faecalibacterium Prausnitzii*. Finally, a diet rich in monounsaturated fatty acids had no effect on individual bacteria but it

decreased total bacterial numbers, TC and, LDL in plasma (108). Moreover, it was observed that consumption of an HFD increases gut permeability, thus leading to the translocation of LPS and metabolic endotoxemia (20). Besides, dietary fat might facilitate the absorption of LPS through chylomicrons. Therefore, it is reasonable to claim that diets rich in fats may increase the risk of metabolic disorders since systemic endotoxemia is the basis for several metabolic diseases (109).

Not only the effect of proteins and fats should be considered when talking about metabolic diseases but also the role of CH. On the one hand, non-digestible CH can resist degradation by intestinal enzymes. Therefore, they act as prebiotics and exert different effects on the organism which have been previously commented. On the other hand, it is known that high sugar diets promote the development of metabolic disorders (1). Nevertheless, it is becoming clearer that artificial sweeteners are not a healthier alternative to increase the sweet taste. Non-nutritive sweeteners (NNS) are artificial sweeteners which became increasingly popular as sugar substitutes since they offer a sweet taste without providing any calories or glycemic effects (110). Despite being the most used additives all around the world (111), there is growing evidence suggesting that NNS consumption can induce metabolic changes which might lead to obesity and T2DM (112). It was observed that consumption of NNS can increase *Firmicutes* to *Bacteroidetes* ratio as it often happens in an obese state (112). Moreover, Suez *et al.* (2014) proved that mice treated with water supplemented with NNS developed glucose intolerance. They obtained similar results with obese mice fed with an HFD and commercial saccharin. However, after the administration of antibiotics, glucose intolerance was reversed. In order to confirm the role of the gut microbiota, they performed fecal transplantations from mice treated with saccharin into germ-free mice, which consequently showed impaired glucose tolerance (111).

DISCUSSION

All the evidence considered in this review suggests that gut microbiota is highly involved in the prevention and the development of metabolic diseases. The main mechanisms which underly such interaction are energy extraction from the diet, low-grade chronic inflammation, and intestinal peptides which can act like hormones.

Not only certain microbial patterns seem to promote metabolic disorders, but also metabolic diseases seem to have an impact on the microbiota composition. One of the main microbial features which characterize obese people is that they show an increase in the *Firmicutes* to *Bacteroidetes* ratio. Some studies do not support this association, but it is important to note that they were conducted with small samples (14,113). Therefore, the vast majority of the evidence suggests that obesity is linked to a higher *Firmicutes* to *Bacteroidetes* ratio, since *Firmicutes* spp. seem to have a greater capacity to extract energy from the diet (12). Interestingly, microbiota composition is also essential when talking about CVD. Bacteria with BSH activity can convert BAs into secondary BAs, leading to higher excretion of these molecules which need to be replenished by the novo synthesis from cholesterol. Hence, cholesterolemia is decreased as well as CVD risk.

On the other hand, low-grade chronic inflammation is the basis of several metabolic diseases, and it is often enhanced by metabolic endotoxemia. One molecule responsible for this metabolic disorder is LPS, which is a component of the cell-wall of gram-negative bacteria. It might seem a paradox since, in an obese state, there is a reduction of *Bacteroidetes*, which are gram-negative bacteria, and an increase of *Firmicutes,* which are gram-positive bacteria (114).

The last notorious mechanism through which gut microbiota plays a role in the prevention and development of metabolic diseases is the generation of several metabolites that can interact with some receptors in the gut and stimulate the synthesis of specific hormones. These gut peptides are

released into the circulation and can exert an effect on the regulation of the host metabolism. For instance, GLP-1 is an incretin hormone whose presence is reduced or absent in T2DM. Nevertheless, SCFA-producing bacteria and bacteria with BSH activity might increase GLP-1 secretion, thus having a positive impact on T2DM. Considering that these microorganisms seem to be reduced in type-2 diabetic patients, it raises the possibility of using probiotics as a potential intervention for T2DM. On the other hand, another bacterial metabolite that should be considered is TMA since it can be converted into TMAO, which is a pro-atherogenic compound that increases the cardiovascular risk (34).

Therefore, it is clear that depending on its composition, gut microbiota may exert a positive or a negative effect on the host metabolism. In view of it, different potential interventions are currently being contemplated in order to modify the gut microbiota. These include probiotics, prebiotics and diet. The main probiotics which seem to have a positive effect on metabolic disorders are *Lactobacillus* spp. and *Bifidobacterium* spp. strains. Regarding prebiotic consumption, clear benefits have been detected in animal studies, but it exists controversy in humans. Some human studies detected no effect on metabolic parameters after consumption of some prebiotics such as GOS or oligofructose (102,103). Although most of the evidence indeed suggest prebiotics as a plausible intervention for preventing metabolic diseases (115), more quality RCTs are needed. On the other hand, diet exerts a direct effect on gut microbiota composition, which is why it should also be considered as a potential intervention.

Altogether, current scientific data suggests an important role of the gut microbiota in the prevention and development of metabolic diseases. Nevertheless, it is important to note that most of the evidence comes from animal studies and some mechanisms need to be better understood. Hence, further studies in humans are needed as well as more RCTs which include larger samples.

REFERENCES

1. Boulangé CL, Neves AL, Chilloux J, Nicholson JK, Dumas ME. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. Genome Med. 2016;8:1.

2. Pushpanathan P, Mathew G, Selvarajan S, Seshadri K, Srikanth P. Gut microbiota and its mysteries. Indian J Med Microbiol. 2019;37:268–77.

3. Sebastián-Domingo JJ, Sánchez-Sánchez C. From the intestinal flora to the microbiome. Rev Esp Enfermedades Dig. 2018;110:51–6.

4. Power SE, O'Toole PW, Stanton C, Ross RP, Fitzgerald GF. Intestinal microbiota, diet and health. Br J Nutr. 2014;111:387–402.

5. Klancic T, Reimer RA. Gut microbiota and obesity: Impact of antibiotics and prebiotics and potential for musculoskeletal health. J Sport Heal Sci. 2020;9:110–8.

6. Abenavoli L, Scarpellini E, Colica C, Boccuto L, Salehi B, Sharifi-Rad J, Aiello V, Romano B, De Lorenzo A, Izzo AA, et al. Gut microbiota and obesity: A role for probiotics. Nutrients. 2019;11:1–27.

7. Patterson EE, Ryan PM, Cryan JF, Dinan TG, Paul Ross R, Fitzgerald GF, Stanton C. Gut microbiota, obesity and diabetes. Postgrad Med J. 2016;92:286–300.

8. Kong LC, Holmes BA, Cotillard A, Habi-Rachedi F, Brazeilles R, Gougis S, Gausserès N, Cani PD, Fellahi S, Bastard JP, et al. Dietary patterns differently associate with inflammation and gut microbiota in overweight and obese subjects. PLoS One. 2014;9.

9. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, et al. A core gut microbiome between lean and obesity

twins. Nature. 2009;457:480–4.

10. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A. 2005;102:11070–5.

11. Bessesen DH. Human gut microbes associated with obesity. Yearb Endocrinol. 2007;2007:163–5.

12. Gomes AC, Hoffmann C, Mota JF. The human gut microbiota: Metabolism and perspective in obesity. Gut Microbes. 2018;9:308–25.

13. Santacruz A, Collado MC, García-Valdés L, Segura MT, Marítn-Lagos JA, Anjos T, MartíRomero M, Lopez RM, Florido J, Campoy C, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr. 2010;104:83–92.

14. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. Am J Clin Nutr. 2008;88:894–9.

15. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, et al. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A. 2013;110:9066–71.

16. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500:541–6.

17. Gérard P. Gut microbiota and obesity. Cell Mol Life Sci. 2016;73:147–62.

18. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027–31.

19. Tanti JF, Ceppo F, Jager J, Berthou F. Implication of inflammatory signaling pathways in obesity-induced insulin resistance. Front Endocrinol. 2013;3:1–15.

20. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, et al. Metabolic Endotoxemia Initiates Obesity and Insulin Resistance. 2007;56:1761–72.

21. Cani PD, Bibiloni R, Knauf C, Neyrinck AM, Delzenne NM. Changes in gut microbiota control metabolic diet–induced obesity and diabetes in mice. Diabetes. 2008;57:1470–81.

22. Wang J, Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012;490:55–60.

23. Zhang X, Shen D, Fang Z, Jie Z, Qiu X, Zhang C, Chen Y, Ji L. Human Gut Microbiota Changes Reveal the Progression of Glucose Intolerance. PLoS One. 2013;8.

24. Tian L, Jin T. The incretin hormone GLP-1 and mechanisms underlying its secretion. J Diabetes. 2016;8:753–65.

25. Brown E, Cuthbertson DJ, Wilding JP. Newer GLP-1 receptor agonists and obesity-diabetes. Peptides. 2018;100:61–7.

26. H. Elrick, L. Stimmler, C.J. Hlad AY. Plasma Insulin Response to Oral and Intravenous

Glucose Administration. J Clin Endocrinol Metab. 1964;24:1076–82.

27. Van Bloemendaal L, Veltman DJ, Ten Kulve JS, Groot PFC, Ruhé HG, Barkhof F, Sloan JH, Diamant M, Ijzerman RG. Brain reward-system activation in response to anticipation and consumption of palatable food is altered by glucagon-like peptide-1 receptor activation in humans. Diabetes, Obes Metab. 2015;17:878–86.

28. Gribble FM, Reimann F. Function and mechanisms of enteroendocrine cells and gut hormones in metabolism. Nat Rev Endocrinol. 2019;15:226–37.

29. Lupien-Meilleur J, Andrich DE, Quinn S, Micaelli-Baret C, St-Amand R, Roy D, St-Pierre DH. Interplay Between Gut Microbiota and Gastrointestinal Peptides: Potential Outcomes on the Regulation of Glucose Control. Can J Diabetes. 2020;44:359–67.

30. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes. 2012;61:364–71.

31. Müller M, Hernández MAG, Goossens GH, Reijnders D, Holst JJ, Jocken JWE, Van Eijk H, Canfora EE, Blaak EE. Circulating but not faecal short-chain fatty acids are related to insulin sensitivity, lipolysis and GLP-1 concentrations in humans. Sci Rep. 2019;9:1–9.

32. Wang Y, Dilidaxi D, Wu Y, Sailike J, Sun X, Nabi X hua. Composite probiotics alleviate type 2 diabetes by regulating intestinal microbiota and inducing GLP-1 secretion in db/db mice. Biomed Pharmacother. 2020;125:109914.

33. Ramírez-Pérez O, Cruz-Ramón V, Chinchilla-López P, Méndez-Sánchez N. The role of the gut microbiota in bile acid metabolism. Ann Hepatol. 2017;16:21-26.

34. Wang Z, Zhao Y. Gut microbiota derived metabolites in cardiovascular health and disease. Protein Cell. 2018;9:416–31.

35. Kitai T, Tang WHW. Gut microbiota in cardiovascular disease and heart failure. Clin Sci. 2018;132:85–91.

36. Kriaa A, Bourgin M, Potiron A, Mkaouar H, Jablaoui A, Gérard P, Maguin E, Rhimi M. Microbial impact on cholesterol and bile acid metabolism: current status and future prospects. J Lipid Res. 2019;60:323–32.

37. Wahlström A, Sayin SI, Marschall HU, Bäckhed F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. Cell Metab. 2016;24:41–50.

38. Chen ML, Yi L, Zhang Y, Zhou X, Ran L, Yang J, Zhu JD, Zhang QY, Mi MT. Resveratrol attenuates trimethylamine-N-oxide (TMAO)-induced atherosclerosis by regulating TMAO synthesis and bile acid metabolism via remodeling of the gut microbiota. MBio. 2016;7:1–14.

39. Villette R, KC P, Beliard S, Salas Tapia MF, Rainteau D, Guerin M, Lesnik P. Unraveling Host-Gut Microbiota Dialogue and Its Impact on Cholesterol Levels. Front Pharmacol. 2020;11:1–15.

40. Groen AK, Bloks VW, Verkade H, Kuipers F. Cross-talk between liver and intestine in control of cholesterol and energy homeostasis. Mol Aspects Med. 2014;37:77–88.

41. Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J Lipid Res. 2006;47:241–59.

42. Wilkins TD, Hackman AS. Two Patterns Of Neutral Steroid Conversion In The Feces Of

Normal North Americans 1. Cancer Res. 1974;34:2250–4.

43. Gérard P, Lepercq P, Leclerc M, Gavini F, Raibaud P, Juste C. *Bacteroides* sp. strain D8, the first cholesterol-reducing bacterium isolated from human feces. Appl Environ Microbiol. 2007;73:5742–9.

44. Lye HS, Rusul G, Liong MT. Removal of cholesterol by *lactobacilli* via incorporation and conversion to coprostanol. J Dairy Sci. 2010;93:1383–92.

45. Antharam VC, McEwen DC, Garrett TJ, Dossey AT, Li EC, Kozlov AN, Mesbah Z, Wang GP. An integrated metabolomic and microbiome analysis identified specific gut microbiota associated with fecal cholesterol and coprostanol in *Clostridium difficile* infection. PLoS One. 2016;11:1–23.

46. Veiga P, Juste C, Lepercq P, Saunier K, Béguet F, Gérard P. Correlation between faecal microbial community structure and cholesterol-to-coprostanol conversion in the human gut. FEMS Microbiol Lett. 2005;242:81–6.

47. Canyelles M, Tondo M, Cedó L, Farràs M, Escolà-Gil JC, Blanco-Vaca F. Trimethylamine N-oxide: A link among diet, gut microbiota, gene regulation of liver and intestine cholesterol homeostasis and HDL function. Int J Mol Sci. 2018;19:3228.

48. Bennett BJ, de Aguiar Vallim T, Wang Z, Shih DM, Meng Y, Gregory J, Allayee H, Lee R, Graham M, Crooke R, et al. Trimethylamine-N-Oxide, a Metabolite Associated with Atherosclerosis, Exhibits Complex Genetic and Dietary Regulation. Cell Metab. 2013;17:49–60.

49. Cho CE, Taesuwan S, Malysheva O V., Bender E, Tulchinsky NF, Yan J, Sutter JL, Caudill MA. Trimethylamine-N-oxide (TMAO) response to animal source foods varies

among healthy young men and is influenced by their gut microbiota composition: A randomized controlled trial. Mol Nutr Food Res. 2017;61:1–12.

50. Romano KA, Vivas EI, Amador-noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from Diet and Accumulation of the Proatherogenic Metabolite. mBIO. 2015;6:1–8.

51. Heianza Y, Ma W, Manson JAE, Rexrode KM, Qi L. Gut microbiota metabolites and risk of major adverse cardiovascular disease events and death: A systematic review and meta-analysis of prospective studies. J Am Heart Assoc. 2017;6.

52. Fu Q, Zhao M, Wang D, Hu H, Guo C, Chen W, Li Q, Zheng L, Chen B. Coronary Plaque Characterization Assessed by Optical Coherence Tomography and Plasma Trimethylamine-N-oxide Levels in Patients With Coronary Artery Disease. Am J Cardiol. 2016;118:1311–5.

53. Karlsson FH, Fåk F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, Bäckhed F, Nielsen J. Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat Commun. 2012;3:1245.

54. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, Li L, Fu X, Wu Y, Mehrabian M. Gut Microbial Metabolite TMAO Enhances Platelet Hyperreactivity and Thrombosis Risk. Cell. 2016;165:111–24.

55. Seldin MM, Meng Y, Qi H, Zhu WF, Wang Z, Hazen SL, Lusis AJ, Shih DM. Trimethylamine N-oxide promotes vascular inflammation through signaling of mitogen-activated protein kinase and nuclear factor-κb. J Am Heart Assoc. 2016;5:1–12.

56. Wang Z, Klipfell E, Bennett BJ, Koeth R, Bruce S, Dugar B, Feldstein AE, Britt EB, Fu X,

Chung YM, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. 2011;472:57–63.

57. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, et al. Intestinal microbiota metabolism of L-carnitin, a nutrient in red meat, promotes atherosclerosis. Nat Med. 2013;19:576–85.

58. Moludi J, Maleki V, Jafari-Vayghyan H, Vaghef-Mehrabany E, Alizadeh M. Metabolic endotoxemia and cardiovascular disease: A systematic review about potential roles of prebiotics and probiotics. Clin Exp Pharmacol Physiol. 2020;47:927–39.

59. Neves AL, Coelho J, Couto L, Leite-Moreira A, Roncon-Albuquerque R. Metabolic endotoxemia: A molecular link between obesity and cardiovascular risk. J Mol Endocrinol. 2013;51:51–64.

60. McIntyre CW, Harrison LEA, Eldehni MT, Jefferies HJ, Szeto CC, John SG, Sigrist MK, Burton JO, Hathi D, Korsheed S, et al. Circulating endotoxemia: A novel factor in systemic inflammation and cardiovascular disease in chronic kidney disease. Clin J Am Soc Nephrol. 2011;6:133–41.

61. ORGANIZATION FAA. Probiotics in food. Chem Funct Prop Food Components, Third Ed. 2006;413–26.

62. Ejtahed HS, Angoorani P, Soroush AR, Atlasi R, Hasani-Ranjbar S, Mortazavian AM, Larijani B. Probiotics supplementation for the obesity management; A systematic review of animal studies and clinical trials. J Funct Foods. 2019;52:228–42.

63. Zhao X, Higashikawa F, Noda M, Kawamura Y, Matoba Y, Kumagai T, Sugiyama M. The obesity and fatty liver are reduced by plant-derived *pediococcus pentosaceus* LP28 in high

fat diet-induced obese mice. PLoS One. 2012;7.

64. Gauffin Cano P, Santacruz A, Moya Á, Sanz Y. *Bacteroides uniformis* CECT 7771 ameliorates metabolic and immunological dysfunction in mice with high-fat-diet induced obesity. PLoS One. 2012;7.

65. Higashikawa F, Noda M, Awaya T, Danshiitsoodol N, Matoba Y, Kumagai T, Sugiyama M. Antiobesity effect of *Pediococcus pentosaceus* LP28 on overweight subjects: A randomized, double-blind, placebo-controlled clinical trial. Eur J Clin Nutr. 2016;70:582–7.

66. Gomes AC, de Sousa RGM, Botelho PB, Gomes TLN, Prada PO, Mota JF. The additional effects of a probiotic mix on abdominal adiposity and antioxidant Status: A double-blind, randomized trial. Obesity. 2017;25:30–8.

67. Kim J, Yun JM, Kim MK, Kwon O, Cho B. *Lactobacillus gasseri* BNR17 Supplementation Reduces the Visceral Fat Accumulation and Waist Circumference in Obese Adults: A Randomized, Double-Blind, Placebo-Controlled Trial. J Med Food. 2018;21:454–61.

68. Pedret A, Valls RM, Calderón-Pérez L, Llauradó E, Companys J, Pla-Pagà L, Morages A, Martín-Luján F, Ortega Y, Giralt M, et al. Effects of daily consumption of the probiotic *Bifidobacterium animalis* subsp. lactis CECT 8145 on anthropometric adiposity biomarkers in abdominally obese subjects: a randomized controlled trial. Int J Obes. 2019;43:1863–8.

69. Minami J, Iwabuchi N, Tanaka M, Yamauchi K, Xiao J zhong, Abe F, Sakane N. Effects of *Bifidobacterium breve* B-3 on body fat reductions in pre-obese adults: A randomized,

double-blind, placebo-controlled trial. Biosci Microbiota, Food Heal. 2018;37:67–75.

70. Chen J, Wang R, Li XF, Wang RL. *Bifidobacterium adolescentis* supplementation ameliorates visceral fat accumulation and insulin sensitivity in an experimental model of the metabolic syndrome. Br J Nutr. 2012;107:1429–34.

71. Kim SW, Park KY, Kim B, Kim E, Hyun CK. *Lactobacillus rhamnosus* GG improves insulin sensitivity and reduces adiposity in high-fat diet-fed mice through enhancement of adiponectin production. Biochem Biophys Res Commun. 2013;431:258–63.

72. Chen JJ, Wang R, Li XF, Wang RL. *Bifidobacterium longum* supplementation improved high-fat-fed-induced metabolic syndrome and promoted intestinal reg I gene expression. Exp Biol Med. 2011;236:823–31.

73. Singh S, Sharma RK, Malhotra S, Pothuraju R, Shandilya UK. *Lactobacillus rhamnosus* NCDC17 ameliorates type- 2 diabetes by improving gut function, oxidative stress and inflammation in high-fat-diet fed and streptozotocin treated rats. Benef Microbes. 2017;8:243–55.

74. Wang G, Li X, Zhao J, Zhang H, Chen W. *Lactobacillus casei* CCFM419 attenuates type 2 diabetes via a gut microbiota dependent mechanism. Food Funct. 2017;8:3155–64.

75. Balakumar M, Prabhu D, Sathishkumar C, Prabu P, Rokana N, Kumar R, Raghavan S, Soundarajan A, Grover S, Batish VK, et al. Improvement in glucose tolerance and insulin sensitivity by probiotic strains of Indian gut origin in high-fat diet-fed C57BL/6J mice. Eur J Nutr. 2018;57:279–95.

76. Jia L, Li D, Feng N, Shamoon M, Sun Z, Ding L, Zhang H, Chen W, Sun J, Chen YQ. Anti-diabetic Effects of *Clostridium butyricum* CGMCC0313.1 through Promoting the

Growth of Gut Butyrate-producing Bacteria in Type 2 Diabetic Mice. Sci Rep. 2017;7:1–15.

77. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Vol. 28, Nutrition. 2012. p. 539–43.

78. Bayat A, Azizi-Soleiman F, Heidari-Beni M, Feizi A, Iraj B, Ghiasvand R, Askari G. Effect of cucurbita ficifolia and probiotic yogurt consumption on blood glucose, lipid profile, and inflammatory marker in type 2 diabetes. Int J Prev Med. 2016;7:30.

79. Tonucci LB, dos Santos KMO, de Oliveira LL, Ribeiro SMR, Martino HSD. Clinical application of probiotics in type 2 diabetes mellitus: A randomized, double-blind, placebo-controlled study. Clin Nutr. 2017;36:85-92.

80. Mobini R, Tremaroli V, Ståhlman M, Karlsson F, Levin M, Ljungberg M, Sohlin M, Forslund HB, Perkins R, Bäckhed F, et al. Metabolic effects of *Lactobacillus reuteri* DSM 17938 in people with type 2 diabetes: A randomized controlled trial. Diabetes, Obes Metab. 2017;19:579–89.

81. Andreasen AS, Larsen N, Pedersen-Skovsgaard T, Berg RMG, Mller K, Svendsen KD, Jakobsen M, Pedersen BK. Effects of *Lactobacillus acidophilus* NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. Br J Nutr. 2010;104:1831–8.

82. Deng B, Wu J, Li X, Men X, Xu Z. Probiotics and Probiotic Metabolic Product Improved Intestinal Function and Ameliorated LPS-Induced Injury in Rats. Curr Microbiol. 2017;74:1306–15.

83. Degirolamo C, Rainaldi S, Bovenga F, Murzilli S, Moschetta A. Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr-Fgf15 axis in mice. Cell Rep. 2014;7:12–8.

84. Jones ML, Martoni CJ, Parent M, Prakash S. Cholesterol-lowering efficacy of a microencapsulated bile salt hydrolase-active *Lactobacillus reuteri* NCIMB 30242 yoghurt formulation in hypercholesterolaemic adults. Br J Nutr. 2012;107:1505–13.

85. Thushara RM, Gangadaran S, Solati Z, Moghadasian MH. Cardiovascular benefits of probiotics: A review of experimental and clinical studies. Food Funct. 2016;7:632–42.

86. Lye HS, Kuan CY, Ewe JA, Fung WY, Liong MT. The improvement of hypertension by probiotics: Effects on cholesterol, diabetes, renin, and phytoestrogens. Int J Mol Sci. 2009;10:3755–75.

87. Seppo L, Jauhiainen T, Poussa T, Korpela R. A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. Am J Clin Nutr. 2003;77:326–30.

88. Aihara K, Nakamura Y, Kajimoto O, Hirata H, Takahashi R. Effect of Powdered Fermented Milk with *Lactobacillus helveticus* on Subjects with High-Normal Blood Pressure or Mild Hypertension. J Am Coll Nutr. 2005;24:257–65.

89. Tuomilehto J, Lindström J, Hyyrynen J, Korpela R, Karhunen ML, Mikkola L, Jauhiainen T, Seppo L, Nissinen A. Effect of ingesting sour milk fermented using *Lactobacillus helveticus* bacteria producing tripeptides on blood pressure in subjects with mild hypertension. J Hum Hypertens. 2004;18:795–802.

90. Donkor ON, Henriksson A, Vasiljevic T, Shah NP. α-Galactosidase and proteolytic

activities of selected probiotic and dairy cultures in fermented soymilk. Food Chem. 2007;104:10–20.

91. Ryhänen EL, Pihlanto-Leppälä A, Pahkala E. A new type of ripened, low-fat cheese with bioactive properties. Int Dairy J. 2001;11:441–7.

92. Ng KH, Lye HS, Easa AM, Liong MT. Growth characteristics and bioactivity of probiotics in tofu-based medium during storage. Ann Microbiol. 2008;58:477–87.

93. Pineiro M, Asp N-G, Reid G, Macfarlane S, Morelli L, Brunser JO, Tuohy K. FAO Technical Meeting on Prebiotics. J Clin Gastroenterol. 2008;42:156–9.

94. Tsai YL, Lin TL, Chang CJ, Wu TR, Lai WF, Lu CC, Lai HC. Probiotics, prebiotics and amelioration of diseases. J Biomed Sci. 2019;26(1):1–8.

95. Queiroz-Monici KDS, Costa GEA, Da Silva N, Reis SMPM, De Oliveira AC. Bifidogenic effect of dietary fiber and resistant starch from leguminous on the intestinal microbiota of rats. Nutrition. 2005;21:602–8.

96. Druart C, Alligier M, Salazar N, Neyrinck AM, Delzenne NM. Modulation of the gut microbiota by nutrients with prebiotic and probiotic properties. Adv Nutr. 2014;5:624-633.

97. Nofrarías M, Martínez-Puig D, Pujols J, Majó N, Pérez JF. Long-term intake of resistant starch improves colonic mucosal integrity and reduces gut apoptosis and blood immune cells. Nutrition. 2007;23:861–70.

98. Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D, De Backer F, Neyrinck AM, Delzenne NM. Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation

and glucose response after a meal. Am J Clin Nutr. 2009;90:1236–43.

99. Vulevic J, Juric A, Tzortzis G, Gibson GR. A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. J Nutr. 2013;143:324–31.

100. Nicolucci AC, Hume MP, Martínez I, Mayengbam S, Walter J, Reimer RA. Prebiotics Reduce Body Fat and Alter Intestinal Microbiota in Children Who Are Overweight or With Obesity. Gastroenterology. 2017;153:711–22.

101. Dehghan P, Gargari BP, Jafar-Abadi MA, Aliasgharzadeh A. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: A randomized-controlled clinical trial. Int J Food Sci Nutr. 2014;65:117–23.

102. Liber A, Szajewska H. Effect of oligofructose supplementation on body weight in overweight and obese children: A randomised, double-blind, placebo-controlled trial. Br J Nutr. 2014;112:2068–74.

103. Canfora EE, van der Beek CM, Hermes GDA, Goossens GH, Jocken JWE, Holst JJ, van Eijk HM, Venema K, Smidt H, Zoetendal EG, et al. Supplementation of Diet With Galacto-oligosaccharides Increases *Bifidobacteria*, but Not Insulin Sensitivity, in Obese Prediabetic Individuals. Gastroenterology. 2017;153:87-97.

104. Cotillard A, Kennedy SP, Kong LC, Prifti E, Pons N, Le Chatelier E, Almeida M, Quinquis B, Levenez F, Galleron N, et al. Dietary intervention impact on gut microbial gene richness. Nature. 2013;500:585–8.

105. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, Hayes P, O'Reilly M, Jeffery IB, Wood-Martin P, et al. Exercise and associated dietary extremes

impact on gut microbial diversity. Gut. 2014;63:1913–20.

106. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, et al. Diet rapidly and reproducibly alters the human gut microbiota. Nature. 2014;505:559–63.

107. Świątecka D, Narbad A, Ridgway KP KH. The study on the impact of glycated pea proteins on human intestinal bacteria. Int J Food Microbiol. 2011;145:267–72.

108. Fava F, Gitau R, Griffin BA, Gibson GR, Tuohy KM, Lovegrove JA. The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome ' at-risk ' population. Int J Obes. 2013;37:216–23.

109. Ghoshal S, Witta J, Zhong J, Villiers W De, Eckhardt E. Chylomicrons promote intestinal absorption of lipopolysaccharides. J Lipid Res. 2009;50:90–7.

110. Pepino MY. Metabolic effects of non-nutritive sweeteners. Physiol Behav. 2015;152:450–5.

111. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, Israeli D, Zmora N, Gilad S, Weinberger A, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature. 2014;514:181–6.

112. Liauchonak I, Qorri B, Dawoud F, Riat Y, Szewczuk MR. Non-nutritive sweeteners and their implications on the development of metabolic syndrome. Nutrients. 2019;11:1–19.

113. Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ. Human colonic microbiota associated with diet, obesity and weight loss. Int J Obes. 2008;32:1720–4.

114. Saad MJA, Santos A, Prada PO. Linking gut microbiota and inflammation to obesity and insulin resistance. Physiology. 2016;31:283–93.

115. Barengolts E. Gut microbiota, prebiotics, probiotics, and synbiotics in management of obesity and prediabetes: Review of randomized controlled trials. Endocr Pract. 2016;22:1224–34.

ANNEXES

Tables:

Table 1 Bacterial phyla and their most predominant genera.

PHYLA	GENERA
Firmicutes	*Ruminococcus, Lactobacillus, Blautia, Clostridium* and *Faecalibacterium*
Bacteroidetes	*Prevotella* and *Bacteroides*
Actinobacteria	*Bifidobacterium,*
Proteobacteria	*Enterobacteriaceae*
Verrucomicrobia	*Akkermansia*
Fusobacteria	*Fusobacterium*

Table 2 Microbiota composition associated to an obese state or a lean state.

OBESE STATE	LEAN STATE
Firmicutes	*Bacteroidetes*
Campylobacter	*Bifidobacterium*
Bacteroides	*Akkermansia muciniphila*
Anaerostipes	*Faecalibacterium*
Dialister	*Coprococcus*
Porophyromonas	*Butyrivibrio Methanobrevibacter*
Parabacteroides	*Lactobacillus*
Staphylococcus	
Ruminococcus	

Table 3 Microbiota composition associated to Cardiovascular disease.

Risk of cardiovascular disease	Reason	Associated gut microbiota
↓	Reduction of total cholesterol	*Bacteroides, Enterococcus*, *Bifidobacterium, Clostridium* and *Lactobacillus.*
↓	Conversion of cholesterol into coprostanol	*Bacteroides spp.* strain D8, different strains of *Lactobacillus, Bifidobacterium, Lachnospiraceae* and *Ruminococcaceae.*
↑	TMAO production	Low gut microbial diversity, *Firmicutes, Proteobacteria*, *Collinsella* and a reduction of *Eubacterium* and *Roseburia.*
↑	LPS	Gram-negative bacteria

↑ = Increase; ↓ = Decrease.

Figures:

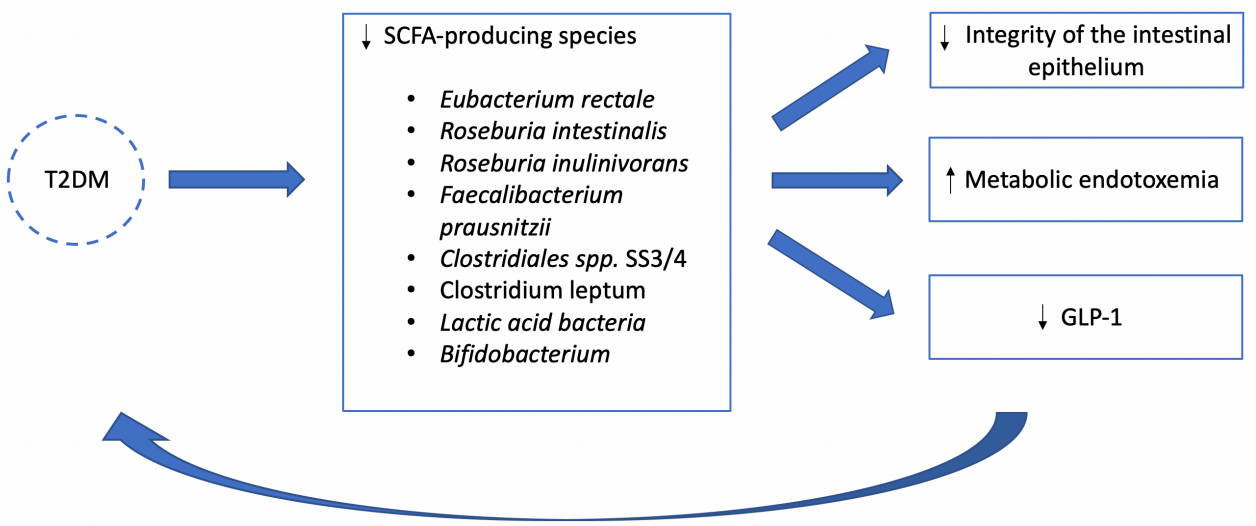

Figure 1 Gut microbiota and type-2 diabetes mellitus.

Type-2 diabetic patients experience a reduction of the abundance of SCFA-producing bacteria, which leads to a decrease of the integrity of the host intestinal epithelium, an increase of metabolic endotoxemia and a reduction of the synthesis of GLP-1. These alterations contribute to the promotion of T2DM.
SCFA= Short Chain Fatty Acids; GLP-1= Glucagon-like peptide-1; T2DM= Type-2 diabetes mellitus; ↑= Increase; ↓= Decrease.

List of abbreviations

BA – Bile acid

BSH – Bile salt hydrolase

CA – Cholic acid

CDCA - Chenodeoxycholic acid

CD36 - cluster of differentiation 36

CH – Carbohydrates

CR – Conventionally Raised

CVD – Cardiovascular disease

CYP7A1 – cholesterol 7α-hydroxylase

CYP27A1 - cytochrome P450 27A1

DCA - deoxycholic acid

EEC - Enteroendocrine cells

FAO - Food and Agriculture Organization of the United Nations

FMO - Flavin monooxygenases

FOS – Fructooligosaccharides

FXR – Farnesoid X receptor

GF - Germ-free

GIP – Glucose-dependent insulinotropic peptide

GLP-1 - Glucagon-like peptide-1

GOS – Galactooligosaccharides

GPCR - G protein-coupled receptors

HFD – High-fat diet

LCA – Lithocholic acid

LPS – Lipopolysaccharide

MACE - Major adverse cardiovascular events

NNS - Non-nutritive sweeteners

SCFA – Short Chain Fatty Acids

Spp. - Species

SR – Scavenger receptor

TC – Total cholesterol

TMA – Trimethylamine

TMAO – Trimethylamine-N-oxide

TLR-4 – Toll-like receptor 4

T2DM – Type-2 Diabetes Mellitus

WHO – World Health Organization