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The role of probiotics on the microbiota: effect on obesity.

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Abstract

The microbiota and the human host maintain a symbiotic association. Nowadays, metagenomic analyses are providing valuable knowledge on the diversity and functionality of the gut microbiota. However, with regards to the definition of a "healthy microbiota" and the characterization of the dysbiosis linked to obesity, there is still not a clear answer. Despite this fact, attempts have been made to counteract obesity through probiotic supplementation. A literature search of experimental studies relevant to the topic was performed in PubMed database with the keywords "probiotic" and "obesity" and restricted to those with "*Lactobacillus*" or "*Bifidobacterium*" in the title. So far, evidence of an anti-obesity effect of different lactobacilli and bifidobacteria has been mainly obtained from animal models of dietary-induced obesity. Using these experimental models, a substantial number of studies have reported reductions in weight gain and in particular in fat tissue mass at different locations following administration of bacteria, compared to controls. Anti-atherogenic and anti-inflammatory effects including regulation of expression of lipogenic and lipolytic genes in the liver, reduction in liver steatosis, improvement of blood lipid profile and glucose tolerance, decreased endotoxemia and regulation of inflammatory pathways are also reported in many of them. The number of human studies focused on probiotic administration for obesity management is still very scarce, and it is too soon to judge their potential efficacy, especially considering the fact that the actions of probiotics are always strain–specific, and the individual response varies according to intrinsic factors, the overall composition of diet and their interactions.

Introduction

Intestinal microbiota and the individual have evolutionarily set up a symbiotic association that allowed them to reach functional stability. The intestinal microbiota performs a series of metabolic functions necessary to the living organism. Among others, microbiota is involved in energy harvest from diet through the utilization of indigestible compounds, vitamin synthesis, micronutrient absorption, xenobiotic biotransformation, immune system stimulation and pathogen resistance[1] . Gut microbiota harbors 10^{14} bacteria, ten-fold the number of human cells, and includes up to 2000 species[2]. The diversity of microbes within a given body habitat can be defined as the number and abundance distribution of distinct types of organisms. A low diversity has been linked to obesity and inflammatory bowel disease, according to evidence derived from modern techniques of 16S ribosomal RNA gene (rRNA) sequencing and metagenomic sequencing[3,4].

While human gut microbiota seems to be fairly stable over time, between-subject variation, even among healthy individuals, is bigger, both in organismal composition and in metabolic function[5] . It has been suggested that human gut microbiomes fall into three distinct types or "enterotypes", robust clusters identifiable by the variation in the levels of one of three genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3)[6] . However, most human gut microbiome data collected to date support continuous gradients of dominant taxa rather than discrete enterotypes[7] . Methods based on16S rRNA sequencing revealed that two bacterial divisions, the Bacteroidetes and the Firmicutes, constitute over 90% of the known phylogenetic categories and dominate the distal gut microbiota[8]. The gut microbial gene catalog

established by metagenomic sequencing as part of the MetaHIT project revealed that the studied cohort harbored between 1000 and 1150 prevalent bacterial species, and each individual at least 160 such species, which are also largely shared[4] . As sequencing and analysis progresses, it seems that a core of microbial species might be common to all individuals and high variability might exist in their specific abundances. In addition, clusters of related species have been found in the MetaHit project which include some of the most abundant gut species belonging to Bacteroidetes and Dorea/Eubacterium/Ruminococcus groups and also bifidobacteria, proteobacteria and streptococci/lactobacilli groups. Among commensals, lactic acid bacteria are species of the acidophilus complex, a diverse group of Gram-positive, non-spore forming, facultatively anaerobic bacteria that have a G+C content of less than 50% and produce lactic acid by fermentation. These includes *Lactobacillus acidophilus*, *Lactobacillus gasseri*, *Lactobacillus johnsonii* and other related lactobacilli which have proven to be microorganisms with probiotic attributes[9] . As commensals, they have the capacity to occupy mucosal niches of humans, including the oral cavity, the gastrointestinal tract and the vagina.

Probiotics are live micro-organisms that, when administered in adequate amounts, have been shown to confer health benefits to the host[10] (FAO/WHO, 2001). In general, those more frequently employed belong to the *Lactobacillus* and *Bifidobacterium* genera, which are common inhabitants of the human intestinal ecosystem. Some of the benefits supported by stronger evidence include: improvements of lactose intolerance and digestive symptoms of discomfort, and reduction of risk of antibiotic-associated diarrhea and necrotizing enterocolitis; and with a lower degree of certainty, some probiotics have been shown to improve immune response to vaccines, infant's eczema,

vaginal infections, and to alleviate allergy symptoms and inflammatory bowel disease[11]. However, scientific agreement on probiotic applications for health is difficult to achieve. In the first place, effects are strain-specific, and secondly, the scientific studies performed on any given benefit for health provide graded evidence that depends on quality criteria applied to human clinical trials, such as demonstration of the benefit in the target population in a well-controlled, blinded, randomized manner. A positive balance between high-quality independent studies showing positive results and those showing no effects or negative results is necessary. Thus, more research should be conducted in the probiotic field in order to understand the potential of these health-promoting microorganisms for humans. A literature search of experimental studies relevant to the topic was performed in PubMed database with the keywords "probiotic" and "obesity" and restricted to those with "*Lactobacillus*" or "*Bifidobacterium*" in the title to focus on the genera that provide the bulk of the information on the topic. Secondly, further relevant articles were retrieved by a broader search with combinations of some of the keywords and adding the term "microbiota".

Interaction of probiotics with host cells and commensal microbiota

To be considered a good probiotic candidate, a bacterial strain should present some characteristics that contribute to indigenous colonization, such as tolerance to low pH, resistance to bile salts and adhesion to the host epithelium[9]. Based on their long tradition of use without any harmful effects on human health, lactic acid bacteria of the *Lactobacillus* and *Bifidobacterium* genera have an established safety record and have been accorded the GRAS (Generally Recognized As Safe) status by the Food and Drug Administration (FDA, USA).

A principal role of the microbiota is the participation in immune system development during the early stages of life[12]. In parallel, immune homeostasis, achieved through interactions with the resident microbiota is fundamental to avoid uncontrolled inflammatory responses and pathologies[13].

Probiotics, either colonizing or in transit, interact with the host in several manners with effects such as: 1) modulation of endogenous microbiota functions affecting its interplay with the host and the competitive exclusion of pathogens, 2) enhancement of epithelial barrier function and other innate immune responses; and 3) modulation of immune cell behavior and cytokine profiles[14]. An important part of the interactions occurs through the microbe-associated molecular patterns (MAMP) in the microbiota, such as cell wall and cytoplasmic membrane anchored molecules (polysaccharides, peptidoglycans, lipoproteins and lipoteichoic acids), which are recognized by pattern recognition receptors (PRRs) expressed in epithelial, immune and other host cells (*i.e.* Toll-like receptors [TLRs])[14]. Although these MAMPs appear to be produced by many different lactobacilli, their chemical structure may still vary between strains in terms of polymer composition, length and substitutions, which may explain part of the strain specificity of probiotics. Lactobacilli, indeed, differ considerably in their ability to trigger TLR2 signaling[15].

Genome sequencing and functional genomics of bifidobacteria have significantly expanded our understanding regarding the roles of gut-derived bifidobacteria in both microbe-microbe and host-microbe interactions through molecules that facilitate their establishment in the human intestine. These colonization factors and their important metabolic abilities render them one of the major microbial players in gut colonization during the first stages of life[16]. Proof of the genomic adaptation to their host is the

identification of a varied arsenal of genes encoding enzymes that are involved in the breakdown of complex carbohydrates derived from the diet (*e.g.*, plant polysaccharides) or from the host (*e.g.*, mucin). These carbohydrates cannot be digested by host-derived enzymes and will thus reach the large intestine in an intact form providing a good environment for bifidobacteria to thrive. Identified genes with a potential role in the processes of colonization and adaptation to the gastrointestinal habitat include those codifying for enzymes conferring bile resistance, enzymes for the utilization of human milk oligosaccharides and dietary complex polysaccharides, adhesins that mediate binding to mucus, plasminogen and other host surface receptors, components of pili, and possibly also exopolysaccharide components, inducible low pH resistance genes, and the LuxS enzyme of the activated methyl cycle of bacteria for recycling of S-adenosylmethionine[17].

Several studies have assessed the changes in microbiota composition in the human gut after probiotic consumption. Most frequently, an increase in the proportion of the supplemented genera was found in feces, but without an effect on the composition and diversity of the main bacterial populations. This was the case in several intervention studies: 1) supplementation with *L. acidophilus* NCFM and *B. lactis* Bi-07 to children with atopic dermatitis led to increases in both species after intervention, indicating survival of the bacteria; however *Bifidobacterium* spp., *L. mesenteroides* and *L. gasseri* determined by qPCR and abundances of the bacterial classes determined by pyrosequencing, including bacteroidetes, clostridia, actinobacteria, proteobacteria and verrucomicrobiae did not change after the intervention, and neither did richness and diversity estimates[18], 2) probiotic cheese administration containing *Lactobacilllus rhamnosus* HN001 and *Lactobacillus acidophilus* NCFM to elderly volunteers resulted

in increased numbers of both species in the feces, while clostridial cluster XIV, *F. prausnitzii* and sulfate reducers measured by qPCR and *Bifidobacterium* genus, the *Bacteroides–Prevotella* group, the *C. histolyticum* group, *A. muciniphila*-like bacteria and the total bacteria analyzed with fluorescent in situ hybridization and flow cytometry did not show any significant changes[19], 3) the synbiotic supplement Gut Balance ™ used in a study with healthy physically active adults increased by a factor of 9 the fecal *L. paracasei* numbers compared to the prebiotic product used as control, but total *Lactobacilli*, *L. acidophilus*, *L. rhamnosus*, *B. lactis* and *E. coli* measured by qPCR remained unmodified[20], and 4) the treatment with *L. reuteri* DSM 17938 in colicky infants (age 10-60 days) did not change the global composition of the microbiota at the phyla and taxa levels by pyrosequencing analysis[21].

On the other hand, other studies have reported wider changes in microbiota composition with probiotic administration. Synbiotic supplementation based in *Lactobacillus acidophilus* NCFM and lactitol in elderly subjects resulted in an increase in total levels of bifidobacteria and lactobacilli[22]. *B. lactis* Bb12 supplementation increased the cell counts of bifidobacteria and reduced the cell counts of enterobacteria and clostridia in preterm infants[23]. Larsen *et al.* [24] described that the ratio of Bacteroides-Prevotella-Porphyromonas group to Firmicutes was significantly increased after administration of *Lactobacillus salivarius* Ls-33 for 12 weeks in obese adolescents, although no statistically significant changes were observed in the number of bacteria of any of the groups analyzed. *Lactobacillus paracasei* subsp. *paracasei* LC01 (LC01), in a 4-week treatment period, reduced fecal *Escherichia coli* and increased *Lactobacillus, Bifidobacterium*, and *Roseburia intestinalis* in healthy adults[25]. *Lactobacillus* GG was shown to act on the dysbiosis found in patients with cirrhosis and minimal hepatic

encephalopathy by reducing Enterobacteriaceae and increasing Clostridiales Incertae Sedis XIV and Lachnospiraceae[26]. Daily ingestion of one or more food products enriched with *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® during 12 weeks increased fecal lactobacilli and bifidobacteria of healthy adults[27]. Other evidences of gut microbiota changes by probiotic feeding have also emerged from animal studies as reviewed by Tsai et al[28].

Evidence from *in vitro* experiments also indicates that the interaction of probiotic bacteria with host epithelial cells promotes phenotypic changes in the bacteria that improve their mutualistic relationship. The contact of *Lactobacillus casei* ATCC 334 with human intestinal epithelial cells promotes functional changes in the bacteria, which acquire a more immunosuppressive phenotype as demonstrated by the ability of *L. casei* to generate functional regulatory T cells ($CD4^+CD25^+FoxP3^+$) and production of the anti-inflammatory cytokine IL-10[29]. Cultivation of *B. longum* NCC2705 with intestinal epithelial cells has been also shown to induce adhesin expression[30].

Relationship between microbiota and obesity. Experimental evidence.

Obesity is one of the main health issues around the world because of the high prevalence and its association with the metabolic syndrome and related pathologies such as hypertension, diabetes, fatty liver disease and cardiovascular disease among others. The negative impact on health is obviously associated with a diminished quality of life and high healthcare costs. In 2008, about one-third of the world's adult population (~1.46 billion) was overweight, whereas the age-standardized prevalence of obesity was

9.8% in men and 13.8% in women; with wide variation between and within countries[31]. The combined effect of genetic and environmental factors on the obesity epidemic is generally accepted, including unhealthy dietary patterns and sedentary behaviors, typical of the obesogenic environment of modern society[32]. However, more recently, gut microbiota dysbiosis has been considered as an additional factor in obesity and type II diabetes mellitus development[33]. Novel methodologies have provided ways to exhaustively study the variety of microbial communities in the intestinal ecosystem and some evidences have emerged linking different microbial phylotypes and different bacterial species to obesity in humans[34] and animals, especially in fecal transplantation experiments performed in germ-free animals[35].

The first metagenomic analysis of human intestinal microbiota reported that obese individuals have lower ratios of *Bacteroidetes* to *Firmicutes*[34]. This was consistent with the results from studies performed in gnotobiotic animals transplanted with the fecal microbiota of obese mice, which led to greater fat deposition compared with control mice transplanted from lean animals[35]. According to the results in animal models, the microbiome of obese animals has an increased capacity for energy harvest[36].

Although the hypothesis exists that microbiota might play a causative role in obesity, the opposite could be true and the dysbiosis might just be the result of the adaptation to a high fat and sugar diet[37,38]. However, both human and animal model studies have yielded conflicting results about the precise nature of the associations between microbiome composition and obesity. Recently, an extensive assessment of the relationship between body mass index (BMI) and the taxonomic composition of the gut microbiome was conducted in the Human Metagenomic Project (HMP) dataset, and the results were compared with those obtained in the other big metagenomic study of gut

microbiota –the MetaHIT study– and also with two other smaller studies that specifically sampled lean and obese individuals[3, 34]. The authors found no association between BMI and stool microbiome taxonomic composition or diversity in the HMP cohort[39]. They also found that inter-study variability far exceeded differences in composition between lean and obese individuals within each study, and concluded by suggesting that no simple taxonomic signature of obesity exists in the gut microbiome. The same conclusion was reached in a meta-analysis searching for indicator taxa in the microbiome and general features of the microbiota associated with obesity[40].

Intervention studies with probiotics in the management of obesity

Based in the idea that different microbiota colonize the gut of normoweight and obese individuals, emphasis has been directed to carry out research studies aimed to provide weight management through probiotic administration, with the advantage that no secondary effects are expected from this kind of interventions; however, most of the research so far has been conducted in animal models of diet-induced obesity. Human intervention studies with the specific aim of obesity management have also been published, although in limited number. In this section we will focus on those studies conducted with bacterial strains of the genera *Lactobacillus* (phylum Firmicutes) and *Bifidobacterium* (phylum Actinobacteria).

Animal studies

Several studies carried out with similar methodologies have shown decreases in the body weight of mice and rats with high-fat diet-induced obesity and administered or

supplemented with one or a number of *Lactobacillus* and *Bifidobacterium* strains. Usually these experimental procedures are also accompanied by a decrease in fat tissue weight at several abdominal locations, and even an improvement of other features associated with obesity, such as insulin resistance, metabolic syndrome, inflammation or liver steatosis. Differences are, however, detected between strains on the results obtained and also regarding the potential mechanisms leading to the observed anti-obesity effects. Modulation of fat absorption and excretion was shown in lean rats[41] , reduction of endotoxemia and inflammation was reported in genetically or diet-induced obese rodents[42,43,44,45,46,47,] and very frequently, a modulation of the expression of genes involved in hepatic lipogenesis and/or adipose tissue lipolysis has been evidenced and suggested as the mechanism for the anti-obesity effects[45,48,49,50,51,52], although the exact genes modulated will depend on the strain used.

Effects on body weight, adiposity and metabolism

In rodent models of obesity, the number of studies showing decreased body weight gain and reduced accumulation of fat deposits with probiotic treatments is much higher than that of studies that report no change in body weight and fat mass. In those studies in which the primary outcome is the anti-obesity effect, the supplementation period is usually longer than 10 weeks.

Lactobacillus plantarum LG42 isolated from Gajami sik-hae, a traditional Korean fermented product, was shown to decrease food intake, weight gain, epididymal and back fat mass and serum and liver triglycerides when supplemented to high-fat diet-induced C57BL/6j obese mice. This supplementation also decreased hepatic lipogenic

genes expression (LXR-α, SREBP-1, and ACC), whereas it increased the hepatic PPAR-α and CPT-1 mRNA levels, which up-regulate the expression of enzymes involved in fatty acid oxidation (Table 1). It also decreased expression of proteins involved in lipid anabolism in adipose tissue, such as PPAR-γ and C/EBP-α [51]. A similar reduction in fat mass and the modulation of lipogenic and lipolytic gene expression in the liver were found in an obesity model of mice fed a high-sucrose diet and supplemented with *Lactobacillus gasseri* BNR17 during 10 weeks[50]. Another supplementation with *Lactobacillus gasseri* SBT2055 for 24 weeks to mice on a high-fat diet during that period resulted in a significant reduction in body weight and fat tissue mass and a relatively lowered level of triglyceride content in the liver, parallel to reduced expression of lipogenic genes, including ACC1, FAS and SREBP-1, while no changes were observed in lipolytic genes (CPT-1-α, PPAR-α, or UCP-2)[52]. On the contrary, in another experiment in which mice received *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 during 10 weeks, CPT-1 and other fatty acid oxidation-related genes were up-regulated, together with a reduction in body weight and adiposity, plasma insulin, leptin and total cholesterol, in comparison with the control group[45]. In healthy mice fed a standard diet, Ji *et al.*[49] found that supplementation during 3 weeks with either *L. rhamnosus* GG or *L. sakei* decreased epididymal fat and the expression of ACC, fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD)-1 genes in the liver. Finally, also in young healthy mice, Angelakis *et al.*[54] described that the intragastrical inoculation of one or two doses of *L. ingluviei* led to increases in weight gain and liver weight and increased the expression of lipogenic markers in liver such as FAS and SREBP-1c, in contrast with the wide majority of findings from probiotic studies performed in obesity-induced models. The efficacy of different *Lactobacillus* species as growth promoters of the young animal in the livestock and

poultry industry has been explored[55] and might be related to these specific and controversial results.

In fact, the work of D. Raoult's team[54,56] has elicited considerable debate among those involved in probiotic research. They support that there is enough evidence to suggest that some probiotic strains of *Lactobacillus* induce weight gain in lean humans and animals while other strains exhibit a weight-loss effect in overweight/obese humans and animals and they advise that it would be a negligence to disregard the hypothesis that probiotics might be linked to human obesity[56,57]. For those opposed to this opinion, the evidence points towards a promotion of growth and lean body mass, but not adiposity[58] and several other weaknesses have been pointed out against the evidence on which the hypothesis was relied on[59].

Other putative mechanisms have been proposed for the anti-obesity effects of *Lactobacilli*. One of them is an increase in the production of ANGPTL4, a lipoprotein lipase inhibitor that controls triglyceride deposition into adipocytes, as supported by the study by Aronsson and colleagues[60]. Feeding *L. paracasei* ssp. *paracasei* F19 to high-fat diet-induced obese mice reduced body fat while increasing circulating ANGPTL4. Other mechanism, suggested by Tanida and colleagues[61], is an enhanced lipolysis linked to increased sympathetic nerve activity in adipose tissue and supported by the increase in plasma free fatty acids; both leading to reduced body and abdominal fat weights in mice fed *Lactobacillus paracasei* ST11. On the other hand, reductions in the absorption of dietary fat have also been reported with probiotic supplementation. In this sense, *L. gasseri* SBT2055 led to a decrease in mesenteric fat weight and cholesterol levels, and an increase in fatty acid excretion in lean rats after 8 weeks of feeding, which the

authors explained through a decreased absorption of dietary fat[41]. This study, however, did not find changes in body fat when the supplementation was made on obese Zucker rats. This result is in contrast with those reported by Miyoshi and colleagues[52] using the same strain; however, Miyoshi's experiment was not performed in genetically obese rats, but in mice fed a high-fat diet, and also the duration of the supplementation was much longer (24 weeks). Decrease in visceral fat and triglyceride in the liver with increased fecal triglycerides in KK-A(y) mice on a high fat diet and supplemented with *Lactobacillus gasseri* NT have also been reported by Yonejima and colleagues, who explain the findings through a reduction of lipid digestion/absorption[62]. Recently, the expression of cloned bile salt hydrolase from *Lactobacillus salivarius* in mice gut has also been demonstrated to regulate transcription of genes involved in lipid and cholesterol metabolism, and has an impact in adiposity and weight control[63].

The existing differences at the strain level are well exemplified in Fak and Bäckhed's study[53] in a mouse model of obesity (*Apoe*-/- mice) in which they tested the anti-obesity, anti-inflammatory and anti-atherogenic effects of three different strains of *L. reuteri*. They found that only one of the strains showed anti-obesity effects, apparently linked to liver lipolytic activity; however, no anti-inflammatory or anti-atherosclerotic effect was achieved irrespective of the treatment.

With regards to bifidobacteria, high-fat or Western-type diets have been shown to reduce their presence in the guts of rodent models of dietary obesity[64]. Supplements of prebiotics, in particular oligofructose and inulin-type fructans, can increase their numbers[64,65,66], and concomitantly reduce body weight and adipose tissue depots and improve glucose tolerance when administered to high-fat diet-fed animals[64,66]. It was suggested that this effect could be mediated by increases in the expression of glucagon-

like peptide 1 (GLP-1)[67]; actually, higher levels of proglucagon mRNA have been found in the guts of prebiotic-treated animals[64,65], as well as higher GLP-1 plasma levels[62]. GLP-1 has been shown to induce satiety[68,69] ; however, in most studies using either prebiotics or bifidobacteria supplements, average caloric intake of the animals did not decrease significantly[64,66,70,71,72,73]. An exception is the study by Bomhof and colleagues[74], where animals fed oligofructose, but not those given *Bifidobacterium animalis* subsp. *lactis*, had overall lower energy intake, body weight gain and fat content. Interestingly, only oligofructose treatment was accompanied by elevated GLP-2 levels.

Other mechanisms have been proposed to explain the anti-obesity effects of bifidobacteria-stimulating prebiotics. For example, Dewulf and colleagues found that inulin supplementation to dietary obese mice increased basal lipolysis in subcutaneous adipose tissue, which had been blunted by the high-fat diet, together with reduced expression of GPR43[66], which is an inhibitor of lipolysis[75] and has been also found to stimulate adipocyte differentiation[76]. In addition, expression of other genes involved in adipocyte differentiation and fat storage, *i.e.*, aP2, CEBPα and LPL, was also reduced after inulin supplementation[66]. The question arises, however, whether the effects of prebiotics are due to increased bifidobacteria populations or the prebiotics themselves, or what the actual role of these bacteria is. For example, in the study by Bomhof, prebiotic supplementation was more efficient than probiotics at changing composition of microbiota in the rats' guts[74].

Direct interventions with bifidobacteria have also rendered significant anti-obesity results. Different studies using several *Bifidobacterium* strains, such as *B. breve* B-3[70] , *B. L66-5*[71], *B. pseudocatenulatum* and *B. longum*[72,43] , *B. adolescentis*[77], *B. lactis* 420[78],

or *B. animalis* subsp. *lactis*[73] have reported reductions in high-fat diet-induced increases in body weight gain and fat content. In addition, most of these studies found as well reductions in circulating levels of glucose and insulin, and/or improved glucose tolerance[43, 70,72,73,74 ,78] . Furthermore, bifidobacteria seem to be effective at ameliorating other features of the metabolic syndrome, like elevated plasma triglycerides[43, 71] and cholesterol[70,71] , and even blood pressure[43] . Some of the mechanisms proposed to explain the beneficial effects of bifidobacteria coincide with those attributed to prebiotics, as mentioned earlier. Kondo[70] found that administration of *Bifidobacterium breve* B-3 (10^9 colony-forming units (CFU)/d) increased proglucagon and Fiaf expression in the gut, and adiponectin expression in adipose tissue. Kondo and co-workers[70] suggested that GLP-1 and -2, together with increased adiponectin expression, might contribute to the enhanced insulin sensitivity observed, while Fiaf could contribute to decrease fat deposition in adipose tissue, as it inhibits LPL. Another mechanism proposed by the authors was the conversion of linoleic acid to conjugated linoleic acid (CLA) by the bifidobacteria, which had previously been reported[79,80], as there is evidence in the literature for an anti-obesity effect of this fatty acid[81] .

It could be argued (and in fact it has been) that the models employed for the study of the effects of probiotics on obesity all introduce a confounding factor in the form of the high-fat diets, as diet is one of the most influential factors for altering microbiota composition. Even though the vast majority of studies have used diet to induce obesity, there are a few cases when obesity has been achieved by other means, and they are worth mentioning. For example, in the work by Savcheniuk and co-workers[82], rats were made obese by injections of monosodium glutamate (MSG) during the first 10 days of life, and fed afterwards on standard chow. Administration of a probiotic mixture of *L.*

casei IMVB-7280, *B. animalis* VKL and *B. animalis* VKB did not change body weight, but partially reduced visceral fat mass and serum triglycerides; it also completely reduced total cholesterol, and partially restored normal levels of the different fractions (VLDL, LDL and HDL cholesterol). In addition, the probiotic normalized serum adiponectin levels and leptin expression in adipose tissue[82]. On the other hand, supplements of either *L. paracasei* (CNCM I-4034), *L. rhamnosus* (CNCM I-4036), *B. breve* (CNCM I-4035), or a mixture of *L. paracasei* and *B. breve* administered to genetically obese Zucker rats did not result in significant reductions of body weight gain, improvement of markers of insulin sensitivity, or any other serum parameter; however, all treatments reduced total lipid content in the liver of the obese rats[47].

It is also worth noting that different effects have been observed depending on the strain used. For example, in the experiment by Yin and colleagues[71], rats with high-fat diet-induced obesity were treated with four strains of bifidobacteria, all obtained from feces of healthy volunteers, and named *L66-5*, *L75-4*, *M13-4* and *FS31-12*. The authors found that *B. L66-5* blunted the diet-induced increase in body weight, while *B. M13-4* further enhanced it. And whereas no differences in relative body fat content, glucose or insulin levels were found between treatments, and all bifidobacteria reduced serum triglycerides, only *B. L66-5* and *B. FS31-12* reduced cholesterol levels as well[71].

Effect on liver steatosis and inflammation

Apart from their effects on body weight and fat, and on glucose and lipid metabolism, probiotics have been found to improve other conditions related to obesity, such as inflammation and liver steatosis. Cani et al. (2008)[33], have provided evidence that gut

bacteria are involved in metabolic endotoxemia and adipose tissue inflammation in obese animals and have also shown that the mechanisms involved in the development of metabolic endotoxemia are associated with an increased intestinal permeability. Activation of Toll-like receptors in macrophage and epithelial cells by LPS is responsible for the induction of inflammation. Thus, improvement in gut barrier function by probiotic bacteria would reduce the chances of endotoxemia and reverse or impede those putative causes of obesity development. In fact, modulation of the expression of inflammatory genes has been analyzed in the high-fat diet-induced obesity model after probiotic supplementation.

Lactobacillus gasseri SBT2055 suplemented to mice on a high-fat diet in the study of Miyoshi *et al.* described previously[52], not only reduced fat tissue and the expression of lipogenic genes but it also inhibited pro-inflammatory CCL2 gene expression in adipose tissue. Similarly, the beneficial findings related to weight, adiposity and fatty acid oxidation-related genes in Park *et al.*'s study[45] with *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032, were accompanied by the down-regulation of pro-inflammatory genes in adipose tissue (TNF-α, IL-1β, IL-6 and MCP-1). Also in a mouse model of fructose-induced obesity, supplementation of *Lactobacillus rhamnosus* GG has proven to restore barrier permeability at the duodenal level, to lower lipopolysaccharide (LPS) in portal vein and to reduce liver steatosis and inflammation, as measured by neutral lipid assay and pro-inflammatory cytokine expression, respectively. Although no differences in weight were observed among groups, there was a trend for a decreased weight gain in the supplemented groups[46]. Reduction in endotoxemia (plasma LPS) was also reported by Naito and colleagues[44] in mice on a high-fat diet supplemented with *L. casei* Shirota despite no changes in intra-abdominal

fat weight, which might perhaps be due to a short duration of the supplementation (4 weeks)[44].

Oral probiotic treatment with VSL#3 significantly improved the high fat diet-induced hepatic NKT cell depletion, insulin resistance and hepatic steatosis in mice[42]. These effects of probiotics are likely due to increased hepatic NKT cell numbers and reduced inflammatory signaling. High-dose VSL#3 (1.5×10^9 CFU/day) was more effective than low-dose VSL#3 (1.5×10^8 CFU/day) and *B. infantis* (1.5×10^9 CFU/day) at improving hepatic NKT cell depletion and steatosis. Weight gain was also significantly reduced in animals fed the high-dose VSL#3 compared to the control. Lipid extracts from VSL#3 also stimulated NKT cells *in vivo* and *in vitro*[83]. The results suggest that specific probiotic strains can have profound effects on hepatic NKT cells and steatosis, and that glycolipid antigens from bacteria can modulate the functionality of NKT cells[83].

A study that measured glucose –insulin homeostasis, hepatic steatosis and the modulation of the structure of the HFD-disrupted gut microbiota by three candidate probiotics using 454 pyrosecuencing of bacterial 16S rRNA genes showed that each strain employed (*Lactobacillus paracasei* CNCM I-4270, *L. rhamnosus* I-3690 and *Bifidobacterium animalis* subsp. *lactis* I-2494) attenuated weight gain and macrophage infiltration into epididymal adipose tissue and markedly improved glucose –insulin homeostasis and hepatic steatosis[73]. Gut microbiota shifted towards that of lean mice fed a normal diet; however, each strain changed a different set of the 49 altered operational taxonomic units (OTUs) defined as functionally relevant by their correlation with metabolic syndrome parameters. *L. paracasei* and *L. rhamnosus* increased cecal acetate but did not affect circulating LPS-binding protein, which is in contrast with the results reported by Plaza-Díaz *et al.* (2014)[47], who found that different strains of the

same species decreased serum LPS in obese Zucker rats. The results support the notion that the beneficial effects on obesity-related comorbidities are mediated through strain-specific impacts on metabolic syndrome-associated phylotypes of gut microbiota[73].

In relation to bifidobacteria, there seems to be a relationship between their presence in the gut and levels of inflammatory markers, in both liver and intestine. It has been proposed that bifidobacteria can stimulate the expression of tight-junction proteins in the epithelium, decreasing the permeability of the gut and thus protecting the organism from pathogens, which leads to increased circulating LPS levels, or endotoxemia, and in consequence inflammation[64,65,84] . Decreased numbers of bifidobacteria associated with high-fat diet feeding have been linked with increased gut permeability and elevated levels of LPS and endotoxemia. The use of prebiotics to increase the numbers of endogenous bifidobacteria[64,65], as well as that of peptides from exogenous bifidobacteria strains, seems to be effective in enhancing the expression of tight-junction proteins, and reducing gut permeability and inflammation[65,84,85]. Administration of prebiotics (oligofructose) has also been shown to reduce plasma levels of cytokines (IL-1β, TNF-α, MCP-1, MIP-1a, IFN-γ, IL-10, IL-15 and IL-18), expression in the liver of markers of inflammation (PAI-1, TNF-α), macrophage infiltration (CD68, TLR4), and oxidative stress (NADPHox, iNOS)[86], and expression of TLR4 and F4/80 in adipose tissue[66].

As with the anti-obesity effects, doubts may arise whether the effects are mediated by the prebiotics themselves or by the bifidobacteria. Again, the work by Bomhof, which compared the effects of prebiotics, probiotics and their combination, showed that only rats receiving oligofructose had significant elevations on tight-junction protein 1 expression, while administration of *B. animals* subsp. *lactis* BB-12 alone did not exert a significant effect on the levels of this protein[74]. However, earlier studies showed the

efficacy of probiotic supplements in reducing endotoxemia[87] and improving mucosal integrity[88]. In models of diet-induced obesity, bifidobacteria have been found to counteract high-fat diet induced hepatomegaly (with a mixture of *B. pseudocatenulatum* SPM 1204, *B. longum* SPM 1205 and *B. longum* SPM 1207[72] and with *B. longum* on its own[77]), and to partially or totally abolish lipid deposition in hepatocytes, contributing to prevent or reverse liver steatosis (with *B. adolescentis*[77,89]; with *Bifidobacteria L66-5*, *L75-4*, *M13-4* and *FS31-12*[71]; with *L. paracasei* CNCM I-4270, *L. rhamnosus* CNCM I-3690 and *B. animalis* subsp. *lactis* CNCM I-2494, independently[73]). Also in non-dietary, genetically obese Zucker rats, reduced triglyceride and total lipid content in liver was observed following administration of *B. breve* CNCM I-4035, alone or in combination with *L. paracasei* CNCM I-4034[47].

In addition, *B. lactis* 420 has been shown to reduce the levels of inflammatory markers in the liver (TNF-α and IL-1β) and muscle (IL-1β) in mice with diet-induced mild diabetes treated with the probiotic[78]. For its part, *B. adolescentis* was reported to reduce neutrophil infiltration and liver damage in the liver of mice made obese by a Western-type diet. And even when no changes in portal levels of LPS were found, expression of TLR4, MCP-1, MIP-3, NF-κB activity, and markers of lipid peroxidation were reduced in the liver of treated animals[89]. Furthermore, amelioration of systemic inflammation, in the form of reduced LPS levels in serum, has been reported after treatments with *B. longum*[43], *B. lactis* 420[78], *B. breve* CNCM I-4035[47], and *B. animalis* subsp. *Lactis*[73]. *B. breve* CNCM I-4035 resulted also in lower levels of serum TNF-α[47]. This effect is likely a consequence of reduced inflammation and/or improved epithelial integrity in the guts of the treated animals. *B. longum* can partially decrease IL-1β expression in small intestine, concomitant with total improvement of its inflammatory status[43]. Chen and

colleagues [43] suggested that it was mediated by elevated expression of Reg-I, which is thought to help repair tissue damage in the small intestine[43,90]. For its part, *B. adolescentis* has been reported to improve the integrity of the gut epithelium as well, through elevated expression of tight-junction proteins, like occludin and ZO-1, in the duodenum[89]. Another mechanism proposed for the protective actions of probiotics in the intestine is the formation of carboxylic acids, like butyrate and propionate, as they may exert beneficial effects on intestinal health, such as increase of the mucosal integrity or reduction of colon cancer risk[91]. However, these effects should be expected to vary greatly depending on the probiotic strain used and on the diet(s) with which they are administered. Nilsson and colleagues compared the effects of combining different prebiotics (inulin, pectin and lactitol) with different probiotics (*B. lactis* Bb-12 and *L. salivarus* UCC500) in the concentrations of carboxylic acids in the intestine of rats. Each combination provided a different outcome; in particular, when administered with inulin, Bb-12 increased carboxylic acids in the cecum, resulting in higher levels of propionate, but decreased their presence in the distal colon, suggesting that Bb-12 induced stimulation of inulin fermentation preferentially in the cecum. In turn, when Bb-12 was provided together with pectin, carboxylic acids in the colon increased, and when given with lactitol, their absorption in the colon was suggested to be enhanced[91]. The precise consequences of these different outcomes for intestinal health need yet to be unraveled, but they highlight the importance of considering the variability and complexity of the relationships established between host and microbiota, between microbiota and diet, and also between the different bacterial populations within an organism.

In this sense, and similarly to what was discussed earlier about the effects of probiotics on body weight and fat, each probiotic strain has a different impact on liver, intestine and inflammatory status of the host. In the study by Yin and coworkers, *B. L66-5*, *B. L75-4*, *B. M13-4* and *B. FS31-12* could all ameliorate liver steatosis, but *B. L66-5* and *B. FS31-12* provided better results. Authors even proposed different mechanisms of action for the different strains: they suggested that while *B. L66-5* could decrease fat deposition in general, the reason why treatment with *B. M13-4* had resulted in higher body weight but reduced liver steatosis was a redistribution of fat deposition away from the liver and towards adipose tissue, highlighting the importance of choosing the appropriate strain depending on the nature of the condition to treat[71]. When comparing *L. paracasei* CNCM I-4270, *L. rhamnosus* CNCM I-3690, and *B. animalis* subsp. *lactis* CNCM I-2494, Wang and colleagues observed that all probiotics reduced the presence of macrophages and crown-like structures in adipose tissue, but only *B. animalis* reduced TNF-α levels in adipose tissue and liver and systemic endotoxemia[73]. Finally, although in the study by Plaza-Diaz *L.*[47] *paracasei* CNCM I-4034, *B. breve* CNCM I-4035, *L. rhamnosus* CNCM I-4036, and the mixture of *L. paracasei* and *B. breve* could all reduce total liver lipids in Zucker rats, liver triglyceride content was decreased by *L. paracasei* and *B. breve*, but not *L. rhamnosus*; and while serum TNF-α and LPS decreased with *L. paracasei* and *B. breve* treatments, serum IL-6 only did with *L. paracasei*[47].

Human studies

Several studies have been performed with *Lactobacilllus gasseri* SBT2055 in Japanese adults. In subjects with large visceral fat areas the intervention with fermented milk

containing 10^8 CFU/day during 12 weeks significantly reduced abdominal adiposity measured by computed tomography, and also other measures such as body weight, BMI and hip and waist circumferences[92,93] . The decrease in weight in 12 weeks was, however, not a big one (1 kg approx.). The same strain tested in non-obese hypertriglyceridemic subjects revealed that 4-week supplementation significantly diminished the postprandial triglyceride and free fatty acid response to an oral fat-loading test in a placebo-controlled, repeated measures trial, without a change in weight[94]. These different outcomes could be due to the normal weight status of the subjects or the shorter duration of the supplementation in comparison with the above mentioned studies. The effect on weight and body composition of these interventions as well as those performed with other strains in humans have been summarizes in Table 2.

A decrease in weight and waist and hip circumferences were observed in Korean adults with high BMI that received *L. gasseri* BNR17 for 12 weeks; however, no differences in these parameters were found between the treated (6×10^{10} CFU/day) and placebo groups[95]. As in the previously mentioned studies, only 1 kg of weight was decreased over the study period. *L. amylovorous* has also shown a significant decrease in total fat mass in overweight subjects that consumed 10^9 CFU/day during 43 days; however, no change in body weight or lean body mass was observed in this study[96].

An interesting follow-up study recently reported the effects on body composition and metabolic markers of an intervention with *L. paracasei* ssp. *paracasei* F19 performed during weaning[97] and evaluated at school age [98]. While lower levels of palmitoleic acid, a marker linked to abdominal fat, were found after the intervention in treated infants, having received LF19 during infancy did not modulate BMI z-score, sagittal abdominal

diameter measured by DEXA, measures of fat and fat-free mass, growth, or any of the assessed metabolic markers at school age. This is interesting as evidence that long-term effect of probiotic administration is not granted once discontinued.

To sum up, results from human intervention studies with lactobacilli of sufficient length and with weight or fat mass as main outcome are still scarce. These mainly used *L. gasseri* strains and suggest an overall modest effect on weight loss, which seems to be due to fat mass loss specifically. However, it remains to be clarified how long should the intervention be and whether the anti-obesity effects are in fact limited to subjects who are obese and/or hyperlipidemic prior to the intervention.

Even fewer studies have been found in relation to the use of bifidobacteria. As observed with animals, some of them do not use directly the probiotics, but prebiotics known to increase bifidobacteria populations in the host gut. These studies, however, vary in their design, duration, and characteristics of the subjects, and of course also in their outcomes. In a study on healthy normoweight adults (5 men and 5 women, 21-38 years old), the effects of a prebiotic supplement (Orafti Synergy1, soluble fibre consisting of a mixture of glucosyl-(fructosyl)$_n$-fructose and (fructosyl)$_m$-fructose extracted from chicory roots) were studied on hunger perception and satiety-related peptides. A dextrin-maltose placebo was used as control, and the treatment lasted 2 weeks. The prebiotic but not the placebo lowered hunger perception 3 hours after the previous meal (breakfast), although no significant changes in food intake were reported for either group. There were also no changes in absolute levels of satiety hormones, but significantly higher relative increases in PYY and GLP-1 were observed in the prebiotic group 15 minutes after breakfast; however, levels in both groups converged shortly

afterwards. Barely any changes were found between groups in markers of insulin sensitivity, only a lower glucose area under the curve after the meal[86].

Parnell and Reimer studied as well the effects of prebiotics on hunger and satiety, but they conducted their study on 48 overweight and obese Canadian volunteers (20-70 years of age). Participants received either a supplement of oligofructose (21 g/d; Raftilose P95, Quadra Chemicals Ltd, Burlington, Canada) or a dextrin-maltose placebo for 12 weeks. At the end of the treatment, levels of PYY in response to a meal tolerance test were increased and those of ghrelin decreased in the prebiotic group when compared to a similar test prior to the intervention, but it was not the case in the control group. This was accompanied by decreased total intake and significantly higher weight loss (due to fat mass, as measured by DXA) in the prebiotic group compared with the control. Absolute concentrations of glucose and insulin were also reduced in the prebiotic group[99].

In relation to interventions with probiotics, in a study with overweight volunteers, researchers compared the effect of a herbal compound (bofutsushosan, or BTS) used in Asia to treat obesity, with and without a combination of probiotics (*Streptococcus thermophilus* KCTC 11870BP, *L. plantarum* KCTC 10782BP, *L. acidophilus* KCTC 11906BP, *L. rhamnosus* KCTC 12202BP, *B. lactis* KCTC 11904BP, *B. longum* KCTC 12200BP and *B. breve* KCTC 12201BP). Thirty-six females (19-65 years) with BMI>25 kg/m^2 and waist circumference >85 cm were given either BTS or BTS+probiotics for 8 weeks. Both groups experienced reductions in body weight, BMI, fat percentage, waist circumference and total cholesterol, and no differences were found between groups. However, while HDL-cholesterol decreased with BTS treatment, it increased with BTS+probiotics. As there was no group with probiotics without BTS, the lack of

differences in body weight and fat between groups in this study does not prove a lack of effect of the probiotics on these parameters, only that the potential actions of BTS and the bacteria mixture were not additive. In fact, the presence of *B. breve* in the gut microbiota showed a negative correlation with endotoxin levels, which in turn were positively correlated with body weight, BMI, and fat mass; and numbers of *B. longum* were negatively correlated with body weight[100].

Also interesting is the study by Ilmonen and colleagues, a study on 185 pregnant Finish women receiving either dietary counselling plus probiotics (*L. rhamnosus* GG and *B. lactis*), dietary counselling plus placebo, or placebo alone. Treatments started during the first trimester of pregnancy and finished at the end of exclusive breastfeeding, maximum 6 months after giving birth. Results showed that probiotics plus counselling reduced the risk of central fat accumulation 6 months after given birth, biceps skinfold thickness and waist circumference at 12 months, and blood glucose levels both at 6 and 12 months post-partum, compared to the other treatments[101].

One important issue to consider when evaluating the theoretical effect of the consumption of a given probiotic is the capacity of response of the individual. In this sense, "the bandwidth of health" paradigm proposes that, despite greater inter-individual variability measured through transcriptomic analyses compared with the change induced by probiotics consumption, the response to probiotics also occurs in a conserved manner across participants; however, the consequences of these responses in terms of their physiological relevance may depend strongly on the basal molecular make-up at the start of the intervention[14,102]. Measuring with current –omics methods the molecular signature of the person provides the way to select individuals most likely to respond to probiotics with a defined biological activity which has been proven either *in vitro* or in

animal models. On the other hand, linking the losing weight effect of a probiotic therapy with a change in the gut microbiota seems like a key point to establish probiotic usefulness against obesity, however, the cross-talk between microbes and host cells in the gut is complex and the mechanisms by which these interactions lead to obesity and metabolic disease are multiple and, so far, only starting to be disentangled (see recent review by Cani & Everard, 2015)[103]. Even when a change in the overall composition of the microbiota is not proven after a dietary intervention, other changes, such as mucin layer depth, antimicrobial peptide production, increased abundance of a single beneficial bacterial species or a change in metabolites in the bacteria or the host cells could influence obesity and metabolic alterations. So far, not a single human clinical trial with a probiotic has found changes in microbiota and weight simultaneously and occurring exclusively in the group receiving the probiotic. Figure 1 depicts potential influencial factors affecting the relationship between changes in microbiota and changes in weight induced by probiotic treatment.

Concluding remarks

While the study of the microbiota has emerged as an outstanding research field with great repercussion for health, it seems to be an intriguing one, difficult to comprehend in order to be able to practice successful, personalized interventions. The number of different microbial species that can be found in the gut is huge and the interactions between them and with the host cells need to be further explained so that probiotic strains can be used with a rationale. As attractive as the use of probiotics may seem to counteract the obesity problem, we are still far from being able to give guidelines for its clinical application. In addition, many more placebo-controlled, randomized clinical

trials are warranted which place the scientific knowledge to a comfortable level of evidence regarding specific strains, length of treatment, and dose that need to be administered. This advice, however, is probably bound to be effective only when taking in consideration the current microbial communities, metabolic alterations and even genetic background of each particular individual, and perhaps, also paying attention to life-style habits that are already known to influence the gut ecosystem.

Table 1. Summary of intervention studies with *Lactobacillus* spp. strains in animal models assessing expression of genes involved in lipid metabolism in the liver.

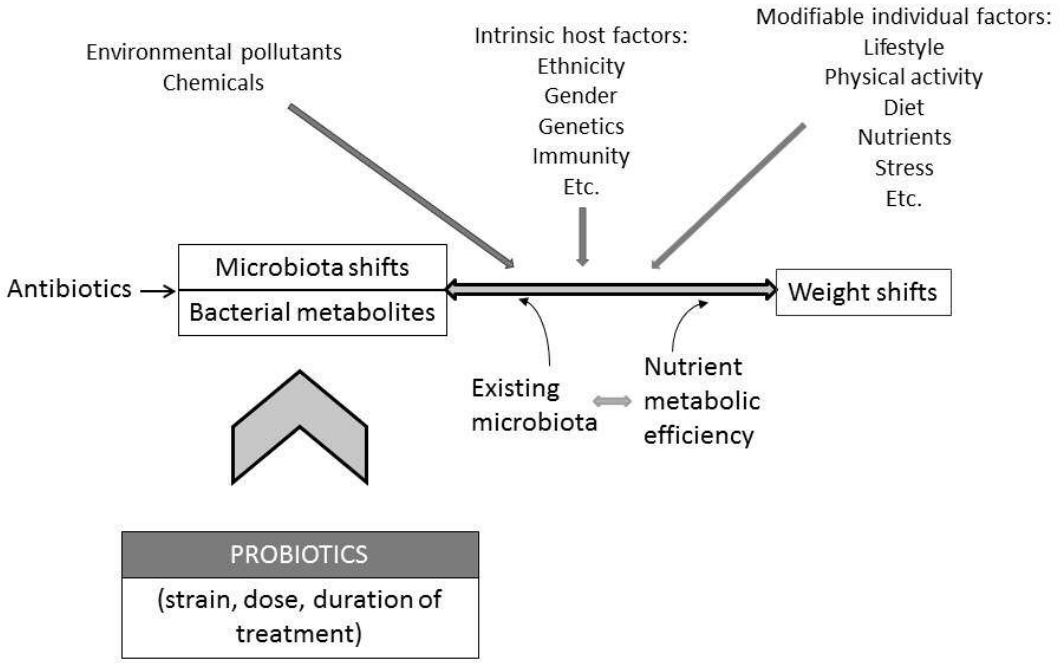
Reference	Experimental model	*Lactobacillus* strain	Change in weight	Change in fat mass	Liver lipolytic genes (mRNA)	Liver lipogenic genes (mRNA)
Park et al., 2014[51]	HFD obesity model, 12 wk.	*Lactobacillus plantarum* LG42	Yes	Yes	>PPAR-alpha and CPT-I	< LXRalpha, SREBP-1, and ACC
Miyoshi et al., 2014[52]	HFD obesity model, 24 wk.	*Lactobacillus gasseri* SBT2055	Yes	yes	No change CPT1-alpha, PPAR-alpha, UCP2	Trends to < ACC1, FAS and SREBP1
Park et al., 2013[45]	HFD obesity model, 8 wk and subsequent probiotic supplementation for 10 wk.	*Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032	Yes	Yes	> PGC1-alpha, CPT1, CPT2, ACOX1	-
Kang et al., 2013[50]	HSD obesity model, 10 wk.	*Lactobacillus gasseri* BNR17	Yes	Yes	>ACO, CPT1, PPARalpha, PPARgamma	< SREBP-1, ACC
Fak and Bäckhed, 2012[53]	HFD + Apoe-/- Metabolic syndrome model, 12 wk.	3 different strains of *L. reuteri* tested separately: *L. reuteri* ATCC PTA 4659, or DSM 17938 or DSM L6798	Yes (only for ATCC PTA 4659)	Yes (only for ATCC PTA 4659)	>CPT1a (only for ATCC)	No change in FAS or ACC
Ji et al., 2012[49]	Healthy mice, standard diet, 3 wk	Two treatments: *L. rhamnosus* GG *L. sakei* NR28	< weight gain (only *L. sakei*).	Yes	-	< FAS, ACC, SCD-1
Angelakis et al., 2012[54]	Healthy 3 wk. old mice, standard diet, (single or double gastric inoculation, days 0 and 7th).	*L. ingluviei* (single or double inoculation)	increased weight gain with *L. ingluviei* inoculation	-	-	> FAS, SREBP-1
Lee et al., 2006[48]	Diet induced obese mice, 8 wk.	*L. rhamnosus* PL60	Yes	Yes	>UCP2	-

Table 2. Summary of intervention studies on the effect of different probiotics on weight and body compositions in humans.

Ref.	Design	Subjects	Probiotic strain and duration of intervention	Anthropometric parameters	Metabolic Changes
92,93	multicenter DBPCR[a]	n= 87 Obese	*S. thermophilus, L. delbrueckii* ssp. *bulgaricus and* *L. gasseri* SBT2055 (10^{11} cfu/d.), 12 wk.	↓Weight, BMI, abdominal visceral fat area and hip and waist circumferences	-
94	SBPC[b]	n=20 Adults with TAG≥200mg/dl, glucose<100mg/dl	*S. thermophilus, L. delbrueckii* ssp. *bulgaricus and* *L. gasseri* SBT2055 (10^{11} cfu/d.), 4wk.	No change in weight or BMI	↓Postprandial serum non-esterified fatty acid (NEFA) and triacylglycerol (TAG) levels
95	DBPCR	n= 62 Obese	*L. gasseri* BNR17 (6 x 10^{10} cfu/d.), 12wk.	↓Weight and waist and hip circumferences	No change in glucose, insulin and lipid profile
96	DBPCR cross-over.	n=28 Obese	Yogurt; yogurt + *L. amylovorous* (LA) (1.39 x 10^9 cfu/d.); yogurt + *L. Fermentun* (LF) (1.08 x 10^9 cfu /d.), 43 d.	↓Total fat mass with LA	↓Abundance of *Clostridial* cluster IV with LA, ↑Abundance of *Lactobacillus* spp. in LF and LA
98	DBPCR follow-up.	n= 179 Children 8-9y	*L. paracasei* ssp. *paracasei* (10^8 cfu/d.), from 4th to 13th month of age	No change in BMI z-score, fat and lean mass (DEXA)	No change in glucose, insulin, lipid profile, hepatic parameters
100	DBPCR	n=36 Overweight females	Herbal compound + (*S thermophilus* KCTC 11870BP + *L. plantarum* KCTC 10782BP + *L. acidophilus* KCTC 11906BP + *L. rhamnosus* KCTC 12202BP + *B. lactis* KCTC 11904BP + *B. longum* KCTC 12200BP + *B. breve* KCTC 12201BP (5x10^{12} cfu/d.)), 8 wk.	↓Weight, BMI, fat mass (%), waist circumference, but also in prebiotic only (herbal compound) group	↓Total cholesterol, but also in prebiotic only (herbal compound) group. ↑ HDL-cholesterol in herbal compound + probiotic
101	DBPCR	n=185 Pregnant women	*L. rhamnosus GG* (ATCC 53103) (10^{10} cfu/d.) and *B. lacti*s Bb12 (10^{10} cfu/d.), from first trimester to end of exclusive breastfeeding	↓Risk of central adiposity 6 mo. postpartum (waist circumference >80cm)	↓Glucose levels at 6 and 12 mo. postpartum

[a]DBPCR: double-blind, placebo controlled, randomized study; [b]SBPC single-blind, placebo-controlled, within-subject, repeated-measures (all subject consumed first the control yogurt and after a wash-out period (4 wk.) the probiotic).

Figure 1. Factors that influence the relationship between changes in microbiota and changes in weight induced by probiotic treatment.



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