Review Article

Probiotics for the control of obesity – Its effect on weight change

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\section*{ABSTRACT}

\textbf{Background:} Obesity is a growing epidemic worldwide. Evidence so far demonstrates that the bacteria that are commonly found in the human gastrointestinal tract affect nutrient acquisition and energy regulation. This suggests that an important role is played by gut microbiota in the development of obesity.

\textbf{Objectives:} Our main goal was to assess if a probiotic diet leads to a significant difference in weight change in non-obese and obese people, and in experimental models.

\textbf{Methods:} Search was undertaken in PubMed, Scopus, ISI Web of knowledge, Cochrane Central Register of Controlled Trials, Google scholar, meta-Register of Controlled Trials, ClinicalTrials.gov and by scanning reference lists of articles, without publication date imposed, for randomised clinical trials studying the administration of probiotics to obese or overweight patients and experimental studies in experimental models and healthy humans. Search terms included probiotics, obesity, weight, BMI, weight gain, weight loss, weight change, probiotic diet and probiotic therapy. In an unblended standardized manner, 2 reviewers analysed the searched studies, using the defined inclusion and exclusion criteria, and performed extraction of data, in an independent way, using predefined data fields.

\textbf{Results:} We've identified, through searching databases specified in methods, 269 records. A total of 4 clinical trials and 14 experimental studies were included in the systematic review. Among the 4 randomized clinical trials only one showed statistically significant results. \textit{L. rhamnosus} CGMCC 1.3724 was efficient in reducing weight in females, but not in males - Mean weight loss 12 week/24 week (kg): Males-probiotic: 4/5.4; Males-placebo: 3/0.54; Females-probiotic: 4.4/5.2; Females-placebo: 2/2.5 (P<0.05 only on females).

\textbf{Conclusions:} In our systematic review, we found that probiotic effect in body weight is species and strain specific. \textit{L. gasseri} BNR17, reduced the weight gain compared to controls; \textit{L. gasseri} L66-5 promoted weight gain, \textit{L. rhamnosus} GGMCC is the only one that had a positive effect in weight loss in humans. Probiotic effect in body weight was species and strain specific. On the other hand \textit{L. plantarum} LG42, \textit{L. gasseri} SBT2055 and \textit{L. plantarum} co-therapy with KY103 and \textit{L. curvatus} HY7601 had an anti-obesity effect in animal models.

\section*{Introduction}

Obesity is a growing epidemic worldwide that has nearly doubled since 1980. In 2008, more than 1.4 billion adults over 20 were overweight, almost 35\% of the population, and about 11\% were obese. More than 40 million children under 5 were overweight in 2011, about 10 million in the developed and 30 million in the developing countries, which demonstrates that this disease is becoming an increasing problem in the latter, that used to fight undernutrition instead. Already being considered the fifth leading risk factor for global deaths, obesity kills at least 2.8 million adults each year.\textsuperscript{1}

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It is a major risk factor for the development of cardiovascular diseases, type 2 diabetes mellitus, musculoskeletal disorders (osteoarthritis) and various kinds of cancer (endometrial, breast and colon).\textsuperscript{2} The enormous social and economic costs of obesity and these associated comorbidities are already threatening to overwhelm health care systems worldwide.\textsuperscript{2}

The World Health Organization (WHO) defines obesity as an abnormal or excessive fat accumulation that may impair health, the result of an energy imbalance between calories consumed and expended. The criteria used by WHO to diagnose obesity is the body mass index (BMI): A person with a BMI greater or equal to 25 is considered overweight; greater or equal to 30 is considered as having obesity. In turn, obesity is also divided into three categories: BMI between 30 and 35 represents 1st degree obesity; BMI between 35 and 40 represents 2nd degree obesity; BMI over 40 represents 3rd
degree obesity. BMI is a useful indicator of overall adiposity, but different fat compartments are associated with differential metabolic risk. Thus, an evaluation of waist circumference results in a more accurate classification of obesity: visceral/central or subcutaneous obesity. A waist circumference over 88 cm for women and over 102 cm for men represents that a person has visceral/central obesity.

However, besides the environmental cause described by WHO, genetic, neural and endocrine factors have been described as causes of obesity, as well as infectious agents. Evidence so far demonstrates that the bacteria that are commonly found in the human gastrointestinal tract, normally referred to as gut microbiota, affect nutrient absorption and energy regulation, while also being different in an obese person, when compared to a lean one. This suggests that an important role is played by gut microbiota in the development of obesity. This information may represent a major advance in obesity therapy, since modifying the gut microbiota, through a diet rich in probiotics (nonpathogenic live microorganisms that, when ingested, confer health benefits to the host), can become an important treatment option for obesity. In fact, multiple studies have been researching the effect of the referred probiotics diet in the organism and its possible contribution to treat obesity as well as other comorbidities. Among the species already studied, the most commonly used are *Lactobacillus spp.*, *Bifidobacterium spp.* and *Enterococcus spp.*

Although there are several studies concerning the effect of different probiotics on weight change and obesity, we were unable to find a systematic review that summarized and discussed them in a global perspective. Thus, the elaboration of this article constitutes a relevant effort to better understand this matter.

**Research question and aims**

Our main goal was to access whether a probiotic diet leads to a significant difference in weight change in experimental models and non-obese and obese people, thus playing a protective and beneficial role in obesity establishment and in the reduction of obesity-related comorbidities, by performing a systematic revision of randomized, controlled trials and experimental studies that assessed the effect of probiotic therapy on weight change in experimental models and non-obese and obese subjects.

This conducts us to our central question: “What’s the effect of probiotic diet on weight change in obese/overweight and/or non-obese subjects?”

**Methods**

Methods of the analysis and inclusion criteria were documented in a protocol before its execution.

**Data sources**

We followed the PRISMA 2009 statement. Studies were identified by searching electronic databases and scanning reference lists of articles. No limits were applied for language and foreign papers were translated. This search was applied to PubMed, Scopus, ISI Web of Knowledge. The last search was run on 3 May 2014. A limited update literature search was performed from 3 May 2014 to 18 November 2014. We searched Google Scholar with several keywords combined to identify grey literature. We also searched for completed and ongoing trials (latest search 7 August 2014) in the following registers: ClinicalTrials.gov (http://clinicaltrial.gov/); and ISRCTN (http://www.controlled-trials.com/isrctn/). The search terms included were: probiotics, probiotic diet and probiotic therapy, Lactobacillus, obesity, weight, BMI, weight gain, weight loss and weight change. There were no new trials identified in the scanning of reference lists of articles. Full details of the search are presented in Table 1.

**Inclusion and exclusion criteria**

Inclusion was limited to randomized clinical trials studying the administration of probiotics to obese or overweight patients, with or without diabetes, cardiovascular diseases, hyperlipidaemia or metabolic syndrome and experimental studies with experimental models and healthy humans. No publication date was imposed. We excluded pregnant women, probiotics given only or simultaneously to the mother, hosts affected by gastro-enteric diseases, such as diarrhoea, colitis or irritable bowel syndrome, any article that evaluated symbiotics or non-direct fed, non-viable or recombiant probiotics, experimental studies in farm animals without clinical purpose, articles with unavailable statistical data, unavailable results or not yet completed, and articles not published in medical sciences journals. Articles without original data, not written in English or Portuguese and double publications were excluded. Articles that we didn’t had access (articles without full text available or of journals not included in the catalogue access of our faculty) were also excluded.

**Data extraction**

Obesity was defined and subclassified according to the WHO definition cited above.

Probiotic was defined as “Live microbial DIETARY SUPPLEMENTS which beneficially affect the host animal by improving its intestinal microbial balance. Antibiotics and other related compounds are not included in this definition” according to MeSH terms (Index Medicus “Medical Subject Heading” terminology).

We analysed, as primary outcomes, differences in weight: significant weight loss; significant weight gain; weight change from baseline, weight at the end of the study, weight/age ratio, BMI changes and weight percentile.

As secondary outcomes, we measured adverse events of probiotic treatment modification on lipidaemia levels, glycaemic levels, blood pressure differences, PCR levels, inflammation levels modification, asthma improvement, other diseases improvement, leptin levels and mortality.

Data retrieved also includes intervention duration, type, dose and frequency; versus placebo or versus of control treatment (duration, type, dose and frequency); outcome duration, improvement in quality of life score [using a validated scale, when there was available information], effect on daily activities, when available; characterization of the publication: year of publication; journal, authors, country of origin, study quality; methodological characteristics such as sample size, study design, control groups and if it is presented the CONSORT statement flow diagram or any written information discriminating the sample in a similar way (http://www.consort-statement.org/); characterization of the participants including gender, age, ethnicity, initial BMI (and consequent classification), initial abdominal circumference, when available.

**Study selection and data collection process**

Eligibility assessment and data extraction was performed in an unblended standardized manner by 2 reviewers that analyzed titles and abstracts of searched studies, using the defined inclusion and exclusion criteria. Discrepancies were discussed between the 2 reviewers and, if required, a third reviewer was involved. This same process, performed after analysis of the entire article, was performed on a data extraction sheet (based on the Cochrane Consumers and Communication Review Group’s data extraction template), and refined after a pilot- test on 5 randomly-selected included studies.

**Risk of bias assessment**

The pair of reviewers, independently, with adequate reliability determined the adequacy of randomization and concealment allo-
tion, blinding of patients, health care providers, data collectors, and outcome assessors; proportion of patients lost to follow-up; stopping of trials early for benefit; if outcome data is complete; whether the analysis followed the intention-to-treat principle; if there’s the possibility of selective outcome reporting and other potential threats to validity of the study. Each criterion for risk bias (based on the Cochrane Consumers and Communication Review Group’s “risk of bias assessment tool”9) was judged as Low, Unclear or High risk.9

Analysis

Individual participants in each clinical trial were the unit of analysis. Results are expressed as mean differences between intervention group and control group with standard deviations, in a summary table, when those values were available.

We performed a meta-analysis for clinical trials only, for weight change and HDL change parameters, and assessed the heterogeneity using the Cochran’s Q statistic with a P value ≤0.1 interpreted as statistically significant. We obtained further information on the impact of statistical heterogeneity on the study results by calculating the I² statistic.9 We used values of the I² statistic above 50% as a cut-off for statistically significant. We used a random-effects model. Meta-analysis was performed using RevMan5.

Had there been a sufficient amount of studies (i.e. at least 10 RCTs, as outlined in Chapter 10 the Cochrane Handbook for Systematic Reviews of Interventions),9 we would have constructed funnel plots to assess for any publication bias.

Results

Study selection

The search identified a total of 269 records, prior to removal of duplicates, with 59 of the records retrieved from the search of MEDLINE, 52 records from SCOPUS, 117 records from ISI, 8 records from CENTRAL, 19 records from Google Scholar, 11 from Clinical Trials.gov and 5 from ISRCTN; 163 records were left after duplicates were removed (Fig. 1). Scanning reference lists of review articles, contacting content experts and contacting authors produced no extra trials. We only included completed trials.

The 16 studies searched in Clinical Trials.gov and ISRCTN were excluded from screening because there were no available results. We discarded 115 after reviewing the abstracts, in its majority for being unrelated to the research question – did not mention researching the effect of probiotics in weight neither in the title, or in the abstract (80) – or for not meeting the study type or the interventions design criteria (24). Others were excluded due to being unavailable to us,4 their target population being pregnant women4 or for being retracted.3 The assessment for eligibility yielded 48 articles. Of these, 30 did not meet the following inclusion criteria: unable to access (21), not referring treatment of obesity,2 not corresponding to the study type we intended to include – not being neither a RCT or an experimental study in experimental models,2 not mentioning the primary outcome searched in this review,2 treatment applied to pregnant animals,3 including in the sample.
Subjects affected by gastrointestinal diseases\(^1\) or unavailable in English or Portuguese.\(^1\) A total of 4 clinical trials and 14 experimental studies were included in the systematic review.

**Study characteristics and results of individual studies**

In Table 2 we describe the data extracted from included human studies. We included four RCTs. All were published between 2013 and 2014. The sample size of these studies ranged from 20 to 50 (total 481 animals). The minimum interventional duration was of 5 weeks\(^14\) and maximum interventional duration was of 24 weeks.\(^15\) Four studies used as experimental models Sprague Dawley albino rats (SDr)\(^1\) (total of 154 rats; 20 female/130 male), one used C57BL/6J db/db mice\(^16\) and the other 10 studies used C57BL/6J mice\(^17\) (total of 337 mice; 24 female/313 male). One study only enrolled female animals – C57BL/6J mice\(^18\) and another enrolled male and female animals – SDr,\(^18\) all the other studies only enrolled male animals. Four studies applied a combination of probiotics.\(^19\) Among these four studies, one used *Bifidobacterium* combination\(^19\) and another also used four *Bifidobacterium* strains but as mono-intervention.\(^20\) The other two used *Lactobacillus* combinations. One other study applied two *Lactobacillus* strains separately.\(^21\)

Among the experimental animal trials, L. *gasseri* BNR17 in one study showed weight loss capability in rats, as well as reduced visceral fat accumulation.\(^22\) In another study, of the same authors,\(^23\) probiotic fed mice also showed reduced body weight. Opposing these results, other authors using the same probiotic were not supportive of these findings, showing instead a non-significant weight gain capability of this probiotic.\(^24\)

**Risk of bias within studies**

Bias assessment for RCT included is described in the Table 4, according to the Cochrane Consumers and Communication Review Group’s “risk of bias assessment tool”, judged as Low, Unclear or High risk.\(^9\)

All the four included RCT as a ‘high’ risk of bias across one\(^9\) or two of the domains. Three of the four RCTs had high risk of bias in blinding of outcome (Fig. 2). The study limitations, differences in probiotics administered and participants, and small sample sizes across the included studies mean that the power to detect a trend of overall effect may be limited and chance findings cannot be excluded.

**Synthesis of results**

**Primary outcome: weight change (Table 5)**

We performed a meta-analysis of weight change. This includes 3\(^10\) of the 4 trials which reported the difference of weight (n=255) in the same measure [difference between baseline body weight and post-intervention body weight in kg]. We included data of 12 and 24 weeks analysis of Sanchez 2014 RCT. One study was only included in the subgroup analysis of female body weight changes (n=164), since it only enrolled female subjects.\(^30\) There was a significant difference
### Table 2
Data extracted from included human studies (4 RCTs).

<table>
<thead>
<tr>
<th>Publication</th>
<th>Methods</th>
<th>Participants</th>
<th>Sample</th>
<th>Criteria</th>
<th>Probiotic</th>
<th>Control</th>
<th>Primary outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study source</strong> (author year)</td>
<td>Location; period of inclusion; mono or multicentre; study design; control groups</td>
<td>Subjects included; age, gender and ethnicity; classification according to initial BMI or initial abdominal circumference</td>
<td>Sample size: eligible (n), randomized (n), allocated for intervention (n)</td>
<td>Inclusion criteria, Exclusion criteria</td>
<td>Duration, type/species, dose and frequency</td>
<td>Present? Type/name, duration, type, dose and frequency</td>
<td>Placebo present?</td>
</tr>
<tr>
<td><strong>Jung 2013</strong></td>
<td>Monocentre; Randomized, Double-Blind Clinical Trial Country: Republic of Korea</td>
<td>Subjects aged between 19 and 60 with body mass index (BMI) ≥23 kg/m² and fasting blood sugar (FBS) ≥100 mg/dL</td>
<td>Eligible (83), randomized (62), allocated for control group (31-1 lost of follow up for primary outcome)</td>
<td>Inclusions: men and nonpregnant women aged between 19 and 60 with body mass index (BMI) ≥23 kg/m² and fasting blood sugar (FBS) ≥100 mg/dL, Exclusions: taking drugs that could affect weight change, including anti-diabetic drugs, lipidlowering drugs, or antihypertrophic drugs, or if they had endocrine, cardiovascular, thyroid, or chronic liver disease, Subjects were excluded if they had received surgery to reduce weight, were taking probiotics or antibiotics within one month, or if their weight had changed over five percent within three months</td>
<td>12 weeks (no wash-out before the initiation of treatment), Lactobacillus gasseri BNR17, 6 capsules per day (2 capsules, 3 times a day), 10^10 cfu of Lb. gasseri BNR17, Excipient: filler powder (50% trehalose, 25% skim milk, and 25% fructooligosaccharide)</td>
<td>Present</td>
<td>Placebo capsules were packaged only with the filler produced by the pharmaceutical factory, 6 capsules per day (2 capsules 30 minutes before breakfast, lunch, and dinner)</td>
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<tr>
<td><strong>Lee 2014</strong></td>
<td>Randomized, double-blinded, placebo controlled study Country: Republic of Korea</td>
<td>Subjects aged 19-65 year old, male and female, obese fasting blood sugar ≥100 mg/dL, Sex (M/F): 0/64</td>
<td>Eligible (64), randomized (50), allocated for intervention (25)</td>
<td>Inclusions: female subjects aged 19-65 years meeting the criteria of BMI&gt;25 kg/m² and waist circumference&gt;85 cm, Exclusions: Hypothyroidism; Cushing’s syndrome; Heart diseases; Cancer; Lung diseases; Severe renal dysfunction (Cr&gt;2.0 mg/dL); Liver dysfunction (ALT, AST 2.5 fold upper limit of normal); Non-insulin dependent diabetes mellitus with FBS&gt;140 mg/dL, neuropsychiatric diseases; Eating disorder; Subjects who underwent anatomical change such as incision; Pregnancy, breast feeding, or planning of pregnancy; Subjects who have lost 10% of body weight within six months of the study</td>
<td>8 weeks, Bofutsushosan (BTS) and probiotics (n=25), Bofutsushosan (BTS) and probiotics (n=25), Streptococ cus thermophiles, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium lactis, Bifidobacterium longum and Bifidobacterium breve, co-administered with BTS extracts, 5×10^8 cells per species, twice per day</td>
<td>Present</td>
<td>Placebo and placebo (n=25). Placebo capsules were identical in appearance, except for the bacterial strains and were also given twice per day, co-administered with BTS extracts. Bofutsushosan (BTS) is an oriental herbal medicine containing 18 components</td>
</tr>
<tr>
<td>Publication</td>
<td>Methods</td>
<td>Participants</td>
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<tr>
<td>Sanchez 2014(^2)</td>
<td>Randomised, double-blind, placebo-controlled trial</td>
<td>Country: Canada; Quebec city; January 2010- May 2012</td>
<td>18 to 55 year old, male and female, obese; Sex (M/F): 48/77</td>
<td>Eligible (153), randomized (153), after randomization they excluded 28 dyslipidaemic participants allocation 125 patients, allocated for intervention (62), allocated for control group (63), loss of follow up in intervention group phase I/phase II (10/7), loss of follow up in control group phase I/phase II (10/5)</td>
<td>Inclusions: age between 18 and 55 years; stable body weight (body-weight change&lt;5 kg for 3 months before screening); BMI between 29 and 41kg/m²; without associated co-morbidities (hypertension 140/90mmHg or more, obstructive sleep apnoea, type 2 diabetes or CVD, or family history of dyslipidaemia); absence of pregnancy, breast-feeding or menopause (determined by the cessation of menstruation); no abnormal thyroid hormone levels; no immunocompromised conditions or anaemia; no use of vitamin and mineral supplementation within 6 months of screening; no use of medication affecting body weight, energy expenditure, or glucose control or antibiotic treatment for the last 3 months; no smoking, drug or alcohol (&gt;2 drinks/d) problem; no consumption of 5 or more cups of coffee/d (1250 ml/d). Participants with allergy to the ingredients in the study product and placebo or experiencing nausea, fever, vomiting, bloody diarrhoea or severe abdominal pain or currently participating or having had participated in another clinical trial during the last 6 months before the beginning of the study</td>
<td>24 weeks (+2 weeks of wash-out before the initiation of treatment); Two-phase intervention protocol: Phase 1: weight-loss period, supervised dietary restriction with or without probiotic LPR supplementation (12 weeks). Phase 2: weight maintenance with supervision of dietary habits without restriction during which LPR or placebo supplementation was continued (12 weeks). Lactobacillus rhamnosus CCM13724 (LPR), 152+108 mg capsules. The probiotic capsules contained a formulation consisting of 10 mg of a LPR powder, 300 mg of a mix of oligofructose and inulin (70:30, v/v) and 3 mg of magnesium stearate. Two capsules per day</td>
<td>Present</td>
</tr>
<tr>
<td>Zarrati 2013(^3)</td>
<td>Randomized double-blind, controlled clinical trial</td>
<td>Country: Iran; Tehran, Department of Nutrition in Tehran University of Medical Science, August 2011-June 2012</td>
<td>20 to 50 years old, male and female, obese and overweight, Sex (M/F): 24/51</td>
<td>Eligible (75), randomized(75), allocated for control group (25), loss of follow-up in intervention group (2)</td>
<td>Inclusions: 75 obese and overweight men and women, aged 20 to 50 years old, who were referred to the Department of Nutrition in Tehran University of Medical Science. Exclusions: clinical signs of acute inflammation, lactose intolerance, cow’s milk allergy, acute gastrointestinal disorders for 3 months before the study initiation, drug and alcohol consumption, diabetes mellitus, pregnancy, lactation, menstruation at the time of blood sampling, history of metabolic disorders, and autoimmune diseases, infectious disease during the study time and having a weight loss diet for 6 months before the study initiation. “Two of the participants became pregnant during the study period and thus were excluded from the trial” - we concluded that this two participants were excluded before randomization - as written in the flow diagram of patient recruitment and randomization process and in the results description.</td>
<td>8 week (+2 weeks of wash-out before the initiation of treatment). Probiotic yogurt was prepared with the starter cultures containing Streptococcus thermophilus + Lactobacillus bulgaricus, without the probiotics in investigation, 8 week, 200mL. Daily Yes. Yogurts had PH range of 4.3-4.5 and a fat content of 1.5%. Group 1 (n=25) who consumed regular yogurt as part of a low calorie diet [RLCD]</td>
<td>Low calorie diet-Yogurt prepared with the starter cultures containing Streptococcus thermophilus + Lactobacillus bulgaricus, without the probiotics in investigation, 8 week, 200mL. Daily Yes. Yogurts had PH range of 4.3-4.5 and a fat content of 1.5%. Group 1 (n=25) who consumed regular yogurt as part of a low calorie diet [RLCD]</td>
</tr>
</tbody>
</table>
Table 3
Data extracted from included non-human studies (14 experimental studies)

<table>
<thead>
<tr>
<th>Study source (journal, authors)</th>
<th>Study design; Country; Study groups</th>
<th>Participants</th>
<th>Probiotic</th>
<th>Control</th>
<th>Primary outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>An 2011&lt;sup&gt;19&lt;/sup&gt;</td>
<td>Experimental study; Country: Republic of Korea; Study groups: The rats were randomly selected and assigned to three groups (12 rats per group) according to the type of diet and test-material. Also tested/controlled the effect of a high-fat diet</td>
<td>Male Sprague-Dawley rats Purchased from Central Lab Animal Inc. (Korea). Animals characteristics: 3 weeks of age and fed a standard diet (5 weeks at the time of intervention); Sex (M/F): 36/0 N=36</td>
<td>5 weeks (+1 week of standard diet +1 week for acclimatizing to the respective diets), B. pseudocatenulatum spm 1204, B. longum spm 1205, and B. longum spm 1207, 10&lt;sup&gt;9&lt;/sup&gt; – 10&lt;sup&gt;10&lt;/sup&gt; CFU (0.2 ml); 1:1:1 ratio; daily Group 3 HFD-LAB</td>
<td>Present</td>
<td>Definition; Results: Global outcome; weight change assessment (unit); average weight/age ratio</td>
</tr>
<tr>
<td>Aroa 2012&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Experimental study; Country: United Kingdom; Study groups: The mice were randomly selected and assigned to two groups (12 rats per group) according to the type of diet and test-material</td>
<td>Male C57BL/6 mice Purchased from Charles River Japan Inc. (Yokohama, Japan). Animals characteristics: 6-8 weeks old; Sex (M/F): 24/0 N=24</td>
<td>8 weeks (+1 week of standard diet), L. acidophilus NCDC 15, 5x10&lt;sup&gt;6&lt;/sup&gt; – 9x10&lt;sup&gt;6&lt;/sup&gt; CFU/ml plus 21% high-fat diet, 6 hours during day-time</td>
<td>Present</td>
<td>Definition; Primary: Body weight, organ weight, fat weight, and food intake Secondary: Total Cholesterol, HDL-C, LDL-C, triglyceride, glucose, leptin, AST, ALT, a-amylase and lipase levels in serum; b-glucosidase, b-glucuronidase, and tryptophanase activities Results: Weight increase; 339.7±26.75 g in the probiotics group 349.1±29.14 g in the control-group (P&lt;0.05)</td>
</tr>
<tr>
<td>Clarke 2013&lt;sup&gt;27&lt;/sup&gt;</td>
<td>Experimental study; Country: Ireland; The mice were randomly selected and assigned to 5 groups (9-10 mice per group) according to the type of diet and test-material Group 1: High fat diet unsupplemented Group 2: High fat diet + Glycopeptide antibiotic vancomycin Group 5: Low fat diet. Also tested the administration of glycopeptide antibiotic vancomycin and controlled the effect of a high fat diet</td>
<td>Male C57BL/6 mice Purchased from Harlan, UK Animals characteristics: 7 weeks of age and fed a standard diet (19 weeks at the time of intervention); Sex (M/F): 45-50/0 N=45-50</td>
<td>8 weeks (+12 weeks of low fat (lean mice) or high fat (diet-induced obese mice) diet), L. salivarius UCC118 Group 3: High fat diet + Bacteriocin producing (Bac+) L. salivarius UCC118</td>
<td>Present</td>
<td>Definition; Primary outcomes: Intestinal microbiota modulation on the progression of obesity under high-fat diet (Whole-body MRI and H magnetic resonance spectroscopy; Adipocyte cell size and number.) Results: Weight increase; No significant weight differences</td>
</tr>
<tr>
<td>Kang 2010&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Experimental study; Country: Republic of Korea; Study groups: The rats were randomly selected and assigned to two groups - control with PBS alone</td>
<td>Female Sprague-Dawley rats Purchased from Koatech Animal Inc. (Pyeongtaek, South Korea) Animals characteristics: (SD) rats; 135 g, 6 weeks of age and fed a standard diet Sex (M/F): 20/0 N=20</td>
<td>12 weeks, Lactobacillus gasseri BNR17 isolated from human breast milk BNR17 suspensions (final, concentration of 10&lt;sup&gt;9&lt;/sup&gt; CFU/0.5 ml) prepared daily in sterilized phosphate-buffered saline. The BNR17 group was administered the BNR17 preparation orally twice a day, while the control group was administered PBS</td>
<td>Present</td>
<td>Definition: Weight gain and microbiota changes (total bacterial numbers in the gut and variation in microbial populations) Results: ΔWeight (g); No significant Differences after week 4 - There is a significant reduction in weight gain in DIO mice at intervention weeks 2-4 (early intervention period) in the Bac&lt;sup&gt;+&lt;/sup&gt; intervention, when compared to Bac- intervention, but this does not persist with time</td>
</tr>
</tbody>
</table>

**Definition:**

- **Primary outcome**
  - Weight increase;
  - No significant weight differences

**Secondary outcomes**

- Total Cholesterol, HDL-C, LDL-C, triglyceride, glucose, leptin, AST, ALT, a-amylase and lipase levels in serum;
- b-glucosidase, b-glucuronidase, and tryptophanase activities

**Results**

- Weight increase; 339.7±26.75 g in the probiotics group 349.1±29.14 g in the control-group (P<0.05)

**Primary:**

- Body weight, organ weight, fat weight, and food intake

**Secondary:**

- Total Cholesterol, HDL-C, LDL-C, triglyceride, glucose, leptin, AST, ALT, a-amylase and lipase levels in serum;
- b-glucosidase, b-glucuronidase, and tryptophanase activities

**Results**

- Weight increase; 339.7±26.75 g in the probiotics group 349.1±29.14 g in the control-group (P<0.05)
Table 3 (cont.)

<table>
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<tr>
<th>Publication</th>
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<th>Probiotic</th>
<th>Control</th>
<th>Primary outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kang 2013</td>
<td>Experimental study; Country: Republic of Korea</td>
<td>Study groups: The mice were randomly selected and assigned to four groups (8 mice per group) according to the type of diet and test-material. Also tested/controlled the effect of a high-sucrose diet</td>
<td>Male C57BL/6j mice (Purchased from Central Lab Animal Inc. (Seoul, South Korea))</td>
<td>10 weeks, L. gasseri BNR17 was prepared fresh daily and orally administered twice per day, (100 or 1000 CFU) Group 3: high-sucrose diet and BNR17 10^9 CFU (HSD+BNR17 hand) Group 4: high-sucrose diet and BNR17 10^9 CFU (HSD+BNR17)</td>
<td>Present Normal diet and high-sucrose diet without BNR17 Group 1: normal diet (ND) Group 2: high sucrose diet (HSD)</td>
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<tr>
<td>Kim 2013</td>
<td>Experimental study; Country: Republic of Korea; Study groups: The mice were randomly selected and assigned to two groups, each with two subgroups (7-8 mice per group) according to the type of diet and test-material. Also tested/controlled the effect of a high-fat diet</td>
<td>Male C57BL/6j mice (Purchased from Hycosan Bioscience (Daegu, Korea))</td>
<td>13 weeks (+1 week of standard diet) Lactobacillus rhamnosus GG (LGG) 1×10^9 CFU per mouse per day Group 1B: normal diet + LGG Group 2B: high-fat diet + LGG</td>
<td>Present Normal diet group with PBS or the high-fat diet group with PBS Group 1A: normal diet with PBS Group 2A: high-fat diet with PBS</td>
<td>Definition: Body weight gain, fat accumulation, food intake, food efficiency ratio (FER) and mRNA expression of obesity-related genes in diet-induced obese mice Results: Final body weight (g): ND: 27.62±1.77 HSD: 30.59±1.46 (P&lt;0.01 versus the ND group) HSD+BNR17(9): 27.58±1.93 (P&lt;0.01 versus the HSD group) HSD+BNR17: 28.35±0.93 (p&lt;0.05 versus the HSD group)</td>
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<tr>
<td>Miyoshi 2014</td>
<td>Experimental study; Country: Japan</td>
<td>Study groups: The mice were randomly selected and assigned to three groups, each with two subgroups (10 mice per group) according to the type of diet and test-material. Also tested/controlled the effect of a 10%-fat diet</td>
<td>Male C57BL/6 J mice (weaned at 3 weeks of age) (Purchased from Charles River Japan Inc. (Yokohama, Japan))</td>
<td>24 weeks Lactobacillus gasseri SBT2055 0.5% of LG2055 cells (5×10^9 CFU/g) was added to the diet of the 10%-fat-LG group Group 3: 10% fat containing the probiotic LG2055 (10% fat-LG)</td>
<td>The groups fed with a 5%-fat diet and with a 10%-fat diet without probiotic 24 weeks Group 1: 5%-fat diet (5% fat) Group 2: 10%-fat diet (10% fat)</td>
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<td>Park 2013</td>
<td>Experimental study; Country: Republic of Korea; Study groups: The mice were randomly selected and assigned to two groups, Normal diet (9 mice) and High fat diet (27 mice), the last group was assigned to two subgroups according to the type of diet and test-material. Also tested/controlled the effect of a high-fat diet</td>
<td>Male C57BL/6j mice (Purchased from Jackson Laboratories (USA))</td>
<td>10 weeks (+1 week for acclimatization of the respective diets + 8 weeks to induce obesity), Lactobacillus curvatus HY7601 and Lactobacillus plantarum KY103 5×10^9 CFU/day each - 27 mice with induced high fat diet for 8 weeks) Sex (M/F): 36/0 N=36</td>
<td>Present PBS without probiotic and non-obese control Group HFD-placebo (PBS) (9 mice) Reference group (9 mice) - sacrificed to determine the accumulation of adipose tissue depot in diet induced obese mice after 8 weeks before probiotic treatment</td>
<td>Definition: Body weight gain, fat accumulation, food intake, food efficiency ratio (FER) and gene expression (mRNA) relating to inflammation in the adipose tissue and lipid metabolism in the liver Results: Final body weight (g): 5% fat: 31.4±2.25 10% fat: 39.15±2.18 10% fat-LG: 35.48±2.26 P&lt;0.05 between the three groups</td>
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<td>Park 2014</td>
<td>Experimental study; Country: Republic of Korea; Study groups: The mice were randomly selected and assigned to four groups, each with two subgroups (10 mice per group) according to the type of diet and test-material. Also tested/controlled the effect of a high-fat diet</td>
<td>Male C57BL/6j mice (Purchased from Charles River Japan Inc. (Yokohama, Japan))</td>
<td>12 weeks (+1 week of standard diet) Lactobacillus plantarum LG42, Group 3: High fat, low dose 1×10^6 CFU (LGLAB) Group 4: High fat, high dose 1×10^8 CFU (HGLAB) Every morning</td>
<td>Present PBS without probiotic and non-obese control Group HFD-placebo (PBS) (9 mice) Reference group (9 mice) - sacrificed to determine the accumulation of adipose tissue depot in diet induced obese mice after 8 weeks before probiotic treatment</td>
<td>Definition: Body weight gain, fat accumulation, food intake, food efficiency ratio (FER), lipids, insulin and leptin serum levels, carnitine serum and liver levels, and mRNA expression of obesity-related genes in diet-induced obese mice Results: Total weight gain (g) at 8 weeks following probiotic treatment, FD-induced body weight gain was 18 percent lower (0.01) in the probiotic group (8.53±0.20g) than in the HFD+placebo group (13.75±1.07 g). After 10 weeks, the average body weight was 11 percent lower (p&lt;0.05) in the HFD+probiotic group compared to the HFD+placebo group</td>
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<table>
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<tr>
<th>Publication</th>
<th>Methods</th>
<th>Participants</th>
<th>Probiotic</th>
<th>Control</th>
<th>Primary outcome</th>
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<tr>
<td>Salaj 2013[8]</td>
<td>Experimental study; Country:</td>
<td>Male and Female Sprague Dawley albino rats, purchased from Central Vивarium,</td>
<td>10 weeks, Lactobacillus plantarum</td>
<td>Present</td>
<td>Definition: Body weight gain, fat accumulation,</td>
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<td>Slovakia; Study groups: The</td>
<td>(Medical Faculty, P. J. Safirik University, Kêsice, Slovakia)</td>
<td>L5/07 and Lactobacillus plantarum Biocenol LP96</td>
<td>Group 1 (C): control group fed conventional laboratory diet (CLD)</td>
<td>fat intake, food efficiency ratio (FER), lipid profiles (seric, hepatic and fecal levels) and activity of beta-glucuronidase</td>
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<td>rats were randomly selected</td>
<td>Animals characteristics: 12 weeks of age and fed a standard diet Sex (M/F):</td>
<td>Groups:</td>
<td>Group 2 (HFD): high fat diet (CLD supplemented with 20% lard)</td>
<td>Results: No significant weight changes</td>
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<td>and assigned to four groups (10 rats per group; 5 male and 5 female)</td>
<td>20/20 N=40</td>
<td>Group 3 (LP1): high fat diet (CLD supplemented with 20% lard) + Lactobacillus plantarum L5/07, 3×10^9 CFU</td>
<td>Group 4 (LPD): high fat diet (CLD supplemented with 20% lard) + Lactobacillus plantarum Biocenol LP96 3×10^9 CFU</td>
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<td>according to the type of diet and test-material. Also tested/controlled the effect of a high-fat diet</td>
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<td>Every day</td>
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<td>Takemura 2010[9]</td>
<td>Experimental study; Country:</td>
<td>Female C57BL6 mice Animals characteristics: 3 weeks of age and fed a standard diet Sex (M/F): 0/20-24 N=20-24</td>
<td>11 weeks, Lactobacillus plantarum strain No. 14 (LP14) Group 2: Normal Fat Diet + LP14 Group 4: High Fat Diet + LP14</td>
<td>Present Control Group Fed with PBS containing branched dextrin (vehicle) Group 1: Normal Fat Diet Group 3: High Fat Diet</td>
<td>Definition and Results: Body weight gain (g) NFD-Control: 4.1±0.5 NFD-LP14: 4.7±0.4 P=0.05 HFD-Control: 7.5±1.3 HFD-LP14: 7.7±0.7 P=0.05</td>
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<tr>
<td>Republic of Korea; Study groups: The mice were randomly selected and assigned to six groups (8 rat per group) according to the type of diet and test-material. Also tested/controlled the effect of a high-fat diet</td>
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<td>Yin 2010[10]</td>
<td>Experimental study; Country:</td>
<td>High-fat diet induced Male Sprague-Dawley rats purchased from Sllacas Lab Animal Ltd (Shanghai, China), weighing 50-70 g Animals characteristics: 3 weeks of age, inbred-specific, pathogen-free, and fed a standard diet (4 weeks at the time of intervention) Sex (M/F): 48/0 N=48</td>
<td>6 weeks (+1 week for acclimatizing to the respective diets), Bifidobacteria (L66-5, L75-4, M13-4, F531-12) 1×10^10 CFU/ml concentration in neutral saline, and 0.4 ml bacterial solution was administered to each rat by intragastric gavage once a day Group 3: HFD + B. L66-5 Group 4: HFD + B. L75-4 Group 5: HFD + B. M13-4 Group 6: HFD + B. F531-12</td>
<td>Present Control groups where given a 0.9% saline by intragastric gavage Group 1: Control group Group 2: high-fat diet (HFD)</td>
<td>Definition: Body weight (BW), obese indexes, oral glucose tolerance test, serum and liver lipid and serum insulin Results: B. M13-4 improved BW gains (264±26.91 vs 212.55±18.54; p=0.001) L66-5 decreased BW gains (188.47±11.96 vs 212.55±18.54; p=0.043) The other two strains had no significant change in BW</td>
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<td>China; Study groups: The rats were randomly selected and assigned to six groups (8 rat per group) according to the type of diet and test-material. Also studied the effect of rosiglitazone</td>
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<tr>
<td>Yun 2009[11]</td>
<td>Experimental study; Country:</td>
<td>Male C57BL/6KSJ/db/db mice Purchased from SLC(Japan) Animals characteristics: 6 weeks of age, inbred-specific, pathogen-free, and fed a standard diet 6 weeks old Sex (M/F): 48/0 N=48</td>
<td>12 weeks, L. gasseri BN17, Group 3: BN17 10^10 CFU/day Group 4: BN17 10^10 CFU/day Group 5: BN17 10^10 CFU/day Group 6: BN17 10^10 CFU/day in 0.3 mL PBS, orally twice a day</td>
<td>Present PBS without probiotic Group 1: control group (excipient suspension in PBS) Other intervention: Group 2: rosiglitazone group (8 mg/kg)</td>
<td>Definition: Body weight gain, fat accumulation, food intake, food efficiency ratio (FER) and lipid profiles Results: Body weight gain (g/day) Control: 0.21±0.09 10^1: 0.22±0.06 10^2: 0.20±0.09 10^3: 0.23±0.08 10^4: 0.25±0.07</td>
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<tr>
<td>Republic of Korea; Study groups: The mice were randomly selected and assigned to six groups (8 rat per group) according to the type of diet and test-material. Also studied the effect of rosiglitazone</td>
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<td>Zhao 2012[12]</td>
<td>Experimental study; Country:</td>
<td>Male C57BL/6j (Sj) SPF mice, high fat diet-induced obese Purchased from Clea Japan Animals characteristics: 6-7 weeks of age and fed a standard diet (12 weeks at the time of intervention) Sex (M/F): 34/0 N=34</td>
<td>8 weeks (+6 week of obesity induction diet) Pedicoccus pentosaceus LP28 with either a high fat diet (LP28-HFD) or a regular diet (LP28-RE), 3×10^10 CFU/day Group 3: HFD + (LP28) Group 4: HFD + (LP28 + SN13T) Group 6: HFD + (RD + LP28) Group 7: HFD + (RD + SN13T)</td>
<td>Present Group 1: RD + RD Group 2: HFD + HFD Group 5: HFD + RD</td>
<td>Definition: Body weight gain, fat accumulation, visceral fat accumulation, plasma lipids, hepatic lipid contents, and the expression of some lipid metabolism-related genes in a high fat diet (HFD)-induced mouse model. Results: Total body weight gain (g) LP28-HFD: 6.4±0.9 Control-HFD: 10.8±1.0 P&lt;0.05 LP28-RD: 0.2±0.8 Control-RD: 11±1.0 P=0.05 LP28 reduced body weight gain and liver lipid contents (triglyceride and cholesterol), in mice fed a high fat diet for 8 weeks (40%, 54%, and 70% less than those of the control group without LAB, and P=0.018, P&lt;0.01, and P=0.021, respectively), whereas SN13T and the heat treated LP28 at 121uC for 15 min were ineffective</td>
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weight loss, with a standardized mean difference of –0.35 kg in the probiotic treatment group (95% CI –0.82 to –0.19) for the subgroup analysis of female participants. In the all gender participants analysis, there was no significant difference between various probiotics and the control group (SMD –0.25, 95% CI –0.73 to 0.23), with results demonstrating considerable levels of heterogeneity (\( \tau^2 = 0.13; \ Chi^2 = 7.22, df=2 (P\ value=0.03); I^2=72% \)).

In this review, it is not sensible to perform any other meta-analysis or to present forest plots due to the very large difference in presentation of outcomes, the variability of the species and strains used, without reproduction in a consistent stratified results related to weight change and due the limited number of studies involved.

**Discussion**

Probiotic effect in body weight is not only species specific, but also strain specific. The 4 clinical trials and 14 experimental used different species, different combinations, had different study durations and, particularly, the experimental studies used different species.
Regarding other probiotic that showed promising results, *L. rhamnosus* GG fed mice with a high fat diet showed reduced weight when compared to high fat diet animals without this probiotic. The same did not happen when animals were fed a normal diet, having instead non-significant results. Although we included 14 experimental studies, the animals species in our analysis, thus the power to detect the trend of overall effect was limited and a chance finding cannot be excluded. Also, participants. Outcomes were not reported in a padronized measure, not allowing to perform other meta-analysis.

Regarding other probiotic that showed promising results, *L. rhamnosus* GG fed mice with a high fat diet showed reduced weight when compared to high fat diet animals without this probiotic. The same did not happen when animals were fed a normal diet, having instead non-significant results. *L. gasseri* SBT2055 was effective in preventing weight gain in 10% fat diet-fed mice. Simultaneous feeding of animals with *L. curvatus* HY7601 and *L. plantarum* KY103 also resulted in a reduced weight gain. *L. plantarum* LG24 fed mice had a smaller weight gain than control animals, with the weight gain being smaller with higher doses of this probiotic. Finally, *Lactobacillus* L66–5 strain also showed a significant body weight decreasing power.

Overall most attempts to use probiotics to reduce body weight, or to slow body weight gain did not have the desired results. Even though several Lactobacillus strains have been studied and some have results that support a possible beneficial weight reducing capability, with only *L. rhamnosus* CGMCC 1.3724 at the moment having positive results in humans.

Probiotics beneficial mechanism in host metabolism is explored Kim 2013, where authors propose that LGG (the probiotic strain used) sensitizes insulin action is co-mediated by an enhanced adiponectin production and AMPK activation and at least in part associated with increased expressions of GLUT4, and lipid oxidative genes in responsible tissues, such as adipose tissue, skeletal muscle and the liver and in Kang 2013, consistent with the hypothesis put forward in Kim 2013, reported that the anti-obesity effect of BNR17 is responsible for the increased expression of fatty acid metabolism-related genes rather than reduced fatty acid synthesis and fat intake in the liver.

It is relevant to mention that there is evidence that the presence of two different bacterial species modifies the mutual metabolic activity. This aspect must be taken in consideration during the establishment of the species that will integrate a probiotic. We emphasize, concerning this topic, the results of Arora 2012, in which the lactobacilli administration promoted an increase of colonization by *Bifidobacterium*, which other studies have demonstrated to be positively correlated with improved metabolic profile and normalization of inflammatory tone.

Prebiotic carbohydrates enhance the survival in the gastric (low pH) and duodenal (bile salts) conditions and proliferation of probiotics, being an useful tool to ensure the presence proliferation in the gut in the desired quantities.

**Potential biases in the review process**

Owing to the limited number of studies involved, it was impossible to detect publication bias. Only four RCTs met the inclusion criteria in our analysis, thus the power to detect the trend of overall effect in humans was limited and a chance finding cannot be excluded. Also, although we included 14 experimental studies, the animals species and the mode of probiotic administration and the specific strains used
Table 6

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<tr>
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<th>Receiving probiotics</th>
<th>Not receiving probiotics</th>
<th>Std. Mean Difference</th>
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<td>Mean</td>
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<td>Jung 2013</td>
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<td>Sanchez 2014 (24 weeks)</td>
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<td>Subtotal (95% CI)</td>
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<td>Heterogeneity: Tau²=0.13; Chi²=7.22, df=2 (P=0.03); #I²=72%</td>
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Test for overall effect: Z=3.17 (P=0.002)**

Female subjects only

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<tr>
<th></th>
<th>Receiving probiotics</th>
<th>Not receiving probiotics</th>
<th>Std. Mean Difference</th>
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<td>Sanchez 2014 (24 weeks)</td>
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Test for overall effect: Z=3.17 (P=0.002)**

Mean = Mean between baseline HDL and post-intervention HDL. Sanchez 2014 (12) in mmol/L – we converted it in mg/dL – we converted it in mmol/L. SD = standard deviation. Heterogeneity was assessed using the Cochran’s Q statistic with a P value ≤ 0.1 interpreted as statistically significant (**). I² statistics was calculated, cut-off for considerable heterogeneity = 50% (§). Standard mean difference was used as effect measure, Confidence interval (95%CI) calculated using inverse variance method and random effects model, not including 0-zero was interpreted as statistically significant (*). Test for overall effect was interpreted as statistically significant (**)
Author contributions

Study concept and design: Rouxinol-Dias, AL. Acquisition of data: Pinto, AR. Analysis and interpretation of data: Rouxinol-Dias, AL, Pinto, AR, Rodrigues, D, Dias, J and Moreira, M. Drafting of the manuscript: Rouxinol-Dias, AL, Pinto, AR, Janeiro, C, Rodrigues, D, Dias, J, Moreira, M, Pereira, P. Critical revision of the manuscript for important intellectual content: Rouxinol-Dias, AL and Dias, J. Statistical analysis: Rouxinol-Dias, AL.

Study supervision: Rouxinol-Dias, AL.

Competing interest statement

All authors have completed the Unified Competing Interest form at http://www.icmje.org/downloads/coi_disclosure.pdf and declare that 1 - no authors have support from probiotics companies for the submitted work; 2 - no authors have relationships with probiotics companies that might have an interest in the submitted work in the previous 3 years; 3 - their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and 4 - no authors have non-financial interests that may be relevant to the submitted work.

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References